Search and we'll start with our first Speaker, Doctor Grace Lee. As many of you may know, she is an assistant professor in endocrine surgery. She received her medical degree from the University of Oklahoma College of Medicine, did her clinical training, residency in Research Fellowship at the Brigham and Women's Hospital at Harvard Medical School, and then did her fellowship in endocrine surgery at the male clinic. Before we were very fortunate, we were able to recruit her here.
Doctor Lee is really an innovative expert in endocrine surgery and is looking at new ways to combat thyroid, parathyroid, cancer among others, and seeing how we can leverage new techniques to improve the care patients. I have a firmer understanding of the disease and Grace. I want to thank you for taking the time to join us for cancer grand rounds and welcome hearing you talk.
I am delighted to be able to present an area of my clinical interest with everyone. Share my screen. Works. Alright, so my name is Grace Lee. I am a newish endocrine surgeon to Yale. I joined Yale earlier this year. Sort of at the height of the pandemic from Tulane Medical Center, down in New Orleans and I have been an early adopter of this new technique, called Radiofrequency Ablation in thyroid at Tulane. So I just wanted to share my experience...
and what I know about where you frequency ablation so hopefully we can also start this treatment.

At Yale as well. I have no disclosures.

My talk will briefly touch on several different areas. I'll just touch on the background of how the RFA works. The physics and mechanism and brief history. Current procedural guidelines. When we are deploying something knew there are governing bodies who. Guide us as far as indications and when not to do it or concerned and will cover why we should be looking into this.
What are the risks and the advantages and will also visit efficacy and safety of this technique as well as I’ll show you a little video clip of how this is done and future directions.

Super Cutaneous Ablation is a technique that obviates the need for a surgery and is often imaging guided. These needle often imaging guided. Way of eliminating undesirable tissue of their multiple different ways of burning or zapping things, we have utilized laser, microwave cryotherapy, freezing it, burning it, even injecting ethanol to a
chemical esclerosis things, or Hifu which is high intensity focused ultrasound.

Today we are going to focus on radiofrequency ablation, which is on the spectrum of these energy harnessing ablation techniques.

Radiofrequency is concentrating heat or thermal energy at the tip of an electrode catheter. By alternating current and this frictional heat that gets generated from ionic molecules will.

Turn into conductive heat from this frictional heat and create...
00:04:30.735 --> 00:04:32.499 this Adelaide of unit.
NOTE Confidence: 0.8005014
00:04:32.500 --> 00:04:35.470 An if you sustain this heat
NOTE Confidence: 0.8005014
00:04:35.470 --> 00:04:37.450 enough in that area,
NOTE Confidence: 0.8005014
00:04:37.450 --> 00:04:39.950 this thermal energy will.
NOTE Confidence: 0.8005014
00:04:39.950 --> 00:04:42.450 Cause irreversible cellular damage,
NOTE Confidence: 0.8005014
00:04:42.450 --> 00:04:44.301 so essentially coagulations
NOTE Confidence: 0.8005014
00:04:44.301 --> 00:04:47.386 necrosis but without charring the
NOTE Confidence: 0.8005014
00:04:47.386 --> 00:04:50.630 tissue or carbonation of tissue.
NOTE Confidence: 0.8005014
00:04:50.630 --> 00:04:52.193 Radiofrequency Ablation in medicine.
NOTE Confidence: 0.8005014
00:04:52.193 --> 00:04:55.640 Interesting Lee has been utilized
NOTE Confidence: 0.8005014
00:04:55.640 --> 00:05:02.845 for a long time.
NOTE Confidence: 0.8005014
00:05:02.850 --> 00:05:06.987 wide cardiology started using
NOTE Confidence: 0.8005014
00:05:06.990 --> 00:05:09.820 It has been around in medicine for over
NOTE Confidence: 0.8005014
00:05:09.820 --> 00:05:12.845 75 years and some of the areas that.
NOTE Confidence: 0.8005014
00:05:12.850 --> 00:05:16.857 regular frequency ablation.
NOTE Confidence: 0.8005014
00:05:16.860 --> 00:05:19.820 Are wide cardiology started using
this for a rythmia vascular surgery?

Surgeons use for varicose veins.

Orthopedic surgeons an for us

there uncle logic purposes for

radiofrequency ablation that you may

be familiar with for either meta,

static or primary liver lesions

in pulmonary Mets, Adrenal Mets,

solitairy,

renal lesions as well as Barrett’s esophagus.

History of thyroid RFA is not

as deep as the history of RFA

in other parts of the body.

was kind of burst here.

I am at year for thyroid RFA.
The paper at the top for Catania’s NOTE Confidence: 0.8005014
radio frequency evolution of the fire. NOTE Confidence: 0.8005014
Guided ultrasound is single animal report. NOTE Confidence: 0.8005014
The animal used was a pig. In Japan. NOTE Confidence: 0.8005014
And that this was just a proof of concept. NOTE Confidence: 0.8005014
Is radiofrequency ablation feasible? NOTE Confidence: 0.8005014
Is this safe? NOTE Confidence: 0.8005014
In subsequently. NOTE Confidence: 0.8005014
It’s interesting that our colleagues at NOTE Confidence: 0.8005014
Interventional radiology and surgery Department got together and NOTE Confidence: 0.8005014
try this RFA in recurrent thyroid tumors. NOTE Confidence: 0.8005014
So this technique has been utilized. NOTE Confidence: 0.8005014
Has been kind of played with NOTE Confidence: 0.8005014
for now nearly two decades. NOTE Confidence: 0.8005014
The person who actually took it
and ran with it is this physician
Interventional radiologist after Beck.
Doctor Beck is located in Austin Medical Center in Seoul,
He himself also had multiple benign thyroid nodules and about 25 years ago he was recommended by Korean surgeons to undergo a thyroid surgery, so he started looking into what other ways?
Are there to eliminate thyroid nodule
and he started to focus on RFA.
Not only he started doing this for.
Patience.
He himself has undergone this treatment so he has been really transparent about the results of RFA and he has been a passionate advocate for how this technique should be utilized efficiently and safely and he has given multiple talks. He’s been the most prolific writer in the RFA literature for this technique. So in 2009, the Korean Society of thyroid radiology came up with recommendations for how Virat RFA should be deployed and in 2012 they came up with the 1st edition they came up with the 1st edition of guidelines for thyroid RFA. In 2017, they revised the guidelines.
and came up with a more updated version of thyroid RFA guidelines. Since then, this technique has been utilized worldwide in multiple hands-on teaching sessions, and workshops have been taught by Doctor Beck in others. So Japan, Taiwan. Obviously, a lot of Asian countries as well as European countries have embraced this technique, and it has slowly gotten to the US as well. So last year this is obviously pre kovid medical conference that we
00:08:51.301 --> 00:08:53.581 don’t have anymore group picture
NOTE Confidence: 0.8512851
00:08:53.581 --> 00:08:56.860 folks from all over the world came
NOTE Confidence: 0.8512851
00:08:56.860 --> 00:08:59.596 together for this Korean Society of
NOTE Confidence: 0.8512851
00:08:59.600 --> 00:09:01.970 ultrasound in medicine conference case.
NOTE Confidence: 0.8512851
00:09:01.970 --> 00:09:04.766 Some insult and I was fortunate
NOTE Confidence: 0.8512851
00:09:04.766 --> 00:09:07.193 to participate in this conference
NOTE Confidence: 0.8512851
00:09:07.193 --> 00:09:10.037 in learn RFA from this conference
NOTE Confidence: 0.8512851
00:09:10.037 --> 00:09:13.540 as well as Doctor Beck afterwards.
NOTE Confidence: 0.8512851
00:09:13.540 --> 00:09:17.660 So there is a great interest and.
NOTE Confidence: 0.8512851
00:09:17.660 --> 00:09:20.192 The things that I have learned
NOTE Confidence: 0.8512851
00:09:20.192 --> 00:09:22.396 in the collaboration in the
NOTE Confidence: 0.8512851
00:09:22.396 --> 00:09:24.546 friendships that I have made.
NOTE Confidence: 0.8512851
00:09:24.550 --> 00:09:27.570 In Korea have been invaluable.
NOTE Confidence: 0.8512851
00:09:27.570 --> 00:09:29.978 And it really has helped me a lot
NOTE Confidence: 0.8512851
00:09:29.978 --> 00:09:32.738 in deploying this technique at
NOTE Confidence: 0.8512851
00:09:32.738 --> 00:09:35.333 my prior institution at Tulane.
So in 2015, Italian radiologists, an endocrinologist, came up with a consensus opinion in the UK. They came together and consolidated recommendation in 2016 and 2017 in Australia. So what about us? Why are we not widely adapting RFA? Why are we just now catching on? There are multiple reasons as to why the US sort of has lagged behind in adaptation of this technique. It’s not that clinicians in the US have not heard of it,
it’s just that we have been a little bit more cautious and.

As of late, just in the past couple years there has been a booming interest in RFA technique by multiple societies who deal with thyroid pathology. An I’m sure that this RFA technique will be widely deployed and widely utilized, and I hope that ye will also start offering this treatment as well soon. So the.

A Sentinel event that kind of took off the interest in the USI believe is this paper which came out of Mayo Clinic in 2018.
The group here are comprised of endocrine endocrinologist who actually do their own.

FN eisen utilizes ultrasound in clinic to assess thyroid nodules as well as. Prominent Interventional radiologists they have. Published this case series comprising 14 patients over the years of 2013 and 2016 in their result. Really has been terrific. They achieve volume reduction by 45% as early as eight months and Durably at couple years out in very safely, so I think this paper actually sort
of put RFA on the map for the US,

00:12:04.090 --> 00:12:05.470 clinicians and audience.

So since then,

00:12:06.850 --> 00:12:09.150 there has been many workshops

00:12:09.150 --> 00:12:10.070 and conferences,

00:12:10.070 --> 00:12:14.998 and as of late I have been getting.

00:12:15.000 --> 00:12:17.196 Presentation notices almost monthly,

00:12:17.196 --> 00:12:21.350 so why are we excited about RFA like?

00:12:21.350 --> 00:12:23.234 What are the advantages?

00:12:23.234 --> 00:12:26.060 So one advantage that I as

00:12:26.160 --> 00:12:28.748 a surgeon really appreciate,

00:12:28.750 --> 00:12:31.930 is that patients have.

00:12:31.930 --> 00:12:35.068 Lower anxiety about undergoing a per

00:12:35.068 --> 00:12:37.160 cutaneous minimally invasive treatment

00:12:37.236 --> 00:12:39.536 rather than undergoing a surgery.

00:12:39.540 --> 00:12:41.564 Most of my patients,
00:12:41.564 --> 00:12:43.588 unlike patients in Korea,
00:12:43.590 --> 00:12:46.632 are a little bit less concerned
00:12:46.632 --> 00:12:48.660 about the cosmetic outcome.
00:12:48.660 --> 00:12:50.992 The scar after surgery,
00:12:50.992 --> 00:12:54.490 but they are still very anxious
00:12:54.604 --> 00:13:00.760 about just the prospect of
00:13:00.760 --> 00:13:02.688 I think this technique,
00:13:02.688 --> 00:13:05.580 if the patient qualifies for it,
00:13:05.580 --> 00:13:07.990 will be highly beneficial from
00:13:07.990 --> 00:13:11.364 and obviously this is
00:13:11.364 --> 00:13:12.807 percutaneous needle poke.
00:13:12.810 --> 00:13:16.690 There won’t be a long.
00:13:16.690 --> 00:13:19.180 Scarred that will be left behind.
Of course, in thyroid treatment world there has been a couple of modalities where we're taking out thyroid through the armpit axilla incision through the mouth transoral. but each one of those scarless or scar hiding surgeries come with either an increased cost of robot deployment or longer surgery, longer recovery, etc. So those are. They probably have a role in the world of Barry.
but they’re still not as optimal if the patient is truly aiming for the best cosmetic outcome, and it’s a needle poke, minimally invasive. There is no hospital admission for some of the thyroid surgeries that we do, of course, are outpatient, but. There is still recovery involved after general anesthesia. There is pain involved. But with this for Catania’s technique of RFA, there is no general anesthesia. There is only one institution in the US currently.
Who's doing this RFA under general anesthesia in Korea? This RFA is actually done in a clinic setting with no Mac sedation just under local anesthesia. It's a relatively short procedure, obviously is volume of the thyroid nodule dependent bigger to nodule, longer to procedure, but it's a relatively short procedure. I would say anywhere from 10 minutes to 30 minutes per nodule. And repeat ablation is a possibility, so you're not burning any bridges if the first go. Does not workout for the patient.
No hyper para no hypothyroidism, which is remarkable because we are only ablating the nodule in preserving the normal parenchyma of the thyroid. Is virtually unheard of to get hypothyroidism after RFA. Treatment in also because it’s nodule specific. There’s no hypoparathyroidism either, so minimal complication risk. There could be voice changes and temporary discomfort, but compared to surgery I would say it’s either equivalent or less and overall lower cost and that comes from.
Not admitting the patient no prolonged or time or anesthesia, etc.

So what are the downsides to RFA the risks? There are several. I just mentioned that repeat procedure is a possibility, but sometimes it’s a necessity. So just one go around. It may not be enough to achieve therapeutic effect, so obviously having to do a repeat procedure is a risk an I would also say that is operator dependent. Many procedures are operator dependent and
that’s why we advocate for. High volume Endocrine Surgeons to be doing endocrine procedures just the same with RFA. The more fast I’ll the operator is with ultrasound and needle guidance, the better the outcome will be in. Some patients may not be able to tolerate a procedure without sedation, whether it’s related to pain or anxiety is. Quite interesting to see them stoic nature of these Korean patients that I observed at Awesome Medical Center last year. I know that 18 gauge needle that’s almost looking like a harpoon hurts, but they were very stoic.
They said that they’re not having any pain and they tolerate it just fine, but when I have been doing fine needle aspiration, thyroid nodule biopsies in clinic setting although is 27 gauge needle much finer. Many of the patients complain of pain, so it will be interesting to see how many patients can actually tolerate this kind of a procedure without sedation. Overall rate is pretty smaller 2 to 3%, which is probably equivalent to a surgical treatment.
There could be dysphonia which is about 1% in this data is coming. This data is coming from multi institutional pulled data so about 1% and none of it was permanent. It was all temporary resolved after. Thyroid is very vascular Schimborn. That’s probably a little bit more prevalent in RFA setting rather than a surgery. There are a couple of lower incident complications such as tumor rupture, which is unheard of in surgery, hypothyroidism, Abscess. Anna couple of anecdotal not
Complications such as tracheal injury and brachial plexus injury when a new technique is being deployed. In multiple people are just trying it out. I think that there will be a variety of complications that may arise, as well as the outcome. So RFA in thyroid. There are guidelines and indications, and most of it is based on 2017 revised Korean guideline. So these are indications that most experts agree that we should be doing this for benign thyroid nodules causing compressive symptoms.
If the patient has cosmetic concern and the nodule should be of at least 2 centimeter or so. An if it’s enlarging but has benign cytology, we have not set any definitive maximum thyroid nodule size for the treatment and Rica. Insist after failure of ethanol. Ablation of experts will agree that ethanol revelation is more studied, more tridan, better method for recurrent cyst or. Cyst of the thyroid treatment rather than rfa.
Autonomously functioning thyroid nodule.

This is toxic nodule indication.

I personally have tried.

Rfa in toxic nodule.

It works beautifully and.

That’s sort of made me a believer

Entirety of nodule should be visible

on ultrasound for safety concerns.

It should not have any substernal

component in.

Nodule should be sufficiently away

from the vital structures in the neck.

As you all know,

there are nerves and.

Vital blood vessels in the neck and.
Since the Heat can transmit, it is important that the structures are constantly being observed and monitored so. We don’t cause any Undesirable damages. RFA is most effective in solid nodules and if the nodule contains macro calcifications, the RFA will not be as effective. Ideal candidate. The pathologic criteria we want to. Be 100% sure that it is benign by either 2 repeated ultrasound guided F na’s and in Korea. What they do is core needle biopsy. This is not universally practiced.
Way of establishing benign status of the thyroid nodule.

But core needle biopsy is an alternative to a fine needle aspiration and we want to make sure that there isn’t any sonographic features or malignancy seen prior to deploying RFA.

And again, we want to make sure that surrounding critical structures are away from harm.

Or if a guidelines so multiple guidelines are coming up with.

Guiding this RFA treatment to be deployed. Obviously, Korean guideline is the most comprehensive.

Italians Australians aace American
Association of clinical endocrinologists.

We have a little bit of more conservative.

Recommendations for RFA.

So we are currently only recommending RF A to be used for benign thyroid nodules that are causing cosmetic or cosmetic concerns or symptoms and should be biopsy proven to be benign.

Contraindications who cannot undergo this procedure.

Pregnant females if the patient has contralateral vocal cord palsy.

If the nodule is not seen in its entire T but the patient has cardiac implanted cardiac device.
We can switch over to bipolar mode instead of regular monopolar electrode or deploy it in shorter bursts in lower energy or if the patient has. Anticoagulation onboard is should be stopped prior to RFA the indeterminate thyroid nodule is a Gray area. There has been a study published in Europe but it’s just a limited number of patients and the Efficacy and the long term outcome of. Doing Arefeen indeterminate thyroid nodule has not been quite established and malignancy. We are pushing a little bit of the boundary on this malignancy.
A territory, so I’ll discuss that briefly, since we are under the umbrella of Smilow Cancer Center, RFA and thyroid. When we are dealing with toxic nodules, Orix beautifully. If we can achieve at least 50% volume reduction of TSH by 12 months, most people are euthyroid, but again. If your desire is to achieve immediate reversal of thyrotoxicosis or hyperthyroidism than RFA is not the way to go. An it may take one more ablation session. Depending on the size,
and oftentimes these toxic nodules tend to be a little bit on the larger side greater than 4 centimeter, so in that case combination of RF NRA I can be entertained. Or large toxic goiters. This is how the electrode of rfa works, and. This is much finer than then liver.

Equipment optimization is what has taken the RF A deployment in thyroid lag behind. Compared to the liver world, so this electrode is internally cooled, is about 18 gauge. Big and seven centimeter in length.
So if we’re dealing with a very big patient that comes into play. So liver has deployment of times whereas.

Rfa in thyroid. We are actually introducing the electrode and. Pulling it out, which is called a moving shot technique. To ablate units, these are circles of ablation zone inside. The thyroid nodule. And you see the triangle containing recurrent laryngeal nerve and esophagus below. This is a brief video of mixed
thyroid nodule that is undergoing.

Radiofrequency ablation you see from the side introduction of the electrode.

We want to be able to see the entire length of the catheter and.

Introduced and.

Treated ablated from posterior.

To anterior and.

Follow up, we do serial ultrasound to make sure there is and there is an adequate resolution of whatever the pathology said is being treated.

In there is a concern for regrowth.
and that is partially due to inadequate ablation at the periphery. So incomplete evolution of the thyroid nodule is being advocated. I'll touch briefly on the Barid malignancy and RFA currently in the US. Not many centers are doing RFA for thyroid malignancy, however it has been done in other countries and more and more folks are pushing the envelope especially in the world of Micropapillary Carcinoma. which is less than one centimeter if the micro papillary thyroid carcinoma is intra fire Riddle and there is no
clinical or ultrasound evidence of.

Lymph nodes spread the current American Thyroid Association guidelines say that you can either observe it with active surveillance or have the patient undergo a thyroid lobectomy overtime. We have found that even the patients who undergo active surveillance, there is about 13% operation rates. So instead of having these patients undergo an operation, why not RFA so? Clinicians overseas have published their data on Micro PTC, and the data is knew and not. Has garnered wider.
Adaptation just yet, but I think it is coming in recurrent thyroid cancer.

As a surgeon, you know redo reop neck or cancer is a bear territory. So if we can safely treat patient with percutaneous method it will be fantastic. Indeterminate Nodules as I briefly mentioned. There is lack of data on whether we should be using this technique for Bethesda three and four, so currently not recommended and this is to be determined. Briefly, just wanted to show you a little graph of how five
year followup of 84 low risk.
NOTE Confidence: 0.8268878

Micro papillary thyroid carcinoma
NOTE Confidence: 0.8268878

had achieved 100%.
NOTE Confidence: 0.8268878

Regression over five years and
NOTE Confidence: 0.8268878

this is just showing that there’s
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no neoplastic change secondary to
NOTE Confidence: 0.8268878

radiofrequency ablation of thyroid nodule.
NOTE Confidence: 0.8268878

It’s mostly just a spin.
NOTE Confidence: 0.8268878

Ion cellular change.
NOTE Confidence: 0.8268878

And because RFA does not alter the
NOTE Confidence: 0.8268878

thyroid capsule or cause neoplastic change,
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it is.
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OK to redeploy RFA if needed or have
NOTE Confidence: 0.8268878

the patient undergo a surgery if needed.
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So I’m going to just wrap up my talk
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by saying that RFA availability an
indications will continue to evolve,
and I think this is not a fad,
but this is a treatment method that
will probably stay and management
decision really should involve
multidisciplinary team of experts and
include the patient in the decision making.
And I think this technique,
given that it’s relatively new to the US.
May involve specialists from the
Interventional radiology surgery in
endocrinology and multiple host of pathology,
and it is fitting for us to be
introducing this technique.
As Smilow and I am a strong proponent of inviting the patient into making what is best for them, so it is important for us clinicians to not introduce our own clinician bias just because I'm a surgeon. I'm not going to advocate a surgery. Ann, this is not a technique that I currently subscribe to 100%, but I do believe that it has a role and I'll be. I'll do my best to be impartial and introduced this technique to Yale, so more research obviously is needed and we are actively doing project
looking at our own institutional data. So I’m just going to wrap up by showing you this quote by Hippocrates,

"those diseases that medicines do not cure or cure by the knife, those that the knife does not cure or cure by fire, those that fire does not cure it must be incurable. But this. Philosopher also said first do no harm, so it is really a responsibility, and it’s imperative that we deploy this technique safely. And I’ll take any questions and..."
00:32:27.822 --> 00:32:30.170 thank you for your attention.
NOTE Confidence: 0.85922
00:32:30.780 --> 00:32:32.108 Or Grace, thank you.
NOTE Confidence: 0.85922
00:32:32.108 --> 00:32:34.100 That was a really terrific summary
NOTE Confidence: 0.85922
00:32:34.160 --> 00:32:36.548 and it’s really wonderful that you’re
NOTE Confidence: 0.85922
00:32:36.548 --> 00:32:38.528 bringing this technology and technique
NOTE Confidence: 0.85922
00:32:38.528 --> 00:32:40.796 to bear for our patients at Yale.
NOTE Confidence: 0.85922
00:32:40.800 --> 00:32:43.306 Just because we’re running a little late.
NOTE Confidence: 0.85922
00:32:43.310 --> 00:32:45.879 I’m gonna. I’m going to suggest that
NOTE Confidence: 0.85922
00:32:45.879 --> 00:32:47.765 anyone has questions should probably
NOTE Confidence: 0.85922
00:32:47.765 --> 00:32:50.645 reach out to grace directly and and turn
NOTE Confidence: 0.85922
00:32:50.715 --> 00:32:52.974 now to our next speaker. And really,
NOTE Confidence: 0.85922
00:32:52.974 --> 00:32:55.116 our next speaker needs no introduction.
NOTE Confidence: 0.85922
00:32:55.120 --> 00:32:57.626 Doctor hyphen Lin Lin, as you know,
NOTE Confidence: 0.85922
00:32:57.630 --> 00:32:59.772 is the Eugene Higgins Professor of
NOTE Confidence: 0.85922
00:32:59.772 --> 00:33:01.822 cell biology, professor of genetics.
NOTE Confidence: 0.85922
00:33:01.822 --> 00:33:02.710 Obstetrics, gynecology,
and Reproductive Sciences and he is the director of the Yale Stem Cell Center. His work since joining Yellen has really taken this center to national and international prominence, his own work focusing on self-renewing mechanisms of stem cells is really informed cell biology and cancer in multiple ways, and I think he’s going to share with us the work that he’s done in terms of a gene family that. He and his lab have discovered and its relevance to cancer, so hyphen thank you very
much for speaking today.

Well, thank you much Charlie for a very kind introduction.

I'm delighted to have this opportunity to give a progress report on my own to my own colleagues and your Cancer Center. Although I've worked for many years on stem cells and developmental biology regarding Cancer Research, I'm completed a new kid on the block. So in the past few years or research on gene regulation or stem cells, let us to the cancer field. So today, instead of talking about my usual type of stem cell research, I'm actually going to share with
you some preliminary results of our recent cancer work. And this actually is my first time talking about these results. I hope to get your expert input advice and potential collaboration. So to begin, let me share my screen sharing. OK can you see my slides? OK, great wonderful yeah. So you know as Charlie mentioned. I will first give a bit background about some of our earlier work to pave the way for my cancer results.
So in 2000. We discovered the Argonauts gene family as the first in family that’s highly conserved during evolution. For its stem cell function and this Infirmary encodes a highly conserved group of proteins, called argument proteins that contains 4 functional domains, among them the PhD domain. Mydomain mines too small Agnes whereas period of mine actually resembles SH. And this gene or protein family has been subdivided into two subfamilies and you probably know very well about Argonaut subfamily. Which plays a central role in
the micro angle and the angle

iron mechanism and these proteins

directly bind to sin is micro honest,

which are in general 21 nucleotides long.

And Becausw are gonna suffer.

My proteins are expressed in

most type of sales.

That’s why I mean I mechanism and

microrna regulation works for all of us

who are doing this type of experiments.

However,

I said stem cell person I was more

interested in the other sub family.

The piece of family cause it’s

mostly expressed in the German in
the most primitive stem cells.

So in 2006 my lap and almost simultaneously three other labs discovered that the piwi subfamily proteins bind to yet another class of non small coding, or paying it for short so paying is just like PV proteins. They’re mostly expressing that you’re mine, and they’re somewhat longer than Micro RNAs or Sir is. So in this slide, just just wanna share with you some salient features of Python is versus Micron is,
which are better known in addition to being a bit longer, pang is a very complex, so in my lab we they have cloned over 16 Millions of these pioneers, which is in sharp contrast to the 1000 species or Micron is that’s known in any Organism. And in addition, the pairing is target. All type of Genomic sequences, again in sharp contrast to microarrays, which mostly target the three point you TR or mature Messenger RNA is. And finally, the Biogenesis is very different.
Hanges are mostly produced from long single stranded RNA’s in conscious to Micro Arnie’s feature. From here penari precursors. So this discovery is very interesting to us because as you all know that the molecule biology field relies on very important principle called the central dogma. Which tells us that genetic information in DNA is transcribed into Messenger RNA’s and then further translated into proteins. And that’s how life process starts. The discovery of Micro Arnesen small Angus like S Ion is further enriched.
this dogma by telling us that now there are two new mechanisms regulating the stability or the translational efficiency of micro earnings. However, when we looked into where these genes encoding pioneers reside in the genome, it to our big surprise they do not reside in this known gene coding region which only represents 1 to 2% of a genome. Instead, they mostly result in this junk DNA we call useful junk represent the vast majority of what you know. And it is those genes they produce these enormous number of Pioneers.
So we were following about this discovery, because if you likens our genome as the world, then the traditional jeans are like the Old World, The. And somehow our research let us lend it to assure of a computer New World.

We spy on agents now many other genes. We didn’t have any product yet, but it’s my first breaking product. And also this discovery was selected by science as one of the 10 breakthroughs in 2006. The only biology breakthrough...
that was selected.

So, this excitement aside, the key question really is.

Does any of these peyronies have any functions?

In past several years my lab is in focusing on these pioneers functions.

It turns out these pioneers and their partner PV proteins.

They can be present sometimes in the nucleus and sometimes in the cytoplasm.

So we wanted to know when these peripatric complexes in the nucleus.

What’s their function?

To address the question,
I’d like to bring your attention to a field called epigenetics. You probably know that epigenetic is a very exciting field that started gene regulation and the focus or current epigenetic studies focus on identifying epigenetic factors and illustrating how they modify the histone ordinate modification status. Therefore, turning on and off the expression of genes. However, to me this epigenetic factors there are so called down molecules. They mostly don’t bind DNA, let alone recognize specific DNA sequences, but somehow they had to be guided
precisely to specific genes in the genome.

For example here, but not here or here.

In order for epigenetic programming to work, and for the life process to unfold.

So to me ascential question in genetics is what are the mechanisms inside the nucleus that can guide these enzymes to the right sites in the genome at the right time in the right type of cells?

When we started our work a few epigenetic factors were illustrated to be guided by a few transcriptional factors to the promoters of a few genes.

And that was exciting by small an exception.
Because for soil represents a feedback mechanism, and Secondly, it was only accountable for very small number of genes in Arduino.

So the big question to me existed and still in a way exist is that what’s the mechanism that guides these epigenetic factors to their target sites?

Fast forward to today after several years old research, we discovered that the PV panel complex it should play a central role, at least in the June sales to guide the epigenetic factors to their target sites.
So this is how it works.

So the peewee protein and its partner apparently will form a complex.

And this complex will bind to Genomic sequences with complementary sequences.

Actually, by binding to Nathan RNA. That's tethered still to the genome.

This then leads to the recruitment of this complex.

Two other epigenetic factors, such as histone here to competent protein, such as histone here to competent protein,

one to this site, which initiated a bit genetic programming

and this complex will further recruit other epigenetic factors such as
Houston Metro transfers to the site, which further then further elaborate epigenetic programming by methylate in this case, Houston H3K9 Residue. So this is what we initially discovered into software and now in human and mammalian systems we found that this complex can also recruit DNA methods to the target size to achieve demethylation. So I’m happy to report that you know, up to now epigenetics study allowed us to discover a major mechanism in the germline cells that will recruit epigenetic factors.
Too many sites in the genome to achieve epigenetic programming. And as you all know, such programming is very important for stem cell self renewal for German development for transposon silencing and so on. So then you might wonder what’s the function of this P. We empowering molecules when they’re in the cytoplasm. So again, just to briefly review what we found in the past few years, we found that they actually play a
very important role in mediating the regulation of transposons and pseudo genes. Thoards protein coding genes. You all know that transposons are viewed as selfish parasites. That invaded our genome and traditionally and still today. Many textbooks regarding. Self is the element without any active function except for potential advantage during evolution. Pseudogenes are viewed as the default caucus of our active genes on their way out during evolution, but we found that transposons, pseudogenes actually play a very
important role in regulating the regular protein coding genes that we know very well, and this regulation actually is mediated by the preparing complex. So, back to the central dogma, again, DNA. For protein coding, gene is transcribed into a pre Messenger RNA which is then Kept tailed and the entrance prized become a mature Messenger RNA. It got exported into the cytoplasm. About two years ago, we discovered that about 50% of genes in human genome.
It should contain transposon sequences in the three point beauty, our or their Messenger on is. That was very surprising finding to us and after that we found that actually, transposons actually expressed a low level, at least in germ cells and primitive and the transposon expressed transcript will be processed into mature. Long is and bound by peewee proteins too. From this peripera complexes. These complexes can then, as you can imagine, beautifully target their corresponding
or complementary transposon sequences.

That's hiding in the three point beauty of material Messenger is to regulate the expression of this Messenger RNA.

In addition, we found that the so called pseudogenes, that copies of our active genes are actually not quite dead. They also have a very important role. They are often expressed into Messenger on is in antisense, and those antisense Messenger honest will be processed by TV proteins.

To become pioneers again and
00:46:15.190 --> 00:46:17.190 form complex with PV protein.
NOTE Confidence: 0.8376487
00:46:17.190 --> 00:46:19.190 And as you can imagine,
NOTE Confidence: 0.8376487
00:46:19.190 --> 00:46:21.812 this antisense RNA will be very
NOTE Confidence: 0.8376487
00:46:21.812 --> 00:46:24.090 capable or targeting the cognitive
NOTE Confidence: 0.8376487
00:46:24.090 --> 00:46:26.766 active genes of the sudo gene.
NOTE Confidence: 0.8376487
00:46:26.770 --> 00:46:29.226 So now we know the gene regulation actually
NOTE Confidence: 0.8376487
00:46:29.226 --> 00:46:31.780 is not a linear way like what’s stated
NOTE Confidence: 0.8376487
00:46:31.780 --> 00:46:34.140 and Pironi Imperial proteins
NOTE Confidence: 0.8376487
00:46:34.140 --> 00:46:35.884 play essential role there.
NOTE Confidence: 0.8376487
00:46:35.884 --> 00:46:37.628 Likewise, if you are aware of new discoveries
NOTE Confidence: 0.8376487
00:46:37.630 --> 00:46:41.126 on some other non coding on is such as long,
NOTE Confidence: 0.8376487
00:46:41.126 --> 00:46:45.056 and Pironi Imperial proteins
NOTE Confidence: 0.8376487
00:46:45.060 --> 00:46:45.474 even the expression of these thousands
NOTE Confidence: 0.8376487
00:46:45.474 --> 00:46:47.958 of Lincoln is regulated by Python is.
And this regulation originates from transposons. Because we found that many linkon is again content transporting sequences. So these sequences, like Trojans horse inside their enemies and the transposon. Derived piony, we’re together with people holding bind to these target armies and regulate this target armies. So, just to summarize this part of my talk as a background for the cancer site. Again, if you like his argeneau as the world, then the traditional jeans that
00:47:28.910 --> 00:47:31.740 we studied most of the time.
NOTE Confidence: 0.8376487
00:47:31.740 --> 00:47:34.477 About 23 thousand of them apart of
NOTE Confidence: 0.8376487
00:47:34.477 --> 00:47:37.058 the small world at the Old World,
NOTE Confidence: 0.8376487
00:47:37.060 --> 00:47:40.132 and even in the Old World we often
NOTE Confidence: 0.8376487
00:47:40.132 --> 00:47:43.039 forget the presence of pseudogenes.
NOTE Confidence: 0.8376487
00:47:43.040 --> 00:47:46.057 In human there are over 14,000 pseudogenes,
NOTE Confidence: 0.8376487
00:47:46.060 --> 00:47:48.658 and the transposons over a million,
NOTE Confidence: 0.8376487
00:47:48.660 --> 00:47:49.740 so transposons.
NOTE Confidence: 0.8376487
00:47:49.740 --> 00:47:51.360 And apparently work.
NOTE Confidence: 0.8376487
00:47:51.360 --> 00:47:54.056 Let us landed ashore of a new world.
NOTE Confidence: 0.8376487
00:47:54.060 --> 00:47:56.328 Now the New World also contained
NOTE Confidence: 0.8376487
00:47:56.328 --> 00:47:58.644 another big class of non coding
NOTE Confidence: 0.8376487
00:47:58.644 --> 00:48:00.474 on is called Inca armies.
NOTE Confidence: 0.8376487
00:48:00.480 --> 00:48:03.252 Implying is actually serve a very important
NOTE Confidence: 0.8376487
00:48:03.252 --> 00:48:05.520 function to connect these two words.
NOTE Confidence: 0.8376487
00:48:05.520 --> 00:48:08.222 I’ve shown you very quickly in the
Schematic Summary Paris can regulate the expression of traditional jeans. Panic and regulate expression of Lincoln engines powering it can regulate the expression of traditional jeans through information from transposon and even pseudo genes. Can you spy on its pathway to regulate the expression of the cognate active genes? So now for this audience you might be very curious about does any of these basic discoveries have anything to do with the clinical side? We reasoned that because all these jeans are so important for stem cell division,
if you delete any of these genes, stem cell with differential into ordinary cell, we wondered if we overexpress these jeans wear that cause malignant professional stem cells. And will there be possibly a cause of cancer? So the question we really ask is is preparing a function related to cancer. So a number of years ago we took some human seminoma cancer patients. Their testicular samples versus testicular samples from normal males and other kinds of testicular cancers derived from non stem cell based malignancy. And we found that a human gene that
we named he means human pee wee is just the overexpressed in seminoma.
Which is known to be a stem cell derived testicular cancer.
This in fact was the first thing to show such a high correlation to this type of cancer,
so encouraged by that in the past few years, we decided to look into other forms of cancer to see if any of these P regions are over expressed in these cancers and we first took M at the breast cancer, especially triple negative breast cancer.
For reasons you all know.
And we first screened through six most representative lines of human breast cancer,
and this is the normal human breasts tissue.

And this is the mouse one.

And we looked for the expression of peewee proteins or genes in this cancer tissues.

There are four human genes encoding period proteins.

They’re called peewee like 123 and four.

And we found out all four overexpressed and especially IPV 4.

In all six breast cancer cell lines before becomes detectable,

expressed and in five out of 6 lines period.

4 become expressed at least 50 times

higher than what’s in the normal tissue.

So encouraged by this cell line based study,

we decided to approach patients
NOTE Confidence: 0.7903517
00:50:55.535 --> 00:50:58.512 directly and we randomly sampled 20 breast cancer patients for the appeal
NOTE Confidence: 0.7903517
00:51:01.254 --> 00:51:03.742 wise tissue between the Nonmalignant was tissue versus their own molecular
NOTE Confidence: 0.7903517
00:51:06.850 --> 00:51:09.430 breast tissue and to our delight, we saw that 10 out of 20 patients indeed showed version overexpression
NOTE Confidence: 0.7903517
00:51:13.243 --> 00:51:16.043 And to further confirm this correlation we went to NCI database and two screens through over 1000 cancer patients whose period expression pattern was known.
NOTE Confidence: 0.7903517
00:51:18.150 --> 00:51:20.304 And indeed we found that for those cancer patients with higher level or PV four expression there much worse prognosis.
NOTE Confidence: 0.7903517
00:51:35.703 --> 00:51:38.258
So based on this very solid correlation, then we wanted to know what’s the role of PV 4IN. Breast cancer. Is this just a passive consequence of cancer development or it could be an active role in promoting cancer formation and development? So in that, to address the question, we first did the cell based experiment. This you probably a very familiar is the triple negative breast cancer cell line. Highly aggressive. When we did the wound healing assay. Normally if you remove this part of the breast cancer cells just within 36 hours, the residual cells will highly proliferate,
highly proliferative and migrate into the central region to see all the void.

However, if you just need to knock down the expression of P V4, then. For long time, much beyond actually 36 hours, these cancer cells fail to proliferate at high speed and they fail to migrate. And we’ve, you know, confirm this asset by chance will essay also indicate that PVL for knockdown affects cancer cell proliferation and migration? Then we wondered through what kind
00:52:52.030 --> 00:52:54.426 of molecular mechanism period 4
NOTE Confidence: 0.7903517
00:52:54.426 --> 00:52:56.526 achieve such a amazing function.
NOTE Confidence: 0.7903517
00:52:56.530 --> 00:53:01.426 And it turns out TV four is very important.
NOTE Confidence: 0.76405585
00:53:01.430 --> 00:53:03.730 In promoting the epithelial tamanika,
NOTE Confidence: 0.76405585
00:53:03.730 --> 00:53:07.517 more transition of this breast cancer cells.
NOTE Confidence: 0.76405585
00:53:07.520 --> 00:53:10.416 Because when we reduce the PV four expression
NOTE Confidence: 0.76405585
00:53:10.416 --> 00:53:13.199 in this triple negative cancer cells.
NOTE Confidence: 0.76405585
00:53:13.200 --> 00:53:15.460 Actually, that alone can revert
NOTE Confidence: 0.76405585
00:53:15.460 --> 00:53:18.235 this cell fate for mesenchyme all
NOTE Confidence: 0.76405585
00:53:18.235 --> 00:53:20.400 state back to epithelial state.
NOTE Confidence: 0.76405585
00:53:20.400 --> 00:53:22.450 So Soon as example here,
NOTE Confidence: 0.76405585
00:53:22.450 --> 00:53:25.276 ecad hearing is a typical epithelial
NOTE Confidence: 0.76405585
00:53:25.276 --> 00:53:28.045 marker and incoherent is Amazon Co
NOTE Confidence: 0.76405585
00:53:28.045 --> 00:53:30.866 marker and this is a loading control.
NOTE Confidence: 0.76405585
00:53:30.870 --> 00:53:31.700 The regular,
NOTE Confidence: 0.76405585
00:53:31.700 --> 00:53:34.190 a triple negative breast cancer cells
are competing between carmalized.

This express very high level, incoherent, but no expressional.

Equal hearing is detectable now if we just knock down PV L4 to 70% or be higher efficiency and the three independent markdown conditions.

Desales, I guess you know, revert back to the procedure failed.

So that was very exciting to us because very few in the cancer literature as you know better than me, why is peewee so important in controlling
the wholesale faith transformation?
And we looked into the molecular pathway to not pee wee.
Four promotes the TGF beta and FDF data signaling pathways in cancer cells.
So these signaling pathways are all highly expressed in breast cancer cells.
But you knock down peewee expression.
In addition to this, we were surprised to find that three or four also inhibit the expression of MHC two complex components in cancer cells.
So this analysis allowed us to propose a model that actually PL-4 is a very assume key regulator.
that promotes breast cancer.

Uncle Genesis,
it does so by promoting these typical
and Progenics signaling pathways.
Activities within promotes
the cancer cell survival,
increase their proliferation and.
Cause them to have EMT.
Meanwhile,
the pee wee molecule will suppress
the host immune system surveillance
allow these cancer cells to
escape the immune surveillance.
So this was the first somatic
and major cancer type that we
looked encouraged by that.

We wondered whether any other piwi protein expression is correlated and possibly a cause of other major type of cancers. Then we looked into a second major type of somatic cancers, namely gastric cancers. And here, as you can see, you probably are very familiar with this Histology. In the left panel this is just a chemical standing with another PV protein standing Brown. You see, in this adenocarcinoma sections
from either the gastric body or the gastric cardia or the gastric trap.

And in turn regions.

There are robust overexpression of PL-1.

And in chronic gastritis, which is a milder form pre cancer condition,

you see also a topic expression of PDL one,

but at much lower level.

P1L1 expression is not there in the normal gastric tissues.

When we quantify that this expression level is nicely correlates to the advancement of the gastric cancer stages from stage one to four,

you see increased expression of
period one and also nicely correlate with the distant metastatic ability of these cancer cells. For you know not to not to multiple distant, not you see. Again these cells show higher level PV one expression and Interestingly its expression level is inversely correlated to the Differentiation State of these cancer tissues or cells. So based on that, we decided that the PO1 must likely also play a role in promoting gastric cancer and two. A test that question in also in addition to the cell biology and cell based assets as I showed you earlier,
we also did the cancelled Genographic transform essay.

So when we transplant these gastric cancer cells into nude mice. It growth, robust tumors, and if you introduce the negative control for knockdown, it does not affect tumor growth. However, if you introduce S Ion1, the specific knockdown, the expression of P V1. In this cancer tissues, you just reduce the tumor formation and when you looked into these two more tissue sections by,
for example, the cell proliferation markers such as PCA, that’s indicating Brown here or Ki 67 you see in the wild type situation. The cells are highly mitotic and prolific. In the controller down you don’t see any big difference. But in the period one knockdown you greatly reduced. The proliferation of these cells and that’s a duplicate sure is the same thing. So based on that? You know we wanted to know. Weather Pang is appeared role in this period. Proteins function in cancers. We expected to see pioneers and
00:58:35.884 --> 00:58:38.020 cancer specific powers to show up,
00:58:38.020 --> 00:58:39.428 but to our surprise,
00:58:39.428 --> 00:58:42.017 when we did deep sequencing for the
00:58:42.017 --> 00:58:44.047 small non coding RNA population,
00:58:44.050 --> 00:58:46.650 we could not detect any pianese we detected
00:58:46.650 --> 00:58:49.478 lots of micro honest which is normally
00:58:49.478 --> 00:58:51.583 a smaller population than pioneers.
00:58:51.590 --> 00:58:54.936 But now even with this big presence
00:58:54.936 --> 00:58:57.470 of microrna population we cannot see
00:58:57.470 --> 00:59:00.580 any or if any just very few pennies.
00:59:00.580 --> 00:59:02.967 That’s where a matching to the high
00:59:02.967 --> 00:59:04.840 level of heavy protein expression
00:59:04.840 --> 00:59:06.388 and let us too.
00:59:06.390 --> 00:59:09.110 Conclude or to hypothesize that
00:59:09.110 --> 00:59:11.830 piwi proteins function in cancer
might be independent of Pianese.

To really demonstrate that’s the case, we generated this mutant PV proteins by mutating three of its residue in the PV domain that you might remember from my introductory slides. When you mutate these three residues, the resulting engineered mutant protein can no longer bind to pioneer.

Then we wanted to see if this. Pilot Binding Deficient Mutant Perior proteins are still oncogenic and turn out that’s the case. So normally in these gastric cancer cells,
if you do transfer your essay for their migratory ability.

Highly migratory.

Now if you knock down the P1 is question, you greatly reduced its migratory ability.

But if you in this knockdown cell now reintroduce into these cells, that mutant PV protein that cannot bind to pay on it, but that’s alone is sufficient.

To restore the majority ability of these cells, so this analysis tells us this pee wee function in gastric cancer,
at least in gastric cancer, is actually very independent.

So our latest results showed that they do not interact with piramis,

but they interact with the nonsense mediated decay immediate regulatory mechanism. An empty to regulate the cancer genome or cancer transcript on what we think is happening is that the piwi protein, when it’s highly expressed in these cancer cells.

It directly target tumor suppressor cells, allowed the uncle genic alarm is to promote her ankle Genesis.
And Meanwhile as you know, some tumor suppressor anger is themselves with directly supports. Cancer grows in the squashing function is not knock down because of PV overexpression. So we’re very excited about these discoveries. In fact, this discovery started from to Safra and now we can always see the hope of approaching clinical site. Of course, I wanted to know whether you know we can use this as a therapeutic target and we have some preliminary
results show that’s quite possible.
So shown here is a new method that’s being developed in my lab.
Is nanoparticles loaded with? This song is against these proteins.
In this case, it’s against another under binding protein called familiar that we
also worked on and has also has a tumor function promoting function.
You can inject these.
Nanoparticles through Kelvin into the mice that contains human cancer.
In this case, colorectal cancer graph.
And 28 days after that. After they closed due to the injection
and after four times or injection then you use city to visualize the tumor growth and the web microangle is and sin is localized. So we label this as an every sci-fi and tumor with luciferase, so by city image Ng just 4024 hours after the first injection. You can see these nanoparticles become highly enriched in the tumor cells. And then if you let these mice continue to grow and you can see normally. If you just in the ceiling control or randomize this, I only control this grafted tumor tissue.
Grow quite robust in this new mice and
also will start to undergo metastasis.
See other spots within 28 days.
But if you do tell,
then injection of these nanoparticles
loaded with anti this particular proteins are as I only you can really control the tumor growth.
So you know,
we really feel like basic research allows us to potentially found the computer novel oncogenic mechanism. That’s not specific to a particular type of cancer, but probably is to multiple type of cancers.
Through our own data,
we’ve shown that in breast cancer, gastric cancer, seminoma and prostate cancer, skin cancer, liver cancer and colorectal cancer. At least subtypes of these cancers. They have high level of period overexpression and period appeared to be a driving mechanism for this type of cancers. So my stream is to use these TV proteins as target. To treat cancer, to develop new treatment for cancer because PV proteins. As I mentioned earlier, there only needed in the germline.
and come to adult states.

They only needed in the male germ line because female germline, namely over does not have stem cells. Only spermatogonia stem cells active here.

So basically you can completely knockout these proteins. The normal self development will not be affected.

We’ve done so now called entire period family in mice, mice happily surviving. The only problem is the male mice and we predict them. Male patient in human, they will temporary loss fertility but
that easily can be solved by stores

and sperm right before the treatment.

So this is my dream and I hope you

know with potential collaboration

with all of you in the future

or some of you we can take,

you know steps forwards and hopefully

there will be a new cancer.

Treatment message will emerge from this,

so I’d like to thank my lab

members who did work and since

I’m running over our stuff here.

Hyphen that was fabulous body of work.

And congratulations on all of it.

And really, as you point out,
it really does Avail a bold new approach of cancer therapy.

I know we’re essentially out of time, but let me just ask one question beyond potentially targeting through SI RNA, are there other potential approaches to target these genes in terms of future Therapeutics?

No, that’s a very good question into it. The answer is yes. Because these proteins, not the atomic structure, have been resolved, so we know exactly how the active side looks like. And so we’re also starting to
01:05:58.276 --> 01:06:00.606 use a small molecule based
NOTE Confidence: 0.8378827
01:06:00.606 --> 01:06:03.076 screen to find these mimics.
NOTE Confidence: 0.8378827
01:06:03.080 --> 01:06:05.270 Of this active site binding substrate,
NOTE Confidence: 0.8378827
01:06:05.270 --> 01:06:07.685 and hopefully those will be specific to
NOTE Confidence: 0.8378827
01:06:07.685 --> 01:06:10.019 target and knock down these molecules
NOTE Confidence: 0.86029005
01:06:10.020 --> 01:06:11.420 too well. That’s exciting,
NOTE Confidence: 0.86029005
01:06:11.420 --> 01:06:13.520 and it sounds like a potentially
NOTE Confidence: 0.86029005
01:06:13.585 --> 01:06:15.129 new area for Therapeutics.
NOTE Confidence: 0.86029005
01:06:15.130 --> 01:06:17.678 Well, I know we’re out of time.
NOTE Confidence: 0.86029005
01:06:17.680 --> 01:06:19.948 I want to thank those who joined
NOTE Confidence: 0.86029005
01:06:19.948 --> 01:06:22.219 us and thank hyphen and Grace
NOTE Confidence: 0.86029005
01:06:22.219 --> 01:06:24.249 for two really superb talks.
NOTE Confidence: 0.86029005
01:06:24.250 --> 01:06:26.530 Great work and thank you everyone
NOTE Confidence: 0.86029005
01:06:26.530 --> 01:06:28.999 and enjoy the rest of your day.
NOTE Confidence: 0.86029005
01:06:29.000 --> 01:06:29.730 Well, thank
NOTE Confidence: 0.86029005

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you very much for your invitation. Bye bye bye bye.