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 today 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 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 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 the American Urology Association, Adams Lectureship. And most recently, and I think a year or so ago,
00:01:37.680 --> 00:01:39.822 election to the National Academy of Medicine and David has really been an incredibly engaged member of our Cancer Center faculty.

00:01:42.027 --> 00:01:43.812 I think David’s leadership, has advanced the cause of our brain tumor program, among other things, David thank you for making the time to share your work with us today.

00:01:55.722 --> 00:01:58.137 to share your work with us today. to present some new unpublished data.

00:02:00.846 --> 00:02:03.870 It’s really a pleasure to be here. And let me turn this on and.

00:02:08.049 --> 00:02:11.786 My cell phone, so I’d like to do today

00:02:14.544 is to present some new unpublished data.
00:02:14.544 --> 00:02:18.541 work which really epitomizes to me of
NOTE Confidence: 0.9125635
00:02:18.541 --> 00:02:21.134 physician scientists of learning from
NOTE Confidence: 0.9125635
00:02:21.134 --> 00:02:24.472 the patient and just in a nutshell,
NOTE Confidence: 0.9125635
00:02:24.472 --> 00:02:27.636 what I’m going to show you is
NOTE Confidence: 0.9125635
00:02:27.636 --> 00:02:29.350 very fundamental question,
NOTE Confidence: 0.9125635
00:02:29.350 --> 00:02:32.416 which is what induces the checkpoint
NOTE Confidence: 0.9125635
00:02:32.416 --> 00:02:35.640 inhibitors particular PD one Tim three lag,
NOTE Confidence: 0.9125635
00:02:35.640 --> 00:02:38.916 3 digit on human T cells.
NOTE Confidence: 0.9125635
00:02:38.920 --> 00:02:40.690 And that's gonna be the nature
NOTE Confidence: 0.9125635
00:02:40.690 --> 00:02:43.118 of the talk that the work has
NOTE Confidence: 0.9125635
00:02:43.118 --> 00:02:44.694 been submitted for publication.
NOTE Confidence: 0.9125635
00:02:44.700 --> 00:02:45.960 It was put online,
NOTE Confidence: 0.9125635
00:02:45.960 --> 00:02:48.284 a bio RX being one’s interest in
NOTE Confidence: 0.9125635
00:02:48.284 --> 00:02:50.480 seeing the paper itself and upfront.
NOTE Confidence: 0.9125635
00:02:50.480 --> 00:02:52.860 I want to really, now Stamos Amita,
NOTE Confidence: 0.9125635
00:02:52.860 --> 00:02:54.220 who really really performed
this work in our laboratory tone
was now an assistant professor
and then pursuing this work.
It wanted knowledge.
My long term collaborator, Vijay Kutru.
Yes, you see a Yale,
a sticker that he was here
helping us recruit students.
Don't tell the people in Boston.
Enjoy dulberg in the Softmod
who did the computational work.
So the question is,
what are the regulatory mechanism
for induction of a Co inhibitory
receptors on human T cells?
But I'll show you is surprisingly type one, interferons induce territory receptors on human T cells, 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 then we identified an in vivo model where abara load strongly correlate's. 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
induces T cell exhaustion. Really first identified by Rafi Ahmed in the HIV system and in CMV infection and that’s associated with expression and Co inhibitory receptors such as PD. One Tim, three lag. Three antigen is interferon signature with the LC MP model suggesting that there may be an Association with type one interferons and these cone hitori molecules so wish to ask do they induce these receptors again here’s why I showed you in terms of mouse. An you know first experiments and when
I googled in 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 and 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 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
NOTE Confidence: 0.7671486
00:07:47.928 --> 00:07:49.107 type One interferon.
NOTE Confidence: 0.7671486
00:07:49.110 --> 00:07:51.385 So these data show that in humans
NOTE Confidence: 0.7671486
00:07:51.385 --> 00:07:53.256 there are two modules regulated
NOTE Confidence: 0.7671486
00:07:53.256 --> 00:07:55.301 by interferon that in fact
NOTE Confidence: 0.7671486
00:07:55.301 --> 00:07:57.360 go in opposite directions.
NOTE Confidence: 0.7671486
00:07:57.360 --> 00:07:58.539 Here’s a kinetex.
NOTE Confidence: 0.7671486
00:07:58.539 --> 00:08:01.290 Overtime the induction of Tim three lag,
NOTE Confidence: 0.7671486
00:08:01.290 --> 00:08:02.074 three PD,
NOTE Confidence: 0.7671486
00:08:02.074 --> 00:08:04.426 one with the decrease in digit.
NOTE Confidence: 0.787109
00:08:06.930 --> 00:08:09.090 So just take a step back.
NOTE Confidence: 0.787109
00:08:09.090 --> 00:08:11.970 Why do we have an interest in Tidjane?
NOTE Confidence: 0.787109
00:08:11.970 --> 00:08:14.202 I mention this because under the
NOTE Confidence: 0.787109
00:08:14.202 --> 00:08:16.093 leadership of Antonio Mora we’re
NOTE Confidence: 0.787109
00:08:16.093 --> 00:08:18.431 about to embark upon a phase one
NOTE Confidence: 0.787109
00:08:18.431 --> 00:08:20.250 clinical trial in patients with
NOTE Confidence: 0.787109
glioblastoma with anti TIGIT or anti PD.

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

So why an interest in 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 RRC expression. And one can see that with anti TIGIT antibody there’s a market loss of these cytokines in culture and if you
00:09:00.345 --> 00:09:03.467 knock down ticket here within SHR Now
00:09:03.467 --> 00:09:05.848 you have market increases engagement
00:09:05.848 --> 00:09:08.824 affair on and decreases dial 10.
00:09:08.830 --> 00:09:10.750 So comparing PD one antigen,
00:09:10.750 --> 00:09:13.249 our hands in human systems been very
00:09:13.249 --> 00:09:15.993 impressed with the effects of ticket and
00:09:15.993 --> 00:09:18.405 also just comparing Ms two glioblastoma,
00:09:18.410 --> 00:09:21.063 there really isn’t a big difference between
00:09:21.063 --> 00:09:24.529 PDL one or PD1 between Ms and brain tumors,
00:09:24.530 --> 00:09:26.828 but there is a virtual absolute
00:09:26.828 --> 00:09:28.360 difference between TIGIT expression,
00:09:28.360 --> 00:09:31.224 typically on the CD 8 cells in patients
00:09:31.224 --> 00:09:33.727 with GBM virtually absent in Ms,
00:09:33.730 --> 00:09:35.944 he was looking at teacher by
00:09:35.944 --> 00:09:37.940 flow and tills versus blood,
00:09:37.940 --> 00:09:40.496 suggesting the potential importance of digit.
NOTE Confidence: 0.787109
00:09:40.500 --> 00:09:42.464 In the central nervous
NOTE Confidence: 0.787109
00:09:42.464 --> 00:09:43.937 system for glioblastoma.
NOTE Confidence: 0.787109
00:09:43.940 --> 00:09:46.220 So first one to work through.
NOTE Confidence: 0.787109
00:09:46.220 --> 00:09:48.445 After that identification of the
NOTE Confidence: 0.787109
00:09:48.445 --> 00:09:50.670 effect of type One interferons
NOTE Confidence: 0.787109
00:09:50.747 --> 00:09:54.261 wanted to work through the in vitro
NOTE Confidence: 0.787109
00:09:54.261 --> 00:09:55.767 transcriptional regulatory network.
NOTE Confidence: 0.787109
00:09:55.770 --> 00:09:58.380 So we use the same model
NOTE Confidence: 0.787109
00:09:58.380 --> 00:10:00.120 that would be regift.
NOTE Confidence: 0.787109
00:10:00.120 --> 00:10:02.856 Near Youssef used in terms of setting up
NOTE Confidence: 0.787109
00:10:02.856 --> 00:10:05.130 identifying the TH17A regulatory network,
NOTE Confidence: 0.787109
00:10:05.130 --> 00:10:09.360 and this is work done by a soft in BJ’s lab,
NOTE Confidence: 0.787109
00:10:09.360 --> 00:10:12.820 so we needed to have higher
NOTE Confidence: 0.787109
00:10:12.878 --> 00:10:14.750 resolution transcriptomic data to
NOTE Confidence: 0.787109
00:10:14.770 --> 00:10:16.547 construct the regulatory network.
NOTE Confidence: 0.787109

17
For those of you who aren’t engaging in terms of looking at RNA now, what we used to do is to take a T cell stimulate, measure the RNA 4 hours later and say this is what it is. We’ve learned that their complex regulatory networks and one needs to really do this. The kinetics overtime to construct a dynamic regulatory network. Such a performance. This network we took dive CD4 CD8 cells, stimulate them, measure them in different time 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, transcription factors induced 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 CD8 T cells, and we as a reality check we asked of these word known. Interferon responsive gene. So here’s the IFN responsive 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
00:13:10.274 --> 00:13:11.870 transcriptional for these
NOTE Confidence: 0.810376
00:13:11.968 --> 00:13:15.200 transcriptional factors in detail.
NOTE Confidence: 0.810376
00:13:15.200 --> 00:13:18.512 So in order to do this and presented dilemma,
NOTE Confidence: 0.810376
00:13:18.520 --> 00:13:20.260 we had to develop new technology
NOTE Confidence: 0.810376
00:13:20.260 --> 00:13:21.997 because I called the Heisenberg
NOTE Confidence: 0.810376
NOTE Confidence: 0.810376
00:13:24.060 --> 00:13:26.208 The process of examining the cell
NOTE Confidence: 0.810376
00:13:26.208 --> 00:13:28.120 with activation perturb the system.
NOTE Confidence: 0.810376
00:13:28.120 --> 00:13:30.376 Some of looking for an electron
NOTE Confidence: 0.810376
00:13:30.376 --> 00:13:32.170 after hitting it with HV.
NOTE Confidence: 0.810376
00:13:32.170 --> 00:13:34.550 So we had to develop a gene
NOTE Confidence: 0.810376
00:13:34.550 --> 00:13:36.879 knockdown the early time points and
NOTE Confidence: 0.810376
00:13:36.879 --> 00:13:38.939 primary T cell without activating
NOTE Confidence: 0.810376
00:13:38.939 --> 00:13:41.879 T cells and again this is all work
NOTE Confidence: 0.810376
00:13:41.879 --> 00:13:44.094 developed by Tomo by Thomas Anita.
NOTE Confidence: 0.810376
00:13:44.094 --> 00:13:46.656 We used an efficient lentiviral vectors
that developed by wearing a green.
And basically one takes a viral like particles V LP’s which is incorporated with TPX, which degrades Sam HD one, removes restrictions, you can transfecT cells with this Sam S1, which now allows transfection with SH RNA, HIV, HIV, lentivirus and all. This can be done in an activated T cells. Could knock down the gene and then do the the incubation. So here we have night CD. Or cells incubated without
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
So let me just say that again, so these are PCA plots.

We knock down each transcription factor and then looked at all the RNA expression and then put that into a principle component.

One in principle component, to what that revealed is that the interferon stimulated genes are positive. Regulated by we call interferon regulated module one, this modulator increased the downstream interferon.

Stimulated genes with module 2 represented 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

the global effects on interferon

thereby directly regulated by

different modules,

Co inhibitory receptors are also

regulated by interferon associate

regulated by interferon associate

transcription factors and which up

regulate 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
00:17:49.238 --> 00:17:51.030 one and three here.
00:17:51.030 --> 00:17:53.202 Positively regulate Tim three
00:17:53.202 --> 00:17:55.917 but negatively regulate PD one.
00:17:55.920 --> 00:17:58.194 So then we performed a hierarchical
00:17:58.194 --> 00:18:00.170 backbone network analysis transcription
00:18:00.170 --> 00:18:02.865 I’ll just go over this very briefly,
00:18:02.870 --> 00:18:05.180 but basically looked at gene expression,
00:18:05.180 --> 00:18:06.338 overtine, differential expression,
00:18:06.338 --> 00:18:07.496 protein, DNA bonding,
a transcription factor database

Those data looked at a rank list of transcription factors which we perturbed and knocked down as I showed you. Integrated those data into refine network model and what we found was at the early and intermediate network contain more upregulated transcription factors. And downregulated in contrast late network had more downregulated up, regulated transcription factors. Involves dominance of the up regulated transcription factors.
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 a 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.
An MIP are intermediate transcription factors and stat one hit 1A and T bet or bimodal transcription factors apart show this it just to get the bigger picture of the what nature does in terms of the biologic complexity of these systems. Abul Abbas would say to me, in Vivo Baratas and then in vitro maybe. So the challenge for us was to find an envy both system which replicate all this lovely in vitro data.
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 $R^2 \approx .9$ seven. 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 CD8 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 CD8 cells this column.

Here are the controls, stable and progressive patients.

So we see this module too.
Upregulated these are highly upregulated.

NOTE Confidence: 0.80999196

PD one Tim, three CTO for lag three.

NOTE Confidence: 0.80999196

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

NOTE Confidence: 0.80999196

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

NOTE Confidence: 0.80999196

So we had a extremely.

NOTE Confidence: 0.80999196

Could the recapitulation what we saw on in vitro.

NOTE Confidence: 0.80999196

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

NOTE Confidence: 0.80999196

Just to summarize,

NOTE Confidence: 0.80999196

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

NOTE Confidence: 0.80999196

whereas TIGIT Slam 6 and layer one all went down.

NOTE Confidence: 0.80999196

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, so this is the regulator which induces lag three and other Co inhibitory molecules while inhibiting.

00:23:19.338 --> 00:23:22.046 Going the opposite direction for ticket.

00:23:25.700 --> 00:23:27.954 And then we looked at the relationship between late faith covid for lag free, that BSL three instaff 3A positive.

00:23:30.050 --> 00:23:32.255 Tim three and PD one and found regulated flag 3 and 10 three.

00:23:32.255 --> 00:23:34.442 looking directly in patients to the SP140B cell three and stat three while elevated in COVID-19 cells, so we’re able to recapitulate what 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.
contributed various parts of this.

My long, long term collaborator, 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 what is clearly a very complex.
and this is sort of my concrete question, which is you know. Obviously you’re sorting through what’s driving expression of Tim. Three lag, three TIGIT and realizing that almost the Holy Grail today is what’s the next PD one? So does this work? Help us understand the relative merits of these targets and in the future of immuno oncology or give us some insight there. Great question. I think the short answer is probably not at one level. It gives us insight, 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
00:27:31.550 --> 00:27:33.618 a Vijay kept saying well Style 27.
00:27:33.620 --> 00:27:35.420 Can’t you find it kept saying?
00:27:35.420 --> 00:27:37.513 Well we keep looking and kept saying
00:27:37.513 --> 00:27:39.064 what you’re doing the experiment
00:27:39.064 --> 00:27:40.858 wrong and I didn’t show them
00:27:40.858 --> 00:27:44.321 we just couldn’t get it to work
00:27:44.321 --> 00:27:45.857 and then we explore different
00:27:45.857 --> 00:27:47.717 like going hit or molecules.
00:27:47.720 --> 00:27:49.616 And then it’s very simple observation
00:27:49.616 --> 00:27:51.200 and actually predicted based on
00:27:51.200 --> 00:27:52.520 all the viral immunology work.
00:27:53.270 --> 00:27:55.769 Yeah, thank you, Ann Habermann has a
00:27:55.769 --> 00:27:58.358 question which is how long does the
00:27:58.358 --> 00:28:00.536 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.

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 as well has really, I think. 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
Co stimulating the innate immune system to treat Melanoma.

So just a few fast facts about Melanoma, it’s a disease of the relatively young, most patients present between age 45 and 55. The incidence has been going up actually for decades already, so just by way of example, in 2003 there were around 54,000 new cases in the United States, and just a decade and a half later it was already up to 87,000. It’s now the fifth most common malignancy among men and the seventh among women, but fortunately most of our patients.
present with stage one disease, so stage one refers to diseases confined to the skin and is. Then stage two is confined to the skin and thicker stage three is disease. It's spread to the lymph nodes and stage four is distant dissemination. And that's essentially what kills patients. So we're really going to be talking about stage four disease today. So for mortality, Interestingly, it was going up as well. So for 2000 three 7600 deaths, So for 2017 ninety 700 deaths. But if you start tracking later on 2019, 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
years at approximately 5 or 7% of patients are still alive. Chemotherapy you actually see a similar kind of a figure, and we don’t think chemotherapy really prolongs survival. Maybe it’s just Natural History of disease that some people live for a long time. Now over here on the right you see the five year survival data from our flagship phase three study of epilim abalon versus nivolumab alone versus the combination thereof at where at five years you see 26% of patients are alive with EPI alone
00:33:14.156 --> 00:33:17.259 44% with anti PD one alone and 52%
00:33:17.260 --> 00:33:19.588 or maybe even higher than that.
00:33:19.590 --> 00:33:22.929 With the combination of the two drugs.
00:33:22.930 --> 00:33:24.610 So what we’re really trying to
00:33:24.610 --> 00:33:26.140 do in the Melanoma field,
00:33:26.140 --> 00:33:27.600 especially the drug development field,
00:33:27.600 --> 00:33:29.256 is to raise the tennis tail
00:33:29.256 --> 00:33:31.109 at the end of the curve.
00:33:31.110 --> 00:33:33.094 So this is a figure that I borrowed
00:33:33.094 --> 00:33:35.192 from one in Microsoft students, Irina,
00:33:35.192 --> 00:33:37.236 who I’ll mention as we go along,
00:33:37.240 --> 00:33:39.240 just showing that targeted
00:33:39.240 --> 00:33:39.277 therapy and chemotherapy.
00:33:39.280 --> 00:33:41.152 You’re very low down here with
00:33:41.152 --> 00:33:42.400 people in Malibu starting
to push up. We're pushing up further with Anti PD one even further with the combination. But really, what we need to do is to get new drugs and drug combinations, so hopefully in the next five years will have a five year survival of 80%. And eventually we'll reach 100%, and until then we still have employment. So what are the limitations of immunotherapy's, the Society of Immunotherapy or City? Which is the big society that Mario presides over recently formed a task force to define to provide 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
00:34:46.937 --> 00:34:48.772 when they’re on monotherapy maintenance,
NOTE Confidence: 0.83978784
00:34:48.780 --> 00:34:50.046 they then progress.
NOTE Confidence: 0.83978784
00:34:50.046 --> 00:34:53.000 Is that resistance to the combination or
NOTE Confidence: 0.83978784
00:34:53.076 --> 00:34:55.736 is that resistance to the monotherapy and
NOTE Confidence: 0.83978784
00:34:55.736 --> 00:34:58.779 all of these things need to be defined?
NOTE Confidence: 0.83978784
00:34:58.780 --> 00:35:00.880 And then how do we define regrowth
NOTE Confidence: 0.83978784
00:35:00.880 --> 00:35:02.290 after patient stops therapy?
NOTE Confidence: 0.83978784
00:35:02.290 --> 00:35:04.246 So we normally treat for a
NOTE Confidence: 0.83978784
00:35:04.246 --> 00:35:06.226 limited period of time being at
NOTE Confidence: 0.83978784
00:35:06.226 --> 00:35:08.347 one years one year or two years.
NOTE Confidence: 0.83978784
00:35:08.350 --> 00:35:10.576 However long we treat for specific disease,
NOTE Confidence: 0.83978784
00:35:10.580 --> 00:35:12.568 if a patient is in off therapy
NOTE Confidence: 0.83978784
00:35:12.568 --> 00:35:14.090 and then has regrowth,
NOTE Confidence: 0.83978784
00:35:14.090 --> 00:35:15.685 does that mean they’re actually
NOTE Confidence: 0.83978784
00:35:15.685 --> 00:35:17.280 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.
00:35:44.486 --> 00:35:45.926 this endeavour with concurrent
NOTE Confidence: 0.83978784
00:35:45.926 --> 00:35:47.366 with the clinical definitions,
NOTE Confidence: 0.83978784
00:35:47.370 --> 00:35:49.986 we really need to work on the science.
NOTE Confidence: 0.83978784
00:35:49.990 --> 00:35:50.620 So really,
NOTE Confidence: 0.83978784
00:35:50.620 --> 00:35:53.140 what I’m going to talk about mostly today
NOTE Confidence: 0.83978784
00:35:53.205 --> 00:35:55.564 is translation going back and forth.
NOTE Confidence: 0.83978784
00:35:55.570 --> 00:35:56.224 So what?
NOTE Confidence: 0.83978784
00:35:56.224 --> 00:35:57.859 Why do patients develop resistance?
NOTE Confidence: 0.83978784
00:35:57.860 --> 00:35:59.500 Or many many potential mechanisms
NOTE Confidence: 0.83978784
00:35:59.500 --> 00:36:01.140 of resistance have been described,
NOTE Confidence: 0.83978784
00:36:01.140 --> 00:36:02.019 and I think.
NOTE Confidence: 0.83978784
00:36:02.019 --> 00:36:04.397 some of the some of these
NOTE Confidence: 0.83978784
00:36:04.397 --> 00:36:06.773 tumors are just desert rumors,
NOTE Confidence: 0.83978784
00:36:06.780 --> 00:36:08.810 lack of till of tumor infiltrating
NOTE Confidence: 0.83978784
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 will cause resistance. 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. 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 examples are MD’s season T regs

which might need inhibition as well.

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 byref mutant and P tenancy. It’s exposed to radiation. You get some sensitivity to anti PD one, but ultimately with time these tumors grow out as well.
So the first question next to asked was what was actually in these in these tumors. All of this work was done by Kurt Perry, who's over here on the right. We can see his picture and he's actually one of the new fellows that match to. Our program will be very thrilled to have him as part of our medical oncology fellowship. So first question that they asked was what was the infiltrating cell type in these mass? In these mass melanomas? And it turns out that the predominant 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 just in a nutshell, the terms that have highlights 6, three like 6 E and low EF 480, or those that are more inflammatory.
in the ones on the right over here are those that are presumed to be more inhibitory.

So at that point they said, OK, we’ve got these terms. We need to try to modulate them, and there are many, many mechanisms for modulating terms. But the ones that they chose to work on with CD 40 agonism, and CSF, one R inhibition, and in the beginning they used a small molecule inhibitor.

So if you take these memory cells and implant them in mice,
00:39:57.410 --> 00:40:00.370 and you treat either with control vehicle or.
NOTE Confidence: 0.83383965
00:40:00.370 --> 00:40:01.554 The CD 40 agonist.
NOTE Confidence: 0.83383965
00:40:01.554 --> 00:40:03.330 You’ll see some some decrease in
NOTE Confidence: 0.83383965
00:40:03.395 --> 00:40:05.222 the size of the tumors with the
NOTE Confidence: 0.83383965
00:40:05.222 --> 00:40:07.521 CD 40 agonist if you give the CSF
NOTE Confidence: 0.83383965
00:40:07.521 --> 00:40:09.266 one receptor inhibitor you get a
NOTE Confidence: 0.83383965
00:40:09.266 --> 00:40:10.696 similar amount of tumor reduction.
NOTE Confidence: 0.83383965
00:40:10.700 --> 00:40:12.416 If you give the two together,
NOTE Confidence: 0.83383965
00:40:12.420 --> 00:40:13.458 you get synergism.
NOTE Confidence: 0.83383965
00:40:13.458 --> 00:40:17.150 As you can see by the red line over here.
NOTE Confidence: 0.83383965
00:40:17.150 --> 00:40:19.341 So to look back into the similar
NOTE Confidence: 0.83383965
00:40:19.341 --> 00:40:19.967 contour plots,
NOTE Confidence: 0.83383965
00:40:19.970 --> 00:40:22.308 what is the content of these different
NOTE Confidence: 0.83383965
00:40:22.308 --> 00:40:24.491 tumors within the mice treated in the
NOTE Confidence: 0.83383965
00:40:24.491 --> 00:40:26.857 graph over here on the left you can
NOTE Confidence: 0.83383965
00:40:26.857 --> 00:40:29.034 see that when you give doublet therapy,

69
the CD 40 agonist in the CSF

one receptor inhibitory,

the main difference is that you get

an increase in this little group over

here on the right in the upper corner,

which are like 60 high and in 480 low and are

presumed to be more inflammatory macrophages,

and that’s essentially

verified on the bar graph.

Over here on the left.

On the right,

at the bottom over here you can

see this to the changes in the in

the immune infiltrating content,

and I think what’s most interesting
over here is that when you give
the CD 40 agonist along with
the CSF one receptor inhibitor,
you do get an increase of
infiltration of T cells.
So possibly we might be able to make
desert those desert tumors more
inflamed by using a regimen such as this.
And in addition you get more
PD one high T cells.
So Catherine Miller Jensen on the main
campus is developed a technology for
single cell site eccentric creation,
and she looked at what the difference of
was between these different treatment
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.
00:42:06.740 --> 00:42:08.972 I'll 12 BIL 6 etc and the same
NOTE Confidence: 0.8104826
00:42:08.972 --> 00:42:11.598 for the panel of the side of kinds
NOTE Confidence: 0.8104826
00:42:11.598 --> 00:42:14.028 of the chemo kinds at the bottom.
NOTE Confidence: 0.8104826
00:42:14.030 --> 00:42:15.715 So essentially the doublet therapy
NOTE Confidence: 0.8104826
00:42:15.715 --> 00:42:17.767 over here is inducing quite quite
NOTE Confidence: 0.8104826
00:42:17.767 --> 00:42:19.307 vast changes in the animals.
NOTE Confidence: 0.8104826
00:42:19.310 --> 00:42:21.850 What does it do to the T cells?
NOTE Confidence: 0.8104826
00:42:21.850 --> 00:42:23.758 What else is important over here?
NOTE Confidence: 0.8104826
00:42:23.760 --> 00:42:26.048 What you see on this figure here is
NOTE Confidence: 0.8104826
00:42:26.048 --> 00:42:28.526 that when you give the doublet therapy,
NOTE Confidence: 0.8104826
00:42:28.530 --> 00:42:30.045 you can actually abrogate the
NOTE Confidence: 0.8104826
00:42:30.045 --> 00:42:32.284 effect if you give anti TNF Alpha
NOTE Confidence: 0.8104826
00:42:32.284 --> 00:42:33.616 or anti interferon gamma,
NOTE Confidence: 0.8104826
00:42:33.620 --> 00:42:35.205 again highlighting the the importance
NOTE Confidence: 0.8104826
00:42:35.205 --> 00:42:38.068 of the T cells in this process as well.
NOTE Confidence: 0.8104826
00:42:38.070 --> 00:42:40.198 So with that at the time we concluded
that CSF one receptor inhibitors in city
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
you’ll see a whole cast of trainees along the way over here.
So Bill 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, the small molecule inhibitor, but rather to move towards and Antibody because of precision of drugging our target. Also 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 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 or 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 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,
you have to have a clinical Pi and a basic science Pi and everything has to have a clinical trial so to go back to the bedside. What are we going to do with these data? So we formed collaborations with Bristol and a company called a passage and that makes a CD 40 agonist and we were able to get them to work together. The problem was that there was no phase one data for the triplet. Now could be oralism AB which is the CSF one receptor antibody and the volume Abbott being given to hundreds of patients in BM S LED studies in
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 done 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 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 40 agonist plus nevala mehrban. The patients had a complete response 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 his 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.

NOTE Confidence: 0.8301139

Ramza, kilogram.

NOTE Confidence: 0.8301139

We escalated the CD 40 agonist very gently,

NOTE Confidence: 0.8301139

so cohort one only had the doublet therapy

NOTE Confidence: 0.8301139

at a tenth of the recommended phase.

NOTE Confidence: 0.8301139

Two dose for the CD 40 agonist within

NOTE Confidence: 0.8301139

escalated by a half a log into cohort

NOTE Confidence: 0.8301139

three in Cohort 5 and concurrently

NOTE Confidence: 0.8301139

added the nevala map on with the goal

NOTE Confidence: 0.8301139

of ultimately reaching cohort six,

NOTE Confidence: 0.8301139

which would be 4 doses at the

NOTE Confidence: 0.8301139

record for of Cabrera.

NOTE Confidence: 0.8301139

Lismer,

NOTE Confidence: 0.8301139

the pic surgeon drug and nivolumab at the.

NOTE Confidence: 0.8301139

Same recommended phase.

NOTE Confidence: 0.8301139

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. 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
of a work in progress,

so we looked at cytokine panels before

and on treatments at 24 hours later,

and you can see nice increasing interferon

The different cohorts are listed over here,

but Code 5 and six are when we hit

them at the recommended phase,

two dose of deep excision drugs,

so that’s where you see most of the activity.

There are other changes in circulating

cytokines and I could spend an

hour just talking about this,

but I selected a few just just

to show you what we’re seeing,

so we’ve got the CL 2,
which is a side kind that’s primarily secreted by dendritic cells and macrophages.

Very high levels of the higher dose levels, same with. P.

10 and then the macrophage colony stimulating factor, also highest levels in Cohort 6 but clear increases.

Across the board, we do have the pretreatment and on treatment specimens show me jessel who supposed dark in my lab is busy analyzing these what you see over here is the basic analysis, so these are just this is just a
munificent staining a CD4 and CD8
before treatment and on treatments
on the left is pre and on the right
You can see an increase in the infiltration of the CD 8
There's also an increase of the CD Force which are in red.
there is much more dense than post treatment.
Increase in the amount of CD 68 on this particular patient,
but 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 that it may not come from this come from the same site, so the pretreatment was a 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
necessarily knock them off completely.

So when we do these experiments in the mice, we were seeing much better activity than the humans, and the question is why?

The dose is selected for the Marin experiments with somewhat random we go based on what is done by other researchers, what’s done by format and the amount that we were giving them was 200MG kilogram.

So we asked the question. Well, what happens if we give them more CSF? One receptor antibody and keep the other two drug steady?
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 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.
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 And 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, and this is our second trial which Kelly and Sarah worked with me to to write. So it’s a phase one study of the CD 40 agonist in combination with epilim urban, the volume app in Melanoma. So just to highlight some of the challenges of a study like this, we know that a polymer mabona volume
app toxicity rate of at least 6570%.

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 then enroll another 46 and at that 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, it does look better than the doublet.
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 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 CT A 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.
00:58:24.035 --> 00:58:25.175 experiment in the mouse.
NOTE Confidence: 0.8341199
00:58:25.180 --> 00:58:26.320 But that’s actually a
NOTE Confidence: 0.8341199
00:58:26.320 --> 00:58:28.030 really good next step to do.
NOTE Confidence: 0.8341199
00:58:28.030 --> 00:58:29.150 It’s a great thought.
NOTE Confidence: 0.8341199
00:58:29.150 --> 00:58:30.830 We should expose the mice to
NOTE Confidence: 0.8341199
00:58:30.893 --> 00:58:32.238 PD one inhibitors and then
NOTE Confidence: 0.8341199
00:58:32.238 --> 00:58:33.939 add on the other ones instead
NOTE Confidence: 0.8341199
00:58:33.939 --> 00:58:35.715 of giving all three up front.
NOTE Confidence: 0.81057096
00:58:36.410 --> 00:58:38.270 And this may be impossible,
NOTE Confidence: 0.81057096
00:58:38.270 --> 00:58:40.400 but is there any consideration of
NOTE Confidence: 0.81057096
00:58:40.400 --> 00:58:42.730 combining all four agents in previously?
NOTE Confidence: 0.81057096
00:58:42.730 --> 00:58:47.200 I mean that is a CSF one R CD40 anti CD L4,
NOTE Confidence: 0.81057096
00:58:47.200 --> 00:58:49.648 GTA 4 and PD one and I realized
NOTE Confidence: 0.81057096
00:58:49.648 --> 00:58:52.029 that’s a smorgasbord of agents,
NOTE Confidence: 0.81057096
00:58:52.030 --> 00:58:54.268 but is that a conceivable approach?
NOTE Confidence: 0.81057096
00:58:54.270 --> 00:58:56.496 We could, we just got it.
We can get through the 1st 3 first, so the CTA for CD for D and P1.

We're doing OK with toxicity. But we are only on the 1st dose level. 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.

So what we're trying to do now is to actually move it forward to the first line, that very last trial that I showed with the CTA for antibody. 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.