I'm iron crop. I'm the CTO director here and it's my pleasure to introduce David Rimm, who has many of you know is the Anthony Brady Professor of Pathology and medicine here. He is a Hopkins alum, did an MD PhD there, then came here for pathology residency and then did a psychopath fellowship at the Medical College of Virginia. He’s actually now been at Yale for almost 30 years. That’s impressive. He’s the director of the Pathology.
tissue service here and serves as director of Translational Pathology.

You know, I think David has been at the forefront of quantitative pathology for many years and he's well known throughout the field. He developed many novel assay techniques for identifying predictive markers to determine which tumors are sensitive to targeted therapies. This has become increasingly important as the number of targeted therapies has increased and our...
use of those drugs has increased. And today he’s going to focus on the development of companion diagnostics for her two directed therapies. I think this is particularly timely as the first her two targeted therapy for non-Fish amplified breast cancers was just approved six months ago and how we identify which patients are going to benefit from this therapy I think is a huge question and the one the field is struggling with and David’s made a lot of inroads into that and I think he’s going to focus on, on that today.
00:01:43.782 --> 00:01:45.780 So thank you for bringing this
NOTE Confidence: 0.66406729
00:01:45.859 --> 00:01:47.560 timely discussion to us.
NOTE Confidence: 0.838330737692308
00:01:51.270 --> 00:01:53.545 OK, great. Thanks, Diane, and thanks to
NOTE Confidence: 0.838330737692308
00:01:53.545 --> 00:01:55.588 the leadership for inviting me today.
NOTE Confidence: 0.838330737692308
00:01:55.590 --> 00:01:56.550 But thanks especially for
NOTE Confidence: 0.838330737692308
00:01:56.550 --> 00:01:57.510 Ian for introducing me.
NOTE Confidence: 0.838330737692308
00:01:57.510 --> 00:01:59.029 He’s a world leader in this space
NOTE Confidence: 0.838330737692308
00:01:59.029 --> 00:02:00.370 That I’m going to talk about.
NOTE Confidence: 0.838330737692308
00:02:00.370 --> 00:02:03.520 That is the her two.
NOTE Confidence: 0.838330737692308
00:02:03.520 --> 00:02:06.586 80C or antibody drug conjugate space.
NOTE Confidence: 0.838330737692308
00:02:06.590 --> 00:02:09.159 I’ll start by my title is precision
NOTE Confidence: 0.838330737692308
00:02:09.159 --> 00:02:10.967 medicine versus persuasion medicine and
NOTE Confidence: 0.838330737692308
00:02:10.967 --> 00:02:12.887 I’ll get to what persuasion medicine
NOTE Confidence: 0.838330737692308
00:02:12.887 --> 00:02:15.185 is a little bit more at the end.
NOTE Confidence: 0.838330737692308
00:02:15.190 --> 00:02:16.718 And reading versus measuring.
NOTE Confidence: 0.838330737692308
00:02:16.718 --> 00:02:20.070 And measuring is what you do quantitatively.
Reading is what pathologists do when they look at slides and difference between subjective and objective assessment of tissue.

Let’s see. Let’s start with my disclosures. As you can see, I do a fair bit of consulting and a lot of the research in my lab, including the work that led to this. Most of what I’m going to present today was sponsored by companies including Sephylon and Kanika Minolta. So today I want to spend the next 55 minutes or so talking about first and quick introduction to the new drugs. If you’ve heard Ian speak,
I don’t need to give this part, but maybe you didn’t a proposed new assay for these new drugs high sensitivity would call the assay high sensitivity or HS or two and then CAP CLIA, what take, what does it take to get something from your research lab so that into a lab where we can deliver information to patients and put the results in the patient’s chart. That’s what cap CLIA is taking the new. Say to the clinic and then finally I’ll talk about precision medicine versus persuasion medicine and try to talk all of you who are oncologists in the room and to not using persuasion medicine.
and focusing on precision medicine.

So this is the, the big drug that got the first standing ovation in 25 years as I understand at ASCO and it’s the same old drug, trastuzumab underneath, but they’ve conjugated 8 topoisomerase inhibitor payloads to the trastuzumab that gives you especially some special tricks. First of all, it brings these highly toxic payloads right to the cell. So it doesn’t have the toxicity that giving the drug and the dosages that would cause. All kinds of toxicity.
But if you bring it right to the salad it causes less toxicity. Whereas if you, and not only that, when it gets uncoupled in the cell it can spill out of the cell and kill neighboring cells. The sort of neighborhood effect or proximity effect of the therapy. It worked really well and that’s why we’ve all heard about it that you can see very few patients were resistant but most patients had some response and there were eleven CR’s in the early trials and in fact it worked at for all patients, especially in patients that
were not amplified for her too.

So the initial trials were all in patients that had her two amplification, but then they started trials on patients with low her two IHC 2 and you can see the curves look pretty similar. And in fact in those low, low patients in the Destiny 4 trial, ultimately the survival curve looks like great, looks like this, which is really a great improvement in the survival curve for advanced breast cancer and changing median survival from 5 to 9 months.
And that’s I think what ultimately has led to the popularization of this drug and the success of the drug.

And they said we concluded a randomized 2 group open label phase two trial with her too low.

What does that mean? So that’s what we’ll examine the rest, but.

Before we go there,

What about her 20? What about if they don’t express any her two at all and can we tell the difference between her 20 and her two low and in fact in her two zeros and this there is a trial going underway that’s her two less
than one but greater than zero.

That’s the Destiny 6 trial hasn’t reported yet.

But there’s also the her 20 equal 0 Daisy trial which was a small trial in France where there was clearly in these waterfall plots clearly patients that benefited from drug even though they had a hurt 2 = 0. So is this the why is it important to understand this and have good diagnostics for it because this drug is the tip of the iceberg. Here’s a list of other drugs which there’s no way you can read,
but all of these drugs are, are all these are targets for ADC’s in clinical trials. So I think ADC’s may become very important for oncology in the next few years and equally important will be companion diagnostics that actually pick the right patients as opposed to giving the drug because unlike when we know the tiger it so well and we know how the drug works. It’s really important to be able to pick the right target or to pick the right patients that express the right amount of target. So what do we do now?
So this is the standard practice guidelines, ASCO CAP guidelines in 2018 and these guidelines are how we practice as pathologists in assessment of HER2 expression. And this is the algorithm for what we look at when we look at the slide circumferential staining that is complete, intense and greater than 10% of the cells. That makes the three plus and then we have a 2 + 1 +. I won’t go through them all, but they’re kind of summarized here where one no staining, no membrane staining observed is a 0 +.
1 is faint partial membrane staining
NOTE Confidence: 0.861392332
and weak to moderate staining is +2.
NOTE Confidence: 0.861392332
That’s kind of subjective. In fact.
NOTE Confidence: 0.861392332
How well can we do that and
NOTE Confidence: 0.861392332
how important is it?
NOTE Confidence: 0.861392332
Well.
NOTE Confidence: 0.861392332
It used to be important to tell
NOTE Confidence: 0.861392332
the heart threes the three
NOTE Confidence: 0.861392332
pluses from the others and the
NOTE Confidence: 0.861392332
two pluses were they the reflex.
NOTE Confidence: 0.861392332
But now it’s important to have this CAD.
NOTE Confidence: 0.861392332
The new category is far too low.
NOTE Confidence: 0.861392332
And how many are there?
NOTE Confidence: 0.861392332
There’s a lot,
NOTE Confidence: 0.861392332
maybe as many as 65 or 70% of the
NOTE Confidence: 0.861392332
patients are thought to fall into this low,
her two low category which means a lot of patients could get drug, but it also means that we need to be as accurate as we can and assigning those patients because we don’t want the her two zeros to get the drug if they aren’t going to benefit. Well, maybe we do. We’ll talk about that later. But so how do we know how, how well do we do at this? Her too. So I’m fortunate to be on the immunohistochemistry committee of the College of American Pathologists.
And so I get access to the surveys that make CLIA labs, what CLIA labs are. That is, for a CLIA lab or clinical lab to return data to the chart, they have to do a survey twice a year to show that they’re competent and effective at doing the assay. And here’s the surveys for anatomic pathology for her two using a tissue microarray. This is her to a 2020. So it was the fall survey or the spring survey from 2020 from the College of American Pilot Pathologists. And you can see my colleagues here,
including myself, who are on this committee.

And when we looked at these surveys, we noticed that four, six and seven,

that is 3 out of 10 did not reach consensus. That means that of the 1400 labs

in the world that did this, they couldn’t come to an agreement. That is,

you need to have 90% consensus to have agreement. In fact, if we look at this one,

it’s interesting. This is one of the cases that didn’t come to agreement and that was

because there was a big discordance
in the number of called 0 versus 1 and there were a fair number that even called it two or three. So that's troublesome. If we're testing these labs twice a year and we're assuring that they're giving the right answer for the patients, how can you have that much difference between zero and one that it's almost 50/50. Since I'm on the CAP committee, I could ask for the data from the last few years. And here's the data from the lab from 2019 and 2020. And of the 80 cases, fifteen of those cases showed a
discordance of greater than 25%. And that’s shown in these pie charts here where the zeros are blue and the ones are red and anything higher than one is black. Two and three, since we’re not going to focus on that. So we did, we thought is this really, you know, this is concerning, but you know what this is tissue microarrays. This is not what happens in the real world. So then we did a study of real world core biopsies.
institutions around the United States and ask them to read actual core biopsies that have been read at Yale.

We collected 170 cases from Yale and had them score them according to the ASCO CAP guidelines before the publication and the popularization of her two one plus versus 0.

So they didn’t know they were just doing the ASCO CAP guidelines as they always have, and scoring 0123.

What they did, what they’re scoring looked like was this. That is, the Blues were the zeros. This is the percent of pathologists. That scored at 0,
so a fair number agreed that there were zeros.

There were 92 cases that were discordant, and of those, 9269 were discordant between zero and one, and only twenty were discordant between 2:00 and 3:00.

So this actually was. Through many reviewers, ultimately got us published in JAMA Oncology.

How come it’s not advancing now? Oh, hold on. I lost my laser pointer. Microsoft doesn’t want me to do this now. It’s the screen has turned gray as it’s saying restart.
I probably shouldn’t.

I should probably wait for the program to respond.

Very sorry about this, but suffice it to say that I’ll skip the next slide so we can keep going.

The next slide was after many review rounds of review, we did get this published in JAMA Oncology, but weren’t allowed to say what we wanted to say, which is that there’s really a great discordance between zero and one and not so much discordance between 2:00 and 3:00.

And I don’t know if we have
00:11:13.399 --> 00:11:14.460 should I restart the program?
NOTE Confidence: 0.85056264125

00:11:14.460 --> 00:11:15.980 I don’t know if we have any IT
NOTE Confidence: 0.85056264125

00:11:15.980 --> 00:11:17.448 people here that or how long we’re,
NOTE Confidence: 0.85056264125

00:11:17.450 --> 00:11:17.886 you know,
NOTE Confidence: 0.85056264125

00:11:17.886 --> 00:11:19.630 we’ll be here for the next 45 minutes
NOTE Confidence: 0.85056264125

00:11:19.679 --> 00:11:21.485 waiting for the computer to come along.
NOTE Confidence: 0.865999128571429

00:11:25.290 --> 00:11:28.076 Wait to respond or restart the program.
NOTE Confidence: 0.865999128571429

00:11:28.080 --> 00:11:29.494 Maybe I should restart the program and
NOTE Confidence: 0.865999128571429

00:11:29.494 --> 00:11:30.910 that will take several minutes too.
NOTE Confidence: 0.74473373

00:11:31.260 --> 00:11:32.646 It’s just it’s just not rebooting
NOTE Confidence: 0.74473373

00:11:32.646 --> 00:11:36.560 the computer, it’s just. Yeah.
NOTE Confidence: 0.914624506666667

00:11:36.560 --> 00:11:40.718 Let’s try again. Yeah, we’re good.
NOTE Confidence: 0.914624506666667

00:11:40.718 --> 00:11:43.094 We’re good. Sorry about that. OK.
NOTE Confidence: 0.914624506666667

00:11:43.094 --> 00:11:45.350 We saw all those things already.
NOTE Confidence: 0.914624506666667

00:11:45.350 --> 00:11:49.480 Let’s go to. The study that was in
NOTE Confidence: 0.914624506666667
JAMA Oncology was here and this is what was the this is the figure and
and Eileen Fernandez was the lead on this study in my lab and she.
Did the analysis that is shown here that shows that there’s a lot more discordance between zero and one than there is between 2:00 and 3:00. And for two we have a solution. For two we can do fish, so we have an orthogonal assay. What do we do between zero and one? Well, we don’t have a solution yet. That’s what I’ll show you in a minute. But also you can look at this analysis which shows you this is a work
done by Jack Robbins in the lab with Eileen Fernandez showing the percentage of people that called 0 versus called one. And so if your pathologist #18 and these are all currently signing out pathologists most with more than five years of experience around the country. So these are not residents or not to say that residents can’t do this just as well. But these are not residents or or or laboratorians. These are sign up with ologists. And if you’re pathologist 18 you only score 15% of the patients with a 0, but if your pathologist number one,
00:12:53.040 --> 00:12:54.090 you have 44%.
NOTE Confidence: 0.914624506666667
00:12:54.090 --> 00:12:56.540 So whether or not you get trustors,
NOTE Confidence: 0.914624506666667
00:12:56.540 --> 00:12:57.130 mab drugs,
NOTE Confidence: 0.914624506666667
00:12:57.130 --> 00:12:59.195 tecan depends on who your pathologist is.
NOTE Confidence: 0.914624506666667
00:12:59.200 --> 00:13:01.846 That doesn’t sound like a great idea to me.
NOTE Confidence: 0.914624506666667
00:13:01.850 --> 00:13:03.810 So what we asked is how many people
NOTE Confidence: 0.914624506666667
00:13:03.810 --> 00:13:05.760 do you need to make sure that
NOTE Confidence: 0.914624506666667
00:13:05.760 --> 00:13:07.570 an assay agrees with each other?
NOTE Confidence: 0.914624506666667
00:13:07.570 --> 00:13:09.826 And we, we invented this with gang hand.
NOTE Confidence: 0.914624506666667
00:13:09.830 --> 00:13:12.630 We invented this system to realize that
NOTE Confidence: 0.914624506666667
00:13:12.630 --> 00:13:14.415 there’s 21,000 pathologists just in the
NOTE Confidence: 0.914624506666667
00:13:14.415 --> 00:13:17.027 US and at that and 100,000 in the world.
NOTE Confidence: 0.914624506666667
00:13:17.030 --> 00:13:18.806 So how many do we need to decide
NOTE Confidence: 0.914624506666667
00:13:18.806 --> 00:13:20.389 whether or not an essay is good?
NOTE Confidence: 0.914624506666667
00:13:20.390 --> 00:13:22.581 And so we there, there is actually
NOTE Confidence: 0.914624506666667
00:13:22.581 --> 00:13:24.089 no statistical method for this.
So we simply decided to plot the overall percent agreement. That’s what overall LPA stands for versus the observers or readers. And what you see is that the more observers you have, the less agreement you have, which makes sense. The more people you ask, the more discordance you’re going to get in your answers, just mathematical truism. And so does this actually work and can this be used to assess assays? So here’s estrogen receptor.
Turns out we’re really good at estrogen receptor. If you have a quartet of pathologists read estrogen receptor, all four of them will agree somewhere between 85 and 95% of the time. How do we do for her? Too well, not so well. This is the plot for her. 2/3 plus or not three plus. We’re really good at that. But if you have a quartet of pathologists decide whether it’s zero or not zero, it’s between 40 and 80 percent. So this is a new method to approach the analysis.
to try to figure out how many we need and how many do we need to make a new assay to make a good study. Well, it’s when it plateaus, so in this case we probably need 9 or 10. And this case, no number is sufficient because it goes all the way down to the baseline to tell ones from, not once. So the point is, I think that. So based on that. We start, we started from the beginning and so that’s what we have done, is propose a new assay that’s measured, not red. And so based on that.
00:14:52.464 --> 00:14:54.347 with cell lines and these cell lines
NOTE Confidence: 0.902744163636364
00:14:54.347 --> 00:14:56.390 are all cell lines that are amplified,
NOTE Confidence: 0.902744163636364
00:14:56.390 --> 00:14:58.330 gene amplified and these cell
NOTE Confidence: 0.902744163636364
00:14:58.330 --> 00:15:00.270 lines are all gene express.
NOTE Confidence: 0.902744163636364
00:15:00.270 --> 00:15:00.848 Her too,
NOTE Confidence: 0.902744163636364
00:15:00.848 --> 00:15:02.582 but are not gene amplified and
NOTE Confidence: 0.902744163636364
00:15:02.582 --> 00:15:04.646 you can see when you plot them.
NOTE Confidence: 0.902744163636364
00:15:04.650 --> 00:15:06.876 If you look with the current FDA
NOTE Confidence: 0.902744163636364
00:15:06.876 --> 00:15:08.863 approved assay you can separate the
NOTE Confidence: 0.902744163636364
00:15:08.863 --> 00:15:11.290 highs from the lows or the negatives,
NOTE Confidence: 0.902744163636364
00:15:11.290 --> 00:15:13.570 but you can’t stratify the negatives.
NOTE Confidence: 0.902744163636364
00:15:13.570 --> 00:15:15.110 Whereas if you do the new assay
NOTE Confidence: 0.902744163636364
00:15:15.110 --> 00:15:16.848 high cut 10 times more antibody,
NOTE Confidence: 0.902744163636364
00:15:16.850 --> 00:15:18.122 pretty simple new assay.
NOTE Confidence: 0.902744163636364
00:15:18.122 --> 00:15:20.382 You can then stratify the low cell
NOTE Confidence: 0.902744163636364
00:15:20.382 --> 00:15:22.686 lines and tell the zeros from the ones.
Essentially if you were reading the cell lines but it was the wrong, the wrong the current assay is the wrong tool for the job. Or as was said by a group in France, The current assay now FDA approved, is like weighing mice on a scale for elephants. And I think this is really good because everybody gets this if you have a skill for elephants, it doesn’t work for weighing mice and it’s all about dynamic range. So here’s the assay we did invented and this is to have
a series of cell lines and then just do like a Bradford assay like we all did in college chemistry for where we make a standard curve. And we used our tissue microarray to make cell lines and with the help of array science made a standard curve. And then with the help of Crotia we figured out how many animals per microgram there were in each of these cell lines and then converted that using Q path to how many animals per square millimeter there are. So now we have an assay. That can tell us animals per square millimeter.
And like all assays, it saturates when it gets too high. So the amplified cases are saturated and we can’t use those. But since we don’t really care about 2 plus and three plus, we got that pathologists can do just fine in telling 3 plus from not three plus. We need an assay to tell 0 from 1. And so that’s this assay works fine. If we get rid of these two, we can now build a very nice standard curve that we can use as a linear assay and then assign each case and animals per square millimeter.
And so just to remind you. I'm going to talk a little bit about limits of detection, limits of quantification, and limits on linearity. A little bit of essay terminology and we want to be in this range, not this range, which is what the saturation range, and this is the range we want to be in, not this range, which is what the current essay really focuses on. Because really the current assay all you need to tell is, Is it saturated or not? For the new assay we need to tell how much they have.
So here's our current standard curve. With the higher two assay and you can see there's two positives and the rest are negative. So it works if you just want to tell amplified from non amplified, but what if you want to tell that low range so you can see the full dynamic range with the new her two low assay antibody concentration or that we're calling high sensitivity HSV or two you can see in that range. So now we have to talk about a little bit of wonky stuff and
that is what are those things.

So what is the limit of detection, what is the limit of quantification or what is the limit of linearity.

So these are the definitions and this is right.

One of the FDA’s handbook on how they advise industry to do this and you can see that the limit of detection is the lowest concentration of the analyte that can be detected but not necessarily quantified that is too low.

So what we really want is to know the limit of quantification because
then we can do it right every time.

But we don’t yet know how much

er two is required to benefit

So we’re going to measure all the

way down to beyond the limits of.

Our essay to to the LD and below and

see what we get and what we got is this.

When we did it on tissue microarray we

could see that the the zeros are blue,

the Reds are one, ones are red, the twos.

This is the pathologist read over here

2 Plus is black and three plus is green,

and most of the Greens are

above our limit of linearity.
But look at how many twos and ones there are in this middle range.

That would be called one.

And I think this is even further evidence that we need a quantitative assay.

We need a measured assay,

tican and surprisingly there are some patients most of whom were called 0, but some were called one or two that are actually below our limit of detection as I’ll show later.
So then we did what you have to do in a clear lab is did 40, you have to do 20 positives and 20 negatives according to Fitzgibbons at all in order to bring your assay to the CLIA lab. But we don’t have positives and negatives. We have a continuous scale. So we did 40 of them and these are actual core biopsies. Now they’re not tissue microarrays, but you can see the same thing. There’s a fair bit of Miss Assignment and in fact summarized here, you can see that there’s zeros and ones, but there’s a broad range of animals per
square millimeter for zeros and ones.

And the two plus not amplified almost

in fact does overlap with the two

plus amplifies and the three pluses,

which we're good at and we're pretty tight.

So how many are there?

Well, in our first forty there

was about 20% of the cases that

appear to be below the limit of

quantification for her two protein,

but potentially present and as

target for TDXD.

So just to summarize to this point,

about 70% of the cases have low her two

defined as above the LQ and below the

levels associated with gene amplification.
00:20:11.000 --> 00:20:12.908 About 8 to 10% are below our LQ or even our LD.
00:20:12.908 --> 00:20:14.840 It’s probably about 6% below our LD.
00:20:14.840 --> 00:20:17.556 Many of the cases that are called her to zero, as many as 60% are in our studies,
00:20:18.600 --> 00:20:19.640 maybe 75% have detectable amounts of her too between 3 and 20 animals
00:20:21.440 --> 00:20:26.394 the quantitative her two asset could be envisioned as a reflex tax.
00:20:23.310 --> 00:20:28.680 and the quantitative her two asset could be envisioned as a reflex tax.
00:20:26.394 --> 00:20:28.680 and the quantitative her two asset could be envisioned as a reflex tax.
00:20:28.680 --> 00:20:31.207 So that if you pathologist reads an IHC equals zero,
00:20:33.196 --> 00:20:34.520 they could then reflex to the quantitative test and the same way we reflex to fish today for A2 plus HC.
OK, so that’s the proposed new assay.

Now let’s take it to the clinic.

So what’s involved in the next step of taking to the clinic? And I like to quote a colleague of mine from Brigham and Women’s who said once you have the essay working in your research lab, you’re 5% of the way there. And I think that’s really true.

Now having brought this assay with hats off to Trish Gal who’s not here and has not left, Nay Chan who was in the audience and Reva come ova who have helped me to bring this assay to the clinical setting.
So the things that you have to do are antibody titration, maximization of signal to noise analytic validation. I'll try to go through this stuff fast because it's a little on the wonky side, performance accuracy, precision, sensitivity and specificity and serial core reproducibility. And then how do we tell our colleagues? What do we tell the oncologists? And then so the reporting is part of this as well. So first of all, we looked at the signal to noise.
and you can see that the peak signal to noise is at 1 microgram per mil for a new antibody. This is a new higher sensitivity antibody for her too than the one that's currently used in the clinic. And we took and we picked the concentration with the maximal signal to noise and then we looked at the accuracy. Our accuracy isn’t great, it's only 87%. Why is that? That's because we're more sensitive than the status quo assay which we had to compare it to which was HC012 and three. But overall we have quite a quite
good concordance especially in the end and more resolution in the low range. And then our intra and intra assay precision is quite high, 10% sounds like it might not be great. To interact assay precision and actually the essay that we just bridged to, we’re now under 10%, we’re now under 10%, it’s acceptable and the intra assay precision, this means to calculate the precision three slides run on separate trays at the same machine is, is about 5%. So these are where we want to be.
Our sensitivity compared to the historical essay as 100% and our specificity is 84%

Why is our specificity low?

Because we're more sensitive and so we call cases positive that we're called negative by IHC.

So here's the proposed clinical future work workflow and this is what we're doing now which is we have we get the labs come to this lab that I've called the qutab lab which is now open and open for business.

And we've now begun to do this.

This is and this is qdap essay #1,
the high sensitivity here.

Two, we batched the stains and do them in our like a bond stainer so that they’re done in an auto stainer and then we read them originally in some old like legacy hardware. But now we’re using this, we just recently completed the bridge study, although our license holder hasn’t signed off yet, he will see it shortly and uses a much more high throughput device. Instead of an hour this machine would take about four minutes to scan a slide.
So we wanted to update our technology a little bit and then we signed it out and Co path as a procedure. And so it ultimately makes it to epic and clinicians can see it, this is what it looks like.

The pathologist has to pick a region. So we’re actually not measuring the entire core biopsy. We’re measuring a region that is quote UN quote representative and that representative region is then looks like this.

This is actually not a brown stain but a pseudo IHC which it shows the pathologist what it looked
like and then the pathologist actually sees the number of fields of view, in this case 23 and the in this case the rare sight score in this case was 15.4 animals per square millimeter. So that will be included in the report. We’d say 15.4 animals require millimeter. We don’t know what that means. We do know that it’s detectable and then we can give a choice in our interpretation that it’s positive for expression high. That is, it’s above our limit of linearity positive expression intermediate.
which means that it’s like a one

or A2 positive for expression low,

which means it’s.

Present, but it might not be reproducible.

That is, it’s above our LOD but

not necessarily above our LOQ

and then negative below the LOD.

And so these are the reports that we’ll

not as as we start to receive specimens.

So far we’ve received a grand total of two.

We hope that after this talk and maybe

in the future and certainly in the more

distant future when we know how much.

Is necessary for patients to respond.

We hope that this essay will

gain some traction.
So our vision we currently offer HSR 2 in the QDAP lab test must be requested by an oncologist and the patients are billed. If the test is requested by an oncologist, there are CD9 codes for all the stuff we’re doing.

We began a prospective study on all breast biopsies so that we have data of a year’s worth of prospective data and we’re about seven months into it now. We offer the essay coalitions from to Yale or elsewhere who want quantitative information, but only two so far to date. And then the discussions of the
What we hope to happen is ultimately it won’t just be yell that can do this, but we’ll license it to some of the big lab companies that provide them the bulk of the service. It’s interesting to know and interesting to me anyway, that only 15% of lab tests in the US are provided by academic labs. The other 85% are provided by private labs. And so clearly if we want to have this effect patients around the world and be useful and needs those discussions are beginning.
So the last thing I want to talk about is the precision versus persuasion medicine. And so our original envision for this essay was that we would need to adjudicate the IHC’s equal 0. And what we would do is we would get all the HC equals zero and we would measure them and then we tell you if you’re above the limit of detection or above the limit of response. We don’t know the limit of response yet. Someday we will and I’ll show you how we intend to get there. But right now we don’t know the limit of response but.
00:26:38.440 --> 00:26:40.421 You would take all the cases that
NOTE Confidence: 0.830076910416667
00:26:40.421 --> 00:26:42.416 were called HC0 and maybe the cases
NOTE Confidence: 0.830076910416667
00:26:42.416 --> 00:26:44.569 that were called HC One and do that.
NOTE Confidence: 0.830076910416667
00:26:44.570 --> 00:26:46.256 But something happened in the last
NOTE Confidence: 0.830076910416667
00:26:46.256 --> 00:26:48.170 three or four months and I haven’t
NOTE Confidence: 0.830076910416667
00:26:48.170 --> 00:26:49.634 been able to document it yet,
NOTE Confidence: 0.830076910416667
00:26:49.640 --> 00:26:51.530 probably because it’s not mature enough,
NOTE Confidence: 0.830076910416667
00:26:51.530 --> 00:26:55.370 but suddenly the IHC equals zero is rare.
NOTE Confidence: 0.830076910416667
00:26:55.370 --> 00:26:57.150 And that’s because pathologists
NOTE Confidence: 0.830076910416667
00:26:57.150 --> 00:26:58.485 are people too.
NOTE Confidence: 0.830076910416667
00:26:58.490 --> 00:26:59.878 Pathologists sometimes might be
NOTE Confidence: 0.830076910416667
00:26:59.878 --> 00:27:01.960 a little more lenient on what
NOTE Confidence: 0.795286742857143
00:27:02.026 --> 00:27:04.098 they call IHC one and this code
NOTE Confidence: 0.795286742857143
00:27:04.098 --> 00:27:05.334 called Sympathy vote because
NOTE Confidence: 0.795286742857143
00:27:05.334 --> 00:27:07.329 then they can get this new drug.
NOTE Confidence: 0.795286742857143
00:27:07.330 --> 00:27:09.087 Here are real quotes that I’ve heard.
I won’t quote the people because to not embarrass them or give them credit, but here’s a real quote.

Hi doctor pathologist. So I see you called Missus X’s biopsy IHC zero. That means I’m going to have to offer her brain radiation. Are you sure it’s not H sequels one? Then I could give her her and her two. Should I go look at that slide again? Does that mean that my first view was not accurate? or was it accurate and maybe I should change my diagnosis?
Because I’m persuaded that that’s better for the patient. I’m not sure that’s a great idea from West Coast director of pathology service. Yeah, we don’t have many IHC’s equal 0 anymore. And from a Midwestern oncologist, I’m not seeing the response rates and in her two patients that they saw in the clinical trial. They’re getting a lot of IHC zeros and maybe IHC zeros really don’t respond. We know that eight to 10% of the cases really don’t express any target and this is a targeted therapy. I mean we don’t definitively
know how the drug works, but we think it’s a targeted therapy. After all, it’s trastuzumab conjugated to toxins. So what’s happened is that really now we need to adjudicate the one pluses what we really need because the zeros have minimized, not, I don’t want to say they’ve gone away. If you ask pathologists, they will sternly tell you yes, of course we still call IHC 0. But. Data will tell us in a year or so from now how
our IC0 calls changed.

But but I see one is now more common

and so if it’s if it’s more common

maybe that’s the one we should be

measuring and in fact that’s the plan.

So there are a few different ways

we’re going to study IHC equals one.

The first is the Qdap Labs

prospective study and this is

copied with me by name by Nate Chan,

who’s the director of the Q dot lab.

And you can see we began August

and we’ll go till July 2023

and today we have 226.

I anticipate we’ll get around 400.

The inclusion criteria will be any
case and the primary objective will be to determine the number of H0 cases that have detectable her two expression. So how many IHC zeros? And this study was designed before everything. Name HC One, but how many HC Zeros have above the limit of detection and how many HC ones have below the limit of detection will be interesting as well. That’s a secondary, that’s a secondary objective of the study and the study is in process and we all just to show you a peak,
we’ve already started doing some quantitative work and in fact you can see from quantitative, this is quantification of prospective tissue from the clinical trial and you can see the lol in this case was 33 and the OD is 3. This is all done on the new platform and you can see that there’s a lot of cases that are called zeros that are above our limit of detection. There’s not as many.

So far it looks like we’re going to not have very many that are below our limit of detection, but time will tell as we get
as the study matures. There’s two other studies that were progressing on one is a TB CRC study report proposal with Ian and Eric’s arrival at Yale, we became part of the Translational Breast Cancer Research Consortium, which is a group of 16 or 17. Now institutions that do studies together on translational research and the goal of this study that is still in proposal stage is to evaluate her two measurement in the one plus metastatic cases. So if we get one plus and we get 2 or
From 17 institutions around the country, we should be able to tell how frequently we see the patients that have one plus actually don’t have any target and vice versa, we should be able to see response since all those patients since they were called one plus will get. Drug will be getting trustors map drugs he can be present in the residency. So here’s the study a draft of the study objectives to evaluate the real world relationship between quantitative her two expression QIF and objective response in patients with her two IHC plus one and
metastatic breast cancer receiving TXT.

And then there’s a number of secondary objectives that are shown here as well.

And then a second study that I, I don’t even have a slide for yet is that we proposed a study led by Merriam Lustberg here of patients who get HC0 and then prospectively giving them TXD much the way the Daisy trial worked on that study is not yet completely designed and not yet completely approved. So I don’t have any slides to discuss it, but I think those are the kinds of studies we need where we have patient response.
Either real-world patient response or clinical trial patient response in order to figure out the animals per square millimeter above which patients benefit. Will it be a cut point? Probably not. Probably there will be patients with high animals per square millimeter that still don’t benefit because there are other mechanisms of resistance. And I have and one of the interesting topics that many labs are working on including my own are what are the mechanisms of resistance beyond just not enough her to present. And hopefully next year or a couple
00:32:15.928 --> 00:32:18.146 years from now I'll come back to you at
NOTE Confidence: 0.833837779090909

00:32:18.146 --> 00:32:19.868 grand Rounds and talk about mechanisms
NOTE Confidence: 0.833837779090909

00:32:19.868 --> 00:32:22.082 of resistance and a more complex assay
NOTE Confidence: 0.833837779090909

00:32:22.082 --> 00:32:25.036 that also doesn’t just assay her too,
NOTE Confidence: 0.833837779090909

00:32:25.040 --> 00:32:26.900 but maybe assays other biomarkers
NOTE Confidence: 0.833837779090909

00:32:26.900 --> 00:32:28.760 that are associated with resistance.
NOTE Confidence: 0.833837779090909

00:32:28.760 --> 00:32:29.546 Or other drugs.
NOTE Confidence: 0.833837779090909

00:32:29.546 --> 00:32:31.380 And in fact the her two trope
NOTE Confidence: 0.833837779090909

00:32:31.441 --> 00:32:33.184 2 assay as well along its way.
NOTE Confidence: 0.833837779090909

00:32:33.190 --> 00:32:35.542 So we can help clinicians decide
NOTE Confidence: 0.833837779090909

00:32:35.542 --> 00:32:37.110 between Saskatoon Vova Tican,
NOTE Confidence: 0.833837779090909

00:32:37.110 --> 00:32:40.652 which is a trope 2 targeting therapy
NOTE Confidence: 0.833837779090909

00:32:40.652 --> 00:32:42.580 versus trastuzumab Drexel can.
NOTE Confidence: 0.833837779090909

00:32:42.580 --> 00:32:44.638 So for that my my last slide,
NOTE Confidence: 0.833837779090909

00:32:44.640 --> 00:32:47.160 overall HSR 2 assay is an LDT,
NOTE Confidence: 0.833837779090909
a lab developed test and not FDA approved.

So if you only do FDA approved tests,

since most of our assays are LDT’s,

but we do have a few FDA approved assays

People don’t realize this,

if you change one step of the protocol of your FDA approved assay,

it is then an LDT and you must thus validate it and so most assays.

We do are not FDA approved.

We might use FDA approved reagents,

but most assays we do are actually LDT’s in
our lab and in all the labs around the world. And that also applies for molecular assays, gene mutation assays. Many of those assays are also not FDA approved assays but rather LDT's. HSR 2 essay is in the correct dynamic range. That is, we’re not weighing elephants on or weighing mice on a scale built for elephants. The level of target required for trustees, mab drugs decan is still unknown and I speak here before you and I don’t want to try to hide that from you. I think it’s very clear that we don’t know the answer to this question yet. But if we waited until we knew the answer to
the question before we started the essay,

NOTE Confidence: 0.811140847333333

we would be years behind as as this essay.

NOTE Confidence: 0.811140847333333

We’ve been working on this essay

NOTE Confidence: 0.811140847333333

for a couple years now to get it

NOTE Confidence: 0.811140847333333

to the point that it’s at.

NOTE Confidence: 0.811140847333333

And so now that we have.

NOTE Confidence: 0.811140847333333

Tools, I am asked that oncologists in

NOTE Confidence: 0.811140847333333

the audience ask for measurements,

NOTE Confidence: 0.811140847333333

not for readings.

NOTE Confidence: 0.811140847333333

And please don’t ask the pathologist

NOTE Confidence: 0.811140847333333

to change their minds.

NOTE Confidence: 0.811140847333333

That’s persuasion Madison,

NOTE Confidence: 0.811140847333333

not precision medicine.

NOTE Confidence: 0.811140847333333

And we all respect our pathology

NOTE Confidence: 0.811140847333333

colleagues and I think we all,

NOTE Confidence: 0.811140847333333

you know,
I know that oncologists really think highly of most of the pathologists they work with. And I think that they don’t realize that when they do pursue persuasion medicine that it’s actually not what the biologist wants to hear. They don’t want to be second guessed. If that was your first impression, don’t change your answer. If that was your first impression, don’t change your answer.
it’s probably your true impression

and probably your best reading.

And so with that,

I just want to thank the people

in lab that do all the work.

I get to talk about it,

but it’s really a crew of people that do

all this stuff that I told you about.

I especially like to point out mirror

to Matafi who started that and started

building this essay in the lab over

two years ago now and then my Yale

collaborators and funding sources etcetera.

And then here’s the the key

group at our last holiday party,

our lab group Aileen has now left.
She was involved in a lot of the analytic stuff. Matt Lou helped out with some of the analytic stuff as well. And then Jack and Katie weren’t at the party, so they got their picture separate. So with now, I’ve also left about 20 minutes for questions. So we have 4 questions in the chat. Maybe while you’re warming up, should I start with those? Oh no, there’s only two.
What about discordance with pathologists reading the same slides after a washout period?

So Manju Prasad, a esteemed pathologist in our department, asks a very pivotal question, that is.

When you're doing any kind of pathologist study, when you read it once, if you're going to read it again, you should have a washout period. That is so you don't remember that case because surprisingly, pathologists have a really good memory for what the morphology of cases look like, and they can also remember the
And so a lot of studies have a washout period. We didn’t need a washout period in this study because they only saw the slides once. So if we’re going to show them to them again and if we’re going to do any kind of intra observer reproducibility, which we didn’t do and some other studies have done, we would need a washout period. But in this case, a washout period was not required. And then Timothy Robinson asks, is heterogeneity within
the tumor important issue?

Is it more important to do a small percentage of cancer cells that express a high amount of heart, too?

Or is it more important to know that a high number of cells expressed at least the minimum amount of her too?

Wow, phenomenal question. That’s Jax three, that’s his. Thesis project, I think that’s a great question.

We obviously don’t know the answer. All the pathologists in the audience know that her two is very heterogeneous. Not only is it heterogeneous from within a slide,
but it’s heterogeneous between cuts. And all the pathologists in the audience know that when we sample one core biopsy that’s less than 1% of the tumor. And so there’s no way for us to actually answer that question about true heterogeneity of the patients tumor. But what we can add, we can ask about heterogeneity on the slide and we can and are asking at that question. That is, how important is high expression in a single cell versus high expression in the average cell?
00:37:27.570 --> 00:37:28.610 We started with the average.
NOTE Confidence: 0.86163855125
00:37:28.610 --> 00:37:30.110 You have to start somewhere,
NOTE Confidence: 0.86163855125
00:37:30.110 --> 00:37:31.454 and I don’t know that the
NOTE Confidence: 0.86163855125
00:37:31.454 --> 00:37:32.750 average is a correct answer.
NOTE Confidence: 0.86163855125
00:37:32.750 --> 00:37:34.325 You could argue because of
NOTE Confidence: 0.86163855125
00:37:34.325 --> 00:37:35.943 the bystander effect of TDXD,
NOTE Confidence: 0.86163855125
00:37:35.943 --> 00:37:37.708 it’s actually the highest ones
NOTE Confidence: 0.86163855125
00:37:37.708 --> 00:37:39.890 that make the most difference,
NOTE Confidence: 0.86163855125
00:37:39.890 --> 00:37:41.370 but we don’t know that.
NOTE Confidence: 0.86163855125
00:37:41.370 --> 00:37:44.670 That’s just speculation at this point.
NOTE Confidence: 0.86163855125
00:37:44.670 --> 00:37:49.398 Let’s see. Now it’s your turn.
NOTE Confidence: 0.86163855125
00:37:49.400 --> 00:37:50.810 It doesn’t.
NOTE Confidence: 0.86163855125
00:37:50.810 --> 00:37:51.240 Say.
NOTE Confidence: 0.87842585
00:37:54.980 --> 00:37:56.140 That’s a great question.
NOTE Confidence: 0.798758137692308
00:37:59.010 --> 00:37:59.882 We can do more.
NOTE Confidence: 0.798758137692308
00:37:59.882 --> 00:38:02.249 So as soon as I had the assay built,
I applied for tissue from AstraZeneca.

And I was rapidly told I would never see that.

And it’s, I don’t fault them for that. They have their own people that can do quantitative work and they have an FDA approval for IHC 0123.

So they don’t want to have to change their FDA approval.

They’re making a lot of money on this drug and it would be detrimental to the shareholders of that company to have me have access to that tissue.

My question was how much heterogeneity do you see in the ATOMAL expression?
Because you’re taking so many fields of view and taking an average, do you see a lot of heterogeneity there or is it? So that’s a great question. Heterogeneity within a core biopsy is quite substantial. And as you know when you read them, you see bright areas and not so bright areas. And you know how do we handle that? Well, someday we’ll know how you know whether it’s the highest sell or the average sell or the lowest sell. That’s most important for response to Trump drug seeking. But we don’t know that yet.
we're just going to take a core biopsy and
say that that represents the whole tumor,
we're going to just take the average and say that that.
Represents the expression of her too.
And the second question
moved for the clinician.
We see situations with heterogeneity
where we have a clear 3 plus tumor where
the patient gets you know trastuzumab
and there's complete response and
there's another tumor which was her
to negative and was zero or you
know one plus which didn’t respond.
So what will these patients benefit
00:39:48.120 --> 00:39:51.630 when they have two distinct?
NOTE Confidence: 0.9305174

00:39:54.780 --> 00:39:57.370 Her two profiles to maybe I can use the
NOTE Confidence: 0.9305174

00:39:57.370 --> 00:40:00.924 to clarify the question because,
NOTE Confidence: 0.71368902

00:40:00.930 --> 00:40:02.530 I mean we wouldn’t use.
NOTE Confidence: 0.837351195714286

00:40:04.690 --> 00:40:06.895 We wouldn’t have used a standard her
NOTE Confidence: 0.837351195714286

00:40:09.077 two therapy if they were one plus.
NOTE Confidence: 0.6665299375

00:40:13.940 Why don’t we choose?
NOTE Confidence: 0.8049838

00:40:22.410 Do we use her two therapy, I mean. Yeah.
NOTE Confidence: 0.832513583333333

00:40:25.428 But the patient?
NOTE Confidence: 0.5355477925

00:40:30.598 Patient had complete response
NOTE Confidence: 0.69599822

00:40:33.874 to that through. So it was our CB0,
NOTE Confidence: 0.69599822

00:40:36.364 but then the tumor which was
NOTE Confidence: 0.69599822

00:40:38.020 one plus still extensive.
Yeah so I mean it depends on the clinical situation. We know pretty clearly now that with the previous generation of her two therapies that you do not see any benefit with non her 2/3 plus or amplified cancer. So the her two lows do not respond to the previous generation any of the previous generation of her two therapies. So but with now with Tristan Madrox taken you know I think you could make a case that you know you might you would see potentially could see benefit both.
And that clearly amplified in the her two lows. But prior to that we would look at a case like that on a case by case basis and say well let’s use the her two therapy to get rid of that usually more aggressive her two her two positive cancer and then we’ll worry about the her two negative or her two low cancer later. But it’s it’s you know again the field is evolving now that we have these drugs that work across different levels of her too. I mean getting to in your earlier point that the two questions were brought
up about her two heterogeneity and I think that’s really interesting. Again with the first generation,
her two therapies, it was very clear. We actually did a prospective, big prospective trial with the other,
the first antibody drug conjugate that doesn’t have bystander effect and in that study a heterogeneous
cancer responded much work much less. Effectively to a heterogeneous cancer than it did to a non heterogeneous
cancer and and quantitatively that the to your specific question what mattered was the percent of her two
negative cells not the intensity

Again with a drug that has bystander effect as as I think David was alluding to that might be switched and maybe just if you just need to have a certain number of her two strongly positive cells to get the drug in. And then the bystander effect will take care of the her two negative cells. We like to test out prospectively with we haven’t. Had the funding yet to do that trial.

Yeah. And I enjoy your talk, David. Is any do you have any information? The conjugate drug can get activated.
In the extracellular and microenvironment of tumor cells.

So I would again defer to Ian, who's much more of an expert on this than I am.

But it's my understanding that the drug, once it comes off, it has to be cleaved inside the cell.

But once it comes off, it survives in the extracellular environment.

And that's how the bystander effect works.

That's how it can kill neighboring cells.

Bystander effect doesn't really require. To go into the cells as long as it's a. Present in the microenvironment of the
tumor cell in the enriched fashion

you will have some activities.

Well the drug is an inhibitor

so it has to get to the nucleus somehow.

I guess to have its effect

Outside of the cells. You don’t have to take

anybody to go inside the cells.

Right, but that but

the antibody doesn’t necessarily

take the the drug I guess can get

into cells without the antibody,

but the reason they conjugated to

antibodies so you can increase
the dose locally to the tumor.
W e have a more protease type.
To break up the linkage between conjugate drug and the conjugate ohh, that’d be fine.
That that means you read that could partly explain why the the heterogeneity potential difficult involvement as well as you may have another interesting parameter to assess now today with the. Mass Effect. We could look into.
Whether they’re saying reached?
Do you conjugate?
Drugs.
And in that case you would argue that it worked.
It would work without trustors maybe even being present.
You could get the deconjugation even if there’s no target present.
That’s why you get a negative.
Right without her two present if it’s a true her the drug could still work because it could get deconjugation and be effective.
Everywhere in the body.
Anywhere in the exercise room.
That particular,
00:45:14.860 --> 00:45:15.500 yeah,
00:45:15.500 --> 00:45:16.496 I think that you know,
00:45:16.500 --> 00:45:18.282 the question is the toxicity
00:45:18.282 --> 00:45:20.150 then and that’s actually the problem.
00:45:20.150 --> 00:45:20.890 I didn’t go into that.
00:45:20.890 --> 00:45:22.451 But one of the problems with this
00:45:22.451 --> 00:45:24.237 drug is it has pulmonary toxicity
00:45:24.237 --> 00:45:26.012 and that patients get interstitial
00:45:26.012 --> 00:45:27.938 lung disease about 10% of the time.
00:45:27.938 --> 00:45:29.118 And that’s another reason why
00:45:29.118 --> 00:45:30.708 you need a companion diagnostic.
00:45:30.710 --> 00:45:33.308 And one wonders if perhaps the
00:45:33.308 --> 00:45:36.069 interstitial lung disease is due to extra,
00:45:36.070 --> 00:45:37.020 you know,
drug even in the absence of her too, although we’ve also found that her two is present. Normal Airways at about the level it is in, or between four and six animals per square millimeter.