And so it’s my great pleasure.

I’m Marcus Bosenberg, one of the Co leaders of the cancer immunology program and we’re actually have our full House of a program Co leaders in person as we speak.

So today’s grand rounds speaker will be David Braun who’s an assistant professor in the Department of Medicine and also has appointments in pathology and is a Lewis Goodman scholar.

So he is also a member of.
The Center for Molecular and Cellular Oncology that Marcus Musham leads at the Yellow Cancer Center and received his PhD in computational biology at NYU and his MD at Mount Sinai and was a resident in Boston and a fellow at Dana Farber prior to coming here. And we're very glad he's come to Yale.

So David, as you will see, really focused initially on renal cell carcinoma. But you know, we'll see where that goes and we're super happy to have him here.
and have them associated with the cancer analogy program.

And David, without further ado, we’ll have to start. That’s perfect.

Thank you so much for the incredibly kind introduction and for that the chance to speak to you all here today and all the people on zoom as well. And So what I’m going to talk about today is some of the determinants of effective antitumor immunity in kidney cancer. And as Marcus mentioned, a lot of this is relevant specifically for kidney cancer and I see patients.
with kidney cancer at smilow.

And so it’s relevant for these, it’s relevant for these patients specifically.

But I hope as well that this could be used as a broader model of human tumor immunology that we might be able to learn, particularly neurologic mechanisms that might be applicable to other cancer types as well. Here my disclosure is not relevant for today’s talk and so I usually like to start off with just a couple of patients just to highlight the challenges within kidney cancer. This is going to be true for a lot...
00:02:01.775 --> 00:02:03.765 of solid tumors and particularly in
00:02:03.765 --> 00:02:05.145 the immune therapy era.
00:02:05.150 --> 00:02:05.874 So the first patient,
00:02:05.874 --> 00:02:06.960 these are two of my patients
00:02:07.001 --> 00:02:08.087 from the last couple of years.
00:02:08.090 --> 00:02:10.016 had a fairly common age for kidney cancer,
00:02:10.020 --> 00:02:12.364 pretty widespread metastatic disease throughout the lungs.
00:02:12.370 --> 00:02:14.785 Thank you.
00:02:14.785 --> 00:02:17.200 The first patient was a
00:02:17.200 --> 00:02:20.720 had metastatic clear cell kidney
00:02:20.720 --> 00:02:23.012 cancer that’s the most common.
00:02:23.012 --> 00:02:26.538 The most common histologic diagnosis of
00:02:26.538 --> 00:02:28.571 kidney cancer and had pretty widespread
00:02:28.571 --> 00:02:31.052 metastatic disease throughout the lungs.
00:02:31.052 --> 00:02:33.492 Lungs actually had a brain metastasis
and so received a standard first line combination of checkpoint inhibitors and the volume animac.

And for those that don’t look at CC’s every day, the primary tumor is outlined in red and we can see compared to prior to therapy, there’s a tremendous shrinkage of that primary.

There’s basically resolution of all metastatic disease.

The the primary, the residual primary was resected. And so this patient is free of disease, potentially cured of their disease has now been off therapy for over a year.
That’s in sharp contrast to a very similar patient, very similar demographic received the exact same therapy for the exact histologic diagnosis. But unfortunately the tumors did not respond to therapy. They all grew and despite this and subsequent lines of therapy, the patient passed away within eight months of diagnosis. And so it’s it’s cases like these, these extreme response phenotypes, the sort of promise of long standing durable responses and resistance.
that sort of drives the questions in my lab and there’s some peculiarities to kidney cancer. That on the scientific front has also been really interesting and fascinating. So historically we think of CDA T cell infiltration as being a positive thing, positive prognostic thing. They’re main factor self or anti tumor immunity. So having a lot of them in the tumor is positive though historical exception is really kidney cancer where over time of the past 20 years or so having a high degree of CD T cell infiltration has been
associated with a worse prognosis.

really in contrast to just about every other solid tumor type.

Further when we think of what our tumor types that typically responsive to new checkpoint inhibitors,

we think of tumor types that are on the far end,

that are on the far end,

targets for the immune system.

They’re potentially more likely to respond.

We have tumors that have high mutation burdens, lots of neoantigens,

so lots of potential antigenic targets for the immune system.

They’re potentially more likely to respond.
So Melanoma, non small cell lung cancer, urothelial cancer, MSI colon cancer and then we see right in the middle is clear cell kidney cancer with a modest mutation burden. Pretty similar to glioblastoma or pancreatic cancer, ovarian cancer. Yet both historically and contemporarily, it’s responsive to immune therapy. And so my hope is we might be able to learn a little bit about why, if we can figure out why this is responsive to immunotherapy, maybe we can apply those lessons elsewhere. And then also for those patients who aren’t lucky to benefit from immune therapy,
the current forms of immune therapy, can we understand mechanisms of resistance that can guide rational combinations of future therapies and so the framework that our lab really uses to. Answer these questions, what are the infiltrating immune cells and what are the antigenic targets in kidney cancer is a pretty simple framework and I’ve sort of outlined it here where we have a tumor cell, the kidney cancer cell, yeah, interacting with an infiltrating T cell and that takes place in the context
of a heterogeneous microenvironment.

And so with this really basic sort of worldview, we can begin to ask focus questions and these are the really the questions that end up guiding a lot of the projects in our lab. So the first is what are the genetic alterations in kidney cancer, how do they potentially impact immune infiltration into the tumor and ultimately how do those intersect or interplay to impact therapeutic response. What are those other immune cells with immune cells within the tumor microenvironment? How do they?
Interact with T cells and impact cell phenotype. And finally when everything goes right and T cells are capable of recognizing the tumor and eliminating it. What is it that it’s recognizing. And so we know that at the heart of this interaction of antigen specific immunity is the tumor cells presenting antigenic peptides and MHC Class 1 molecules being recognized by the cognate T cell receptor and for for kidney cancer and for most of their I would say solid tumors,
we don’t actually know what those antigens are.

We know sometimes for high mutation burden tumors that can be classic in the antigens, but for things that are modest mutation burdens, tumors like kidney cancer, it’s much less clear. And so these are the sort of three fundamental areas that the lab is currently working on and we’ll kind of step through each one, maybe talking a little bit about some prior work over the last couple of years when I was
back in in Boston and then some ongoing efforts now in the lab.

So the first is really what are the mutations in kidney cancer that might impact immune infiltration and ultimately response to therapy. And I would say broadly, we use a lot of classic genomic techniques, whole exome sequencing, RNA sequencing to really approach these questions. And so a lot of the motivation for this came from an early study just a few years ago from Ellie van Allen’s group at Dana Farber,
where he looked at a small phase one trial of nivolumab in kidney cancer, the checkmate O 9 trial and it was only about 35 patients that had genomic data available, but for those 35. Patients asked a pretty simple question, what are the mutations that are recurrent in kidney cancer among those 35 patients and out of those recurrent mutations which are actually significantly impacting response to therapy and that’s on the X axis. And now those recurrent mutations which are actually significantly impacting response to therapy and that’s on the X axis. And now those recurrent mutations which are actually significantly impacting response to therapy and that’s on the X axis. And now those recurrent mutations which are actually significantly impacting response to therapy and that’s on the X axis. And now those recurrent mutations which are actually significantly impacting response to therapy and that’s on the Y axis. And we can see there’s only one loss of function mutations in the Pfaff matrix.
complex member PBR one was associated with improved response and we see the bottom also improve survival in this small cohort of 35 patients. So really building off of this initial funding that we were through partnership with Bristol-Myers able to sequence not only the phase one data but the tumors from the phase two and phase three trials of nivolumab in kidney cancer. And looking specifically at the phase three trial which is the Checkmate O25 trial, we were able to confirm that yes, loss of function mutations and BM
one were associated with improved response and in this case progression free and overall survival with nivolumab with immune therapy though we can see the effect size is fairly Modest. So it’s really, you know, it’s something that’s there, but it’s certainly not the whole picture. And so this was a very focused sort of validation question, but we really want to look at this much more comprehensively. And So what we did was again in partnership with Bristol-Myers, we’re fortunate to have access
to tumors from the phase one, to phase two and phase three trials of nivolumab in kidney cancer. The phase three trial is really the pivotal trial that led to the first checkpoint inhibitor approval within kidney cancer. And we’re lucky to benefit as well from that phase three trial also having a control arm and mtor inhibitor of your elymus. And so we could see if there’s anything that we find that might be associated with response or resistance. Is that something that’s specific for
immune therapy or is that something that’s perhaps more prognostic that we see with old therapies, including one with an independent mechanism of action like an mtor inhibitor. So we performed whole exome sequencing, RNA sequencing and CD8 immunofluorescence to really look at the immune infiltration of the broad immune phenotype of these tumors. At the time this was the largest study of advanced kidney genetic which was really a foundational work really skews towards earlier stage tumors. Only around 10 to 15% of the kidney
cancer tumors in TCG are advanced stage.
And so by sequencing this we can really get an understanding of first the genetic landscape of advanced kidney cancer and there’s some interesting findings in here, some enrichment and clinically unfavorable aggressive mutations and copy number variants, things like NF2 mutations, a loss of.

But really our primary question was how does this ultimately impact response to immune therapy? And so the first thing we did was look at some of the classic markers of somatic
alteration burden that is present in other tumor types.

So we know that there’s a Histology agnostic approval for the immunotherapy drug pembrolizumab based purely on mutation burden and we know that in many tumor types there’s an association with high mutation burden response to therapy.

And so for kidney cancer, we looked at total mutation burden, neoantigen load we inferred.

You want to drive from frameshift insertion, insertion deletions which create new open reading frames and no metric.
00:10:16.765 --> 00:10:18.245 of somatic alteration burden was
NOTE Confidence: 0.865333130588235
00:10:18.245 --> 00:10:19.910 at all associated with response.
NOTE Confidence: 0.865333130588235
00:10:19.910 --> 00:10:21.772 So again this is in sharp contrast
NOTE Confidence: 0.865333130588235
00:10:21.772 --> 00:10:23.546 to Melanoma and non small cell
NOTE Confidence: 0.865333130588235
00:10:23.546 --> 00:10:25.076 lung cancer and bladder cancer.
NOTE Confidence: 0.865333130588235
00:10:25.080 --> 00:10:27.540 Here mutation burden really does not
NOTE Confidence: 0.865333130588235
00:10:27.540 --> 00:10:29.930 associate or predict response to therapy.
NOTE Confidence: 0.865333130588235
00:10:29.930 --> 00:10:32.072 When we then looked across each
NOTE Confidence: 0.865333130588235
00:10:32.072 --> 00:10:33.143 individual recurrent mutation
NOTE Confidence: 0.865333130588235
00:10:33.143 --> 00:10:35.478 and tried to see which one might
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00:10:35.478 --> 00:10:37.135 be associated with resistance or
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00:10:37.135 --> 00:10:39.067 response in this much expanded cohort,
NOTE Confidence: 0.865333130588235
00:10:39.070 --> 00:10:40.690 again we found only one in
NOTE Confidence: 0.865333130588235
00:10:40.690 --> 00:10:41.500 this pooled analysis.
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00:10:41.500 --> 00:10:43.633 So again it was only PBR on one which
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A very common mutation is a president perhaps up to 30 to 40% of kidney cancer tumors that was associated with improved response and overall survival. And again here we really could benefit and see that this response, this impact on response and survival was unique to the patients tree with immune therapy and was not seen in patients treated with the control arm with an M Tor inhibitor. So that’s uh mutations.

What about the immune landscape and how that might impact kidney cancer? We know that prognostically having a lot of CDT cells might be a negative thing,
but how does that impact response
to immunotherapy?
And so the first thing we did
was characterize these tumors
broadly into 3 immune phenotypes.
And these are types you might be familiar
with the classic immune infiltrated
where there’s lots of CDT cells,
immune deserts,
where there’s made perhaps lots of
the tumor center and have a factor
and then in Munich excluded tumors
and really unable to infiltrate
the tumor center and have a factor
function that’s a potential mechanism
NOTE Confidence: 0.865333130588235
resistance and you know other solid
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tumor types including a common mechanism
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of resistance in bladder cancer.
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And so when we looked at
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our kidney cancer tumors,
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our advanced kidney cancer tumors,
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the first thing we observe
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our kidney cancer tumors,
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our advanced kidney cancer tumors,
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the first thing we observe
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was that immune occlusion is
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really not a common phenotype.
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It’s not looking to be a predominant
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mechanism of resistance in kidney cancer.
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We see here only about 5% of
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these tumors are Munich excluded.
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This is in contrast to some like
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bladder cancer where up to 50%
of metastatic bladder cancers. Would have this immune exclusion phenotype.
The other thing we can observe is by and large these are heavily CD8 infiltrated tumors.
About 3/4 of these tumors are highly infiltrated by CD T cells.
But again in contrast to a lot of other solid tumors, we’re having a lot of CD T cells might positively impact response.
Here it had no impact on response and we can see that for patients I’m showing here treated with immune therapy that regardless
00:12:31.585 --> 00:12:33.796 of whether you had an immune excluded
NOTE Confidence: 0.918145438
00:12:33.796 --> 00:12:36.267 tumor and infiltrated tumor or desert tumor,
NOTE Confidence: 0.918145438
00:12:36.270 --> 00:12:38.391 all of those had roughly the same
NOTE Confidence: 0.918145438
00:12:38.391 --> 00:12:40.109 response to therapy and survival.
NOTE Confidence: 0.918145438
00:12:40.110 --> 00:12:42.384 And so looking at genetics alone
NOTE Confidence: 0.918145438
00:12:42.384 --> 00:12:44.330 you know didn’t yield much.
NOTE Confidence: 0.918145438
00:12:44.330 --> 00:12:45.920 Looking at the immune phenotype
NOTE Confidence: 0.918145438
00:12:45.920 --> 00:12:47.907 alone really doesn’t tell us which
NOTE Confidence: 0.918145438
00:12:47.907 --> 00:12:49.627 tumors are responsive to therapy.
NOTE Confidence: 0.918145438
00:12:49.630 --> 00:12:51.640 Is there perhaps some interaction
NOTE Confidence: 0.918145438
00:12:51.640 --> 00:12:53.455 or interplay between them and
NOTE Confidence: 0.918145438
00:12:53.455 --> 00:12:54.890 so the first thing we did was.
NOTE Confidence: 0.918145438
00:12:54.890 --> 00:12:55.228 Really,
NOTE Confidence: 0.918145438
00:12:55.228 --> 00:12:57.256 just look at among the infiltrated
NOTE Confidence: 0.918145438
00:12:57.256 --> 00:12:58.710 and non infiltrated tumors.
NOTE Confidence: 0.918145438
00:12:58.710 --> 00:13:00.440 Are there different mutational landscapes?
Are there different driver mutations that might be present in one or the other? And the answer was yes. And here again it was only one that it was actually the immune desert tumors, the ones that lack CD infiltration that were really enriched for these clinically favorable CDA T cells that nearly half of those immune desert tumors had mutations in people on one, whereas less than 1/4 of the immune infiltrated ones did. So my mutations, we have enrichment of clinically favorable PR1 mutations within desert tumors.
How about within infiltrated tumors, particularly looking at copy number alterations? And here there was a different picture. There was actually a lot more copy number alterations within these infiltrated tumors potentially indicating these might be perhaps more chromosomally unstable having a higher copy number burden in general than non infiltrated tumors. We looked systematically which copy number alteration was associated with was increased and infiltrated tumors and that’s on the X axis.
And then out of those infiltrated tumors which copy number alteration might be associated with altered response or survival either positively or negatively. And that’s on the Y axis. And again only one came out deletions of nine P 21.3 which contain genes like CDKN 2A, CDKN 2B M tap loss of function. The loss single copy loss of nine was associated with worse survival and worse response. And looking at whether this effect would really specific to NTP one treatment or a broad prognostic effect.
we could see that really loss of nine

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P 21.3 within these infiltrated tumors

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was associated with worse progression.

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Green overall survival

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really only with anti PD,

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one treatment with immune therapy on

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the left and not with mtor inhibition

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of control arm shown on the right.

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So what is it that’s impacting this response?

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Well, how is 9 P 21.3 actually acting to,

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to lessen response to immune therapy?

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That remains an open question.

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We we took a a first look at

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least some activated pathways by

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integrating the RAC data and really

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looking at which pathways might be
00:15:04.675 --> 00:15:06.984 enriched in these nine P 21.3 tumors.

00:15:06.984 --> 00:15:09.252 And there’s some potentially initial hits.

00:15:09.260 --> 00:15:12.062 There’s certainly more angiogenesis

00:15:12.062 --> 00:15:14.017 and hypoxia on those tumors.

00:15:14.020 --> 00:15:16.498 mtor signaling.

00:15:15.672 --> 00:15:16.498 And so at least some initial hints

00:15:16.500 --> 00:15:18.145 There’s definitely more increased

00:15:18.145 --> 00:15:20.300 and so at least some initial hints

00:15:20.300 --> 00:15:22.963 But there’s a lot of mechanistic

00:15:22.963 --> 00:15:23.439 work that still needs to be done.

00:15:23.440 --> 00:15:25.512 And so the initial model we put

00:15:25.512 --> 00:15:27.192 forward for this is that yes,

00:15:27.192 --> 00:15:29.236 in theory CD and infiltration should be

00:15:29.236 --> 00:15:30.937 associated with better response to PD,

00:15:30.940 --> 00:15:33.796 one therapy just like in other tumors.
But what we have here is overlying the genetics of the tumor, these non infiltrators. Infiltrate tumors enriched for loss of function P brown one mutations, these clinically favorable mutations that bring the response rates up and the infiltrated tumors are enriched for these clinically unfavorable deletions of NI P 21.3 and again dragging the response rates down. And so this is the work that was done now published a couple of years ago. And while at the time this was a large sequencing effort, it was about 454 tumors that
underwent whole exome sequencing. It turns out that that is only enough to capture you essentially fairly common. Mutations and kidney cancer that might be associated with immune infiltration or response that you actually need much, much larger numbers to really saturate and really get a better sense of the full landscape of of genetic alterations within kidney cancer. And so in efforts that we’re leading together with Allie Van Allen’s lab, we’ve put together in our cohort of just about 2000 patients that were treated with immune therapy.
This is from a series of phase three trials including that checkmate O25 trial, but other more modern combination therapies phase three trials of.

Pure immune checkpoint inhibitors like the volume Applebaum lab or immune therapy plus antiangiogenic inhibitors.

And the reason we're doing this is sort of demonstrated by this simulated power calculation which I've shown here, which is basically for the number of patients we have in our cohort, of patients we have in our cohort,

which is shown on the X axis, what frequency of mutation are we actually powered to detect.

So if we look at our original paper.
from a couple of years ago now, we were actually powered to detect exactly what we found things that are really quite common in this case. Number one, mutations was present in nearly half of responsive patients and a little bit less than 1/4 of non responsive patients, very, very common mutations. That’s all that we were power to detect. Now that we have a much more substantial cohort of over 2000 out of which about 1500 were treated with immune therapy, we’re now powered to detect a much
broader range of mutations that might impact response or resistance, things that might be present in as low as 5% of responders. And so really getting a much broader land idea of the genetic alterations and how they might be associated with. Resistance and so our questions driving this project are one just about kidney cancer genetics. What are the long tail of mutations, we know the the most common mutations from TGA, but what are the long tail of driver mutations and kidney cancer? Do those fall within common pathways
that might actually lead us to better understand kidney cancer biology? What is the connection between the somatic alterations and immune infiltration? We saw some interactions between PT Barnum one or deletions of nine P 21.3, but again are there others? And then finally how do these intersect? Or interplay to ultimately impact response. So that’s a large ongoing project, but I think the use of whole exome sequencing and an RNA sequencing is really applicable to answer a number of other focus questions. And I think as we think about these,
it's also important.

We're really obviously tumor focused, but also to integrate what's happening in the host immunity as well, for instance, soluble circulating factors in the plasma or circulating immune cells as well. And so just a little bit of a hint of some of the things that we've been working on over the past year. One is a partnership with Random McKay at UCSD who ran a phase two trial of another immune therapy drug atezolizumab plus bevacizumab. This was actually non clear cell kidney cancer less common variants.
And what we could see by looking at circulating factors by plasma cytokines that is actually a highly correlated module of inflammatory cytokines that are present in a variety of these patients with non clear cell disease and what we call the systemic inflammatory module. This was actually associated with worse response and worse survival within these patients.

We’ve talked mostly about the genetics, but we know that the RNA sequencing can also be leveraged to really understand some of the molecular subtypes of kidney cancer.
There was really nice work done from the Genentech group and work that was initially led by Bob Motzer and Brian Reaney where they broke down kidney tumors from a phase three trial and kidney cancer into different molecular subtypes. And actually we're able to see differential response to therapies was actually predictive of whether a patient would respond to therapy A or therapy. Which is a really exciting sort of idea, biomarker driven selection of of therapy for patients with kidney cancer. However that was from patients treated with drugs that are not FDA approved. It was overall a negative phase three trial.
And so in work led by Renee Maria Saliby in my lab we’ve actually used a random forest model to now classify tumors in a FDA approved regiment, a value map plus axitinib and actually look at whether these are associated with response or resistance in a really FDA approved. Measurement really.

Can you use this for for treatment selection? The answer is probably not.

And finally, we know that some patients as I showed not just respond to immune therapy but really have exceptional response. They really have long term durable

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response that goes on for years or tremendous tumor shrinkage.

And so how can we learn what might drive not just responsive theory but exceptional response.

And this is together with Suchat Shukla’s lab at MD Anderson. We’ve partnered to look at a handful of these exceptional responders both from courts we have but also again in partnership with industry and are able to identify certain features, the presence of high clonal neoantigens and actually.
with these exceptional responders, ones that really have response that lasts for years. And so overall, our lab is really focused on using a lot of these classic genomic and transcriptomic tools to understand response resistance to therapy. But we know these are sort of broad tools, classic genomic tools that to understand really what’s happening in the tumor microenvironment and the tremendous heterogeneity both in T cell phenotypes but also in other cells with immune system,
00:21:25.524 --> 00:21:27.420 we need finer tools and that
NOTE Confidence: 0.795158911666667
00:21:27.487 --> 00:21:29.084 we’ve heavily relied on single
NOTE Confidence: 0.795158911666667
00:21:29.084 --> 00:21:30.714 cell RNA sequencing for this.
NOTE Confidence: 0.795158911666667
00:21:30.720 --> 00:21:32.848 And so our past work really asked a
NOTE Confidence: 0.795158911666667
00:21:32.848 --> 00:21:34.131 pretty basic question independent
NOTE Confidence: 0.795158911666667
00:21:34.131 --> 00:21:36.539 of therapy which was as you advance
NOTE Confidence: 0.795158911666667
00:21:36.539 --> 00:21:38.774 along disease stage as you go from
NOTE Confidence: 0.795158911666667
00:21:38.774 --> 00:21:40.584 a relatively normal kidney or at
NOTE Confidence: 0.795158911666667
00:21:40.584 --> 00:21:42.044 least non malignant kidney to
NOTE Confidence: 0.795158911666667
00:21:42.044 --> 00:21:43.360 early stage kidney cancer.
NOTE Confidence: 0.795158911666667
00:21:43.360 --> 00:21:44.750 To locally advanced kidney cancer,
NOTE Confidence: 0.795158911666667
00:21:44.750 --> 00:21:46.566 to metastatic kidney cancer,
NOTE Confidence: 0.795158911666667
00:21:46.566 --> 00:21:49.290 how does the immune microenvironment change?
NOTE Confidence: 0.795158911666667
00:21:49.290 --> 00:21:50.430 How do the T cells change?
NOTE Confidence: 0.795158911666667
00:21:50.430 --> 00:21:52.358 How do the myeloid cells change and are
NOTE Confidence: 0.795158911666667
00:21:52.358 --> 00:21:54.169 there any interactions between them?
And to do this, we prospectively collected fresh tumor specimens from different patients with either early stage locally advanced or metastatic disease and perform single cell RNA in T cell TCR sequencing. Overall, we had a pretty good balance. We sequence about 165,000 cells from a little over a dozen patients heavily skewed towards sequencing the immune compartment. And so now armed with this data set, we can begin to ask questions what are the T cell compartment look like and how.
does that evolve with progressive with advancing disease stage and ask similar questions of the myeloid compartment.

For the T cell compartment, we can see heavy infiltration by CDT cells. Largely there’s a huge component of terminally exhausted CD8T cells, but we see a variety of T cell phenotypes ranging from resident memory like cells to classic T regs. And so when we classify these different cell populations, these T cell clusters and organize them about organize them in a way to see which might be increase in advanced disease stage,
we begin to see highlighted in red that there are few T cell clusters.

There really seems to be enriched in more advanced disease.

Now, at least for me, it’s a little bit unwieldy to look at so many different cell populations.

And So what we did was brought more broadly, classify them just using standard hierarchical clustering.

We can construct this dendrogram where transcriptionally related groups of cells are near each other on this dendrogram and once that
transcriptionally different are far apart.

And what we can see is now instead of, you know, 18 or 19 different clusters, we can see really 2 broad groups in red T cell CD. She sells that broadly have markers of T cell exhaustion, expression of talks, high expression, multiple inhibitory receptors and then everything else. All of the other T cells which are shown in blue, and now with this much more simplified definition, we can see a pretty striking pattern.
that terminally exhausted or exhausted CD8T cells progressively increase with advancing disease stage. They’re essentially absent a normal kidney president, very low levels in early stage disease and progressively increasing more. Against disease stages and that we by contrast see relatively few non exhausted CD T cells with an advanced disease stage. So that’s the T cell compartment. We have this progressive exhaustion with advancing disease stage. What about the myeloid compartment?
And for the myeloid compartment, it's often harder to put these cells into discrete buckets.

You know, for T cells were labeled them as either AT RAG or a CDA T cell that's exhausted. You put them in some of these discrete buckets or clusters.

Myeloid cells as we know can exist much more along a phenotypic spectrum and so for this sort of analysis, using a trajectory inference analysis. Is a really nice approach.

It doesn’t force you to put things into discrete buckets.
It allows cells to exist on a phenotypic spectrum.

And so when we do that for our myeloid cells we see a actually this interesting, this nice interesting branching pattern, which I think recapitulates a lot of sort of known biology.

We have classic monocytes at the root and then branching either into non classical monocytes on the left or into macrophages on the right.

And if we look at where these individual cells are coming from, those myeloid cells that are present in normal kidney.
I should say normal with a caveat. They’re adjacent non malignant kidney. So it’s from a cancer patient might not be totally normal, but we see that they’re largely classical monocytes and non classical monocytes, very few macrophages in these non malignant kidneys. Now if we look at myeloid cells from different tumor types, there’s just a lot more macrophages across different disease stages. The first thing that might catch your eyes, there’s many more macrophages that’s the right-hand branch and across different disease stages. there’s just a lot more macrophages.
than there are normal kidney.

But if we actually hone in on that right branch,
we see again a different pattern between early stage disease,
locally advanced and metastatic and early stage disease.
Those myeloid cells,
locally advanced tumors are kind of spread throughout that branch or spread throughout what we call pseudo time.
And if you look at the metastatic tumors at the bottom, those macrophages are all the way at the end of that branch, all the way at the end of pseudo time. And so what are the genes and gene programs that are really driving these trajectories?

It looks like a switch from a more pro-inflammatory state to an imperfect term but a more immune suppressive or pro-tumorigenic state that we see if we look at signatures.
NOTE Confidence: 0.80934627625
00:26:29.356 --> 00:26:31.666 those are really those peak and are
NOTE Confidence: 0.80934627625
00:26:31.666 --> 00:26:33.570 really high early on in pseudo time
NOTE Confidence: 0.80934627625
00:26:33.628 --> 00:26:35.698 at that right hand branch where
NOTE Confidence: 0.80934627625
00:26:35.698 --> 00:26:37.370 those early stage macrophages are.
NOTE Confidence: 0.80934627625
00:26:37.370 --> 00:26:39.435 And by contrast we look in an
NOTE Confidence: 0.80934627625
00:26:39.435 --> 00:26:40.320 anti-inflammatory signature that
NOTE Confidence: 0.80934627625
00:26:40.377 --> 00:26:42.142 really peaks later corresponding to
NOTE Confidence: 0.80934627625
00:26:42.142 --> 00:26:43.554 where those metastatic macrophages
NOTE Confidence: 0.80934627625
00:26:43.554 --> 00:26:45.147 are if we look at individual.
NOTE Confidence: 0.80934627625
00:26:45.150 --> 00:26:46.474 Means prone inflammatory genes,
NOTE Confidence: 0.80934627625
00:26:46.474 --> 00:26:48.644 aisle 1, beta TNF, aisle 6.
NOTE Confidence: 0.80934627625
00:26:48.644 --> 00:26:51.518 Those are all relatively absent in those
NOTE Confidence: 0.80934627625
00:26:51.518 --> 00:26:54.238 metastatic macrophages outlined in pink.
NOTE Confidence: 0.80934627625
00:26:54.240 --> 00:26:56.816 Whereas if we look at genes that
NOTE Confidence: 0.80934627625
00:26:56.816 --> 00:26:58.343 are typically associated with
NOTE Confidence: 0.80934627625
00:27:00.118 --> 00:27:02.815 things like C163 of the folate receptor,
NOTE Confidence: 0.80934627625
00:27:02.820 --> 00:27:05.385 those are significantly enriched expression
NOTE Confidence: 0.80934627625
00:27:05.385 --> 00:27:07.437 in those metastatic macrophages.
NOTE Confidence: 0.80934627625
00:27:07.440 --> 00:27:09.092 I should say not shown here those
NOTE Confidence: 0.80934627625
00:27:09.092 --> 00:27:10.613 really do express high levels of
NOTE Confidence: 0.80934627625
00:27:10.613 --> 00:27:12.137 complement genes as well and and
NOTE Confidence: 0.80934627625
00:27:12.137 --> 00:27:13.692 trimmed 2 which has been described
NOTE Confidence: 0.80934627625
00:27:13.692 --> 00:27:14.439 by other groups,
NOTE Confidence: 0.80934627625
00:27:14.440 --> 00:27:17.930 these trimmed 2 positive macrophages.
NOTE Confidence: 0.80934627625
00:27:17.930 --> 00:27:20.352 And so we’ve looked independently at T
NOTE Confidence: 0.80934627625
00:27:20.352 --> 00:27:22.230 cells independently and myeloid cells.
NOTE Confidence: 0.80934627625
00:27:22.230 --> 00:27:23.174 The natural question is,
NOTE Confidence: 0.80934627625
00:27:23.174 --> 00:27:24.590 are those independent events or are
NOTE Confidence: 0.80934627625
00:27:24.633 --> 00:27:26.085 they actually talking to one another?
NOTE Confidence: 0.948506981428571
00:27:26.090 --> 00:27:27.749 And to begin to look at this,
we inferred cell cell interactions using the transcriptomic data.

And the idea is fairly simple. We use a tool called cell phone DB, and the idea is if one group of cells is expressing a ligand and another group of cells is expressing the known receptor complex for that ligand, you might infer that they're interacting or talking to one another. And by randomly permuting the labels, you can actually get some statistics. And say, is this something that we expect more than by chance? And what’s shown here is a heat map.
of the number of interactions between different cell populations and this couple different patterns you might see. There’s the darker blue area that’s an area of relatively low number of cell interactions between different cell types and that’s between usually between different cell populations. In red in the upper left corner, you see a lot of interactions mostly between different myeloid cell populations. And then outlined in Black was a particular area that caught our eye, which are. High number of interactions between T cells and myeloid cells and when we zoom
and look on exactly what populations, it's actually these M2 like these trim positive macrophages interacting with these terminally exhausted CD T cells at a high degree. So these are inferred interactions. We obviously have to make sure they're actually present in the same sample. They have to be in the same tumor to physically interact. And so we'll look at the relative proportion of these different populations. We can again see they're really highly correlated.
00:28:45.750 --> 00:28:47.022 That's a strong correlation
NOTE Confidence: 0.948506981428571
00:28:47.022 --> 00:28:48.930 between the presence of these CDT.
NOTE Confidence: 0.948506981428571
00:28:48.930 --> 00:28:51.435 Cells and these tumor associated
NOTE Confidence: 0.948506981428571
00:28:51.435 --> 00:28:52.437 macrophage populations.
NOTE Confidence: 0.948506981428571
00:28:52.440 --> 00:28:54.780 And So what are these interactions?
NOTE Confidence: 0.948506981428571
00:28:54.780 --> 00:28:56.145 They appear to be interactions
NOTE Confidence: 0.948506981428571
00:28:56.145 --> 00:28:57.510 that are bidirectional and really
NOTE Confidence: 0.948506981428571
00:28:57.562 --> 00:28:59.058 support these different phenotypes.
NOTE Confidence: 0.948506981428571
00:28:59.060 --> 00:29:00.648 So these tumor associated
NOTE Confidence: 0.948506981428571
00:29:00.648 --> 00:29:02.236 macrophages are producing ligands
NOTE Confidence: 0.948506981428571
00:29:02.236 --> 00:29:04.219 for inhibitory receptors on T cells,
NOTE Confidence: 0.948506981428571
00:29:04.220 --> 00:29:06.266 things we already know about and
NOTE Confidence: 0.948506981428571
00:29:06.266 --> 00:29:08.810 target like PD1 and PDL 2 for PD one,
NOTE Confidence: 0.948506981428571
00:29:08.810 --> 00:29:10.268 but things we don’t yet target
NOTE Confidence: 0.948506981428571
00:29:10.268 --> 00:29:10.997 and kidney cancer,
NOTE Confidence: 0.948506981428571
00:29:11.000 --> 00:29:13.072 things like PVR and actin 2 for
TIGIT ligands for Tim three and other inhibitory checkpoints. But it’s not all myeloid cells inhibiting T cells, those T cells terminally exhausted CD T cells are also producing factors like myth or. Uh, CS, CSF one that support this more CSF one that support this more M2 like polarization and so it’s really a bidirectional sort of immune circuit that seems to be present in advanced disease stages. This is of course all inferred
from gene expression.

So can we gain a little bit more confidence that this is true, this is real and we have a couple of different validation exercise.

One is are these actually expressed at the surface of the protein level and these different populations and we can use flow cytometry to look at that are these actually present in the same tumors and physical proximity? In situ within the tumor itself and we can use Multiplex immunofluorescence and then ultimately is is actually true.
not just in this small discovery

cohort but also more broadly in other larger patient cohorts.

And so briefly to step through this in partnership with Arlene Sharps lab.

We looked at these different terminal exhaust C8T cell populations and CD163 positive macrophage populations and ask the question do the T cells express the ligands we think they should or the receptors we think they should and do the macrophages express? Well, I guess we think they should and the answer was yes, they do and they’re higher.
in advanced disease stages.

Partnering with Sabina Signer Ideas Lab performing Multiplex immunofluorescence,

we looked at whether these myeloid cells,

these CD163 positive macrophages were actually physically interacting in advanced tumors.

With these terminal exhausted T cells and again in multiple metastatic tumors,

we can see evidence for insight 2 interactions.

And then finally looking at other external cohorts,

we first looked at a previously published saitov cohort from Burn.
00:30:56.990 --> 00:30:58.720 Bodenmiller’s Group and re analyze
00:30:58.720 --> 00:31:00.800 that data to specifically look for
00:31:00.800 --> 00:31:02.576 our T cell and myeloid populations,
00:31:02.580 --> 00:31:03.836 our CD163 myeloid populations.
00:31:03.836 --> 00:31:05.720 And again could see this pattern
00:31:05.771 --> 00:31:07.775 where the proportion of these cells
00:31:07.775 --> 00:31:09.460 increase with advancing disease stage.
00:31:09.460 --> 00:31:11.650 And finally we derived a gene
00:31:11.650 --> 00:31:13.110 expression signature representing that
00:31:13.165 --> 00:31:14.429 interaction between those myeloid
00:31:14.429 --> 00:31:17.008 cells and T cells and use that gene
00:31:17.008 --> 00:31:18.563 signature to interrogate the TCA
00:31:18.563 --> 00:31:20.838 and again found this pattern of.
00:31:20.838 --> 00:31:22.622 Increasing signature of interaction
00:31:22.622 --> 00:31:24.380 with advancing disease stage.
Now that we have this RNA seek signature, we actually ask the question is this interaction signature actually associated with worse outcomes with worse survival. And the answer was yes. If we look at the TCG a data having a high expression of this T cell interaction signature was really associated with worse overall survival. But again we have to be cautious that might just be because it’s enriched in stage four disease. And so if we look specifically at those patients with stage four disease, it
again we see the same.

Effect having a high number,

higher number of those interactions

associated with worse overall survival.

And if we look at again our initial cohort,

our checkmate cohort that we previously

reported on again having a high expression

of those interaction signatures

associated with a horse survival.

And so the model we would put forward would

be that with advancing disease stage we

have this progressive T cell exhaustion,

this switch to more M2 like this

anti-inflammatory macrophage

population and that critically in

population and that critically in
advanced disease states that they’re really talking to one another, they’re interacting in a way that we hope is therapeutically. So up until now I’ve talked broadly about kidney cancer as if it’s one entity, but I’ve been sort of misleading you. It’s actually many, many different diseases. And So what we’ve been talking about is really clear cell kidney cancer which is shown here histologically which is looks clear under the microscope where it gets its name. But actually this is a host of over 20
different disease with more entities being described each WHO update and there’s a huge proportion of, I apologize, should be about 25% non clear cell which is. So it was bad to be labeled by what you’re not, but these are variant histologies of clear cell. The more common ones are papillary chromophobe accounts for about 5% rarer types including translocation and then hereditary forms including FH deficient really aggressive disease biology that often affects people in their 30s.
And so while all of our efforts have been really focused on clear cell kidney cancer and that’s where a lot of the clinical data is as well. We know that these non clear variants really aren’t unmet clinical and scientific need. We really need to understand their biology and how to treat them because most of their treatment is really extrapolated from our experience in clear cell. And so our first sort of approach to this is really in the chromophobe space. So chromophobe is really along a spectrum of these renal oncotic neoplasms that range...
from pretty benign tumors,
renal oncocytoma which never metastasize,
I should say never virtually
They really have limited
They are true malignant disease.
They have multiple whole chromosome
losses and then there’s also these
emerging entities in between low
and high grade unconscious tumors
which have variable potential to
actually invade and metastasize.
And so one of the key things
about these chromophobe tumors is unlike clear cell kidney cancer, these don’t do well with immune therapy. And so there’s always going to be exceptions. But both in in clinical trial data of immune therapies where they’ve included chromophore patients, the response rate is typically less than 10%. And if we look at these chromophobe tumors, this is our own data partnership with the international Metastatic Database consortium, Danny hangs group at University of Alberta and we looked at now hundreds of patients treated with new checkpoint inhibitors. Real world data that are the clear cell
or non or chromophobe and the chromophobe patients really don’t do well here. This is in sharp contrast to in other treatment varieties chromophores typically shape a better disease prognosis and so really this is an area of unmet need. Why aren’t chromophobe tumors responding to immune therapy. And so to begin to look at this and again these are rare tumor types we we were able to identify a handful of patients, about five patients that really represent this disease spectrum and again perform single cell. Kinda sequencing to look at the
immune microenvironment and also the tumor and stromal components as well. And really our focus questions were why aren’t these responding to immune therapies and going in. We had a couple of hypotheses. So one is maybe it’s just a lack of immune infiltration. If you don’t have cells there to begin with, then it’s hard to get any immune response. The second is perhaps they’re exhausted in ways that don’t rely on PD1. So perhaps these have some severely exhausted or dysfunctional program that are really incapable of being reinvigorated.
00:35:45.326 --> 00:35:47.549 by our current immune therapies.

00:35:47.550 --> 00:35:49.638 And the last possibility is maybe

00:35:49.638 --> 00:35:51.858 those are just bystander T cells

00:35:51.858 --> 00:35:53.778 where they’re not actually infiltrated

00:35:53.778 --> 00:35:56.310 by tumor specific T cells that are

00:35:56.310 --> 00:35:58.290 required for true anti tumor efficacy.

00:35:58.290 --> 00:36:00.145 And so with these sort of focused

00:36:00.145 --> 00:36:01.814 hypotheses we can begin to look

00:36:01.814 --> 00:36:03.440 at these different areas and see

00:36:03.440 --> 00:36:04.857 which of them actually are.

00:36:04.860 --> 00:36:06.228 Able to chromophobe tumors.

00:36:06.228 --> 00:36:08.280 So the first is immuno filtration.

00:36:08.280 --> 00:36:10.716 Here we did very basic CD 45

00:36:10.716 --> 00:36:11.760 immunohistochemistry just to

00:36:11.824 --> 00:36:13.779 look at the immune infiltration,
broad immune infiltration of these tumors and what we can see is on the right you see clear cell and the immunohistochemical stain for CD45. These are really heavily immune infiltrated T cells, immune infiltrated tumors as we saw in our previous study. By contrast these oncocytic neoplasms, oncocytoma is low grade oncocytic tumors, these have really low degree of immunity. Centration and we can see that sort of characterized statistically on the right. And so that seems to be one problem. They just aren’t a lot of immune cells.
So we’re going to improve immune responsiveness of these tumors. One will be driving, driving immune cells actually into the tumor itself. The second is, are these cells that are there just too exhausted to respond. And to begin to look at this weekend, turn to our single cell data and look to the CDA T cell populations and saw that they express markers of immune exhaustion and the transfer is no, those were not exhausted T cells.
So if we look at clear cell which on the left versus chromophobe on the right, we see as we know the ones in the clear cell tumors are often exhausted terminally exhausted CDT cells. That was not the case for chromophobe tumors and if we look now at the TCGA data, we see a very similar pattern that chromophobe tumors have relatively low levels of exhaustion markers, expression of exhaustion markers that’s compared to clear cell disease. And so these are not exhausted T cells. They seem actually like they’re cytotoxic. They seem like they should
be functional.
The ones that are there, why aren’t they actually doing the job. The last part is maybe they’re not tumor specific and so there’s ways to formally prove this by actually TCR sequencing these, that’s what needs to ultimately be done. Our first take out is is to use the single cell TCR data that we have to try to infer specifics and we did this in two different ways. One is by taking those TCR’s and
mapping them to known to TCR’s.

I’ve known viral specificity.

Those are usually specific for

things like CMB or EB or flu,

common viruses that have

nothing to do with these tumors.

And we can see that the the T cells

and chromophobe kidney cancer

mapped out a much more significant

degree to these viral specific.

They have a viral specificity so much

more likely to be bystander T cells.

The other approach we used

was to take signatures,

gene expression signatures defined by

both Kathy who’s group but also Steve
Rosenberg’s group at the NCI that are signatures of tumor specificity including neoantigen specificity. See what that expression looks like in these different tumor types. And we can see for clear cell kidney cancer in red that those have a high degree of tumor specificity and for chromophobe that’s substantially less. And so overall this is our first sort of attempt to really characterize what is an uncommon and rare tumor type and really try to understand its immune biology.
It looks like it has a lack of immune infiltration. It looks like the T cells that are there are probably fully functional but lack of tumor specificity. So that’s the work we’ve largely done the work that was published last year and the work on chromophobe tumors that’s been really over the past year. But now what we want to do is move from just characterizing the disease biology to really understanding how these different tumor microenvironments might impact response or resistance if therapy.
And ultimately how can we functionally evaluate these, how can we actually go from a laundry list of potential sell interactions to ones that actually might be therapeutically targetable in the clinic and that’s really what the focus is. We’ve started this process, but over the next year. The sort of idea behind this is shown here. This is a perspective we publish a couple of years ago now which is to really try to integrate these.
tumor biopsies for fresh tissue collection for single cell and sequencing into clinical trials.

And obviously this is expensive, this is technically difficult this feasibility issues.

But if you could do this even for a handful of patients, a small discovery cohort and really go into a lot of depth for small number of patients then you can actually learn some lessons like we did in our prior work and try to then use more. Conventional tools, standard exome sequencing, standard exome sequencing,
NOTE Confidence: 0.66308334
00:40:20.706 --> 00:40:21.304 immunofluorescence,
NOTE Confidence: 0.66308334
00:40:21.304 --> 00:40:22.500 amnestic chemistry,
NOTE Confidence: 0.66308334
00:40:22.500 --> 00:40:26.148 then try to apply that to a larger
NOTE Confidence: 0.66308334
00:40:26.148 --> 00:40:27.060 validation cohort.
NOTE Confidence: 0.66308334
00:40:27.060 --> 00:40:29.538 And so this is our attempt,
NOTE Confidence: 0.66308334
00:40:29.540 --> 00:40:31.116 this is our basic schema that we try
NOTE Confidence: 0.66308334
00:40:31.116 --> 00:40:32.725 to take patients who are responsive
NOTE Confidence: 0.66308334
00:40:32.725 --> 00:40:34.471 and non responsive to immune therapy.
NOTE Confidence: 0.66308334
00:40:34.480 --> 00:40:36.181 We try to get biopsies before treatment
NOTE Confidence: 0.66308334
00:40:36.181 --> 00:40:38.417 as much as possible is often challenging.
NOTE Confidence: 0.66308334
00:40:38.420 --> 00:40:41.062 We try to get biopsies on treatment
NOTE Confidence: 0.66308334
00:40:41.062 --> 00:40:42.817 or at least that progression,
NOTE Confidence: 0.66308334
00:40:42.820 --> 00:40:45.540 very variable success on that.
NOTE Confidence: 0.66308334
00:40:45.540 --> 00:40:47.076 And then to perform single song
NOTE Confidence: 0.66308334
00:40:47.076 --> 00:40:48.432 RNA sequencing to really uncover
NOTE Confidence: 0.66308334
what are the cell type differences, cellular composition differences, phenotypic differences and ultimately what are the differences in cell interactions and so we’ve. Uh Bin lucky again. This is through partnership with a number of academic collaborators, but also industry collaborators preparing to a number of phase two and phase three trials that we’ve been able to collect fresh tissue from a total of 96 tumors that were treated with immune therapy. And we’ve performed enzymatic association single cell RNA sequencing
on these on these tumors really to understand what is the difference in the immune landscape between responsive and nonresponsive tumors. And we’ve put through a partnership with AKOYA began to look at what is the orientation of the physical location of these tumor types, these different immune populations in space using these high dimensional platforms. This is an example of one of our tumors from a responsive patient showing actually a high number of traditional lymphoid structures.
And so is this actually feasible, are we actually able to collect these cryopreserved specimens from different clinical trials and get viable cells out of this? Our first attempt at this was on a small number of patients was just on 13 patients. This is a collaboration with Kathy still with MM Atkins at Georgetown and with Kelly St who runs a computational group at USC where we looked at the small number of cryopreserved tumors and said, are we able to get viable cells out of this? The short answer was yes.
that we’re able to get actually really good. Representation of both tumor cells, immune cells and also stromal components and actually even with this small cord of end up being about 13 samples that were suitable for analysis. After passing quality control, we can actually begin to see are the differences in immune microenvironment between responsive and resistant tumors. And so again this is a trajectory inference analysis, this time it’s for T cells. And again we see a branching structure, but here are fairly interesting one,
one that starts with a root of naive

T cells and branches either into terminally exhausted CD8T cells.

Those are the same ones we saw in our prior work across disease stages or to these still having an exhaustion phenotype but these slam F7 positive CD8T cells.

And we look, when we look specifically at which immune populations are associated with resistance or with altered survival, it turns out that the slam of seven positive CDT cells again in this very small cohort only 13 patients, but associated with progressive
disease and with worse progression.

And so this is our sort of initial 13 patients.

We’re now parsing through the sequencing data from our 96 patients to really get a better handle on what are the different human populations that might exhibit this sort of behavior.

But we also have to move beyond immune profiling.

We might get a sense of what are the immune populations that are relevant, and what are the immune interactions, which could play a role.
But we actually have to nominate individual targets for the clinic.

We actually have to be able to test this.

And then kidney cancer is a unique opportunity to do this.

Part of it is just purely practical.

You can have a 6 1/2 centimeter tumor that’s a stage one tumor and it’s not uncommon for these tumors to extend to exceed 10 centimeters.

And so there’s just lots of material to be able to extract individual immune populations.

Individual tumor populations and actually functionally test which
interactions might actually play a role and we’ve begun to do this. We’ve been able to take these primary tumors and I should mention Cat sudakin in the lab is really spearheaded this process of identifying patients as in collaboration with Mike Hurwitz who runs the Gu tumor bank with Debo Adeniran and Peter Humphrey and Pathology and Pat Kenny and Urology where we’re able to routinely on just about every nephrectomy that’s done at the hospital. Here, collect fresh tumor for this sort of work.
And so we’re able to extract both immune cells and tumor cells and for a subset of patients we’re actually able to grow out tumor cell lines. This is really a valuable resource for thinking about autologous coculture experiments with T cells. Our sort of overall plan for functional validation is sort of two phases. Again this is led by a few people in the lab.
Uh Katrina and Hannah is to basically break this down into individual cell populations. So to associate those tumors into single cell suspension, isolate individual cell populations of interest, coculture just those populations of interest with a therapeutic drug with an inhibitor of a particular interaction. And then be able to measure changes in T cell function, basic flow cytometry for intracellular cytokine production collaboration assays, expression of cytotoxicity markers like...
granzyme and then we begun to implement.

These model antigen systems where we have now T cells that we engineer with a specific TCR, TCR against NY, so one or against WT1 and we have tumor cells that express those antigens and express luciferase and we can actually now test have a model antigen system for for testing these impact on cytotoxicity.

Again, there’s limitations of a reductionist model. And so in work that’s done in collaboration actually now with AstraZeneca and and a lot of
00:46:01.868 --> 00:46:03.320 mentorship from Marcus here,

00:46:03.320 --> 00:46:05.420 we also have begun to implement these

00:46:05.420 --> 00:46:07.389 tumor fragment models where we try

00:46:07.389 --> 00:46:09.074 to recapitulate the 3D microenvironment.

00:46:09.080 --> 00:46:10.455 We actually cut the tumor

00:46:10.455 --> 00:46:11.555 into these various fragments.

00:46:11.560 --> 00:46:13.716 We embed them in a collagen matrix.

00:46:13.720 --> 00:46:15.561 We float that in media where we

00:46:15.561 --> 00:46:16.350 can add various

00:46:16.414 --> 00:46:18.451 perturbations and we can see that over

00:46:18.451 --> 00:46:20.679 the course of its short-term culture,

00:46:20.680 --> 00:46:21.832 three to five days,

00:46:21.832 --> 00:46:23.560 we really can recapitulate the Histology,

00:46:23.560 --> 00:46:25.030 the architecture of these clear cell.

00:46:25.030 --> 00:46:27.094 Consumers and preserve a lot of
the immune microenvironment both
t cell and myeloid components.
And so with this we can actually
use this to actually function
We’ve done some toy experiments,
low dose or higher dose IL two and
can show that we can impact the
t cells that are there just as a
I’m showing one where we’ve added
low dose or higher dose IL two and
can show that we can impact the
t cells that are there just as a
But now together with AstraZeneca
really looking at some of these
interactions that we found in our
original cancer cell paper TIGIT
and others and seeing whether
inhibition of those inhibitory interactions might actually impact. Tumor killing and cell function. And so that second piece is really the tumor microenvironment and how that changes with advancing disease stage and now how we can use that to understand response and resistance to therapy. The final aspect of our lab focuses on is really trying to identify what are the relevant antigens in kidney cancer and how we might be able to therapeutically target them with antigen directed therapy. And in order to do this,
in my mind we need sort of two pieces.

We need a toolkit of experimental

And so the experimental tool toolkit

Computational tools includes

Physical tools for antigen detection.

These are immuno epidemics where we

can actually immunoprecipitate off

Class 1 molecules from tumor cells,
elute peptide and and actually physically detect the presence of individual peptides. This was done in collaboration with Steve Carr’s group at the Broad. And then TCR tools and these are now pretty established tools, tools for single cell TCR sequencing where we know the full alpha, beta paired TT cell sequence, but also the ability to then reconstruct them in primary healthy T cells and actually probe their specificity. So we do have a good toolkit for antigen discovery now we need the samples and I think clinical trials,
particularly early phase clinical trials
are really wonderful platform to be
able to do this really in-depth analysis.
And so I’m going to give a little
bit of a vignette of work.
That’s wrapping up from my time
at Dana Farber.
This is a clinical trial,
a phase one trial that led together
with Tony Sherry and Patrick OTT of
neoantigen vaccination in kidney
that I think has some
interesting findings in of itself,
but really also serves I think,
as a platform for answering some
of these questions.
So this was a trial that took stage three or stage four patients with kidney cancer. They had to be fully resected at the time of surgery. So they had no evidence of disease and these were clear cell only and we treated 5 patients, vaccine or local. Pipeline maps or ctla 4 inhibitor given at the vaccine site or vaccine alone and the basic process we would take their tumor and then perform whole exome sequencing and RNA sequencing on the tumor.
We'd identify tumor specific mutations, we ensure that they're actually expressed at the RNA level. We'd use some of our tools that I just described to infer what are likely to be HLA binding peptide, so likely to be antigens and then we'd actually get together in a spin a room. And turn it over zoom, but we get together as an epitope selection board to actually pick which of the mutations we want to target and design synthetic long peptides anywhere between 20 to 25 more typically in order to actually physically synthesize them.
We then partnered with a GMP peptide manufacturer to actually synthesize these peptides up to 20 representing different neoantigens, pulled that together with an immune adjuvant Poly ICLC and then that delivered that to the patient. We treated 9 patients overall. It’s the sort of standard demographics you’d expect for kidney cancer. Majority were stage three. There were a couple of stage four patients as well. And in every patient we were able to identify enough mutations to actually target.
So median of 13 unique mutations per patient were targeted with 15 different peptides. Kidney tumors have a lot more frameshift mutations than a lot of other tumor types. So we're able to target a lot of frame shifts. I think interestingly, we're actually able to target in the majority of patients actually driver mutations within kidney cancer. And it turns out those when we look back ends up being the most immunogenic immunogenic peptides, the ones that represent driver mutations.
any sort of clinical data here just
because it’s it’s nine patients,
but they’re,
yeah,
at least encouraging that was certainly safe.
No one,
everyone did well on the trial and
there have been no clinical relapses,
I would say in this population probably
at this point somewhere around 1/3
third to half might have relapsed.
And so the fact that there have been no
disease recurrences is at least encouraging.
But again,
a big part of this was really the
Biospecimen collection and these were really generous patients that went through a lot for this trial. And so for each of these patients and they went through the vaccination itself, which were five priming doses of the course of three weeks and two booster shots at week 12 and week 20. They had multiple skin biopsies prior to and after vaccination to look at whether they infiltrated immune populations within the skin. We obviously have the tumor tissue itself, but we need lots of circulating blood cells as well to perform immune monitoring and so we perform leukapheresis.
Often required a central line placement before and after treatment and weeks here and week 16 and pretty regular 200ML blood draws, regular 200ML blood draws, really look at what are the circulating immune populations and our questions, we’re really trying to look kind of end to end what’s happening at the skin as we move to the circulating immune system. Ultimately are we getting tumor reactivity, it’s nice to get reactivity against the vaccine itself, but it’s actually not impacting tumor reactivity. We haven’t done too much.
We haven’t actually been helpful. And so just to briefly walk through this, this is what a typical actual patient one. So this is the prior vaccine scars and what the vaccine site looks like two to three days after vaccination. We can perform enzymatic dissociation, CD45 isolation and single cell RNA sequencing identifying really high populations of myeloid and lymphoid cells. I would say predominantly it happens.
With vaccination, we're not seeing huge differences with the addition of epilimnion map. Moving on to the circulating immune system, really the workhorse for this was interferon gamma Ellie spots. These are taking peripheral T cells out of a patient peripheral blood cells, putting them into a dish and stimulating them with the same vaccine peptides and seeing whether those T cells release interferon gamma as a marker of antigen reactivity and would see it week one prior to vaccination. Basically none of the neoantigen pools,
00:52:58.120 --> 00:53:01.074 the 1st 4 rows had any reactivity,
NOTE Confidence: 0.782859847894737
00:53:01.080 --> 00:53:03.936 but we get pretty strong reactivity
NOTE Confidence: 0.782859847894737
00:53:03.940 --> 00:53:06.614 with vaccination and that when we do.
NOTE Confidence: 0.782859847894737
00:53:06.620 --> 00:53:08.198 Close cytometry and it’s Cellular said
NOTE Confidence: 0.782859847894737
00:53:08.198 --> 00:53:10.184 to kind of standing we actually see that
NOTE Confidence: 0.782859847894737
00:53:10.184 --> 00:53:11.858 these are not largely polyfunctional,
NOTE Confidence: 0.782859847894737
00:53:11.858 --> 00:53:15.890 that they T cells not only produce interferon
NOTE Confidence: 0.782859847894737
00:53:15.970 --> 00:53:18.746 gamma but things like aisle 2 and TNF.
NOTE Confidence: 0.782859847894737
00:53:18.750 --> 00:53:19.554 And finally,
NOTE Confidence: 0.782859847894737
00:53:19.554 --> 00:53:21.564 moving beyond just vaccine reactivity,
NOTE Confidence: 0.782859847894737
00:53:21.570 --> 00:53:23.376 are we getting actually tumor reactivity.
NOTE Confidence: 0.782859847894737
00:53:23.380 --> 00:53:25.342 And So what we can do is again take
NOTE Confidence: 0.782859847894737
00:53:25.342 --> 00:53:27.247 some of these post vaccine T cells,
NOTE Confidence: 0.782859847894737
00:53:27.250 --> 00:53:29.350 stimulate them with one of our vaccine
NOTE Confidence: 0.782859847894737
00:53:29.350 --> 00:53:31.529 peptides in this case against a driver
NOTE Confidence: 0.782859847894737
00:53:31.529 --> 00:53:33.761 mutation PR one and then coculture with
that same patients autologous tumor and see whether those PBR one specific T cells actually recognize tumor.

And the answer is, is yes, that we are able to actually get tumor reactivity. It’s not for all patients, but for the majority of patients we’re able to get evidence of tumor. The activity with vaccination and directed therapy and kidney cancer, but I think neoantigens are a good place to start.
But I think those are clearly not going to be the whole story. And kidney cancer we know that there's not an association between high neoantigens and response to therapy. So we have to look beyond this initial neoantigen focused world and really look at other sources of antigens as well. Very briefly this is work large in collaboration with Bill Kaylan’s Group and Steve Carr’s group. We’re using the same cohort of patients, the same tumors to actually look at endogenous retroviruses as
00:54:24.472 --> 00:54:26.059 potential antigenic targets.

00:54:26.060 --> 00:54:27.194 These are ones that are aberrantly expressed in a few different tumor types, specifically kidney cancer has a high expression of these endogenous retroviruses.

00:54:28.780 --> 00:54:31.062 but specifically kidney cancer has a high expression of these endogenous retroviruses.

00:54:31.062 --> 00:54:33.380 So we can again use our computational tools to predict antigens, potential ER derived antigens and use mass spec based approach to actually physically detect those antigens.

00:54:33.380 --> 00:54:35.620 So we can again use our computational tools to predict antigens, potential ER derived antigens and use mass spec based approach to actually physically detect those antigens.

00:54:35.620 --> 00:54:37.180 And in this first patient, this patient 110 from our original trial, we see that there were seven ERV Derived peptides that were present.

00:54:37.180 --> 00:54:39.184 And in this first patient, this patient 110 from our original trial, we see that there were seven ERV Derived peptides that were present.

00:54:39.184 --> 00:54:41.383 mass spec based approach to actually physically detect those antigens.

00:54:41.383 --> 00:54:42.999 physically detect those antigens.

00:54:42.999 --> 00:54:44.860 And in this first patient, this patient 110 from our original trial, we see that there were seven ERV Derived peptides that were present.

00:54:44.860 --> 00:54:47.317 this patient 110 from our original trial, we see that there were seven ERV.

00:54:47.320 --> 00:54:49.210 we see that there were seven ERV. Derived peptides that were present.

00:54:49.210 --> 00:54:50.745 Derived peptides that were present.

00:54:50.745 --> 00:54:52.941 on tumor but on a normal normal
tissue and when we take one of those, the one highlighted in pink and actually test those for reactivity in peripheral blood cells from that patient, we can see that those patients actually capable of mounting a low level but a response to that peptide. So just a initial proof of concept that these RV’s can actually be antigenic and now we can actually do this much more systematically look across all patients and all of their endogenous retroviruses that are presented and look for antigenicity. Again, these are very focused approaches.
neoadigen.

Uh or endogenous retroviruses, the last thing we want to do is ultimately build systems and collaborate with groups that are interested in more broad antigen discovery efforts for things that we’re not thinking of. And so we recently entered a partnership with Remedy Bio, a biotech company based in Ireland, which has a novel platform, a nano reactor platform that actually allows you to coculture individual T cells and tumor cells within each of these wells,
but actually measure which of those wells are reactive to tumors,
use a pneumatic system to extract viable.
These cells tumor reactive T cells and be able to sequence their TCR,
so really be able to understand much more systematically what is the repertoire of tumor reactive T cells in kidney cancer.
And so overall our kind of hope with this branch of the lab is really to move beyond our classic tools for immunomodulation to add the steering wheels rather than only looking at the inhibitory checkpoints or the sort of go signals for your
immune cells to actually be able to add a component of an antigen directed therapy really focus on HLA restricted antigens. And that’s where the model for the lab, it’s been a busy but a great year. That’s been a wonderful, a wonderful time. I felt incredibly welcome here at Yale and been lucky to have remarkably energetic and kind group of people joined the lab and really focus on sort of this model that we start with the patient. We try to learn things from their tumor, from their immune system.
We have a lot to go and a lot of open questions about sell sell interactions and about antigenic targets, but always with an eye to try to bring that into improved diagnostics, actually improve therapeutics. And try to bring that back into early phase trials like I showed with our neoantigen trial and then to continually iterate to try to get a little bit better each time that we do this. And so with that, thank you again for the opportunity to speak and a lot of people, both my lab and collaborators, but most importantly the patients.
and their families.
And this time I'm happy
to take some questions.
Alright, just one question.
In general, most of them are.
There must be some difference. Responded.
Yeah.
The CDA looks great based on
responding to non response.
Within infiltrated tumors,
it's a good question.
I think that's where our sort
of larger collection of this 90
of larger collection of this 90
tumors

single cell sequence of 96 tumors
will I think be very helpful.
If I were to answer this six months ago, I would have said it’s going to be impacted largely by the myeloid component as well. And I think that’s still probably true that we kind of showed in our original study that even though we’re thinking we’re measuring CDT cells, likely what we’re actually capturing is interactions between those terminally exhausted CDT cells and the myeloid. Component and that. Historically, we’ve only targeted 11 branch of that. We’ve only targeted the T cell compartment.
and not the myeloid compartment. I think that’s going to be one big piece of it. The second piece which we weren’t expecting to find is this particular phenotype of slam of seven positive CDT cells that requires a lot of validation both that they’re actually real, but then that they have a functional role. I think that’s going to be the other sort of component. Are there different actually the cell phone even though they’re broadly infiltrated by similar numbers of CDT cells,
are those CDT cells of a different phenotype that actually might be perturbed? In some way and one nice thing is there are even you know FDA approved antibodies like elotuzumab for slim F7. So one can see actually a pathway those end up being true to to the clinic. So that’s that’s kind of ongoing work. Now actually a rotation student in the lab is putting some of seven into some of these T cells and we’re actually seeing whether this impacts cytokine production proliferation, tumor killing. Yeah. Ohh. And. 27. Thank you.
Yeah, it’s a great question.

So the question was just about the stromal component fibroblasts and other stromal and kidney can’t strictly anthelion cells as well which these are heavily vascular tumors.

I would say our first study really didn’t we weren’t we, really didn’t look at it at all because our protocol really enriched for immune cells.

I think now with not only the chromophobe, but also this these you know larger 96 samples we actually much more broadly.
Capture cancer, associated fibroblasts and epithelial cells. I will say that first 13 patients we didn’t see any that were specifically associated with response or resistance in this very broad look. But that obviously doesn’t mean they’re not important actually driving either T cell or myeloid biology. And so that’s something that I think we need to look into in more depth, but we don’t have, we don’t know quite yet but actually now I think. Yeah. Now we actually have the tools that
I would be able to look at it. You know. Really the only type that sold?

Obesity and commonly treated with cycling inhibitors.

I'm wondering if you have any hints in your data as to the role of metabolism in the micro environment.

Yeah, it's a great question. The shortening I'll give is, is not yet, but I'd love to be able to support it and look at it because it's some fascinating parts about kidney cancer as well.

So there's something where even
though it’s you’re more likely to get it the incidence is higher in obese patients. Those patients who are obese who have metastatic disease do better. Something called the obesity paradox within kidney cancer. And we know that there’s some hints that these are in general really metabolically active. There’s really excellent work from Jeff Rathmell and Jim Rathman’s group that looked at what are the primary consumers of for instance glucose and the micro environment. And since that’s not the tumor cells,
it turns out it’s mostly the myeloid compartment that’s a primary drive the primary consumer. But T cells are are still consuming a lot. How those actually ultimately I think impact the function of T cells, I think we haven’t looked at all, but it would be great to be able to explore especially with some of these models where we’re you know have them in. Really nutrient rich, metabolically favorable conditions and xvo actually would be nice to recapitulate some of the nutrient limitations that are present in the tumor itself.
Yes.

Well, thank you,

David.

I thank you all for also coming here in person and we’ll look forward to grand rounds next week.

Thanks so much.