The hearts go out to his wife.

Doctor Kellie Martin is two children tests and Jacob and just take a moment just to silence, just to recognize Tony’s legacy.

Well, thank you, so let’s now turn to our first of two great speakers. We were very fortunate this year to recruit Doctor Jeffrey Ishizuka. Jeff is an assistant professor of medicine. And Jeff’s work. Previously at Harvard was was focused on the biology of T cells.

Discovering knew better understanding
00:00:46.162 --> 00:00:48.790 of that biology and and and

00:00:48.857 --> 00:00:50.417 ultimately leveraging that science

00:00:50.417 --> 00:00:53.708 to what is likely to be the next

00:00:53.708 --> 00:00:55.460 generation of amino therapies.

00:00:55.460 --> 00:00:58.001 And we’re really very fortunate to have

00:00:58.001 --> 00:01:01.381 Jeff as one of our physician scientists in

00:01:01.381 --> 00:01:04.380 the center of molecular Italian Colosseum,

00:01:04.380 --> 00:01:06.510 member of the Melanoma program,

00:01:06.510 --> 00:01:09.276 and physician scientists in general at.

00:01:09.280 --> 00:01:10.920 At Yale and Smile also,

00:01:10.920 --> 00:01:13.528 Jeff really excited to hear about your work.

00:01:13.530 --> 00:01:17.040 Turn it over to you.

00:01:17.040 --> 00:01:17.370 Thank

00:01:17.370 --> 00:01:19.536 you so much Charlie, really appreciate

00:01:19.536 --> 00:01:22.659 it and let me just project my slides.

2
How there we go?

Yes, thank you so much and thank you for the opportunity to speak today.

Today I’m going to be talking to you about some of the work we’ve done.

This is my disclosure slide.

I wanted to begin with the overall survival curves from the Checkmate 067 trial, which is likely familiar to this audience.

These curves represent survival in advanced Melanoma by patients treated with immune checkpoint blockade.
In this case, with antibodies targeting PD-1C, TL A4 or the combination, I wanted to start here because Melanoma has been something of a touchstone for the use of checkpoint blockade in solid tumors. First indication approved and remains one of the indications in which immune checkpoint blockade is most effective in these data are outstanding, particularly when compared with the preimmunotherapy standard of Care Dakar Buisn, which had an overall survival of five to 10% at five years.
However, even in this disease, large proportion of patients don’t experience durable benefit. The situation which is actually more challenging in other diseases where responses are less good. And this is really the focus of our work to improve responses in this disease and in others.

Certainly, however, if you check, my blockade is rapidly reshaping the landscape of cancer care across indications. I was preparing for this talk and I had to go through and update this slide because indications have nearly doubled.
since its original publication by Tony Ribas and Jed will Chuck in 2018. Although many of us have followed this emerging data very closely, I have to admit that it gave me pause to consider the pace of change in this field. The advancement of PD one access approvals continues through lymphomas and solid tumors of desperate tissue origins. Combination approaches have also proliferated, including approvals in music, leoma, breast cancer, and others. Successful combinations include combinations of checkpoint inhibitors.
with other checkpoint inhibitors,
CHEMOTHERAPIES AND TOURISTING
kinese inhibitors,
notably many here at Yale,
have played critical roles in this dance.
Still, for all the advances,
there have been a lot of failures and
there remain a lot of ongoing challenges.
For most, many patients don’t respond,
indeed, considered across all indications,
most patients don’t respond in a few
of the response rates listed are
really based on earlier trials that
likely overestimated response rates.
Many of them also include
biomarker cutpoints,
00:04:02.680 --> 00:04:07.189 PDL 1 positive ITI and this sort of thing.

00:04:07.190 --> 00:04:09.233 And in my mind there are really a couple

00:04:09.233 --> 00:04:11.319 of big areas in which we can improve.

00:04:11.320 --> 00:04:14.365 1st for all of the new indications,

00:04:14.370 --> 00:04:16.710 few combinations involving novel

00:04:16.710 --> 00:04:19.050 targets have been approved.

00:04:19.050 --> 00:04:21.918 2nd, we have a limited mechanistic

00:04:21.918 --> 00:04:24.849 understanding of how these agents work.

00:04:24.850 --> 00:04:25.130 Accordingly,

00:04:25.130 --> 00:04:27.090 the biomarkers that we used to deploy

00:04:27.090 --> 00:04:29.009 them lack sensitivity and specificity,

00:04:29.010 --> 00:04:31.810 and there’s not a great way to rationally

00:04:31.810 --> 00:04:33.488 prioritize combinations with anti PD one.

00:04:35.570 --> 00:04:37.418 So it’s worth considering for a moment what

00:04:37.418 --> 00:04:39.139 we’ve learned about response and resistance,
not so much in the interest of an extensive overview for which we wouldn’t have time today, but in terms of the pathways that have given the strongest clinical signals to date. The data shown here are from the study by Merck of over 300 different patients across 22 different tumor tissue types. These figures show responses. Non response defined as CR or PR versus no CR PR. When graphed with tumor mutational burden on the Y axis and a gene expression profile representing tumor microenvironment, inflammation kind of T cell inflammation on the X axis.
The genes in this profile are listed in the upper right here and notably include PDL, one among them as well as several MHC related genes and kind of T cell related genes. Tumor mutational burden, as you know, is often used as a surrogate for too many antigens and the gene expression profile really points to information of the tumor, micro environment and the authors make two points that are important here. First, that these are two of the strongest predictors they could find. Reviewing one of the largest and most comprehensive datasets
that existed at the time.

Really, it’s telling us in second

that they appear to predict response independently of one another.

That is to say that although the best responses are in that kind of.

Upper right quadrant that you actually get a good number of responses in a T cell.

Inflamed only micro environment, or in TMB only TB high only tumors.

For the sake of time today, I won’t spend a lot of time on TMB or antigen load,

so it’s obviously an important consideration.

Instead, I’m just going to talk about tumor microenvironment information,
which is really the focus of our lab.

Aside from the work by the Merck Group A number of lines of evidence have established inadequate tumor microenvironment information.

As one of the most prominent mechanisms of resistance to me, no therapy. Most dramatically, this occurs in immune desert type tumors, which entirely lack T cell infiltrate, as depicted here. However, it can also occur in a different phenotype. The so-called immune excluded tumors which have anti tumor immune cells at the site of the tumor,
although they are excluded from the tumor core, either by physical barriers or by immune signaling. Finally, we believe that there is the T cell inflamed type of tumor that have diffuse infiltration of T cells that tend to be PD L1 positive, and these are the ones that we believe respond best to immunotherapy. To date, there's been progress in identifying therapeutic strategies to enhance this tumor microenvironment information, many of which involve either real
or simulated infection of the tumor to trigger anti-tumor immunity, and I think about them in kind of two big buckets. The first is the provision of exogenous sources that mimic nucleic acid ligands to tumors. This includes Sting agonist, MDA 5 or rig I agonist, double stranded RNA sensing pathways and unlytic viruses. The other is the induction of endogenous sources of nucleic acid ligands, primarily endogenous retroviruses, although others have been published recently.
alu repeats in humans.

And examples of this include a deciding in CDK 46 inhibitors.

So my interest in turning these cold microenvironments hot and kind of providing these logins to tuners really developed out of work in the Canings lab was finishing my postdoctoral work through the type of experiment that I’m showing here on the left, you have kind of a transplantable tumor cell line, something like a B16 Melanoma, and the way the experiment works is to, in fact, that cell line with a library of CRISPR CAS 9 guides that knockout thousands of.
Immunologically relevant genes. In the genome and then to kind of select those guides until you have a pool of knockout tumor cell lines that is then implanted into mice under increasing immune selective pressure from extremely immunodeficient mice that lack T cells to mice with an intact immune cell system. 2 mice treated with immunotherapy. In this case, the irradiated GM CSF secreting whole tumor cell vaccine GBX, plus anti PD one kind of strong immunotherapy treatment regimen.
Would grow these tumors for about 2 weeks and then remove them. Harvested tumors and sequence the sequence.

The barcodes sequence the guides using them as barcodes and quantitating. Enrichment and depletion of each guy and the way we interpreted this experiment was to compare high to lower mean selective pressure.

So immunotherapy treated to immunodeficient mice, for example, comparing height alone, selective pressure represented Jews that, when deleted, convert sensitivity.
To the mean system, and therefore potential targets for combination therapy.

In contrast, guides that were enriched under strongly selective pressure suggested to US jeans that were lost made tumors resistant to new therapy.

And a lot of the targets that we found this way actually ended up in the kind of realm of double stranded RNA sensing or antiviral triggering, and this is really the area that I focused on throughout my time.
what we know about viral infection comes from the study of exonerees viruses. But of course the genome is comprised largely of repetitive elements that have the potential to form double stranded RNA. These could be small interspersed nuclear elements and obvious retrovirus. Endogenous retroviruses are long interspersed nuclear elements or or others. And so we considered that that we’ve co evolved with these elements. With these kind of viral remnants in many cases and ourselves have developed systems to regulate double stranded RNA sensing to distinguish between double stranded RNA.
That’s a result of normal cellular activity and exogenous viral threats. And so we thought that by targeting some of the genes that control this regulation, we might sensitize tumor cells to tumor therapy. Trigger this kind of anti virus state. And the top hits that we discovered through this process in the antiviral sensing arena was this paid. R18 R is an adenosine deaminase that acts on double stranded RNA. It has a long cytoplasmic P-150 isoform. That’s interferon inducible and a short. Constitu Tively Express P110I support him.
The main known function of edar is to catalyze the conversion of adenosine to in a scene and double stranded RNA. And it's thought that in so doing it prevents double stranded RNA sensing in the triggering of antiviral immunity. Kind of autoimmunity. Accordingly, there is an autoimmune syndrome called Acardi Goutieres syndrome that is associated with biallelic mutations of a Darwin on the catalytic domain. It can be quite severe effects and mimics viral infection. However, Interestingly, the parents of affected patients...
who have monolith mutations in the catalytic domain have evidence of increased signatures of interferon gene expression in the blood, but have no detectable disease phenotype, suggesting that there’s a gene dose effect. So to begin to validate our one as a potential drug target for combination immunotherapy. We created dedicated knockout tumor cell lines again using the B16 Melanoma model. This transplantable tumor model and we implanted these into mice under increasing selective pressure. It means selective pressure starting.
with the extremely immunodeficient

nods give Gamma mice that entirely

lack adaptive immunity and have

only impaired innate immunity.

In these mice,

looking at the 8 Arnold tumors,
either P-150 knockouts in Orange,
compared to controls and Gray,
looking at tumor volume on the top,
or survival in the bottom,
you can see a sort of minimal
decrease in the growth of the
Darnell tumors compared to controls.
And a minimal increase in survival.
In contrast,
when planted these tumors into wild type mice with an intact immune system, you see a significant decrease in the growth of tumors in a significant survival advantage for the mice. Finally, when we implemented these tumors into mice and treated with anti PD one, we saw a near 100% cure rate for mice treated that were a Darnall and almost no cures in the control chambers. So to start to understand the mechanism of this, we looked at the tumor micro environment of untreated a Darnall and control.
tumors 14 days after implantation, and we did this using immuno histochemistry and as you can see on the left in control tumors you have the immune desert type phenotype. Almost no CD8T cells infiltrating. In contrast, in a Darnall tumors we saw this T cell inflamed phenotype with diffuse infiltration of CD8T cells. Quantitative here on the right.

To understand this more deeply, we next perform flow cytometry. Again with tumors 14 days after implantation in the untreated setting, and as you might predict,
we saw an increase in CD 45 positive immune cells and a Darnell tumors compared with controls.
And then looking within the CD 45 compartment we saw increases in CD 3 positive T cells, CD 4 positive T cells, CD 8 positive T cells, gamma Delta T cells and NK cells.
In contrast, when we looked at immunosuppressive populations, including mdse and tumor associated neutrophils, we saw significant increases in control tumors relative to a Darnell tumors. Finally, to probe the micro
environment yet more deeply, we perform single cell RNA sequencing. These are the populations we recovered with myeloid populations in the upper right and T cell populations in the bottom left. As you can see, using these density plots that we adapted for this purpose, you get a strong signal from suppressive myeloid populations and to like macrophages and mdse in control tumors. But a weaker signal from inflammatory monocytes and CD8 T cells. In contrast, in the 8 Arnold tumors you
have hardly any signal from the suppressive minded populations and enrichment of single from inflammatory monocytes and CD8T cells.

To understand what’s driving this change in the micro environment, we wanted to study the double stranded RNA sensing pathways that we thought could be associated with the phenotypes we’d observed. Specifically, we wanted to understand the role of protein kinase are an MD5 rig, I and nouns which are both associated with his internal sensors of nucleic acids in double stranded RNA,
specifically protein kinase power

is associated with translation

arrest in a pop ptosis.

Upon binding double stranded RNA.

Where is MD5 regarding mass induced type

one interferon in the antiviral state?

to test the role of each of these sensors,

we generated a series of double

and triple knockout tumor cell

lines and probe some of the in

vitro phenotypes that we previously

specifically, we looked first at growth inhibition.

So when you stimulate control

with interferon in vitro,
there's a slight defect in growth that's magnified when you knockout eight R1.

Looking at our double knockouts, we saw no effect of knocking out rig. I MDA 5 or Mens but saw that knocking peak PQR reduced the phenotype to the levels observed in control tumors, suggesting that PQR was alone responsible for the in vitro growth defect that we'd observed.

We next looked at interferon beta production. And this was again an in vitro Aliza and tumor cells stimulated with interferon.

Control tumors produce no 30 peak PQR out peak PQR reduced the phenotype to the levels observed in control tumors.
detectable interferon, whereas a Darnall tumors produces significant quantity. This is maintained from the loss of Rig I suggesting that guy is not involved in the phenotype. However, following the loss of MDA, Five Man’s or PK are you see a significant reduction suggesting that all three of these sensors, or these two sensors in this adapter have a role to play in phenotype. We next wanted to understand which of these double stranded RNA sensing pathways was required for the in vivo phenotype of
sensitization to whom checkpoint blockade. So we took our double and triple knockout tumor cell lines and implanted them into mice, treating the mice with PD one. Antibodies targeting PD one, and as you can see in our control experiment, control tumors continue to grow out as they did previously for us in the eternal summers respond well to, you know, therapy. This phenotype persisted following loss of PQR, suggesting that PQR is alone not required for the phenotype. It persisted following loss of MD5,
suggesting MDA 5 alone does not explain the phenotype. However, following the deletion of both PK are in MDA 5 together with eight or one we no longer observe any difference between the growth of eight R1 knowledge control tumors treated with immunotherapy. Together, these results suggested to us that growth inhibition by PQR or antiviral sensing by MDA 5 amounts sufficient mediate sensitivity to no therapy but that at least one is required. We next wanted to understand which
double stranded RNA sensing pathway was required for the enhanced community filtration for the inflammation in the tumor microenvironment that we’d observed. And so we again used our double and triple knockout tumor cell lines. In this time return to our habit of looking at the tumor microenvironment, dissecting the tumors out, separating out the cells, and quantitating them. To look which sensor was was required. In our control tumors, you see a relatively low infiltration of immune cells that significantly
increased following loss of eight R1.

And Interestingly, this phenotype is, if anything exaggerated following loss of protein kinase are however attenuated following loss of MBA 5 and oblatab following the loss of the two senses together.

A similar pattern followed when we looked at the proportion of the 45 positive immune cells that was comprised of CD8T cells, again, increases in eight are null that persisted following loss of PQR was attenuated following loss
00:19:31.905 --> 00:19:34.234 of MD5 with loss following the
NOTE Confidence: 0.782579
00:19:34.234 --> 00:19:36.339 loss of both sensors together.
NOTE Confidence: 0.782579
00:19:36.340 --> 00:19:39.175 When we look at a immunosuppressive mdse,
NOTE Confidence: 0.782579
00:19:39.180 --> 00:19:41.265 we saw the opposite pattern
NOTE Confidence: 0.782579
00:19:41.265 --> 00:19:43.350 increases in control that persisted
NOTE Confidence: 0.782579
00:19:43.417 --> 00:19:45.267 or work were even increased.
NOTE Confidence: 0.782579
00:19:45.270 --> 00:19:47.825 Further following loss of PQR and no
NOTE Confidence: 0.782579
00:19:47.825 --> 00:19:50.211 loss of the phenotype following loss
NOTE Confidence: 0.782579
00:19:50.211 --> 00:19:53.800 of MDA 5 for the two sensors together.
NOTE Confidence: 0.78578705
00:19:56.900 --> 00:19:58.482 This suggested to us that MBA five
NOTE Confidence: 0.78578705
00:19:58.482 --> 00:20:00.179 may be playing the predominant role.
NOTE Confidence: 0.78578705
00:20:00.180 --> 00:20:02.304 And inducing tumor microenvironment
NOTE Confidence: 0.78578705
00:20:02.304 --> 00:20:04.428 inflammation may danel tumors.
NOTE Confidence: 0.78578705
00:20:04.430 --> 00:20:05.567 To confirm this,
NOTE Confidence: 0.78578705
00:20:05.567 --> 00:20:08.220 we looked at the production of interferon
NOTE Confidence: 0.78578705
beta interferon gamma in the tumor

microenvironment of the eternal jiggers.

And we saw a similar pattern again

increases in a terminal tumors that persisted following loss of PQR but was lost after law after loss of MD5 or the two sensors together in the same pattern.

Looking at tumor lysate interferon gamma.

So haven’t seen having seen this powerful dual mechanism for sensitizing tumors to immunotherapy.

We asked whether loss of eight R1 was sufficient to overcome commonly acquired mechanisms of resistance to amino therapy, including genetic aberrations.
that have been identified as enriched when comparing discordant, responsive, pretreatment, and resistant posttreatment lesions. Matched with the same patient. Known mechanisms that fit this description include the loss of MHC one through mutations of HLA or beta 2M, loss of targeting, children expressing through editing mutations and interferon sensing pathways including interferon gamma receptor, the Jackson, the stats.
And we focused first on the loss of MHC one, as mediated by loss of data to microblogging which has been repeatedly identified as important in challenging form of resistance. To create this model we again use CRISPR CAS 9. This time deleting beta 2 microglobulin and eight are together. Along with creating match control tumor cell lines. To validate our model of resistance, we compared control in beta two of null tumors in the untreated that is dashed line state versus the treated state. That’s the solid lines using again, this strong immunotherapy treatment.
00:21:50.922 --> 00:21:54.000 regimen of GBX and PD one.

00:21:54.000 --> 00:21:56.485 And we did this because the normal control chambers responded very poorly.

00:21:58.113 --> 00:22:02.851 to PD one and we wanted to make sure that we could see a response in control tumors and then validate that it was lost in the beta two unknown tumors.

00:22:08.621 --> 00:22:11.179 That’s what we did see you can see the control tumors respond albiately transiently.

00:22:13.391 --> 00:22:15.428 do grow out to this strong unit therapy treatment regimen.

00:22:16.480 --> 00:22:18.010 but made it to heaven.

All tumors hardly respond at all.

And sure enough, and sure enough, and sure enough.
We next looked at Darnall tumors.

This is our positive control experiment using strong again with therapy treatment regimen.

We got a great response to treatment.

The untreated tumors grow out, albeit more slowly than controls.

Strikingly, however, this sensitivity persisted following loss of beta two microglobulin, suggesting that loss of a Darwin in tumors is sufficient to overcome this mechanism of resistance.

This result was a bit surprising actually. At first, as it suggests that CD8T cell recognition with MHC one in
00:22:54.240 --> 00:22:56.221 tumors is not in all cases required
00:22:56.286 --> 00:22:58.380 for the response to amino therapy.
00:22:58.380 --> 00:23:00.515 It also raises the question as to
00:23:00.515 --> 00:23:02.593 whether it could be possible to
00:23:02.593 --> 00:23:04.423 target tumors that entirely lack
00:23:04.423 --> 00:23:06.319 high quality CDH cell antigens.
00:23:06.320 --> 00:23:07.769 A lot of ongoing work in the
00:23:07.769 --> 00:23:09.354 lab is focused on dissecting the
00:23:09.354 --> 00:23:10.566 mechanism of this finding,
00:23:10.570 --> 00:23:12.270 and one of the first
00:23:12.270 --> 00:23:13.970 things we wanted to know.
00:23:13.970 --> 00:23:15.920 Is whether antigenic vaccine GBX,
00:23:15.920 --> 00:23:17.472 which was unsuccessful in
00:23:17.472 --> 00:23:19.024 translating to human use,
00:23:19.030 --> 00:23:22.520 was required for this response.
This is actually pretty new data or afraid with PD one alone, and found that indeed you still get great responses in a Darwin all tumors.

Even without the gmax.

To start to understand this mechanism further, we again looked in the tumor microenvironment, this time focusing on our beta 2M null compared to control tumors. And so, as you would expect, increased immune infiltration increased immune infiltration and cytotoxic populations and these
include granzyme B positive CD4 positive T cells and NK cells.

With the hypothesis that perhaps these cells which don’t require MHC one for recognition of tumor cells.

May be involved in the phenotype we’ve observed.

We’ve also begun to dissect the cytokinin kyma kind drivers,

by which these populations may be recruited and activated.

These graphs are from side to kinda be Teresa Beta to null and a Darnall tumors.

The two prominent chemo kinds were identified so far.
00:24:30.760 --> 00:24:33.790 CX CL 10 in CCL 5.
NOTE Confidence: 0.792159180909091
00:24:33.790 --> 00:24:35.170 Which are both significantly
NOTE Confidence: 0.792159180909091
00:24:35.170 --> 00:24:37.240 increased in our beta to emulate
NOTE Confidence: 0.792159180909091
00:24:37.297 --> 00:24:38.989 our one all tumors compared with
NOTE Confidence: 0.792159180909091
00:24:38.989 --> 00:24:41.030 beta to a control control tumors.
NOTE Confidence: 0.76325333
00:24:43.550 --> 00:24:45.614 Notably Ehrenring here at Yale has
NOTE Confidence: 0.76325333
00:24:45.614 --> 00:24:47.284 described a similar phenotype of
NOTE Confidence: 0.76325333
00:24:47.284 --> 00:24:48.730 being able to overcome the loss
NOTE Confidence: 0.76325333
00:24:48.730 --> 00:24:50.631 of MHC one using a modified I’ll
NOTE Confidence: 0.76325333
00:24:50.631 --> 00:24:52.305 18 side kind that he designed.
NOTE Confidence: 0.76325333
00:24:52.310 --> 00:24:54.360 So this remains another possibility
NOTE Confidence: 0.76325333
00:24:54.360 --> 00:24:56.410 that we haven’t yet explored.
NOTE Confidence: 0.76325333
00:24:56.410 --> 00:24:58.524 However, we think this type of study
NOTE Confidence: 0.76325333
00:24:58.524 --> 00:25:00.136 is important ’cause articulating the
NOTE Confidence: 0.76325333
00:25:00.136 --> 00:25:02.404 general principles by which loss of MHC
NOTE Confidence: 0.76325333
00:25:02.404 --> 00:25:04.931 one can be overcome could lead to new
treatment approaches to target tumor specific immune evasion mechanisms.

In summary, I hope I’ve convinced you have several points.

First aid are one loss over improves the response to me to therapy. Specifically, it can overcome the lack of evidence.

Plain tumor, micro environment and the loss of antigen presentation by image C1.

Additionally, this phenotype is driven both by tumor microenvironment,

inflammation mediated by MDA

Interferon driven by PK are.

Finally, and I think this may be important.
Tumor cells contain sufficient innate lightning into drive therapeutic information. If they are in need. Nucleic acid sensing checkpoints are disabled. And what we think this implies is that there may be other similar innate immune checkpoints that limit the sensing of double stranded RNA or other nucleic acid ligands that we could think about as therapeutic targets. And really, those questions inform the rest of the work that the lab is doing. I've mentioned already a focus on double stranded RNA and eight R1. We're also applying functional genomics.
to try to identify other novel targets.

Really, with the insight that we have to focus on turning on some of these pathways of double stranded RNA sensing or micro violent information.

And then we’re involved in human translation, doing kind of in depth tumor microenvironment investigation across several different tumor indications.

We’re always looking for new collaborators there.

And all of this comes under the rubric of therapeutically targeting the information in the tumor microenvironment.

In just the last couple of minutes here,
I want to quickly mention some of the ongoing projects in the lab that I haven’t talked about this far.

First, I mentioned just the project. Describing how to Riker environment inflammation can overcome the loss of MHC one. This is being led by Jessica Way, but she’s Additionally leading a project. Looking at human tumors and trying to turn these pathways on in ex vivo samples as well as doing deep dissection of the micro environment. Where we go is working on novel strategies to detect double stranded RNA and to mimic the sensors of double
stranded RNA that we believe will be compatible with functional genomic screening in the identification of novel cancer immunotherapy targets. And finally, even Kim who is in the lab focused on the comparison of discordant response lesions. So responsive and resistant lesions. From the same patient trying to understand novel mechanisms of resistance to new therapies so that we can focus on overcoming. With that I want to thank everybody in our lab as well as our collaborators and mentors here at, you know,
have been fantastic.
I also wanted knowledge at Nikki Ning my form. Enter drumming.
So much of the work that I presented early derives from studies with them, and of course our funding here at the Cancer Center and the International Research Alliance.
With that, I will wrap up.
Thank you so much for the chance to present, and I'm happy to take questions.
Jeff, thank you. That's just terrific work and really exciting.
And we have folks can submit questions.
We have one question.
So given the response in eight R1 knockouts in the absence of MHC class one, do you think that’s function of CD4T cells or NK cells, or both? Or some other mechanism? Yeah, I think that’s a great question and we definitely would love to know that answer. Best hypothesis Now is that partially based on some of the work that Ehrenring is presented in Marcus Bosenberg. NK cells could be an important player there. Certainly there increased and we started to see some cytokines in Kemah kinds that may activate them further, but you know, we don’t even know for
sure that CD8T cells aren’t important. That’s an experiment we’re doing now. We just know they’re not recognizing the tumor, but could they be activated through cross presentation or another means is another question that we’re investigating. And then you know, in related work. Obviously Akiko, Saki, and Anna Pile of working independently on Rig I are iguana, which which it is. But which obviously is not necessarily related to the function vadar one, and you know how? How do you see those two with those two sort of bodies of work relating? Yeah, so this is
A great question Charlie and actually Akiko is one of my mentors here and. Collaborators and we’ve talked about this. We’re actually in the process of testing. 

Are a guy at. Egotist with the innate arnolin control tumor cell lines and you know the colloquial way we we thought about this is kind of as a maximum inflammation bomb because what we’ve shown is that any interferon producing stimulus can trigger this Arnold amplification of sensing, and so our hypothesis would be that if you initiate signaling through a guy, even if there a guy is not involved.
00:30:22.502 --> 00:30:24.899 in the pathways we’ve described here,
NOTE Confidence: 0.7803051
00:30:24.900 --> 00:30:26.475 you basically create a massive
NOTE Confidence: 0.7803051
00:30:26.475 --> 00:30:27.424 amplification of interferon,
NOTE Confidence: 0.7803051
00:30:27.424 --> 00:30:29.320 buy by further knocking out eight
NOTE Confidence: 0.7803051
00:30:29.320 --> 00:30:31.534 R1 so that remains to be seen,
NOTE Confidence: 0.7803051
00:30:31.534 --> 00:30:33.430 but that’s what I would hypothesize.
NOTE Confidence: 0.8670604
00:30:33.830 --> 00:30:34.892 Yeah, that’s interesting.
NOTE Confidence: 0.8670604
00:30:34.892 --> 00:30:36.662 It sounds like a great
NOTE Confidence: 0.8670604
00:30:36.662 --> 00:30:38.158 opportunity to look at that.
NOTE Confidence: 0.8670604
00:30:38.160 --> 00:30:41.157 Well, I want to keep us on time,
NOTE Confidence: 0.8670604
00:30:41.160 --> 00:30:42.400 so Jeff, thank you.
NOTE Confidence: 0.8670604
00:30:42.400 --> 00:30:44.260 I know there are other questions
NOTE Confidence: 0.8670604
00:30:44.326 --> 00:30:46.258 coming in and people should certainly
NOTE Confidence: 0.8670604
00:30:46.258 --> 00:30:48.490 reach out to you directly, Jeff.
NOTE Confidence: 0.8670604
00:30:48.490 --> 00:30:50.870 But thank you for a superb presentation
NOTE Confidence: 0.8670604
00:30:50.870 --> 00:30:53.807 and let me now turn to our second speaker,
00:30:53.810 --> 00:30:56.130 doctor Robert Bone and Bob Bone is a
00:30:56.130 --> 00:30:58.138 professor of medicine in hematology,
00:30:58.140 --> 00:31:00.831 and recently the past year joins us as the
00:31:00.831 --> 00:31:03.128 director of the Benign Hematology program,
00:31:03.130 --> 00:31:05.846 as well as the medical director of
00:31:05.846 --> 00:31:07.540 the Hemophilia Treatment Center.
00:31:07.540 --> 00:31:09.156 Prior to joining Yale,
00:31:09.156 --> 00:31:11.176 Bob was founding faculty member
00:31:11.176 --> 00:31:13.745 and leader at the Frank Netter
00:31:13.745 --> 00:31:15.835 School of Medicine at Quinnipiac,
00:31:15.840 --> 00:31:18.784 as well as a professor of medicine at
00:31:18.784 --> 00:31:21.416 the University of Connecticut School of
00:31:21.416 --> 00:31:24.140 Medicine and Bob throughout his career,
00:31:24.140 --> 00:31:27.101 really has been a leader in in the
00:31:27.101 --> 00:31:29.615 clinical care and sort of advancing
00:31:29.615 --> 00:31:32.055
work in hemostasis thrombosis as well as benign hematologic conditions. And we’re really very fortunate Bob to. That Bob, now leading this section and sharing with his work with us. So Bob thank you.

Thank you, Charlie for that introduction and for the opportunity to speak today. Let me just share my screen here. So good afternoon everybody.

And what I would like to do in the next 25 minutes or so is discuss with you some of the advances that have a curd in the treatment of hemophilia.
and what I hope to show you is that over the past five years there have really been significant and substantial advances which came in the background of really several decades of really only modest advances in therapy.

So just as a brief review here, these are excellent disorders, mostly affecting men, but can also affect women who might have low factor levels due to unequal X chromosome inactivation, hemophilia A&B or deficiencies in factor 8 or 9 respectively. They are clinically identical disorders.
and the severity of the disease is really relies primarily on the residual factor that is remaining in the blood with those with severe. And moderate disease having less than 5% of factor 8 or factor 9 and those with mild disease having a higher value and morbidity and mortality is due to spontaneous and trauma induced bleeding, including bleeding into joints which can cause a hemophilic arthropathy which we can be quite disabling. The history of hemophilia and just the treatment in the last century is seen briefly on this slide.
and at the end of World War Two blood or plasma transfusions were largely ineffective, is only small amounts of factor 8 or factor 9 could be transfused in the 1960s. Cryoprecipitate was discovered as a source of Factor 8, and that quickly gave way to the use of factor concentrates either factor 8 or factor 9. Purified from the plasma of 10s of thousands of donors. And of course, while this advanced care,
it also exposed individuals to a number of viral particles and hepatitis C and HIV became a very significant problem in this population. And then in the early 90s recombinant factors 8 and 9, or produced and for the developed world, where economically this was allowable of the treatment of hemophilia with recombinant factors 8 and 9. Became really the standard of care up until very recently. There are now about 145 federally funded hemophilia treatment centers and of course jeliz is one of those centers.
And the therapeutic.
The approach in clinical issues are outlined here.
Patients with hemophilia can either be treated in what’s known as on-demand or episodic factor replacement,
which is the treatment with Ivy Factor 8 or factor 9 to treat a bleed or prophylactic therapy.
An inhibitor development, that is an Allo antibody directed against Factor 8 or less commonly, factor 9 is a significant problem for patients and may occur in 30 or 40% of individuals with hemophilia A.
and makes treatment very difficult

and the goals of therapy are really here to prevent any bleeding. If possible,

prevent joint disease and optimize a quality of life for these individuals.

And the infusion of factor 8 or factor 9 by patients is traditionally given at home intravenously.

Patients from a very young age learn to start an Ivy and infuse factor 8 or factor 9,

but because of the short half-life of these drugs,

about 12 hours for factor 8 and 18 to 24 hours for factor 9,
three to sometimes four times per week to keep the factor levels in a range that will prevent bleeding. So this is an onerous thing for patients to do. And any advances here would be greatly appreciated by them. So here’s the obligatory coagulations slide that I would like to show to reinforce and emphasize the role that Factor 8 and factor 9 having blood coagulation. So what we’re seeing here is the tissue factor initiated pathway and activation of factor 10 by tissue.
factor 7A or activation by factor 9 to 9 A by tissue factor 7A.

And 9A is also able to activate all important enzyme factor 10A,

and in this latter reaction factor 8 serves as a cofactor for the enzyme factor 9A.

To act on its substrate factor 10 and increases the rate of reaction hundreds of 1000 fold when factor 8 is able to align the substrate and enzyme on a phospholipid surface in the correct fashion.

One other thing to mention about Factor 8 before we get into some of
the details of the advances is that factor 8 travels if you will in the blood bound to von Willebrand factor. Von Willebrand factor is seen here in this linear structure at the bottom, factor 8 is the yellow diagram above, and the binding of factor 8 von Willebrand factor enhances the half life of factor 8 from about 2 hours to about 12 hours. So this is a very important interaction. And just to point out here, ’cause this will become important later is that the binding site on von Willebrand factor is these.
two protein domains,

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designated D prime and D3,

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and another important point is there

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appears to be a large portion of the

NOTE Confidence: 0.85170436

factor 8 molecules termed the B domain,

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which is not required for factor 8 function,

NOTE Confidence: 0.85170436

so you could remove that domain

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and in fact factor 8 has a similar

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activity than it does with that domain.

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So the advances in care of hemophilia

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really over the past five to six

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years come into three different areas.

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One is extended,

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half-life factor concentrates,

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allowing for patients to infuse

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less frequently.
The development of non factor 8 or 9 therapeutics, and then gene therapy and we’ll go through these individually in the next 15 minutes or so. So the extended Half-life products have been produced by manipulating the recombinant factor eight or nine in a number of different ways, many of which are familiar to you by either adding polyethylene glycol or conjugating the factor to the FC portion of immunoglobulin or albumen, to improve half-life, or, in the case of factor 8,
to remove that B domain, which causes a slight increase in the half life. And there are now a number of products that have been approved for use at our extended Half-life products, and I'll draw your attention to the last three on this list. These are factor 9 products which have been manipulated by these methods, and the half life of these products has been extended from 18 to 24 hours to upwards of 90 or 100 hours. So this is allowed patients with factor 9 deficiency or hemophilia B to be treated. Once a week, once every 10 days and in some circumstances,
even once every two weeks. So a significant advance for people needing to give intravenous therapy themselves at home. The advances in hemophilia A with factor 8. However, a much more modest with this type of manipulation, and it turns out that the the degradation in the catabolism and clearance from the circulation of factor 8 is much more linked to the clearance of von Willebrand factor, the protein that it’s bound to. So making modifications in the FAQ.
After 8 molecule has really had minimal effect up until recently. So an interesting construct has been devised, and it’s shown on the top panel here. In this construct the D prime and D3 regions of von Willebrand factor, the binding region to factor 8, is linked to an FC portion of an immunoglobulin and linked to the B domain less factor 8 molecule, which also has linked on at this hydrophilic polypeptide, which also can extend the half life. So this product has been called bib 001. And was treated with.
Was used to treat a handful of patients in a safety study, and those results were reported in the New England Journal of Medicine earlier this year, and patients were either treated at two different doses of this new product and the factor clearance from the circulation was compared to the typical factor 8 clearance seen in the lighter blue bars here and what you can see I think, is that the half life of this newer product is now about two days increased, about five or six fold the half life.
00:41:15.166 --> 00:41:17.548 of the standard factor 8 product.
NOTE Confidence: 0.85449994
00:41:17.550 --> 00:41:19.895 So this product is now in
NOTE Confidence: 0.85449994
00:41:19.895 --> 00:41:21.797 large scale clinical trials and I
NOTE Confidence: 0.85449994
00:41:21.797 --> 00:41:24.202 think in the next year or two we
NOTE Confidence: 0.85449994
00:41:24.202 --> 00:41:26.227 should have some more information,
NOTE Confidence: 0.85449994
00:41:26.230 --> 00:41:28.774 and this may be an advanced
NOTE Confidence: 0.85449994
00:41:28.774 --> 00:41:31.860 for some of our patients.
NOTE Confidence: 0.85449994
00:41:31.860 --> 00:41:33.636 So let me shift for a minute for
NOTE Confidence: 0.85449994
00:41:33.636 --> 00:41:35.799 the non factor product for
NOTE Confidence: 0.85449994
00:41:35.799 --> 00:41:37.832 the treatment of hemophilia and I
NOTE Confidence: 0.85449994
00:41:37.832 --> 00:41:41.370 think their significant advance
NOTE Confidence: 0.85449994
00:41:41.370 --> 00:41:43.350 three drugs that will talk about
NOTE Confidence: 0.85449994
00:41:43.350 --> 00:41:45.428 will really focus primarily on this
NOTE Confidence: 0.85449994
00:41:45.428 --> 00:41:47.450 first drug which is called EMAS
NOTE Confidence: 0.84887415
00:41:47.511 --> 00:41:50.895 ISM AB. A nemesis Omab is a
NOTE Confidence: 0.84887415
00:41:50.895 --> 00:41:52.668 bispecific monoclonal antibody.
NOTE Confidence: 0.84887415
00:41:52.670 --> 00:41:56.086 That binds the factor 9 and factor 10,
NOTE Confidence: 0.84887415
00:41:56.090 --> 00:41:59.514 so it simulates the activity of Factor 8.
NOTE Confidence: 0.84887415
00:41:59.520 --> 00:42:01.974 Remember that factor 8 is able
NOTE Confidence: 0.84887415
00:42:01.974 --> 00:42:04.580 to colocalize factor 9 and factor
NOTE Confidence: 0.84887415
00:42:04.580 --> 00:42:06.790 10 on a phospholipid surface.
NOTE Confidence: 0.84887415
00:42:06.790 --> 00:42:10.024 This antibody is able to bind factor
NOTE Confidence: 0.84887415
00:42:10.024 --> 00:42:14.057 9A and factor 10 in the circulation an
NOTE Confidence: 0.84887415
00:42:14.057 --> 00:42:17.529 again simulate the activity of Factor 8.
NOTE Confidence: 0.84887415
00:42:17.530 --> 00:42:21.148 So this drug is not exactly like Factor 8.
NOTE Confidence: 0.84887415
00:42:21.150 --> 00:42:21.952 There are.
NOTE Confidence: 0.84887415
00:42:21.952 --> 00:42:23.957 There are certain differences here.
NOTE Confidence: 0.84887415
00:42:23.960 --> 00:42:26.774 It binds to factor 8 and nine
NOTE Confidence: 0.84887415
00:42:26.774 --> 00:42:27.980 in the circulation,
NOTE Confidence: 0.84887415
00:42:27.980 --> 00:42:30.386 not just on the phospholipid membrane.
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It has different infinities

for the substrate and enzyme,

and whether or not that becomes an

issue for this drug will only know

as more experience is accumulated.

But nonetheless,

this drug is really shown dramatic activity,

so this is a study that was

published a few years ago in the


Here we had patients who have hemophilia

A with inhibitors to factor 8,

so a challenging group of patients to

treated either with their

typical regimen of recombinant factor

7A or factor 8, bypassing activity,
or with Emma system AB given by subcutaneous injection once a week and the annual bleeding rate. Is been been described on this slide here and you could see if we just look at these blue histograms for a minute here. The annualized bleeding rate in the EMA system app Prophylaxis Group was about five or six and it was almost 30 in the standard of care. Treatment of patients with hemophilia A and inhibitors. So a really significant advantage for these individuals. And then a second study was published.
with looked at patients with hemophilia

A without inhibitors and these.

This was a randomized trial.

Patients were treated with one

of two doses of Emma’s is a map

either given weekly or every other

week by subcutaneous injection,

compared with no prophylaxis.

About 100 patients in the trial,

and again the annual annualized

bleeding rate went from about

to about one or two.