I'm, 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. And this has become increasingly important as the number of targeted therapies has increased.
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 exactly 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 that today.
So thank you for bringing this timely discussion to us.

OK, great. Thanks, Diane, and thanks to the leadership for inviting me today.

But thanks especially for Ian for introducing me. He's a world leader in this space that I'm going to talk about.

And reading versus measuring. 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. Today I want to spend the next 55 minutes or so talking about first and quick introduction to the new drugs.
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, 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 would be given cause 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 so 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, but 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 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 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 her two 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 Clea 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 fall survey or the spring survey from 2020 from the College of American Pilot Pathologists. So it was the fall survey or the spring survey from 2020.

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. 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
00:09:15.698 --> 00:09:17.329 discordance of greater than 25%.
00:09:17.329 --> 00:09:19.243 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.
00:09:21.063 --> 00:09:22.911 Two and three, since we’re not going to focus on that.
00:09:22.978 --> 00:09:25.066 So we did, we thought is this really, you know, this is concerning, but you know what this is tissue microarrays.
00:09:25.070 --> 00:09:27.210 And enrolled 18 pathologists from 15 world core biopsies.
00:09:27.210 --> 00:09:28.154 So then we did a study of real world core biopsies. And enrolled 18 pathologists from 15
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.
00:10:11.894 --> 00:10:14.030 so a fair number agreed that
00:10:14.030 --> 00:10:15.438 that there were zeros.
00:10:15.438 --> 00:10:18.370 There were 92 cases that were discordant,
00:10:18.370 --> 00:10:19.822 and of those,
00:10:19.822 --> 00:10:23.210 9269 were discordant between zero and one,
00:10:23.210 --> 00:10:24.875 and only twenty were discordant
00:10:24.875 --> 00:10:26.207 between 2:00 and 3:00.
00:10:26.210 --> 00:10:28.438 So this actually was.
00:10:28.438 --> 00:10:30.109 Through many reviewers,
00:10:30.110 --> 00:10:34.457 ultimately got us published in JAMA Oncology.
00:10:34.460 --> 00:10:35.978 How come it’s not advancing now?
00:10:38.430 --> 00:10:43.566 Oh, hold on. I lost my laser pointer.
00:10:43.570 --> 00:10:45.386 Microsoft doesn’t want me to do this now.
00:10:45.390 --> 00:10:46.960 It’s the screen has turned
00:10:46.960 --> 00:10:48.530 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 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 people here that or how long we’re, you know.
NOTE Confidence: 0.85056264125

00:11:15.980 --> 00:11:17.448 we’ll be here for the next 45 minutes
NOTE Confidence: 0.85056264125

00:11:17.450 --> 00:11:17.886 waiting for the computer to come along.
NOTE Confidence: 0.865999128571429

00:11:25.290 --> 00:11:28.076 Maybe I should restart the program and that will take several minutes too.
NOTE Confidence: 0.865999128571429

00:11:28.080 --> 00:11:32.646 It’s just it’s just not rebooting
NOTE Confidence: 0.74473373

00:11:31.260 --> 00:11:32.646 We’re good. Sorry about that. OK.
NOTE Confidence: 0.74473373

00:11:36.560 --> 00:11:40.718 Let’s go to. The study that was in
NOTE Confidence: 0.914624506666667

00:11:40.718 --> 00:11:43.094 We saw all those things already.
NOTE Confidence: 0.914624506666667

00:11:43.094 --> 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 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
NOTE Confidence: 0.914624506666667
00:12:21.286 --> 00:12:24.221 done by Jack Robbins in the lab with
NOTE Confidence: 0.914624506666667
00:12:24.221 --> 00:12:26.120 Eileen Fernandez showing the percentage
NOTE Confidence: 0.914624506666667
00:12:26.120 --> 00:12:29.080 of people that called 0 versus called one.
NOTE Confidence: 0.914624506666667
00:12:29.080 --> 00:12:30.886 And so if your pathologist #18 and
NOTE Confidence: 0.914624506666667
00:12:30.886 --> 00:12:32.772 these are all currently signing out
NOTE Confidence: 0.914624506666667
00:12:32.772 --> 00:12:34.872 pathologists most with more than five
NOTE Confidence: 0.914624506666667
00:12:34.872 --> 00:12:37.056 years of experience around the country.
NOTE Confidence: 0.914624506666667
00:12:37.060 --> 00:12:39.274 So these are not residents or not to say
NOTE Confidence: 0.914624506666667
00:12:39.274 --> 00:12:41.599 that residents can’t do this just as well.
NOTE Confidence: 0.914624506666667
00:12:41.600 --> 00:12:43.365 But these are not residents
NOTE Confidence: 0.914624506666667
00:12:43.365 --> 00:12:44.777 or or or laboratorians.
NOTE Confidence: 0.914624506666667
00:12:44.780 --> 00:12:46.640 These are sign up with ologists.
NOTE Confidence: 0.914624506666667
00:12:46.640 --> 00:12:48.999 And if you’re pathologist 18 you only
NOTE Confidence: 0.914624506666667
00:12:48.999 --> 00:12:51.657 score 15% of the patients with a 0,
NOTE Confidence: 0.914624506666667
00:12:51.660 --> 00:12:53.040 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.

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. I’ve I hope that I’ve convinced you that we need a new assay. And so based on that. We start, we started from the beginning
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 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 we 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 the current our 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 that 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

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 but and reliably to strings

from zero 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
her two is required to benefit
from trust who’s mab drug stcan.
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
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,

We need a measured assay,

not a red.

Say,

in order to pick the right patients for trastuzumab drugs,

patients most of whom were called 0,

but some were called one or two

that are actually below our limit

of quantification or even 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 a 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.
About 8 to 10% are below our LQ or even our LD. It’s probably about 6% below our LD. Many of the cases that are called her to zero, as many as 60% are in our studies, maybe 75% have detectable amounts of her too between 3 and 20 animals and the quantitative her two asset could be envisioned as a reflex tax. So that if you pathologist reads an IHC equals zero, 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 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 and 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%,
but 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.

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 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. 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 they what it looked
like and then the pathologist actually sees the number of fields of view, in this case 23 and 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 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 issue 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
license we will.

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 to make it into private labs and 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.
You would take all the cases that were called HC0 and maybe the cases that were called HC One and do that. But something happened in the last three or four months and I haven’t been able to document it yet, probably because it’s not mature enough, but suddenly the IHC equals zero is rare. Pathologists sometimes might be a little more lenient on what they call IHC one and this code called Sympathy vote because then they can get this new drug. 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 two. Should I go look at that slide again? Does that mean that my first view was not accurate? of that slide 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...
00:30:09.540 --> 00:30:10.320 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 pluses and we get 2 or 61
00:30:41.314 --> 00:30:43.730 from 17 institutions around the country,
NOTE Confidence: 0.833837779090909
00:30:43.730 --> 00:30:46.334 we should be able to tell how
NOTE Confidence: 0.833837779090909
00:30:46.334 --> 00:30:48.120 frequently we see the patients
NOTE Confidence: 0.833837779090909
00:30:48.120 --> 00:30:50.130 that have one plus actually don’t
NOTE Confidence: 0.833837779090909
00:30:50.130 --> 00:30:52.207 have any target and vice versa,
NOTE Confidence: 0.833837779090909
00:30:52.210 --> 00:30:53.666 we should be able to see response
NOTE Confidence: 0.833837779090909
00:30:53.666 --> 00:30:55.016 since all those patients since they
NOTE Confidence: 0.833837779090909
00:30:55.016 --> 00:30:56.591 were called one plus will be get.
NOTE Confidence: 0.833837779090909
00:30:56.600 --> 00:30:58.217 Drug will be getting trustors map drugs
NOTE Confidence: 0.833837779090909
00:30:58.217 --> 00:31:00.246 he can and be present in the residency.
NOTE Confidence: 0.833837779090909
00:31:00.250 --> 00:31:02.610 So here’s the study a draft of the
NOTE Confidence: 0.833837779090909
00:31:02.610 --> 00:31:04.441 study objectives to evaluate the
NOTE Confidence: 0.833837779090909
00:31:04.441 --> 00:31:06.013 real world relationship between
NOTE Confidence: 0.833837779090909
00:31:06.013 --> 00:31:07.977 quantitative her two expression QIF
NOTE Confidence: 0.833837779090909
00:31:07.977 --> 00:31:09.772 and objective response in patients
NOTE Confidence: 0.833837779090909
00:31:09.772 --> 00:31:12.522 with her two IHC plus one and

62
00:31:12.522 --> 00:31:14.612 metastatic breast cancer receiving TXT.

00:31:14.620 --> 00:31:17.077 And then there's a number of secondary objectives that are shown here as well.

00:31:17.077 --> 00:31:21.174 And then a second study that I, 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.

00:31:21.180 --> 00:31:23.524 I don't even have a slide for yet.

00:31:23.524 --> 00:31:26.388 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.

00:31:26.388 --> 00:31:28.508 giving them TXD much the way the Daisy trial worked on that study is not yet completely designed and not yet completely approved.

00:31:31.396 --> 00:31:46.009 but I think those are the kinds of studies 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
grand Rounds and talk about mechanisms of resistance and a more complex assay
that also doesn’t just assay her too,
but maybe assays other biomarkers that are associated with resistance.
So we can help clinicians decide between Saskatoon Vova Tican,
and in fact the her two trope assay as well along its way.
So we can help clinicians decide between Saskatoon Vova Tican,
and in fact the her two trope assay as well along its way.
So we can help clinicians decide between Saskatoon Vova Tican,
and in fact the her two trope assay as well along its way.
So we can help clinicians decide between Saskatoon Vova Tican,
and in fact the her two trope assay as well along its way.
So we can help clinicians decide between Saskatoon Vova Tican,
and in fact the her two trope assay as well along its way.
So we can help clinicians decide between Saskatoon Vova Tican,
and in fact the her two trope assay as well along its way.
So we can help clinicians decide between Saskatoon Vova Tican,
and in fact the her two trope assay as well along its way.
So we can help clinicians decide between Saskatoon Vova Tican,
and in fact the her two trope assay as well along its way.
So we can help clinicians decide between Saskatoon Vova Tican,
and in fact the her two trope assay as well along its way.
a lab developed test and not FDA approved. So if you only do FDA approved tests, you probably don’t do them here since most of our assays are LDT’s, but we do have a few FDA approved assays and many 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,
We’ve been working on this essay for a couple years now to get it to the point that it’s at.
And so now that we have.
Tools, I am asked that oncologists in the audience ask for measurements,
not for readings.
And please don’t ask the pathologist to change their minds.
That’s persuasion Madison,
not precision medicine.
And we all respect our pathology colleagues and I think we all,
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.
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 and 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, if there are questions. Thank you very much. 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, an 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
00:36:12.714 --> 00:36:14.320 patient’s name on the label.
NOTE Confidence: 0.86163855125
00:36:14.320 --> 00:36:16.322 And so a lot of studies have
NOTE Confidence: 0.86163855125
00:36:16.322 --> 00:36:17.180 a washout period.
NOTE Confidence: 0.86163855125
00:36:17.180 --> 00:36:19.196 We didn’t need a washout period in this
NOTE Confidence: 0.86163855125
00:36:19.196 --> 00:36:21.179 study because they only saw the slides once.
NOTE Confidence: 0.86163855125
00:36:21.180 --> 00:36:23.241 So if we’re going to show them to them
NOTE Confidence: 0.86163855125
00:36:23.241 --> 00:36:25.063 again and if we’re going to do any
NOTE Confidence: 0.86163855125
00:36:25.063 --> 00:36:27.079 kind of intra observer reproducibility,
NOTE Confidence: 0.86163855125
00:36:27.080 --> 00:36:28.532 which we didn’t do and some
NOTE Confidence: 0.86163855125
00:36:28.532 --> 00:36:29.500 other studies have done,
NOTE Confidence: 0.86163855125
00:36:29.500 --> 00:36:30.718 we would need a washout period.
NOTE Confidence: 0.86163855125
00:36:30.720 --> 00:36:31.960 But in this case,
NOTE Confidence: 0.86163855125
00:36:31.960 --> 00:36:33.820 a washout period was not required.
NOTE Confidence: 0.86163855125
00:36:33.820 --> 00:36:36.620 And then Timothy Robinson asks,
NOTE Confidence: 0.86163855125
00:36:36.620 --> 00:36:37.715 is heterogeneity within
NOTE Confidence: 0.86163855125
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 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
00:39:44.828 --> 00:39:48.120 from a second round of DXD or
NOTE Confidence: 0.9305174
00:39:48.120 --> 00:39:51.630 when they have two distinct?
NOTE Confidence: 0.9305174
00:39:51.630 --> 00:39:54.780 Her two profiles to maybe I can use the
NOTE Confidence: 0.9305174
00:39:54.780 --> 00:39:57.370 microphone since there’s 71 people online.
NOTE Confidence: 0.71368902
00:39:58.240 --> 00:39:59.934 Yeah, when I but I, I do want
NOTE Confidence: 0.71368902
00:39:59.934 --> 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
00:40:06.895 --> 00:40:09.077 two therapy if they were one plus.
NOTE Confidence: 0.6665299375
00:40:13.180 --> 00:40:13.940 Why don’t we choose?
NOTE Confidence: 0.8049838
00:40:24.600 --> 00:40:25.428 But the patient?
NOTE Confidence: 0.32513583333333
00:40:29.210 --> 00:40:30.598 Patient had complete response
NOTE Confidence: 0.5355477925
00:40:30.610 --> 00:40:33.874 to that through. So it was our CB0,
NOTE Confidence: 0.69599822
00:40:33.880 --> 00:40:36.364 but then the tumor which was
NOTE Confidence: 0.69599822
00:40:36.364 --> 00:40:38.020 one plus still extensive.
NOTE Confidence: 0.69599822
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 positive cancer and then we’ll worry about the her two negative or her two low cancer later. But 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
00:41:38.606 --> 00:41:40.328 up about her two heterogeneity and
00:41:40.378 --> 00:41:41.978 I think that’s really interesting.
00:41:41.980 --> 00:41:43.520 Again with the first generation,
00:41:43.520 --> 00:41:45.655 her two therapies, it was very clear.
00:41:45.660 --> 00:41:47.150 We actually did a prospective,
00:41:47.150 --> 00:41:51.098 big prospective trial with with the other,
00:41:51.100 --> 00:41:53.390 the first antibody drug conjugate
00:41:53.390 --> 00:41:55.680 that doesn’t have bystander effect
00:41:55.748 --> 00:41:58.172 and in that study a heterogeneous
00:41:58.172 --> 00:42:00.530 cancer responded much work much less.
00:42:00.530 --> 00:42:02.315 Effectively to a heterogeneous cancer
00:42:02.315 --> 00:42:05.016 than it did to a non heterogeneous
00:42:05.016 --> 00:42:07.131 cancer and and quantitatively that
00:42:07.131 --> 00:42:09.621 the to your specific question what
00:42:09.621 --> 00:42:12.302 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 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, 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. And that’s how the bystander effect works. Bystander effect doesn’t really require presence in the microenvironment of the cell as long as it’s a.
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

of the cells. You don’t have to take anybody to go inside the cells.

Anybody to go inside the cells.

Take the the drug I guess can get

into cells without the antibody.

but the reason they conjugated to

antibodies so you can increase
NOTE Confidence: 0.786440327826087
00:43:59.416 --> 00:44:01.684 the dose locally to the tumor.
NOTE Confidence: 0.786440327826087
00:44:01.690 --> 00:44:02.920 Michael environment.
NOTE Confidence: 0.786440327826087
00:44:02.920 --> 00:44:07.240 We have a more protease type.
NOTE Confidence: 0.786440327826087
00:44:07.240 --> 00:44:10.648 To break up the linkage between
NOTE Confidence: 0.786440327826087
00:44:10.648 --> 00:44:13.192 conjugate drug and the conjugate ohh,
NOTE Confidence: 0.786440327826087
00:44:13.192 --> 00:44:14.668 that’d be fine.
NOTE Confidence: 0.786440327826087
00:44:14.668 --> 00:44:18.353 That that that means you read that
NOTE Confidence: 0.786440327826087
00:44:18.353 --> 00:44:22.656 could partly explain why the the
NOTE Confidence: 0.786440327826087
00:44:22.656 --> 00:44:25.014 heterogeneity potential difficult
NOTE Confidence: 0.786440327826087
00:44:25.014 --> 00:44:28.706 involvement as well as you may
NOTE Confidence: 0.786440327826087
00:44:28.706 --> 00:44:31.250 have another interesting parameter
NOTE Confidence: 0.786440327826087
00:44:31.250 --> 00:44:35.938 to assess now today with the.
NOTE Confidence: 0.786440327826087
00:44:35.940 --> 00:44:40.148 Mass Effect. We could look into.
NOTE Confidence: 0.786440327826087
00:44:40.150 --> 00:44:42.779 Whether they’re saying reached?
NOTE Confidence: 0.786440327826087
00:44:42.780 --> 00:44:44.520 Do you conjugate?
NOTE Confidence: 0.786440327826087
Drugs.

And in that case you would argue that it worked.

It would work without trustors maybe even being present.

You could you get the deconjugation even if there's no target present.

That's that's why you get a negative.

Right without her two present if if it's a true her 20 the drug could still work because it could get deconjugation and be effective.

Everywhere in the body.

Anywhere in the exercise room.

That particular,
I think that you know, the question is the toxicity then and that's actually the problem. I didn't go into that. But one of the problems with this drug is it has pulmonary toxicity and that patients get interstitial lung disease about 10% of the time. And that's another reason why you need a companion diagnostic. And one wonders if perhaps the interstitial lung disease is due to extra,
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, low in low in about at about 

1/4 UN quote one plus level, or between four and six animals per square millimeter.