Good morning, everybody.
Thank you for being here.
Welcome to Grand Rounds.
This is the Grand Rounds in a special location, obviously, because we are linked today to the first of what we hope will be a really successful series of annual translational science retreats meant to highlight the amazing resources that are present at Yale Cancer Centre for people who do translational science and also to
highlight some of the amazing stories that have come out of this work. And no one better to be our inaugural speaker than Doctor Katie Politi. Katie studied biology at the University of Pavia in Italy and then moved to New York, obtaining her PhD in genetics at Columbia University. She then joined Harold Varmus’s lab at Memorial Sloan Kettering and began her life’s work on the molecular basis of lung cancer. She continues this work at Yale, now as a professor in the Departments of Pathology and Internal Medicine in the section of Medical Oncology.
Her laboratory is focused on studying the biology of lung cancer and uncovering mechanisms of resistance to targeted therapies and immunotherapies in this disease. She’s also got a keen knowledge of essentially every mutation that’s ever been described in lung cancer. And I know that doctors often call her up and say what drug should I use. She Co leads the cancer signaling networks research program. She’s the scientific director of the Center for Thoracic Cancers, Co Director of the Yale Sport in.
00:01:43.900 --> 00:01:45.597 Lung Cancer and recently elected
NOTE Confidence: 0.919951375
00:01:45.597 --> 00:01:47.799 to the ACR Board of Directors.
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00:01:47.800 --> 00:01:50.010 So we’re really appreciative that
NOTE Confidence: 0.919951375
00:01:50.010 --> 00:01:52.999 you’re going to kick us off today
NOTE Confidence: 0.919951375
00:01:53.000 --> 00:01:56.968 the the ID number there is to record
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00:01:56.968 --> 00:01:59.145 your attendance and then we’ll
NOTE Confidence: 0.919951375
00:01:59.145 --> 00:02:01.515 have questions both in the room
NOTE Confidence: 0.919951375
00:02:01.520 --> 00:02:05.360 and online when we’re done.
NOTE Confidence: 0.919951375
00:02:05.360 --> 00:02:05.680 Thank you.
NOTE Confidence: 0.956115768
00:02:10.200 --> 00:02:11.880 Thank you very much, Barbara,
NOTE Confidence: 0.956115768
00:02:11.880 --> 00:02:14.600 for that wonderful introduction
NOTE Confidence: 0.956115768
00:02:14.600 --> 00:02:16.615 and thank you very much for
NOTE Confidence: 0.956115768
00:02:16.615 --> 00:02:18.360 having me as a speaker today.
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00:02:18.360 --> 00:02:20.640 It really always is, I think,
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00:02:20.640 --> 00:02:23.650 very special to speak at one’s own
NOTE Confidence: 0.956115768
00:02:23.650 --> 00:02:25.574 institution and then especially
Also associated with this first translational science retreat.

So I’m really excited about this.

And today what I’m going to do is I’m going to tell you about some of the work that we’ve been doing over the past few years in the laboratory.

These are my disclosures.

So we have a long standing interest in the lab on studying lung cancer.

And as all of you know,

there are several histological subtypes of lung cancer.

But one of the things that we’ve learned over the past 20 or so years is that
lung cancer is not one entity and that there are in addition to different histological subsets of the disease, there are also a variety of laser pointer of molecular subsets and in particular in lung adenocarcinoma. Through various sequencing efforts, different mutations in genes that encode either receptor tyrosine kinases or downstream signaling components of receptor tyrosine kinase signaling pathways that regulate cell proliferation and cell survival have been identified as you can see here in this pie chart. And I think one of the things to
really highlight is what we've learned over the years is that these mutations are in addition to being molecular to establishing molecular subsets of the disease. They really also are clinically relevant because different targeted agents have been developed that can be used to block the activity of these mutated oncogenic drivers. And in particular and in the work that I'll tell you about today, mutations were found 20 years ago now in Exxon's encoding the kinase.
00:04:20.788 --> 00:04:22.990 domain of the epidermal growth factor
NOTE Confidence: 0.924020505
00:04:23.049 --> 00:04:28.399 receptor after in about 15 to 4050% 
NOTE Confidence: 0.924020505
00:04:28.399 --> 00:04:31.394 of lung and nocarcinomas depending 
NOTE Confidence: 0.924020505
00:04:31.394 --> 00:04:34.640 on which population you look at. 
NOTE Confidence: 0.924020505
00:04:34.640 --> 00:04:38.960 And these are mutations that 
NOTE Confidence: 0.924020505
00:04:38.960 --> 00:04:41.470 confer sensitivity to EGFR tyrosine 
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00:04:41.470 --> 00:04:42.474 kinase inhibitors. 
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00:04:42.480 --> 00:04:44.080 But there are many other 
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00:04:44.080 --> 00:04:45.360 targeted therapies as well. 
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00:04:45.360 --> 00:04:48.948 So you can have rearrangements in 
NOTE Confidence: 0.924020505
00:04:48.948 --> 00:04:51.720 the anaplastic lymphoma kinase and 
NOTE Confidence: 0.924020505
00:04:51.720 --> 00:04:53.645 targeted therapies that are effective 
NOTE Confidence: 0.924020505
00:04:53.645 --> 00:04:57.047 in that and so on for a number of 
NOTE Confidence: 0.924020505
00:04:57.047 --> 00:04:59.520 different oncogenic drivers and lung cancer. 
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00:04:59.520 --> 00:05:02.160 And so this has really transformed the field. 
NOTE Confidence: 0.924020505
00:05:02.160 --> 00:05:06.870 And so if we look at this diagram here of
approved FDA approvals for lung cancer in,
in recent years,
what you’ll see is it really has been an explosion in FDA approvals,
especially from the early 2000s in the 2000 and 10s and approvals now also in the first part of the twenty 20s. Most of these agents that were approved recently have been targeted agents and that really is linked to the discoveries of these molecular subsets of the disease. But also do I think one of the things that has been emerging also in the past 10 to 15 years really are the
approvals of immunotherapies that we hear a lot about agents that are targeting immune checkpoints like the anti PD1, anti PDL ONE Access and CTLA 4. And so this has really been transformative in a lung cancer. And I’d like just like to point out how in recent analysis what we’re seeing is that there’s actually a decrease in mortality from lung cancer in recent years. And in the study published in the New England Journal of Medicine a few years ago, it was really shown that the decrease in mortality from lung
cancer can’t be accounted for just because of a decrease in incidence of the disease. But is likely reflects advances in the care and in the new therapeutics that have emerged, including in particular in the years that were studied in this paper for targeted agents. And so I think this is a really nice example of how what we’ve learned over the years from the biology and from the genetic studies of tumors really is having a profound impact for patients with this disease.
And of course I would be remiss if I didn’t point out how immunotherapies have also been transformative. And I think the continued decrease in mortality that we are continuing to see is actually going to show how it isn’t only the targeted therapies but also the immunotherapies that are really contributing to this decrease in mortality from lung cancer. So if you know you look at this, there’s really these advances have been tremendous. But what we do know is that both primary and acquired resistance
immunotherapies are common. And here you can see an example of scans from a patient with a tumors with AK Ras G12C mutation treated with AK Ras G12C inhibitor and you can see the tumor regresses but then comes back and you have this is acquired resistance. And here if we look at this plot taken from a review looking at studies of immunotherapies, at studies of immunotherapies, you can see that across various different indications but including in lung cancer here that in clinical studies of immunotherapies,
the response rates or to immune
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checkpoint inhibitors are not super high.
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We’re not talking 70%-80% the way we’re
talking with some targeted therapies.
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Not only that,
but also we see acquired resistance
commonly emerging.
So there’s a lot of work that needs
to be done to really understand and
optimize treatments for both targeted
agents and immunotherapies and to
understand mechanisms of sensitivity
and resistance to these agents.
And So what do we do in my lab?
And as part of the research program,
we are really interested in understanding
mechanistically biological processes that are involved in cancer. We like to integrate these with studying and addressing clinical challenges and investigating specimens and data from patients with cancer. And really the hope is that the work that we do collectively as a group, this is work that we do with many different people is to discover things that will discover findings that will lead to clinical trials and new therapeutic approaches to patients. Central to our research program is the use of biological specimens from
00:09:23.488 --> 00:09:26.800 patients and analysis of these specimens.
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00:09:26.800 --> 00:09:28.632 And I think this slide is also going
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00:09:28.632 --> 00:09:30.825 to be showed later in the day as an
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00:09:30.825 --> 00:09:32.560 example of one of the resources that
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00:09:32.560 --> 00:09:35.250 we have as part of the lung cancer
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00:09:35.250 --> 00:09:39.560 program to really be able to collect
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00:09:39.560 --> 00:09:42.360 and use specimens from patients.
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00:09:42.360 --> 00:09:44.232 And this is just one of the examples
NOTE Confidence: 0.929720887619048

00:09:44.232 --> 00:09:46.154 of one of the resources I think
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00:09:46.154 --> 00:09:47.544 you’ll hear about a couple
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00:09:47.605 --> 00:09:49.075 of others later on as well.
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00:09:49.080 --> 00:09:51.194 But really an effort that started many,
NOTE Confidence: 0.969507246923077

00:09:51.200 --> 00:09:54.692 many years ago working initially
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00:09:54.692 --> 00:09:57.834 with Scott Genger and Anna
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00:09:57.834 --> 00:10:00.198 Wertz and Roy Herbst and many,
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00:10:00.200 --> 00:10:03.160 many people in this room now with
Sarah and many of all of the thoracic oncologists on the team and pathologists. Kurt for example, really working on collecting specimens from patients who have advanced lung cancer through treatment, especially at the time of resistance. So that then we can take these specimens and analyze them, generate patient derived models. And really these have contributed extensively to the work that I will tell you about today. And so I put a little cryovile here. And So what I'm going to do through the talk is when you see a cryovial on the slide,
it actually is an example of data that we've been able to analyse and use because of the specimens that were collected through this approach.

So what am I going to tell you about today. So I think as most of you know we have a long standing interest in studying the biology of EGF receptor driven lung cancer. And so when patients and really the focus that we’ve had at least in the past or until recently has really been and because of the sort of the clinical landscape has
00:11:18.250 --> 00:11:19.960 really been on advanced metastatic
NOTE Confidence: 0.969507246923077
00:11:19.960 --> 00:11:22.560 EGF receptor driven lung cancer.
NOTE Confidence: 0.969507246923077
00:11:22.560 --> 00:11:26.032 And so when patients are diagnosed
NOTE Confidence: 0.969507246923077
00:11:26.032 --> 00:11:28.600 with EGF receptor driven lung cancer,
NOTE Confidence: 0.969507246923077
00:11:28.600 --> 00:11:32.602 now they’re mostly treated with tyrosine
NOTE Confidence: 0.969507246923077
00:11:32.602 --> 00:11:34.612 kinase inhibitors most recently and
NOTE Confidence: 0.969507246923077
00:11:34.612 --> 00:11:37.296 in the United States especially the
NOTE Confidence: 0.969507246923077
00:11:37.296 --> 00:11:39.316 tyrosine kinase inhibitor awesome.
NOTE Confidence: 0.969507246923077
00:11:39.320 --> 00:11:41.936 Merton if this is one of the newer
NOTE Confidence: 0.969507246923077
00:11:41.936 --> 00:11:44.128 generation of agents that has more
NOTE Confidence: 0.969507246923077
00:11:44.128 --> 00:11:46.368 activity on mutant EGFR compared
NOTE Confidence: 0.969507246923077
00:11:46.368 --> 00:11:47.712 to wild type.
NOTE Confidence: 0.969507246923077
00:11:47.720 --> 00:11:49.745 So hopefully decreasing its toxicity
NOTE Confidence: 0.969507246923077
00:11:49.745 --> 00:11:52.850 and has been shown to have superior
NOTE Confidence: 0.969507246923077
00:11:52.850 --> 00:11:55.365 progression free survival and overall
NOTE Confidence: 0.969507246923077
survival compared to standard of care earlier generation tyrosine kinase inhibitors in this disease.

And so this was an important advance in the field. However, what we do know is that still resistance or acquired resistance to asamertinib occurs almost inevitably and it actually isn’t very commonly associated with on target EGFR mutations. And this is different from some of the earlier generations of tyrosine kinase inhibitors that instead where we saw commonly one most frequently observed on target EGF receptor mutation,
the T79 TM mutation. But you see additional mechanisms of resistance met amplification for example, so a bypass signaling pathway being one of the more common. Then we see a histologic changes in the tumors that occur quite frequently, but then most of the mechanisms of resistance are really not known and poorly understood. And so one of the things that we’ve been interested from when as we think about these problems is really, really understanding these tough challenges like really understanding
this part of the pie chart, right.

What are these mechanisms of resistance,

What is happening in these tumors where we don’t really have a key genetic alteration that has changed that or clear process that is happening that we can target.

And so just a couple of thoughts that sort of guide our thinking.

Targeted agents are probably not sufficient.

We need to discover new untapped vulnerabilities of oncogene driven lung cancers and then the tackling resistance requires new knowledge of the links between cancer cell plasticity and the tumor microenvironment and tumor heterogeneity.
And so these are some of the low hanging fruit but the fruit really at the top of the tree that we’re trying to really grasp and understand when we. And really if we look at EGF receptor driven lung cancer and we think about it, one of the things that we know is that with the targeted agents that I’ve told you about today is that we do see this acquired resistance. But not only that. We also know that when we use the targeted agents they don’t completely eradicate all the tumor cells and
there’s variability in the depth and duration of responses in patients.

And you can see this really in this waterfall plot where there’s some tumors that shrink dramatically and others that shrink less.

And so we’ve been interested in the question of what accounts for this heterogeneity and disease progression and sensitivity to tyrosine kinase inhibitors.

And so the first thing that I’m going to go through is some of the work that we’ve done to study how different EGF receptor mutations can actually have distinct properties.
I've sort of told you about EGF receptor mutations and one could think, oh, we can lump them all together. But in reality, what we do know and what is becoming increasingly clear in recent years is that you have different EGF receptor mutations and not only that, the different EGF receptor mutations have different properties both biological, biochemical and also in terms of TKI sensitivity. And so when we look at EGF receptor mutations, there are two major categories of mutations.
There’s the L858R point mutation and then there’s a set of small in frame deletion, some of them more complex and Exon 19. The most common of these is this E 746 to a 750 mutation. But then there are these other in Dells that are found at, you know, variable frequencies in these tumors, but they exist. And So what does it mean? Are all these mutations alike? Well, one of the things that we know is that even if you just broadly categorize the L858R mutations and the e.g FRXN 19 deletion mutations and you look at the
survival curves on osumertinib from the trial of frontline osumertinib, you see that even just the Exxon 19 deletion mutations, the overall survival is about 40 months in that study. But for the L858 Rs, it’s about 33 months. And this is consistent over across different tyrosine kinase inhibitors that are used. So the L858R subset does worse with TKIS compared to the Exxon 19 subset. We also found several years ago in work that we did together with Sarah.
Goldberg and Mark Lemon is that that
there's a small in frame deletion
in a Proline insertion mutation and
one of the Exxon 19 deletions that
actually if you look at that mutation
and you look in upon treatment with
Erlotinib this was a few years ago.
So one of the early generation
tyrosine kinase inhibitors that the
progression free survival duration
of a treatment overall survival were
all worse for the for Erlotinib in
that subset compared to the more
common Exxon 19 deletion mutation.
And this along with some laboratory
studies really piqued our interest in
studying these differences a little bit more.

And here you see the cryovile appear.

This is also work that was Zenta Walther

was really central to helping us identify these patients for this study.

And so working with lots of different groups here we were able to show that this proline insertion for example what you see in Western blots is when you treat with tyrosine kinase inhibitors, it’s less sensitive to various
tyrosine kinase inhibitors compared to the canonical e.g.

FRXN 19 deletion mutation.

Not only that,
when you actually go and look biochemically,

and this is work that was spearheaded by a former student that Mark Lemon and I shared.

Eris von Alderweil, von Rosenberg showing that this proline insertion mutation has AKM for ATP that is more more closer to the wild type in contrast to some of the other variants that instead are more sensitive to tyrosine kinase inhibitors.

So really is that affinity of the kinase for ATP that is probably rendering it more resistant to these tyrosine kinase inhibitors.

So really from the clinical observations,
00:18:50.620 --> 00:18:52.160 studies going to the biochemistry,
00:18:52.160 --> 00:18:54.834 we’re really able to figure out what
00:18:54.834 --> 00:18:56.960 was happening with this variant.
00:18:56.960 --> 00:18:59.936 And this led to work that we did
00:18:59.936 --> 00:19:02.649 together with Mike Grant and Sarah
00:19:02.649 --> 00:19:05.880 Goldberg really putting together a multi
00:19:05.880 --> 00:19:09.040 institutional cohort of patients with e.g.
00:19:09.040 --> 00:19:10.930 Fr XL19 deletion mutations treated
00:19:10.930 --> 00:19:13.190 with asumertinib because we wanted to
00:19:13.190 --> 00:19:15.032 look at the tyrosine kinase inhibitor
00:19:15.032 --> 00:19:16.679 that was really clinically relevant
00:19:16.679 --> 00:19:19.017 for patients right now and that was
00:19:19.017 --> 00:19:21.130 being used to see what outcomes
00:19:21.130 --> 00:19:23.564 were for patients with this Proline
00:19:23.564 --> 00:19:25.920 insertion mutation with asumertinib.
It’s pretty rare. So you have to really work together and put together a cohort from various institutions. Mike and Sarah assembled this cohort including data from our Yale cohort and actually showed that in patients whose tumors have this proline insertion mutation treated with ossomatinib, you have worse progression free survival. Then if you look at the common e.g. Fr XM19 deletion mutation, the overall survival isn’t quite statistically significant, but you can see that there is a trend in worse outcomes there as well.
And so what does this mean? What does this make us think? I think the message here is that not all mutations are the same. And now we have the tools and the drugs to better match mutations with therapies. We aren’t the only ones who are thinking about this. There’s some other work from Jacqueline Robichaud and John Haymack’s group at MD Anderson, work from Christine Lovely at Vanderbilt, all really pointing in this direction. We need to know about the biology, the biochemistry of the mutations,
and that can help us think about perhaps how to better optimize these therapies now that we have them. Another point, yeah, the structural and biochemical understanding of the effects of the mutation can guide predictions for TKI sensitivity and resistance. And of course, the other question that comes along is how do we translate to the clinic this to the clinic now what? What are the next steps that we can take? So we can test trials of like optimal TKI. So now we have all these reagents, we can test other agents and other.
NOTE Confidence: 0.965352661666667
00:21:13.878 --> 00:21:15.393 drugs on these different variants
NOTE Confidence: 0.965352661666667
00:21:15.393 --> 00:21:17.905 and see if there’s some that are more
NOTE Confidence: 0.965352661666667
00:21:17.963 --> 00:21:20.318 effective for specific mutational subsets.
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00:21:20.320 --> 00:21:21.796 But then the other question is,
NOTE Confidence: 0.965352661666667
00:21:21.800 --> 00:21:24.464 are there other agents that we
NOTE Confidence: 0.965352661666667
00:21:24.464 --> 00:21:26.896 should be thinking about for certain
NOTE Confidence: 0.965352661666667
00:21:26.896 --> 00:21:28.864 subsets of the disease in combination
NOTE Confidence: 0.965352661666667
00:21:28.864 --> 00:21:30.080 with also Mertinib?
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00:21:30.080 --> 00:21:31.816 And I think this will be a
NOTE Confidence: 0.965352661666667
00:21:31.816 --> 00:21:33.259 recurring theme throughout the talk.
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00:21:33.259 --> 00:21:34.946 So for example, you know,
NOTE Confidence: 0.965352661666667
00:21:34.946 --> 00:21:37.184 should we be thinking about specific
NOTE Confidence: 0.965352661666667
00:21:37.184 --> 00:21:39.172 antibody drug conjugates or other
NOTE Confidence: 0.965352661666667
00:21:39.172 --> 00:21:41.524 approaches to target tumors with that
NOTE Confidence: 0.965352661666667
00:21:41.524 --> 00:21:43.718 don’t do as well with monotherapy?
NOTE Confidence: 0.965352661666667
00:21:43.720 --> 00:21:44.580 Awesome.
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00:21:44.580 --> 00:21:47.565 Or so after you know thinking
NOTE Confidence: 0.965352661666667
00:21:47.565 --> 00:21:48.840 about the different.
NOTE Confidence: 0.965352661666667
00:21:48.840 --> 00:21:51.878 So we talked about how different EGF
NOTE Confidence: 0.965352661666667
00:21:51.878 --> 00:21:55.772 receptor mutations themselves can
NOTE Confidence: 0.965352661666667
00:21:55.772 --> 00:21:57.437 but what about Co mutations?
NOTE Confidence: 0.965352661666667
00:21:57.440 --> 00:22:01.306 How can Co mutations influence tumor
NOTE Confidence: 0.965352661666667
00:22:01.306 -- > 00:22:04.636 progression but also TKI sensitivity.
NOTE Confidence: 0.965352661666667
00:22:04.640 --> 00:22:06.956 And so many years ago now,
NOTE Confidence: 0.965352661666667
00:22:06.960 --> 00:22:09.252 I probably started working on this
NOTE Confidence: 0.965352661666667
00:22:09.252 --> 00:22:11.697 actually almost exactly 20 years ago
NOTE Confidence: 0.965352661666667
00:22:11.697 --> 00:22:14.235 when EGF receptor mutations were discovered.
NOTE Confidence: 0.965352661666667
00:22:14.240 --> 00:22:18.060 I think it was May 2004 that I started
NOTE Confidence: 0.965352661666667
00:22:18.060 --> 00:22:20.120 generating these mouse models.
NOTE Confidence: 0.965352661666667
00:22:20.120 --> 00:22:23.824 We generated genetically engineered
mouse models of EGF receptor driven lung cancer in which we could express the EGF receptor mutants inducibly in the lung epithelium. And this was really to be able to study the biology of the disease. And we’ve used these mice extensively over the years to study signaling by mutant EGF receptor discover resistance mutations to tarsine kinase inhibitors, identify therapeutic strategies to overcome or prevent drug resistance and study the effects of targeted therapies on
the immune microenvironment.

And here you can see MRI images.

We use MRI imaging for our mice to look at the lungs and see or you can see lungs full of tumors you treat with a tyrosine kinase inhibitors, the tumors shrink and go away.

Over time the tumors come back and we can study those resistant tumors.

So a few years ago we decided to upgrade our mouse model and use a slightly different system that would allow us then also to be able to modulate other genes.

Because we know that EGF receptor mutations and lung cancer don’t occur in a vacuum.
There are other mutations in the tumors there and we wanted to be able to model that. So we decided to take this still this tetracycline inducible EGFR allele across it to another mouse. That in which using Cree recombinase you can then turn on expression of the reverse tetracycline transactivator which can bind the tetromotor in the presence of doxycycline and induce expression of EGF receptor. And we also crossed it to AP phloxed allele.
we deliver it with a Lantivirus
into the lungs of mice,
turn on mutated EGF receptor.
Simultaneously we can delete P53.
And here's some images,
these are the lungs of mice.
You can see the by MRI,
you can see here by Histology and a a
these are the lungs of mice.
You can see the by MRI,
you can see here by Histology and a a
bigger magnification of the Histology.
So we said OK,
so we have this mouse model with now
EGFR and mutants and P53 deficient tumors.
The P53 deficient tumors are higher grade,
they're nastier.
I see Rob Homer here.
He has helped us extensively over the
Years characterize and study these tumors. And so one of the questions that we had is well in addition to P53, what role do other mutations in EGF receptor play in EGF receptor driven lung cancer? How do they affect tumor progression? How do they affect TKI resistance and how do they affect the molecular properties and phenotypes of the tumors? And So what we did is we worked with a colleague at Stanford University, Monty Winslow, who had developed an approach in and used it in K Ras driven tumors to
really be able to inactivate using CRISPR,
CAS 9 technology, different tumor suppressor genes
simultaneously in the lungs of mice. So not all of them in the same cell,
then you can deliver this kind of pool of lentiviruses and in different
cells you can then inactivate
different tumor suppressor genes.
And then you can use a computational approach that he developed called
tumor barcode sequencing which
based on various controls that are
spiked in and based on barcode IDs.
You can actually look and quantify the effect of inactivating that tumor
suppressor gene on the number and size of tumors in a screen.

It’s essentially a way of doing an in vivo screen.

And so we applied, we took this pool of lentiviruses targeting different tumor suppressor genes that were frequently altered in lung cancer, not necessarily in EGF receptor driven lung cancer but in lung cancer and he had used it in the K Ras model previously and so we applied it to our e.g. FRL 850 at RP53 model and in particular we had also crossed the model that I just told you about with one.
00:26:34.180 --> 00:26:36.037 that has an inducible CAS 9 Ileo.
NOTE Confidence: 0.82526931

00:26:36.040 --> 00:26:38.596 So these are experimental animals here.
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00:26:38.600 --> 00:26:39.612 These are controls because
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00:26:39.612 --> 00:26:40.877 they don’t have CAS nine.
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00:26:40.880 --> 00:26:43.896 You can’t do CRISPR CAS 9 mediated genome
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00:26:43.896 --> 00:26:46.398 editing when you don’t have CAS 9:00.
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00:26:46.400 --> 00:26:50.080 So we transduced the lungs of the mice,
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00:26:50.080 --> 00:26:53.160 waited 11 weeks and then took the lungs
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00:26:53.160 --> 00:26:56.599 of the mice and did tumor barcode
NOTE Confidence: 0.82526931

00:26:56.599 --> 00:26:58.104 sequencing in our control animals.
NOTE Confidence: 0.82526931

00:26:58.104 --> 00:26:59.580 When you look at the relative
NOTE Confidence: 0.82526931

00:26:59.632 --> 00:27:01.117 tumor size compared to controls,
NOTE Confidence: 0.82526931

00:27:01.120 --> 00:27:03.120 you don’t really see any.
NOTE Confidence: 0.82526931

00:27:03.120 --> 00:27:04.488 The tumor suppressor gene
NOTE Confidence: 0.82526931

00:27:04.488 --> 00:27:06.198 inactivation doesn’t have any effect,
NOTE Confidence: 0.82526931

00:27:06.200 --> 00:27:08.120 but that’s because you don’t have CAS 9,
so you shouldn’t see anything.

So that was reassuring.

What do we see in the mice with CAS 9?

So one of the things that we saw is

that when you inactivate APC from the wind signaling pathway RBM 10 and RB1, these three tumor suppressor genes when inactivated had the biggest effect on tumor growth.

So the tumors grew faster when you were inactivating these tumor suppressor genes compared to controls.

We also noticed interestingly that SET D2 and LKB 1, both of these putative tumor
suppressor genes I'd say actually had a negative effect on tumor growth, which was quite interesting and is and I'll go, I'll tell you a little bit more about that in a minute, but it's a topic of interest, interesting work that we're doing. And then there were a number of tumor suppressor genes that really had no effect on tumor growth. We went ahead and we validated these using single SGRNAS. This is towards APC and this is to RBM 10 which is an RNA binding protein and a splicing factor.
And you can see that when you inactivate them you see these bigger tumors and tumors progress faster than in the EGF receptor P53 model. So what does this mean though in the context of human cancer? And so if we, what we did at that time is we actually interrogated the ACR Project Genie database, which is a large data set that has a lot of mutational information that has been contributed to this data set from various institutions that are from their tumor sequencing.
efforts at their institutions.

And when we look in this data set at e.g. F RP53 driven tumors and we look at the frequency with which there are alterations in this Co occurring tumor suppressor genes, you actually see that the top hits RBM 10 RB one and APC are where the top hits in our functional screen in mice. So we think that our screen in mice is actually telling us something about the functional relevance of these alterations in the human tumors and arid 1A didn’t come out in our screen at 11 weeks, but we actually did another time.
point at 19 weeks and it popped up. So perhaps it’s more important later and interestingly Genes SDK 11 is LKB one, it’s really not frequently altered and that was the one that I showed you seemed to have a negative effect in our in vivo screen. So we’ve actually, this has been a really powerful system and we’ve actually been able to do broader screens with more genes and try to learn a little bit more about what genes are important for the progression of these tumors.
And I'd just like to highlight an example of work that we did continuing this effort with D2G Oncology, a company that was founded by our collaborators Monty Winslow and Dmitry Petrov. And we work together on doing this screen of additional tumor suppressor genes in the context of EGFR tumors but also in the context of K Ras driven tumors for example. And you know I just like to go back to LKB one for example showing how this has a negative effect on EGFR driven tumors. It's not really a contributing,
it doesn’t really Co occur mutationally with EGFR driven tumors. So it seems to be like a synthetic lethality with these tumors. But it’s an amazing contrast with what we see in Keras driven tumors where it is one of the major drivers of tumor growth. And so this is I think telling us and it’s frequently mutated with Keras in human tumors. So we’re really think that this is a cool system to be able to understand how Co occurring alterations impact the fitness of tumors.
And Fran Exposito in the lab is really working a lot to understand this synthetic lethality and is doing experiments to knock it LKB one out and established EGF receptor tumors and see what happens and also to understand mechanistically what is happening in these tumors. So stay tuned for for data on these studies that I think will be really fascinating. And there are some other targets that we’re studying along these lines as well. So I think a very powerful system. We’ve also used this approach not just to study mechanisms of tumor progression,
but also use this type of approach to really understand what genes can modulate the sensitivity to tyrosine kinase inhibitors. So we did the same experiment and instead of just waiting and collecting the tumors, what we did is we also had an arm where we treated for two weeks with a tyrosine kinase inhibitor osumertinib. You see here the tumors go away or they’re shrinking mostly. They’re not completely going away at two weeks, but you do see a response. And so we did the same tumor bar code sequencing and what we found
here is so this is the plot that I showed you earlier looking at what is affecting tumor growth. Well, when we add Asamertinib, one of the things that we saw is that keep 1 the tumor suppressor gene, keep one that really didn’t have much of an effect on the growth of the tumors in the absence of drug now limits the sensitivity to Asamertinib. In other words, the tumors aren’t shrinking as much as wild wild type or control tumors do when keep one is present.
What do we think is happening here?

Well, we know that keep one is important to sequester NRF 2 in the cytoplasm.

When you knock out KEEP 1, NRF 2 can then go into the nucleus and activate antioxidant response elements and those gene expression programs that allow cells to really withstand oxidative stress.

And when we take our mice and we just use an individual SGR and a targeting keep one, an individual SGR and a targeting keep one, the tumors go away. And when we take our mice and we just use Asamertinib, the tumors go away.

these are the control mice that don’t have CAS nine, you use Asamertinib, the tumors go away.

you don’t really see anything left in the lungs.
But if you have the experimental mice that have CAS 9 and you use the SGR and a targeting keep one treat with Asamertinib, you see tumors are still left over. And so again, what does that mean for patients? So at the time what we did is we worked with Jessica Hellier and Heather Wakeley at Stanford University who had a collection of data from patients with e.g. F RP53 driven lung cancer and looked at whether there were mutations in genes in the keep one access in these tumors. And you can see here in this blue line, the patients who had mutations in the keep One access in their tumors had
a shorter time to treatment failure
compared to controls suggesting that if
you have alterations in this program,
this antioxidant response program,
you’re going to have limited sensitivity
to tyrosine kinase inhibitors.
We were interested in looking at the tumor suppressor genes is that when you do have mutations or you have alterations that occur
with EGF receptor and with EGF receptor
these can modulate both the growth and sensitivity to these agents.
We were interested in looking
00:35:06.190 --> 00:35:09.100 further and in work that Paul
NOTE Confidence: 0.749463982631579
00:35:09.100 --> 00:35:12.206 Stockhammer who was a resident is
NOTE Confidence: 0.749463982631579
00:35:12.206 --> 00:35:15.426 now a hospitalist here and is an
NOTE Confidence: 0.749463982631579
00:35:15.426 --> 00:35:18.780 incoming he monk fellow did recently.
NOTE Confidence: 0.749463982631579
00:35:18.780 --> 00:35:23.820 He looked at both our Yale internal data
NOTE Confidence: 0.749463982631579
00:35:23.945 --> 00:35:26.260 from our tissue collection program.
NOTE Confidence: 0.749463982631579
00:35:26.260 --> 00:35:28.560 You see the cryovial here,
NOTE Confidence: 0.749463982631579
00:35:28.560 --> 00:35:32.322 but also at the ACR project gene data set
NOTE Confidence: 0.749463982631579
00:35:32.322 --> 00:35:37.525 and looked at outcomes for patients on
NOTE Confidence: 0.749463982631579
00:35:37.525 --> 00:35:41.234 tyrosine kinase inhibitors whose tumors
NOTE Confidence: 0.749463982631579
00:35:41.234 --> 00:35:44.319 had different combinations of mutations.
NOTE Confidence: 0.749463982631579
00:35:44.320 --> 00:35:46.588 And I think the take away here is he
NOTE Confidence: 0.749463982631579
00:35:46.588 --> 00:35:48.938 was able to look at tumors that had
NOTE Confidence: 0.749463982631579
00:35:48.938 --> 00:35:51.555 mutations in a subset of tumor suppressor
NOTE Confidence: 0.749463982631579
00:35:51.555 --> 00:35:54.084 genes because tumors had been analyzed
NOTE Confidence: 0.749463982631579
00:35:54.084 --> 00:35:57.400 across a wide variety of different platforms.
So we had to sort of focus in on the the common subset of tumor suppressor genes that were looked at across platforms. But essentially if tumors had both P53 mutations and a mutation, at least one of these tumor suppressor genes that he looked at, they had worse outcomes. These are EGFR mutant tumors even compared to mutations that just had TPF 3 mutations and were wild type for those different tumor suppressor genes. And So what does that mean? Again, I think we’re identifying a subset of tumors where there may be a benefit.
from adding a different therapy or it should be at least be investigated from the get go because they are likely to have worse outcomes with monotherapy tyrosine kinase inhibitor treatment.

And this is very relevant right now at least in the field of EGF receptor driven lung cancer because there are studies of chemotherapy plus asamartinib in the first line that are positive.

But people are very reluctant to give that combination to everybody. If we can identify people who might benefit more or might need it more than that could be really helpful for deploying these different strategies in the clinic.
And then I think another point is that we're really learning the Co mutations can affect therapeutic sensitivity and it isn't only in the context of EGFR tyrosine kinase inhibitors. This is happening in multiple contexts and with multiple agents. So here an example, I'm just giving you a few examples. There are many more in the literature. But if we look at alterations seem to have been negative for response rates to Sotirasip in K Rash G12C driven lung cancer.
Worse, you know higher local recurrence with chemo radiation in the context of immunotherapy LKB 1 mutations actually seem to be worse confer, you know be worse for or describe, define a word a subset that does worse with immunotherapy. And so in conclusion for this part of the talk, the nature of the oncogenic mutation and Co occurring mutations effects sensitivity to Tkis and mechanisms of resistance. We’ve developed a new generation of genetically engineered mouse models that can be used to study these complex genotypes.
And I’d like to point out that really we have a lot of work that is happening now studying these individual different components. Mariana Do Carmos, an MD, PhD student in the lab. She’s studying the role of RBM 10 in EGF receptor driven lung cancer working with Luisa escobarahoyos lab. Because we really can join forces and Luisa is an expert in splicing and this is really important gene protein that is involved in splicing.
So we’re doing that.

I told you about Fran’s work.

We have Kita who’s working on KMT 2D, another potential target that came out of this screen.

So really we can really study these different genotypes and understand the biology of these different complex genotypes, which is really exciting.

We have found out that an activation of these different tumor suppressor genes can have different effects on both tumor growth including positive and negative effects and TKI sensitivity.
depending on the oncogenic context.

We showed that keep one loss limits sensitivity to osmertinib in mice and think that this is really potentially a bad actor if there’s Q1 alterations either at the genetic level or also alterations in the pathway.

The pathway can be modulated in many different ways, and tumor suppressant gene mutations can be used to identify patients, subsets of patients who are likely to have worse outcomes and could be considered for additional
therapeutic interventions.

So in the last part of the talk, I’d like to tell you about some other work that we’ve been doing more recently to study non mutational mechanisms of resistance and I’d say also of persistence.

So on tyrosine kinase inhibitors. And So what are some of the things that we’re thinking about broadly in the lab when we think about this problem of this 50% of tumors that we don’t know why a resistance emerges.

So some of the things that we’re really interested in in understanding
and studying are how the tumor microenvironment effects resistance and persistence.

And this is work that we’re doing collaboratively, Jake Schillo in the lab doing collaboratively working with Don Nguyen’s lab.

We are studying lineage plasticity and tumor heterogeneity.

And I’ll tell you about an example of this that was just recently published this month and that comes out of work studying mechanisms of tumor persistence.
And of course another area that we're really interested in is while we've talked a lot about genes and mutations and genetics here, but are there ways of reading out pathways and learning about how pathways are altered in tumours which might be an important way. And so one of the non-mutational mechanisms that we recently discovered and published on, I'm not going to tell you about that today because I don't really have time is that we identified a role.
for the ATP as of the SLY sniff
complex in mediating resistance
to tyrosine kinase inhibitors and
SMARCA 4 is actually usually lost,
you have loss of function mutations
in tumors.
One of the things that we found
which was really interesting is that
actually it seems to be important
for the resistance phenotype because
in resistant tumors it actually can
promote accessibility of chromatin
at both cell proliferation genes but
also at genes it are NRF 2 low size
so that allow activation of genes
that are antioxidant genes with that.

So it links to that keep one,

keep one finding that we had in

So I’m not going to tell you about this,

but I did want to highlight it

as as one of the some of the work

that we have done recently on non

mutational mechanisms of resistance.

What I really wanted to focus the last

few minutes of the talk on is telling

you about some work that we’ve been

doing to study tolerance and persistence

to tyrosine kinase inhibitors.

And you saw this waterfall plot earlier.

But one of the and one of the
questions that we’ve had and I think that is a prominent question in the field is why aren’t all cells eradicated upon TKI treatment, right, Because if we could get rid of all of the cells from the get go, we wouldn’t have the problem of acquired resistance. And here’s some scans. You see the tumor and you see several months later the tumor is still there, there still is some residual tumor leftover. So what is the biology of residual disease? Well, we decided and this is work from a former graduate student in the lab, Boom Yao,
who is now in Arno Osher's lab

And I think Boom Yao is here.

And So what Bom Yao did is he took advantage again of our collection of specimens from patients.

And he said, well, what happens if I implant these PDXS that we’ve generated, treat them with a tyrosine kinase inhibitor and then look at residual disease?

We can harvest that.

You know, we take it at a plateau, right?

Once the tumors aren’t shrinking anymore,
And can we really hard to study residual disease in patients. We can’t really easily do biopsies on treatment, but this is as a surrogate of that. So here are some examples of the PDXS that Boom Yao studied. So he took these PDXS, treated them and then took what was leftover after four to six weeks of treatment when they plateaued. And you can see in all of the cases there was tumor leftover after treatment, varying amounts of tumor and in some very little.
very small islands of tumor,

but there was tumor leftover.

And I’d like to highlight an example

of one of the things that we found

from one of these PDXS that we

studied in a little more detail.

We found that in one of them we

saw up regulation of Ascl 1.

ASCL one is a basic Helix loop

Helix transcription factor.

It has a role in neuronal differentiation

and its expression actually identifies

a subset of small cell lung cancer.

So it was really up in the residual

disease in this tumor and not only

disease in this tumor and not only

was it up at the transcriptional
level and the signature was was enriched in the residual disease,
but it’s downstream targets rat BCL two and DLL three were also all turned on in
the residual disease in that tumor.
Ossumertinib was working really well. You can see phospho EGFR is gone here.
And so this was really interesting to us because we know that a subset of
EGFR driven tumors when they’re treated with osumertinib can actually undergo
neuroendocrine differentiation and transformed to small cell lung cancer,
a subset of which are ASCL 1 positive. And so this kind of piqued our interest.
And so one of the first questions that we had was are these ASCL one cells present in the tumor pretreatment. When we looked and we did single cell RNA sequencing, we actually saw that the if you look at the pretreatment specimen here in blue, there is a subset of these cells that is ASCL 1 positive. So we think that those cells were present beforehand. Whether other cells then turned it on, we can’t really tell from the types of experiments that we did. But we do know that there was a population that was there pretreatment.
And so our next question after that was well how is ASCL 1 conferring TKI tolerance, what is happening.

And so we said OK, let’s turn to our human EGF receptor driven cell lines and let’s express ASCL one in these cells.

And so one of the first things that we did, we expressed ASCL one in the cells and you can see here in this HCCA 27 cell line, we did this across in another cell line.
and we saw no effect of ASCL one expression.

And so this was also interesting and we said,

OK, so why does ASCL one having a phenotype has a phenotype in one cell line but not the other.

We did gene expression profiling and what we saw is that in the permissive cells, these HCC 827 cells, you actually saw that ASCL one could lead to an EMT gene expression program was it had no effect at all in the PC-9 cell line.

And we went on and we looked with ataxiq at chromatin accessibility at EMT genes and we see that upon ESAS CL1 expression,
00:47:54.361 --> 00:47:57.403 you do see changes in chromatin accessibility at both epithelial genes and mesenchymal genes when you put Ascl one into these HCC 827 cells that are permissive, but you don’t see any changes in the PC-9 cells.

And So what do we think is happening? So we think that when you have, when you don’t have ASCL 1, the TKI can work and you see death of the EGF receptor driven cells. If you have a permissive cellular context what happens is that one can turn on ASCL one can turn on
or can lead to an EMT program and we

NOTE Confidence: 0.901540450357143

know that that is associated with

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resistance to tyrosine kinase inhibitors.

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In a non permissive cellular

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case though that you don’t have,

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you don’t turn on this program so

NOTE Confidence: 0.901540450357143

context though that you don’t have,

NOTE Confidence: 0.901540450357143

you don’t have a difference in ASCL 1

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expressing versus non expressing cells.

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We also found that pre-existing

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cells with transcriptional features

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of drug tolerant cells are present

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in the untreated tumors.

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And I think one of the questions that

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we’ve we’re really interested in is you

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know why are some cells permissive or not.

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I think this is sort of one of
the major problems in cancer, one of the things that has been a mystery in cancer over all of the years. Why do you see certain phenotypes when you have certain settings and not others? And in the case of ASCL one, this is very reminiscent of reprogramming because it’s known, for example, that you can put ASCL one into fibroblasts and reprogram them to neurons, but you put them when you put them in keratinocytes. You can’t and this has been shown to be due to actually the chromatin
landscape at Ascl, one target genes in the different cells. So could something like that be happening in the cancer cells as well? And one of the other questions of course that we have is since Ascl one is important for and neuronal differentiation, it’s associated with neuroendocrine differentiation, Is it poising these cells? We didn’t see any other, you know, neuroendocrine markers on, but is it poising the cells to undergo that type of change? And so, some of the things that we’re thinking
00:50:12.152 --> 00:50:14.597 about now and we have experiments ongoing,
00:50:14.600 --> 00:50:17.344 we have Mark Wieshofer in the lab
00:50:17.344 --> 00:50:19.705 who’s been thinking about this and
00:50:19.705 --> 00:50:22.295 working about on this in the context
00:50:22.372 --> 00:50:24.672 of both prostate cancer where very
00:50:24.672 --> 00:50:27.360 similar things happen and lung cancer.
00:50:27.360 --> 00:50:29.208 We’re asking how does a chromatin
00:50:29.208 --> 00:50:31.426 state of a cancer cell affect
00:50:31.426 --> 00:50:33.716 responsiveness to therapy and plasticity.
00:50:33.720 --> 00:50:35.360 And so you can have these different cells,
00:50:35.360 --> 00:50:37.012 you add ASCL one and you can
00:50:37.012 --> 00:50:38.393 see different things happen in
00:50:38.393 --> 00:50:39.320 these different cells.
00:50:39.320 --> 00:50:41.000 And why is that happening?
00:50:41.000 --> 00:50:42.694 And is there something that we can
learn from these cells that then we can apply to human tumors and could we use this information?

I'm thinking far a little bit far ahead, but it’s something that’s in the back of the, my mind is can we predict how a tumor will evolve on treatment with this knowledge. So finally a couple of final thoughts.

baseline mutations and Co mutations can affect disease progression, drug sensitivity and mechanisms of drug resistance and how can we incorporate this knowledge into clinical investigation and practice. This is something that we think about a lot.
There's a vast heterogeneity and complexity of non-mutational resistance and persistence mechanisms. We're working to identify them, establish when they are relevant for specific tumors and find vulnerabilities of these. I'm happy to talk more about these thoughts throughout the day.

Today there are a lot of people to acknowledge. Here are some pictures of lab members throughout the years. This was a particularly fun one.

85
for a closer to free team that so

I thought that was pretty cool.

These are Halloween,

All of the lab has contributed
tremendously to all of these
efforts over the years,

and I'm so grateful to have

the opportunity to work with

so many talented people.

There are lots of people to acknowledge

who have contributed to this work

in addition to members of the lab,

so many collaborators outside of Yale,

but in particular everybody here at Yale,
which I, I, I really, I hope everybody is on this slide. It’s one of the things that I was worried about but want to make sure that everybody is acknowledged here because of the tremendous contributions that makes it such an amazing place to work together. A couple of things that I’d like to say, we have a retreat too on thoracic cancers. On Monday, it’s retreat season. We have a team that has been working. Sarah’s in here, I think.
Sarah Goldberg, Justin Blasberg.

We have Glynis Arnold and Melody MENA who’s been working to organize this retreat. So we hope you can join us and then save the date for our annual lung cancer workshop on June 12th and 13th. It is also going to be at West Campus here and it’s particularly special this year because we are going to be recognizing the 20th anniversary of the discovery of EGF receptor mutations and lung cancer, which has really transformed the field. It’s near and dear front to my heart as you can imagine from the talk,
but it’s really going to be I think a spectacular event with lots of people coming from all over to mark this,

And so we hope that you can participate in that too.

Thank you very much and I’ll be happy to take questions.

Thank you so much, Katie.

Are there questions in the room?

Maybe I’ll start as a person who knows more about squamous cell cancers than adenocarcinomas.

When you talk about P53 mutations,
00:54:24.720 --> 00:54:26.970 are they always the same
NOTE Confidence: 0.893251607333333
00:54:26.970 --> 00:54:28.320 in adenocarcinoma patients?
NOTE Confidence: 0.893251607333333
00:54:28.320 --> 00:54:29.811 And we spend a lot of time
NOTE Confidence: 0.893251607333333
00:54:29.811 --> 00:54:31.140 in the squamous world talking
NOTE Confidence: 0.893251607333333
00:54:31.140 --> 00:54:32.280 about disruptive mutations,
NOTE Confidence: 0.893251607333333
00:54:32.280 --> 00:54:36.120 gain of function mutations. Yeah,
NOTE Confidence: 0.918960678888889
00:54:36.120 --> 00:54:40.314 we have, I think there's a wide variety of
NOTE Confidence: 0.918960678888889
00:54:40.320 --> 00:54:44.000 P53 mutations that you see in lung cancer.
NOTE Confidence: 0.918960678888889
00:54:44.000 --> 00:54:46.760 So they’re like different types and
NOTE Confidence: 0.923658161111111
00:54:46.760 --> 00:54:48.704 have you dissected out if they
NOTE Confidence: 0.923658161111111
00:54:48.704 --> 00:54:49.676 have different implications.
NOTE Confidence: 0.923658161111111
00:54:49.680 --> 00:54:51.534 We think the gain of function
NOTE Confidence: 0.923658161111111
00:54:51.534 --> 00:54:53.239 mutations don’t lead to as much
NOTE Confidence: 0.923658161111111
00:54:53.240 --> 00:54:55.160 genomic instability for example. Yeah,
NOTE Confidence: 0.882860934444444
00:54:55.160 --> 00:54:56.980 those are things that we
NOTE Confidence: 0.882860934444444
00:54:56.980 --> 00:54:58.436 haven’t studied that much.
I think Paul had looked at the different mutations a little bit in terms of outcomes, Paul Stockhammer and I don’t think he had found differences in terms of outcomes with Tkos with the different classes mutations. So is the polycommers suppressor name screen that your biggest hit at least in one of the assays was loss of RB, but it looks like in the in the cancers RB loss was relatively infrequent. Does it does that suggest or have you looked at whether there’s other
dysregulators of the RB pathway that are more common in lung cancer like the Cyclone CDK pathway and that’s a potentially targetable approach?

Yeah, that’s a great question.

So it’s interesting because RB as you said RB one loss is one of the biggest drivers of tumor growth in our screen. It is also if you look at how frequently it occurs with EGFR and P53 mutations, it’s one of the tumor suppressor genes that is most frequently co altered. So none of them go really above the like 10% threshold.

We do know, we haven’t really looked at other ways in which the P50 in which the
RB pathway could be altered in tumors. We haven’t really looked at that. What we do know is that if you have tumors with e.g. F, RP53 and RB alterations, those are the ones that have the highest likelihood of undergoing neuroendocrine differentiation. And so like 1/4 of those will undergo the neuroendocrine differentiation. Any other questions from.