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 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 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 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 the American Urology Association, Adams Lectureship. And most recently, and I think a year or so ago,
NOTE Confidence: 0.879088
00:01:37.680 --> 00:01:39.822 election to the National Academy of
NOTE Confidence: 0.879088
00:01:39.822 --> 00:01:42.027 Medicine and and David has really
NOTE Confidence: 0.879088
00:01:42.027 --> 00:01:43.812 been an incredibly engaged member
NOTE Confidence: 0.879088
00:01:43.812 --> 00:01:45.870 of our Cancer Center faculty.
NOTE Confidence: 0.879088
00:01:45.870 --> 00:01:47.354 I think David’s leadership,
NOTE Confidence: 0.879088
00:01:47.354 --> 00:01:49.588 I think, has advanced the cause
NOTE Confidence: 0.879088
00:01:49.588 --> 00:01:51.448 of our brain tumor program,
NOTE Confidence: 0.879088
00:01:51.450 --> 00:01:52.605 among other things,
NOTE Confidence: 0.879088
00:01:52.605 --> 00:01:55.722 an David thank you for making the time
NOTE Confidence: 0.879088
00:01:55.722 --> 00:01:58.137 to share your work with us today.
NOTE Confidence: 0.9125635
00:01:59.550 --> 00:02:00.846 Thank you Charlie.
NOTE Confidence: 0.9125635
00:02:00.846 --> 00:02:03.870 It’s really a pleasure to be here.
NOTE Confidence: 0.9125635
00:02:03.870 --> 00:02:08.049 And let me turn this on and.
NOTE Confidence: 0.9125635
00:02:08.050 --> 00:02:11.786 My cell phone, so I’d like to do today
NOTE Confidence: 0.9125635
00:02:11.786 --> 00:02:14.544 is to present some new unpublished
NOTE Confidence: 0.9125635
work which really epitomizes to me of
physician scientists of learning from
the patient and 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.
And 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
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.

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 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,
NOTE Confidence: 0.9125635

three lag three and TIGIT on C4 and CD8

NOTE Confidence: 0.9125635

cells that their modulated together.

NOTE Confidence: 0.9125635

And that this is a new spot.

NOTE Confidence: 0.9125635

We have the induction of Tim 3

NOTE Confidence: 0.9125635

not so much PD one but lag three

NOTE Confidence: 0.9125635

and TIGIT by I'll 27 you knock

NOTE Confidence: 0.9125635

down aisle 27 the mouse you lose

NOTE Confidence: 0.9125635

the induction by aisle 27.

NOTE Confidence: 0.9125635

That’s the upregulation and

NOTE Confidence: 0.9125635

downregulation by the knock down.

NOTE Confidence: 0.9125635

Now it’s been known for a long time.

NOTE Confidence: 0.9125635

That type one interferon signatures,

NOTE Confidence: 0.9125635

or enriching chronic viral infection,

NOTE Confidence: 0.9125635

and both mouse and humans,

NOTE Confidence: 0.9125635

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 with the LC MP model suggesting that there may be an Association with type one interferons and these cone hitori

00:05:22.870 --> 00:05:24.542 One Tim, three lag.

00:05:24.542 --> 00:05:26.632 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

00:05:26.632 --> 00:05:29.024 molecules so wish to ask do they induce these receptors again here’s why I showed you in terms of mouse.

00:05:29.024 --> 00:05:31.757 An you know first experiments and when

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

00:05:34.547 --> 00:05:37.828 molecules so wish to ask do they induce these receptors again here’s why I showed you in terms of mouse.
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 this. Here’s how the experiments were done. So now we go into more depth to show this. By interference. 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 4%.

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
type 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,

three PD, 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.

NOTE Confidence: 0.787109

One or a combination of the two, working with Jemal eternal

NOTE Confidence: 0.787109

and lead in my lab.

NOTE Confidence: 0.787109

By Liliana Luca.

NOTE Confidence: 0.787109

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

NOTE Confidence: 0.787109

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

NOTE Confidence: 0.787109

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

NOTE Confidence: 0.787109

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,
suggesting the potential importance of digit.

In the central nervous system for glioblastoma.

So first one to work through.

After that identification of the effect of type One interferons

wanted to work through the in vitro transcriptional regulatory network.

So we use the same model

that would be regift.

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

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

so we needed to have higher resolution transcriptomic data to construct the regulatory network.
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, and 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. 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. We so 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 CD4 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. So here's the IFN responsive gene score overtime and then the green represents regulators for Co inhibitory receptors until the yellow. 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 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
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 Sam HD one, removes restrictions, you can transfect primary human T 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.
00:14:21.664 -- 00:14:24.010 CD3 CD 28 with this procedure,
NOTE Confidence: 0.810376
00:14:24.010 -- 00:14:26.170 knocking down the different genes
NOTE Confidence: 0.810376
00:14:26.170 -- 00:14:28.827 and then there is stimulated with
NOTE Confidence: 0.810376
00:14:28.827 -- 00:14:31.335 and without interferon beta and then
NOTE Confidence: 0.810376
00:14:31.335 -- 00:14:33.589 measured five days later and then
NOTE Confidence: 0.810376
00:14:33.589 -- 00:14:36.098 we perform fax GFP of we sort of
NOTE Confidence: 0.810376
00:14:36.098 -- 00:14:38.246 the GFP positive cells were knocked
NOTE Confidence: 0.810376
00:14:38.246 -- 00:14:40.665 down and did bulk RNA sequencing
NOTE Confidence: 0.810376
00:14:40.665 -- 00:14:43.197 and you can see very efficient
NOTE Confidence: 0.810376
00:14:43.197 -- 00:14:45.516 knockdown in the GFP positive cells.
NOTE Confidence: 0.810376
00:14:45.520 -- 00:14:47.470 With these different transcription factors.
NOTE Confidence: 0.810376
00:14:47.470 -- 00:14:51.040 This is a monumental amount to work.
NOTE Confidence: 0.810376
00:14:51.040 -- 00:14:52.168 Performed by tomo.
NOTE Confidence: 0.810376
00:14:52.168 -- 00:14:54.048 So we perform principal component
NOTE Confidence: 0.810376
00:14:54.048 -- 00:14:56.408 analysis to changes in the total
NOTE Confidence: 0.810376
00:14:56.408 -- 00:14:58.343 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.

00:15:25.075 --> 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

NOTE Confidence: 0.79101753
regulated the interferon interferon genes.

So to go into more detail,

we first have the interferon regulated module one,

so a something that a transcription factor that knocks down the gene will lead to decreased expression,

which means as positive regulating.

So the interferon regular module one regulates the conical interferon stimulated genes over here.

Where is interferon regulated module two over here regulates these non Canonical jeans?

Interferon stimulated genes perhaps a greater interest was looking at the 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.

The data just showed you, which is the effect of these different Co inhibitory receptors.
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, transcription factors and then 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 one and three here.

00:17:49.238 --> 00:17:51.030 Positively regulate Tim three

00:17:51.030 --> 00:17:53.202 but negatively regulate PD one.

00:17:53.202 --> 00:17:55.917 So then we performed a hierarchical backbone network analysis transcription factors.

00:17:55.920 --> 00:17:59.710 I’ll just go over this very briefly,

00:17:59.765 --> 00:18:00.170 but basically looked at gene expression,

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 overture, differential expression,

00:18:06.338 --> 00:18:07.496 protein, DNA bonding,
a transcription factor database
NOTE Confidence: 0.79101753
is integrated.
NOTE Confidence: 0.79101753
Those data looked at a rank list
NOTE Confidence: 0.79101753
of transcription factors which we
NOTE Confidence: 0.79101753
perturbed and knocked down as I
NOTE Confidence: 0.79101753
showed you integrated those data
NOTE Confidence: 0.79101753
into refine network model and what
NOTE Confidence: 0.79101753
we found was at the early and
NOTE Confidence: 0.79101753
intermediate network contain more
NOTE Confidence: 0.79101753
up regulated transcription factors.
NOTE Confidence: 0.79101753
And downregulated in contrast late
NOTE Confidence: 0.79101753
network had more downregulated in up,
NOTE Confidence: 0.79101753
regulated transcription factors and
NOTE Confidence: 0.79101753
interferon induced differentiation.
NOTE Confidence: 0.79101753
Involves dominance of the up
NOTE Confidence: 0.79101753
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.

Where is the purple circles represent transcription factors that differential expressed in all transcriptional waves. So Cal offense tattoo are early intermediate transcription factors.
Math blimp one?

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.

So a dear friend of mine, somebody may know of one of the great.

Textbook authors of immunology.

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.

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 $R^2$ of .97.

So nature had accidentally given us a in vivo model of type one interferons in their effect on T cells.

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, so then we wish to ask. 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 this column. Here are the controls, stable and progressive patients. So we see this module too.
00:21:43.430 --> 00:21:45.890 Upregulated these are highly upregulated.
NOTE Confidence: 0.80999196
00:21:45.890 --> 00:21:49.826 PD one Tim, three CTO for lag three.
NOTE Confidence: 0.80999196
00:21:49.830 --> 00:21:53.106 Precisely what we saw in vitro in
NOTE Confidence: 0.80999196
00:21:53.106 --> 00:21:58.159 CD4 and CD8 cells, whereas module 1.
NOTE Confidence: 0.80999196
00:21:58.160 --> 00:22:01.470 Which led to downregulation again
NOTE Confidence: 0.80999196
00:22:01.470 --> 00:22:06.010 of TIGIT BTL ACD 160 and such.
NOTE Confidence: 0.80999196
00:22:06.010 --> 00:22:10.850 So we had a extremely.
NOTE Confidence: 0.80999196
00:22:10.850 --> 00:22:12.578 Could the recapitulation what
NOTE Confidence: 0.80999196
NOTE Confidence: 0.80999196
00:22:14.740 --> 00:22:17.140 Here’s expression of Co inhibitory receptors
NOTE Confidence: 0.80999196
NOTE Confidence: 0.80999196
00:22:19.920 --> 00:22:21.216 Just to summarize,
NOTE Confidence: 0.80999196
00:22:21.216 --> 00:22:25.109 here’s like 3 going up to three going up,
NOTE Confidence: 0.80999196
00:22:25.110 --> 00:22:27.270 whereas TIGIT Slam 6 and
NOTE Confidence: 0.80999196
00:22:27.270 --> 00:22:29.430 layer one all went down.
NOTE Confidence: 0.80999196
00:22:29.430 --> 00:22:33.798 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, 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 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
00:25:05.940 --> 00:25:08.020 contributed various parts of this.

00:25:08.020 --> 00:25:08.852 My long, long term collaborator, PJ Kutru Shadow Bergen is.

00:25:08.852 --> 00:25:10.100 collaborator PJ Kutru Shadow Bergen is.

00:25:10.100 --> 00:25:12.284 off Marty and also wondering knowledge.

00:25:12.284 --> 00:25:15.089 The covered work led by audio Untermann with Tomo Jonas Scoop.

00:25:15.090 --> 00:25:17.430 The covered work led by audio Untermann with Tomo Jonas Scoop.

00:25:17.430 --> 00:25:19.514 and enough Tally Kaminski.

00:25:19.514 --> 00:25:21.326 So I’ll stop there and take any questions.


00:25:24.660 --> 00:25:25.490 What an incredible body of work and

00:25:26.100 --> 00:25:27.318 What an incredible body of work and

00:25:27.318 --> 00:25:30.160 congratulations on sorting through

00:25:30.240 --> 00:25:32.264 congratulations on sorting through

00:25:32.264 --> 00:25:35.300 what is clearly a very complex.

00:25:35.300 --> 00:25:37.420 Regulatory system, let me ask,
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 an 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
00:26:53.590 --> 00:26:57.000 see that over 50% of the T cells.
NOTE Confidence: 0.83932835
00:26:57.000 --> 00:26:59.275 In this DSL or PD,
NOTE Confidence: 0.83932835
00:26:59.280 --> 00:27:00.868 one positive high expression
NOTE Confidence: 0.83932835
00:27:00.868 --> 00:27:02.853 digit in three with spontaneous
NOTE Confidence: 0.83932835
00:27:02.853 --> 00:27:04.429 production of gamma interferon.
NOTE Confidence: 0.83932835
00:27:04.430 --> 00:27:07.244 So I think each organ and that’s
NOTE Confidence: 0.83932835
00:27:07.244 --> 00:27:09.968 why I showed the Ms GBM data.
NOTE Confidence: 0.83932835
00:27:09.970 --> 00:27:12.874 I think looking at what is expressed in
NOTE Confidence: 0.83932835
00:27:12.874 --> 00:27:15.119 tumors compared to autoimmune disease,
NOTE Confidence: 0.83932835
00:27:15.120 --> 00:27:17.454 which goes the opposite direction may
NOTE Confidence: 0.83932835
00:27:17.454 --> 00:27:21.060 give us insight as to what is the next
NOTE Confidence: 0.83932835
NOTE Confidence: 0.83932835
00:27:23.040 --> 00:27:25.368 I think that would be perhaps
NOTE Confidence: 0.83932835
00:27:25.368 --> 00:27:28.219 the best way of addressing it.
NOTE Confidence: 0.83932835
00:27:28.220 --> 00:27:29.720 And this is more mechanistic,
NOTE Confidence: 0.83932835
00:27:29.720 --> 00:27:31.550 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

NOTE Confidence: 0.83932835
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 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 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 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 adaptive immunity to treat Melanoma.

So just a few fast facts about Melanoma, so 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 in the United States, 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.

Interestingly, it was going up as well.

So for mortality, it was going up as well.

So for 2000 three 7600 deaths,

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
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% of
NOTE Confidence: 0.8806305
00:33:11.828 --> 00:33:14.156 patients are alive with EPI alone
NOTE Confidence: 0.8806305

55
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. This is a figure that I borrowed from one of 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 over 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,

does that mean 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 is translation going back and forth.
is is translation going back and forth.
So what?
Why do patients develop resistance?
Or many 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 the some of these
tumors are just desert rumors,
lack of till 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, beta, two microglobulin in the tumor cells 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 one. Inhibition isn’t going to do very much over there. And then the other costimulatory
or Co inhibitory molecules

00:36:42.575 --&gt; 00:36:44.645 Lag 3 might also be present,

00:36:47.611 --&gt; 00:36:47.611 and maybe it’s just not sufficient in

00:36:51.000 --&gt; 00:36:53.730 And finally there there are many other

00:36:56.356 --&gt; 00:36:58.432 focus on in the tumor microenvironment,

00:37:00.810 --&gt; 00:37:00.810 and sometimes those might just be

00:37:03.278 overpowering the role of the T cells.

00:37:10.890 --&gt; 00:37:10.890 So when we started putting

00:37:12.890 --&gt; 00:37:12.890 together the renewal of the spore,

00:37:14.726 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 a gym model. It's byref mutant and P tenancy. 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 Y ammer 1.7. You get some sensitivity to anti PD one, but ultimately with time these tumors to grow out as well.
So the first question next to asked was what was actually in these in these tumors. So 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 tumor content 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 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.
NOTE Confidence: 0.8146802
00:39:27.834 --> 00:39:30.080 in the ones on the right over here
NOTE Confidence: 0.8146802
00:39:30.080 --> 00:39:31.724 are those that are presumed to
NOTE Confidence: 0.8405346
00:39:31.779 --> 00:39:32.820 be more inhibitory.
NOTE Confidence: 0.83383965
00:39:35.970 --> 00:39:38.500 So at that point they said, OK, we’ve got.
NOTE Confidence: 0.83383965
00:39:38.500 --> 00:39:39.620 We’ve got these terms.
NOTE Confidence: 0.83383965
00:39:39.620 --> 00:39:41.587 We need to try to modulate them,
NOTE Confidence: 0.83383965
00:39:41.590 --> 00:39:43.246 and there are many, many mechanisms
NOTE Confidence: 0.83383965
00:39:43.246 --> 00:39:44.680 out there for modulating terms.
NOTE Confidence: 0.83383965
00:39:44.680 --> 00:39:46.661 But the ones that they chose to
NOTE Confidence: 0.83383965
00:39:46.661 --> 00:39:48.332 work on with CD, 40, agonism,
NOTE Confidence: 0.83383965
00:39:48.332 --> 00:39:49.737 and CSF, one R inhibition,
NOTE Confidence: 0.83383965
00:39:49.740 --> 00:39:51.786 and in the beginning they used
NOTE Confidence: 0.83383965
00:39:51.786 --> 00:39:53.150 a small molecule inhibitor.
NOTE Confidence: 0.83383965
00:39:53.150 --> 00:39:55.316 So if you take these memory
NOTE Confidence: 0.83383965
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,
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
the immune infiltrating content,
and I think what’s most interesting
00:40:57.730 --> 00:40:59.946 over here is that when you give
NOTE Confidence: 0.83383965
00:40:59.946 --> 00:41:01.638 the CD 40 agonist along with
NOTE Confidence: 0.83383965
00:41:01.638 --> 00:41:03.650 the CSF one receptor inhibitor,
NOTE Confidence: 0.83383965
00:41:03.650 --> 00:41:05.618 you do get an increase of
NOTE Confidence: 0.83383965
00:41:05.618 --> 00:41:06.930 infiltration of T cells.
NOTE Confidence: 0.83383965
00:41:06.930 --> 00:41:09.378 So possibly we might be able to make
NOTE Confidence: 0.83383965
00:41:09.378 --> 00:41:11.076 desert those desert tumors more
NOTE Confidence: 0.83383965
00:41:11.076 --> 00:41:13.820 inflamed by using a regimen such as this.
NOTE Confidence: 0.83383965
00:41:13.820 --> 00:41:15.668 And in addition you get more
NOTE Confidence: 0.83383965
00:41:15.668 --> 00:41:17.420 PD one high T cells.
NOTE Confidence: 0.8104826
00:41:19.820 --> 00:41:22.214 So Catherine Miller Jensen on the main
NOTE Confidence: 0.8104826
00:41:22.214 --> 00:41:24.475 campus is developed a technology for
NOTE Confidence: 0.8104826
00:41:24.475 --> 00:41:26.430 single cell site eccentric creation,
NOTE Confidence: 0.8104826
00:41:26.430 --> 00:41:29.038 and she looked at what the difference of
NOTE Confidence: 0.8104826
00:41:29.038 --> 00:41:30.812 was between these different treatment
NOTE Confidence: 0.8104826
00:41:30.812 --> 00:41:33.791 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 take 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? What you see on this figure here is 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 and you'll see a whole cast of trainees along the way over here. off for residency.
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, the small molecule inhibitor, but rather to move towards 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 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,
NOTE Confidence: 0.8090304
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
00:44:53.540 --> 00:44:55.262 So we formed collaborations with Bristol
NOTE Confidence: 0.8090304
00:44:55.262 --> 00:44:57.381 Myers Squibb and a company called a passage
NOTE Confidence: 0.8090304
00:44:57.381 --> 00:44:59.447 and that makes a CD 40 agonist and we
NOTE Confidence: 0.8090304
00:44:59.447 --> 00:45:02.590 were able to get them to work together.
NOTE Confidence: 0.8090304
00:45:02.590 --> 00:45:04.550 The problem was that there was no
NOTE Confidence: 0.8090304
00:45:04.550 --> 00:45:06.388 phase one data for the triplet.
NOTE Confidence: 0.8090304
00:45:06.390 --> 00:45:08.366 Now could be oralism AB which is the
NOTE Confidence: 0.8090304
00:45:08.366 --> 00:45:10.329 CSF one receptor antibody and the
NOTE Confidence: 0.8090304
00:45:10.329 --> 00:45:12.447 volume Abbott being given to hundreds
NOTE Confidence: 0.8090304
00:45:12.511 --> 00:45:14.615 of patients in BM S LED studies in
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 CD40 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 study of APX. In other words, the CD40 agonist plus nivo in Melanoma and lung cancer started at around that time and we rolled a
NOTE Confidence: 0.8090304
00:45:43.220 --> 00:45:45.566 good number of patients there and
NOTE Confidence: 0.8090304
00:45:45.566 --> 00:45:47.130 actually saw phenomenal responses.
NOTE Confidence: 0.8090304
00:45:47.130 --> 00:45:49.418 So this is an example of a patient
NOTE Confidence: 0.8090304
00:45:49.418 --> 00:45:51.508 treated by doctors know who had
NOTE Confidence: 0.8090304
00:45:51.508 --> 00:45:52.597 a mucosal Melanoma,
NOTE Confidence: 0.8090304
00:45:52.600 --> 00:45:54.484 which tends to be very resistant
NOTE Confidence: 0.8090304
00:45:54.484 --> 00:45:56.469 to implement map in the volume.
NOTE Confidence: 0.8090304
00:45:56.470 --> 00:45:58.645 Evan the patient indeed had
NOTE Confidence: 0.8090304
00:45:58.645 --> 00:45:59.950 progressed on there.
NOTE Confidence: 0.8090304
00:45:59.950 --> 00:46:02.030 So we put the patient on the CD
NOTE Confidence: 0.8090304
00:46:02.030 --> 00:46:03.858 40 agonist plus nevala mehrban.
NOTE Confidence: 0.8090304
00:46:03.860 --> 00:46:05.612 The patients had a complete response
NOTE Confidence: 0.8090304
00:46:05.612 --> 00:46:07.604 and remains of therapy couple of years
NOTE Confidence: 0.8090304
00:46:07.604 --> 00:46:09.403 later we have four of these patients
NOTE Confidence: 0.8090304
00:46:09.460 --> 00:46:11.686 and others and implement Melbourne Nivolumab.
NOTE Confidence: 0.8090304
We don’t actually see this, so maybe this is the answer to Charlie’s question is what’s the next anti PD? Why? 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 died already did it already defined 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. 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
00:48:18.076 --> 00:48:21.140 with the Phase one trial which is an
NOTE Confidence: 0.8301139
00:48:21.140 --> 00:48:23.597 integral part of the sport project.
NOTE Confidence: 0.8301139
00:48:23.600 --> 00:48:25.790 We have completed the Phase 126
NOTE Confidence: 0.8301139
00:48:25.790 --> 00:48:27.758 patients in total have been
NOTE Confidence: 0.8301139
00:48:27.758 --> 00:48:29.923 enrolled sarahs busy preparing the
NOTE Confidence: 0.8301139
00:48:29.923 --> 00:48:32.433 publication for this and that should
NOTE Confidence: 0.8301139
00:48:32.433 --> 00:48:34.904 be going out over the coming weeks.
NOTE Confidence: 0.8301139
00:48:34.910 --> 00:48:37.790 Overall it was reasonably well tolerated.
NOTE Confidence: 0.8301139
00:48:37.790 --> 00:48:38.982 It certainly wasn’t candy,
NOTE Confidence: 0.8301139
00:48:38.982 --> 00:48:41.194 though we saw a lot of periorbital
NOTE Confidence: 0.8301139
00:48:41.194 --> 00:48:43.378 edema as well as diffuse edema
NOTE Confidence: 0.8301139
00:48:43.378 --> 00:48:45.328 elevations in CPK AST and a Lt,
NOTE Confidence: 0.8301139
00:48:45.330 --> 00:48:47.208 but those didn’t appear to be
NOTE Confidence: 0.8301139
00:48:47.208 --> 00:48:48.147 particularly clinically significant.
NOTE Confidence: 0.8301139
00:48:48.150 --> 00:48:49.406 Fevers Insider Kind release,
NOTE Confidence: 0.8301139
00:48:49.406 --> 00:48:50.976 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 gamma as well as in TNF Alpha.

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,
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
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.
Increase in the amount of CD
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 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?

So 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, 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 well, is the CSF one receptor
the optimal second target, the optimal second target,
in addition to CD 40 agonist in addition to CD 40 agonist
and PD one inhibitors? and PD one inhibitors?
So it’s possible, So it’s possible,
at least theoretically, at least theoretically,
that CTA for is a better target because that CTA for is a better target because
CTA for new mission is is really CTA for new mission is is really
key for dendritic cell activation. key for dendritic cell activation.
So Kelly Alina, So Kelly Alina,
who’s one of our wonderful who’s one of our wonderful
surgeons in the Melanoma group surgeons in the Melanoma group
and also surgeon scientists, and also surgeon scientists,
is doing work in the lab. is doing work in the lab.
It, primarily Marcus is lab where she 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 --> 00:53:33.100 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
app toxicity rate of at least 65.70%.
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.
How do we design a study like that? So how long? How do 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
It took 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. Two more patients have consented and...
00:55:46.896 --> 00:55:48.840 we’re looking for the six patient,

00:55:48.840 --> 00:55:51.374 but they all have to be monitored

00:55:51.374 --> 00:55:54.308 for 9 weeks before we can proceed.

00:55:54.310 --> 00:55:56.494 So I’m going to conclude there that

00:55:58.552 --> 00:55:58.552 Co targeting the innate and adaptive

00:56:00.676 --> 00:56:00.676 immune system with the CSF one

00:56:02.482 --> 00:56:02.482 receptor inhibitor or antibody plus

00:56:04.911 --> 00:56:04.911 CD 40 agonist results in better anti

00:56:06.616 --> 00:56:06.616 tumor activity than either alone.

00:56:08.923 --> 00:56:08.923 It also increases the CD 8 tumor

00:56:11.001 --> 00:56:11.001 content in animals if we treat

00:56:12.711 --> 00:56:12.711 mice bearing PD one resistant

00:56:14.828 --> 00:56:14.828 tumors with all with these drugs

00:56:16.880 --> 00:56:16.880 in combination with anti PD one,

00:56:19.274 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 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 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.
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. So far 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. Mostly because of memory. So if you take patients
00:59:30.079 --> 00:59:32.019 with her previous settling for,
NOTE Confidence: 0.81057096
00:59:32.020 --> 00:59:34.484 you can get additive toxicity over there.
NOTE Confidence: 0.8556669
00:59:36.850 --> 00:59:39.940 But that’s a really good idea to do that in
NOTE Confidence: 0.8556669
00:59:39.940 --> 00:59:41.180 the mouse. Thank you.
NOTE Confidence: 0.8556669
00:59:41.180 --> 00:59:43.045 Yeah, well, I know where I
NOTE Confidence: 0.8556669
00:59:43.045 --> 00:59:44.880 know we’re just we’re out of.
NOTE Confidence: 0.8556669
00:59:44.880 --> 00:59:47.211 We’re a little past the hour and I want
NOTE Confidence: 0.8556669
00:59:47.211 --> 00:59:49.520 to be sensitive to everyone’s time.
NOTE Confidence: 0.8556669
00:59:49.520 --> 00:59:50.732 So Harriet and David.
NOTE Confidence: 0.8556669
00:59:50.732 --> 00:59:52.920 Thank you both for really exceptional talks.
NOTE Confidence: 0.8556669
00:59:52.920 --> 00:59:54.152 Congratulations on all your
NOTE Confidence: 0.8556669
00:59:54.152 --> 00:59:55.692 work and everyone in attendance.
NOTE Confidence: 0.8556669
00:59:55.700 --> 00:59:57.007 Thank you for joining us and enjoy your day.
NOTE Confidence: 0.8556669
00:59:57.010 --> 01:00:00.900 Thanks. Bye bye.