[MUSIC PLAYING]

JONAS T. JOHNSON: Welcome to our program. I'm delighted that you were able to come and join us today. So this is a very special program, which is actually underwritten in part by the Marion Mosites family in their fund for personalized medicine in head and neck oncology. So Dr. Duvvuri is a member of our faculty. He is the medical director for our Collaborative for Research Educational and Technical Enhancement in Surgery. He's the Chief of Robotics Surgery for UPMC, and he's a funded investigator. So Dr. Duvvuri will take the floor now and walk you through personalized medicine, what it means, and where it could go. I think this is pretty exciting.

UMAMAHESWAR DUVVURI: Thank you, Dr. Johnson, and thanks to everyone for taking the time to be here. I look forward to taking you through this topic, which is near and dear to my heart. And so let's start by talking about head and neck cancers. Head and neck cancer-- and some of you may have seen some of these slides in the past, so I apologize if there's a bit of redundancy. But head and neck cancer is a disease that afflicts people throughout the world.

It is one of the leading causes of death in the world. It is not as common in the US as it is in some of the other countries of the world, partly because we have a better control over the exposure agents that cause head and neck cancers, such as tobacco and alcohol. [INAUDIBLE] the most common. In the US, we think that there are about 50,000 new cases a year, although this number seems to be increasing, and most of these tumors are derived from the tissues that line the head and neck region, the inside of the mouth, the nose, the throat, et cetera.

We also know that head and neck cancer is not one disease. It's a disparate disease, and there are a couple of different types of head and neck cancer, which have come to light recently. And I'll talk to you about those, and we'll share some information about them. But really it is a disease that is most commonly associated with tobacco and alcohol use, as you can see here.

So what about this thing that we everybody's talking about, personalized medicine? What does that mean? What it is? So the definition of personalized medicine as is commonly accepted in the vernacular now is this is a method of tailoring the treatment to the individual characteristics of a patient. So in the context of cancer, there is great opportunity for us to tailor and define the treatments for these patients. Because as the field of medicine evolves, we now know that cancer is not just one disease. It's not just one entity.

There are lots of different kinds of cancer. In the old days, we'd use to say, well, somebody's got cancer. But now we know that there are different types of cancers. There's lung cancer. There's colon cancer. And each of these cancers behave differently. They're caused by different things, and they respond in different ways to treatment. And so head and neck cancer, also, is a general term, which is now being refined more and more as we learn more about the disease.

I like to say that the treatment of cancer, especially head and neck cancer, is a three legged stool. So we have what we call the triangle of therapies. That is surgery. You basically remove the tumor with surgical techniques. You effectively-- pardon the vernacular-- you cut it out. You cut out the tumor. You can also treat these patients with chemotherapy, which is used to attempt to cure the tumor in a non-surgical way. And, finally, we treat these patients sometimes with radiotherapy. So radiation, or radiotherapy, is another modality that's used to-- I'm sorry-- that's used to treat these patients.

And for many head and neck cancer patients, they require all three modalities. It's a tri-modality. So they may have surgery first, and then chemotherapy with radiation therapy. And in general for most patients with head and neck cancers, which is derived from this squamous mucosa, chemotherapy in and of itself doesn't work, so surgery and radiation remain the mainstays of treatment for these patients.

Now the problem is that the responses to the treatment are variable. And so not every patient that gets a particular type of treatment is able to enjoy the same type of response. In other words, some people get cured, and some people don't. And we don't really understand why that happens.

So the promise of personalized therapy, or personalized treatment, is that we will be able to theoretically find a magic bullet, right? To find a particular Achilles heel for some of these tumors. And then if you could target that Achilles heel, you would be able to cure these patients without having to give broad spectrum therapy. Because as you can imagine, surgery is a morbid procedure. It's going to cause pain. You get a scar. You can't have surgery without a cut. And with that cut, you have a scar. And so we would love to find ways to treat people in a less invasive way and in a more targeted fashion. And so this is the promise of targeted therapies and their potential to find what we call the magic bullet.

Now we know that, again, cancers are sub-classified these days. It's not just head and neck cancer. There are different types. And in fact, one of the main distinctions that we have is the breakdown between tumors that are caused by smoking and drinking, carcinogen-induced tumors, and those tumors that are associated with exposure to the human papillomavirus. I know you guys have probably heard of this in the late press, and we'll talk a little bit about that here, as well. So you've got tumors that are driven by this human papillomavirus, HPV. We have, now, vaccines for it, et cetera. And we have other tumors that are driven by smoking and drinking. And these tumors are characterized by different genomic and phenotypic profiles, which means that their genome is different. Their genes are different in these different types of tumors, and they behave differently to treatment. And we'll talk a little bit about that.

So talking about human papillomavirus and associated cancers, because this is very important currently. It's one of the leading causes of cancer in America. It has overtaken cervical cancer, in fact, as the leading virally associated cancer. We can see from these graphs that the oropharyngeal cancers associated with the human papillomavirus virus-- they're showing blue-- are sort of crossing. They're getting bigger. It's growing, as opposed to other tumors that are decreasing. And this, for example, is the line that shows cervical cancer in women. And as we get better methods, like pap smears and vaccinations, et cetera, the cervical cancer rates are actually decreasing. Unfortunately, the HPV associated cancer rates are increasing, both in men dramatically, somewhat in women. And here is the total, so it continues to increase over time.

What's also true is that people that have human papillomavirus associated cancers enjoy really good survival. In other words, we're able to treat these patients for these cancers and keep them alive. And so here you can see the prevalence and the incidence of this disease, and you can see how from 1980 to 2010, the number of people that have this disease keeps going up. And the median age of diagnosis for these kind of cancers is decreasing, so they're getting-- the patients are younger and younger. But also what's very interesting is that there is an 18-fold increase in the frequency of 5 year survivors between the 1980s and 2010s. What this means is that more people are alive after being treated for this cancer.

So now we're very interested in trying to figure out, well, what is the quality of life for these long term survivors? It's not enough just to say, well, I've survived the cancer. It's not as useful to have the longevity of life if you don't have good quality of life, if you can't eat, if you can't swallow, if you can't enjoy working or spending time with your family, et cetera. So quality of life is so very important for these patients. And this is why, as we move towards personalizing the care for these patients, we want to make sure that not only are we keeping people alive longer, but that we're keeping people alive with a better quality of life. And Dr. Johnson has really led the way in this with the survivorship [INAUDIBLE], which seeks to optimize quality of life for these patients.

Now, remember we talked about this idea of targeted therapy and trying to de-escalate and trying to treat these patients better to have a better quality of life. So these tumors are driven by certain molecules that promote tumor cells to grow. And one of these molecules is called EGFR, the epidermal growth factor receptor. And EGFR was thought to be a very, very important driver of cancer based on a lot of work that happened in laboratory, and so companies came out with molecules that could now target this EGFR. One of these molecules is called cetuximab, shown here.

So in an effort to try to improve the quality of life for these patients, because we thought, well, look, these HPV positive cancer patients seem to survive. They have an 18-fold increase in survival over five years. So, jeez, let's try to de-escalate therapy for these patients. A huge study was done where we compared patients with oropharyngeal cancer treated with radiation, which we know works for them in combination with either cetuximab, which is a targeted therapy, trying to personalize the care for these patients, or standard chemotherapy called cisplatin. And cisplatin is a standard toxic chemotherapy. It makes people feel not so good. Sometimes it causes problems, like kidney failure and other things that we can find with chemotherapy. And so we wanted to see whether cetuximab could be given to these patients in an effort to avoid the toxicity of chemotherapy.

So the hypothesis here was that patients treated with cisplatin would have the same survival as those treated with cisplatin but would have better quality of life. They would have less complications. They are going to have less side effects. They would not be admitted to the hospital as much, et cetera. And so there were two studies that were done on this. One in North America, led by [INAUDIBLE] Gillison, and this is the data that I'm showing. It was funded through a trial called the RTOG, Radiation Therapy Oncology Group 1016. Another was run by our colleague [INAUDIBLE], based in UK, looking at the European cohort.

And so what was really interesting is after randomizing, taking almost 1,000 patients and breaking them in and trying to break them out into these groups and then giving them these treatments, what came out was something really shocking. What came out was that people that were treated with cisplatin and radiation-- that is the blue line-- had a much better survival than those patients treated with cetuximab. So not only did these patients not have a change in their quality of life, which I'm not showing here, but that was the data that was seen, but people actually died more when they were treated with this targeted therapy. So this was a failure of our attempts to personalize the therapy for these patients. And it was such a failure, in fact, that they stopped the trial early. And they said, we shouldn't keep going with this. This is not right. This is unethical. And they halted the trial, published the data, and we now no longer use cetuximab for these patients by and large.

Now, another way of attempting to deescalate therapy for these patients would be to perform surgery. So if the cetuximab thing didn't work, well, people say, well, maybe there's a way that we could use surgery to reduce the toxicity for these patients. And this is where robotics surgery came into the field. And I'm not going to spend too much time on it. I just want to introduce to you guys this concept that we can use surgical robots. We can put them into the mouth, and we can tackle these tumors.

And here's a brief video of a patient that has a tumor in the back of the throat. This is a breathing tube. This is the back of the tongue. And we now use these surgical robots with these little-- with these instruments, using a cautery, for example, to basically resect these tumors. We cut these tumors out, and we make sure that we remove it completely. And then that also leads to an attempt to reduce the treatment for these patients, because if you can remove these tumors completely, we think that we would have helped these patients. And so this is an example of removing one such tumor through the mouth in an attempt to reduce the toxicity profile for these patients.

I'm sorry. So what we have now is this concept called surgical de-intensification. So can you do surgery to take out the tumor, and then based on the pathology, appropriately treat these patients so that we're not giving them all three legs of that three legged stool-- surgery, chemo, and radiation. Can we avoid one of them or more than one? So the concepts have now been put into action, and this is the schema that we have of this concept called surgical de-intensification.

Patients with oropharyngeal tumors usually caused by the human papillomavirus undergo surgery. Once they have surgery, if they are deemed to be low risk based on the pathology, they get nothing else. They just get observed. So just surgery, no chemo, no radiation, nothing else. And that should be pretty good. If patients have some risk factors-- maybe they have some lymph nodes that have popped up or we didn't get as big of a margin of the tumor as you would like-- so margins, this is a concept that we have that tumors are sort of like weeds, and they have roots.

So if you just pluck the top of the weed off, well, it'll grow back. So what you want to do is to remove the weed with the roots. So you need to take out some of the normal soil around the tumor, and that's called a margin. But if you didn't get enough of a margin because the tumor was in a critical place or you just didn't know, maybe these people could be treated with some kind of therapy afterwards, which we call adjuvant therapy.

But maybe they don't need chemo and radiation. They're just adjuvant. And then there's another cohort of patients who would have high risk features where the tumor was maybe very infiltrated, very sort of creepy crawly for lack of a better word. Maybe they have a lot of lymph nodes that the tumor has spread to. Maybe they have other bad features. And these patients would then get adjuvant therapy with chemo and radiation.

And so several trials are ongoing or have recently been completed, including one called ECOG 3311. This is the Eastern Cooperative Oncology Group. That's one of the groups, like the RTOG I just spoke about, that allows us to do large trials. I'll show you some of that data. My colleague, [INAUDIBLE], in Lausanne in Switzerland has started a trial called Best Of, where he's trying to determine whether radiation and chemo or surgery, of which one is better. And it's called the Best Of trial. Another one called PATHOS, which is led by my colleague, Terry Jones, who's in Liverpool, UK, which seeks to, again, do surgery and then stratify patients and deescalate therapy for these patients in an appropriate fashion.

And so this is the results of this trial ECOG 3311 that was run out of the University of Pittsburgh. Rob Ferris, Cancer Center Director, is the first author. And this is some data that was presented at the American Society of Clinical Oncology last year where we showed the initial data on this. This paper is currently written up and is currently awaiting publication. But basically what this trial did-- this was a huge trial looking at over 500 patients over many, many different university hospital centers throughout the country.

And what happened-- the way that this trial was set up was that we took patients that had small volume disease, so early stage disease, so T1, T2. This is the tumor stage. One and two are early. Three and four are advances. S we didn't do advanced patients. [INAUDIBLE] we did earlier patients with small lymph nodes in the neck. They all had surgery. And then after they had surgery, those that had low risk features would be observed. Those that had high risk features would get chemotherapy and radiation therapy.

Those that were intermediate-- like I talked about before, maybe close margins, maybe a couple of lymph nodes positive, et cetera-- well, those patients got randomized. They got a coin flip, and we decided either to give them radiation with the standard dose, which is called 60 Gray or deescalated radiation to 50 Gray. And this was really the experimental arm. This is the question that was being asked in this particular study.

Now, surprisingly, what we found-- I shouldn't say surprisingly, but interestingly what we found is that the patients that were in that sort of arm A that were just observed, they had a 94% survival. Really good, only 6% of people failed. Those that were arm B or C, so that deescalated radiation treatment after surgery-- all those people had pretty good survival, as well, 95% to 96%. And those that were in arm D, those that had bad features, multiple lymph nodes, maybe these infiltrated tumors, they had worse survival. 90%, even though they got surgery, chemotherapy, and radiation.

So this tells us that just throwing the kitchen sink at people that have these advanced tumors is not going to work. It's not really getting this 90% up to 94% or 95%. And even those people that seemingly have small volume disease that would do well, they still do have a 5% to 6% rate of failure. So there is a finite failure rate associated with treatment of these patients, and we don't really understand why that happens. So this is what we're trying to move forward to in the next phase of our investigations in the context of this disease.

So the question is, what is it about those patients that fail treatment? Whether it's surgery alone, surgery and radiotherapy, or surgery and chemo radiotherapy, why do these people fail? What is the biological underpinning of that? And that's what we're trying to figure out. So we asked the question, can we use next generation sequencing-- this is genomic sequencing-- to evaluate the people who are poor responders so that we can identify differences between these patients and try to figure out how to maybe target them better.

So the cetuximab thing didn't work, because we just thought it was EGFR. Maybe we were wrong. Maybe it's not EGFR. Maybe there's other stuff happening in there we need to think about. So this was a collaboration that I undertook with a dear colleague of mine, Dr. Eddie Méndez who is shown here at the bottom left hand corner to the left of the picture, and his resident, Alex Harbison. Alex is now a trained [INAUDIBLE]. He finished at the University of Washington. Eddie and Alex were both at the University of Washington. Unfortunately, about two years ago, Dr. Méndez succumbed to liver cancer, and he himself died from liver cancer. So this work was completed posthumously, but we continue on this endeavor in honor of Eddie's legacy.

So Alex has really done the yeoman's work in this [INAUDIBLE] work for this particular project. But what we did is we collaborated across the coast from Pittsburgh to Seattle. We identified a cohort of patients who had HPV positive cancer who were all early stage, and then we identified those 5% of people that failed. So you can imagine we had to do a lot of work, find 100 patients to find five that failed. So we needed large numbers to identify these people that didn't do well. But we did.

We identified cohorts of patients, and then we painstakingly tracked down their tissues. So we identified the tissues from the initial biopsy, and if they failed when they had a biopsy, to document the recurrence of a failure, we got that tissue, as well. And then we were able to do sequencing on these paired specimens. So we identified a cohort of patients who never failed, which are shown here. Non-recurrent is what we called them. Then we identified a cohort of patients who did fail.

We had 15 at Pitt and four at University of Washington. And this is really bias, not because we have more failures here, but because we have a very, very robust database that has been supported through funds from the [INAUDIBLE] Foundation at the Hillman Cancer Center that has tracked patients all the way back from the mid-80s. So we have 30 plus years of data on these patients. We have a huge database, one of the biggest in the country, which allows us now to identify patients to ask these very interesting questions.

And then we found the primary tumor, what we call the index tumor, and the recurrent tumor, and then we [INAUDIBLE] sequencing. And the first thing we did was we sequenced the DNA. So DNA, as you guys might recall, is the linchpin of the genome. So this is the stuff that we get from our mom and dad, and this is the genomic material that is present and forms the core of our genomic programs. So the DNA can be sequenced much more easily than RNA, which is the product of DNA. So DNA gets transcribed into RNA, and then RNA gets converted to proteins. So proteins actually do the work. But the DNA is the code that provides the blueprints to make the proteins. Now, DNA is also more stable, so some of these patients with older tumors had-- we needed to use the DNA first to make sure that the technique validated and that it was working. So that's what we did.

We found these non-recurrent and recurrent tumors. We did the sequencing on them. And, interestingly, what we found was that when we looked at the mutations within these tumors, there were certain sets of genes that seemed to be uniquely mutated only in the patients that recur, but not in the patients that didn't. So we looked at the patients, again, that HPV-related that did not recur. Those that did recur, we did a comparison to a larger data set from the National Cancer Institute called the TCGA, The Cancer Genome Atlas.

And by comparing our cohort of patients with patients that were already sequenced, we did some really fancy statistical analyses to see where these-- what genes are really important or what kind of pathways are being dysregulated, because we now know from the EGFR story that it's very unlikely for there to be one single molecule that drives cancer. If you block that one molecule, you're going to cure it. That's what we want. We'd love to have that. But that's probably not the way this cookie is going to crumble. So we know that we need to look at multiple pathways to see which genes are fitting into pathways so that we can target those pathways.

Interestingly, what we found was that tumors that recurred behave more like HPV unrelated cancers. They behave more like smoking-related cancers, even though these patients may not be smokers. That means that these tumors are mutating. The DNA is changing. And with these mutations, they're acquiring a phenotype condition that makes them seem more like HPV negative cancer. And that's really what this graph here is showing us. So this is HPV unrelated so this is more like an HPV unrelated. Less like that, the [INAUDIBLE] that did not recur were here, and the ones that did recur tend to move towards that HPV related or HPV unrelated states, so more at the smoking related state.

So that's-- we found that quite interesting. And we could make these sort of calculations based on a whole exome sequencing. So we sequenced all the genes that we could find in the human genome, and then we did large data crunching to be able to get to this. And this required some significant collaboration between us and the Fred Hutch Cancer Center in Seattle and others.

Now, what we also realized is that when we kind of look now at these HPV negative or these HPV positive tumors, and we try to figure out what pathways really specifically mutate. Are any of these actionable? We found that, lo and behold, none of them really jumped out as being an actionable or targetable pathway. There was no single pathway that we could say, well, if we give this drug, we can make this work, based on the DNA. And that's really what we're finding here. So although this is a landscape-- although we were able to see what the DNA mutation profile looked like, we were not able to say, well, aha, we have a smoking gun that we can now pinpoint.

So what we then did was we moved to the next phase, which is called RNA. So DNA gets converted to RNA to try to figure out, well, if these genes get transcribed into proteins, can we find some additional piece of information that we could now use to target it? This is some work, again, that's currently in review. We hope that it will be published very soon. Again, graciously supported by the Society's Fund and philanthropy from [INAUDIBLE] Foundation.

So now what we did is we identified a bigger cohort of HPV-related tumors, and we looked at the primary and the recurrence. And then we did this RNA sequencing on them. And when we did that, interestingly, we found a gene signature that came out called NRF2, or the nuclear respiratory factor two. So this is a gene that's associated with metabolism. So this gene regulates how tumors make ATP, which is one of the fundamental molecules that gives cells energy. So the tumors that were recurrent seem to have a much higher signature of this NRF2.

And, in fact, when we went back to the TCGA or the cancer genome data, we looked at human patients who had high levels of NRF2 versus those that had basal levels. You can see that these patients survived less. This is called a Kaplan-Meier survival curve. So every time somebody dies, there's a little step off, unfortunately. So what we can see is that this is a curve that shows that high NRF2 patients die more quickly than those that have low NRF2. So this really made us think, aha, there might be something interesting, that we should look into this. But we can't just use this phenomenologic data and say, this is, therefore, worthwhile and you should spend a lot of time and effort on this, because that's exactly what we did with that cetuximab EGFR story. We had a little bit of information. Everybody was so excited to try to treat patients better. We jumped into treating thousands of patients without appropriate data. And that's why we had this bad outcome, which we did not expect. We never want to have that.

So here we took a step back and we said, well, how about we take tumor cells and we manipulate this NRF2 gene? So we took HPV positive tumor cells, which we grew in the lab in a Petri dish. We forced NRF2 to be over expressed in these tumors, and then we put them into mice. And these mice then grow tumors, and you can see here that control tumors do make cells-- do make tumors, but they're small. But when we over express this NRF2, you can see how much bigger these tumors are. They grow a lot more. And so this tells us that, actually, NRF2 is doing something, and it's not sort of a bystander effect. It's not just an artifact of the statistics. It's a real phenomenon.

And then, interestingly, we identified a new drug. This is a drug called IACS. [INAUDIBLE] just as a number, because it's not yet quite ready for prime time, although this drug has been used in a couple of really early phase clinical trials. It is essentially similar to metformin, which is used to treat diabetes. So it was very exciting that we could repurpose or use a drug to potentially treat these tumors. And so what we find here is that tumors that have high levels of NRF2 actually die more quickly or die at a lower concentration of 1.6 nanamolar, as opposed to almost nine, so fourfold more deaths at the same concentration of these drugs.

So we think that this drug is going to be useful, and we're moving this forward to do more animal experiments to validate this before we take this to humans. So that's very exciting, and so this tells us that in the near future, we may be able to sequence patients with this HPV positive disease. Look not just at a particular gene but at the whole system, program of NRF2 mediated signaling or mediated pathways, and then hopefully treat these patients in a more definitive way.

Now, just to be very, very frank about it. I don't think this is going to cure cancer. I think this may help a certain percentage of people that have high NRF2. We hope, and we have to do the studies. But it's unlikely to-- and a bit naive for us to think, well, this is going to fix everybody. It's probably not. But in fact, when you look at patients with HPV positive versus HPV negative disease, when patients recur-- when tumors come back, if you have HPV positive disease, you actually still do better. You live longer than those that have HPV negative disease that recurs. So these are the smokers, and these are the nonsmokers, if you want to think of it that way.

So HPV negative disease has a much worse median survival time of only six months after recurrence. It's a devastating disease. And you see some data from-- again, from Ethan Argiris, who used to be one of our faculty members here. And so what we know is that these people have a really bad prognosis. Now one thing that we also know is that many times, patients with this bad prognosis who have these sort of advanced tumors, these tumors seem to escape the body's own checkpoints, regulatory points that prevent tumors from growing.

Not to scare anybody, but the reality is that all of us are making thousands of cancer cells in our body a day. It just happens by statistics and by probability. However, when these cancer cells form, before they actually turn into a true cancer, the body's immune system kills them. And it does it through these things called CD8 T cells. So these are T cells-- that white blood cells that go into to kill cancers-- cancer cells, I should say. Now when tumors form and they become a tumor mass, we know that these tumors start expressing molecules on their surface, like here. We put this one here called PD-L1, programmed death-ligand 1. And when these tumors express PD-L1, they inactivate-- they bind to this thing called PD-1. This was programmed death receptor 1 on the T cell, and when it touches-- when these two things touch, this signal comes off into the T cell. And these T cells basically die, or they stop working. They become anergic.

Now, this is a normal checkpoint in the body. The body needs to have checks and balances. Otherwise, if your immune system goes haywire, you can get things like lupus. You can get things like rheumatoid arthritis. You can get multiple sclerosis. These are all conditions where the body's own immune system is fighting the body. And so the body doesn't want that. You have to balance these checkpoints. And so these are normal, and they're healthy. They should be there. But in the context of cancer, they become dysregulated.

So Jim Allison, who is a very famous scientist at MD Anderson Cancer Center, recently won the Nobel Prize last year-- not this year, but the year before-- for discovering this pathway and for coming up with drugs that can block this PD-1, PD-L1 interaction. Here are some of the drugs-- nivolumab, pembrolizumab, which block the anti-PD-1 part of this arm. And others, like, avelumab and durvalumab, et cetera that block the PD-L1 part, so they block the tumor cell part. Either way, it's a lock and key. Either you block the lock, or you block the key. But as long as the lock and key don't fit, we should be able to get some significant responses. And in fact, this is what we are seeing.

And, again, another beautiful piece of work from Dr. Ferris. He published this in *The New England Journal* a couple of years ago, showing that patients who had already recurrent metastatic tumors that were heavily through treatment, had failed surgery, failed chemotherapy, failed radiation, maybe failed another round of chemotherapy with different agents, who were just not doing well. When we gave them this nivolumab, a certain percentage of them were alive up to 16 months, a year and a half, 18 months after treatment. So this is what we call a tail on the curve. And this is remarkable, because almost nobody lives for this long after having recurrent metastatic disease that's been previously treated. So this was very exciting, and this tail on the curve is really what got everybody excited about this idea that you can use immune therapies now to treat patients.

However, if you look at the percentage of patients that actually have this-- come across here, you can see it's only about 20% to 30%. And if you look at progression free survival-- those people in whom the tumor never progressed-- it's even less. It's only about 10%. It's a good 10%. If you're in that 10%, it's great. But who are the people that will fall into that 10% versus who are the people who will fall into the 90%? Can we use these drugs in a more rational way so that we give the therapy and get the benefit from it without having all the downsides of it?

So that's really important, and that's really what we're moving on and/or how can we make this 10% higher? What if we could get this 10% to be 20% or 30%? That would be a huge benefit, as well. So this is where personalized medicine is going in the future. And so, while there is a cohort of patients who do very well with this therapy, there's also a large percentage of patients who do not do well. And that's why you can either be a glass half full or a glass half empty.

Again, going back to this idea of, OK, well, we know HPV positive cancers are pretty good, in terms of survival. We know-- we think we have some targets to go forward there. My lab sort of switched gears and started asking the questions about HPV negative cancers, which tend to be more lethal and tend to kill people. So we know that these tumors are driven by many, many genomic events that characterizes HPV negative disease. And one of this is-- we'll call amplification, so a little bit of DNA gets repeated over and over and over again. And when this bit of DNA gets repeated multiple times, the genes in that DNA get expressed at high levels. And we think that these over expressive genes are bad actors and therefore contribute to cancer.

And one of the genes that we've been very interested in my laboratory for the better part of a decade now is this molecule called TMEM16A, and I'm not going to go into the details of it to bore you guys with the very, very basic science of it. But suffice it to say that this is a molecule that is very important in neuroscience. It's very important in lung physiology. This is also the molecule that prevents multiple sperm from fertilizing a single ovum, a single egg. That is a reason why one sperm enters the egg to initiate life, to initiate the creation of what we call a zygote. The other sperm in that area can't get in again, so that the genomic material isn't duplicated or triplicated or quadruplicated and cause really bad problems.

So this channel plays a lot of interesting and important roles in fundamental biology. But surprisingly, it's over expressed in cancers, especially head and neck cancers. And we didn't know why. We were very curious about why this would be. And so at that time in my lab, it was sort of like who do you want to be? The guy in the boat or the guy who was stranded on the island? We had this interesting target. We found this thing that seemed very, very cool. But we didn't really know what it was all about.

And so we started looking more and more into the biology of what this channel does, and it turns out that this channel is very important for regulating an organelle on the cell called the lysosome. And lysosomes are basically the trash men of the cell. So lysosomes do a lot of things. One of the things that they do is that they eat dead proteins in the cell through a process called autophagy. Auto, cell. Phagy, to eat. So it eats itself.

They also regulate how these little peptides-- these little bits of proteins that get chewed up, get pushed out of the cell. They're the garbage men. They clean things out of the cell. And in doing this, what we call exocytosis, pushing these molecules out of the cell, they also regulate how different molecules get to the membrane of a cell and get back out, kind of like the PD-L1 and PD-1 story, right? If you mess up the pathways that allows molecules to get to the membrane, you may actually disrupt the function of those molecules and therefore disrupt the tumor cells, also.

So we've made some fundamental descriptions on this, and we did a lot of this work with one of my colleagues at the Department of Biological Sciences here at the School of Arts and Sciences, [INAUDIBLE] who's a world expert in lysosomal trafficking and lysosomal biogenesis. And so he and I kind of started collaborating. And what we found, interestingly, is that this molecule, TMEM16A, which is over expressed in cancers, also drives the biology of the lysosome.

I'm not going to go to the details of this, but suffice it to say that when you over express or you knock out this molecule, TMEM16A, the amount of lysosomal function either goes up or goes down. This is a control when we over expressed the TMEM16A. There are more lysosomes. There's more function. This is, again, control level. When we knock it out, you actually decrease the functions. So very interesting stuff, and this is data that's currently, also, in review for publication at one of the top journals.

Now, because I said that this lysosome regulates these trafficking of various proteins, we asked the question, well, what about the trafficking of one of these molecules like PD-L1? What if tumors that have high levels of this TMEM16A somehow escape the immune system? Could this be a reason why these tumors are over expressed in this molecule? So we, again, went back to our gracious tissue bank, data that we've collected over decades of patient information, as well as samples obtained from their surgical specimens. These are, again, all funded through philanthropic efforts, because this is not the kind of stuff that external government funding sources want these. These are resources-- programmatic resources that you have to have to do good science. But the science is actually what's funded.

And so what we did was we went back to the tissue bank, pulled out these specimens, and then we stained them for this molecule TMEM16A. And we found certain tumors that were lower, and certain tumors that were higher of this. And it turns out that when you look at tumors that have high expression of TMEM16A, they uniformly have lower expression of this CD8 T cells. And again, these are the T cells that kill cancers. So high TMEM expressing tumors somehow have formed a way to form a shield, if you want to think of it that way, and they keep the immune cells away from it. They push them out so the immune cells don't get into the tumor to kill the tumor. Fascinating. So we thought that was fascinating.

And, in fact, this correlates with outcomes to patients treated with this anti-PD-1 therapy. And we even showed that. I don't think I have that data here, but we did find that this regulates the outcome of patients who had their cancer treated with PD-1 therapy. Interestingly, what we also then showed is that this was not just a phenomenon, but that it was actually real and that we dig into the mechanism of this a little bit.

So we took some head and neck cancer cells, and we used this genetic technology called CRISPR, which you may or may not have heard of. CRISPR is a way that you can actually knock out proteins, genes, in a cell. And this comes from some very fascinating biological observation of how bacteria actually prevent themselves from undergoing infection with viruses, called bacteriophages. And so bacteria, like staph, for example-- you guys may have heard of Staph aureus, a very common bacterium. Staph aureus makes a molecule called Cas9 that literally chews up the nucleic acids and viruses. And so some very smart scientist, Jennifer Doudna and Feng Zhang, identified that if you could capture this Cas9, and you could target it to a specific portion of the DNA, it would chop up the DNA. It would cut it up like molecular scissors. And you would knock out that gene, disrupt that gene.

And so that's what we did. We knocked out TMEM16A and B cells, and this is what we mean. KO is knockout. NT is non-targeting, so we use a version of Cas9 that is inactive so that it can't do its job, as a control. And then what we were also able to do was rescue the expression of TMEM16A, and we're putting the gene back into the cell, again, with sort of viral CRISPR based technologies. So we can now really cleanly show that when you knocked out this molecule, TMEM16A, the PD-L1 expression went up. Not tremendously, but by about two to three-folds [INAUDIBLE]. And when you have the rescue, it actually comes back down. Very interesting. And this is interferon gamma. Interferons are molecules that cause PD-L1 to go up. And so this is what we call a positive control, which proves that when you put interferon in, you get a dramatic increase in the PD-L1 expression, as expected. So this tells us that our experiment is working.

Now, it turns out that you can actually regulate the lysosomal function with small drugs, with small molecules. Here's one called bafilomycin. There's another one called MLSA1. These came from various different labs throughout the country, so we collaborated and we got these drugs. And, in fact, when we knock out or disrupt lysosomal function by creating cells with either bafilomycin or MLSA1, you can see that there is an increase in the PD-L1, as expected. So this is very-- this made sense to us.

And in fact, you can activate the lysosomal function in these cells by using this other molecule called PIKfyve, and when you really disrupt that PIKfyve that way, you actually see a decrease in PD-L1. So you can really show that you can manipulate these levels, and this is not just an artifactual phenomenon. This is real. We're actually doing this properly, and we can prove that there is some linkage between the lysosomal manipulations and PD-L1 expression.

So you guys might be thinking, oh, this is all very interesting, but what does this mean? And what does this have to do with patients? Well, it turns out there's another way to block the lysosome. Something that's been in a lot in the lay press recently, and that's hydroxychloroquine. So hydroxychloroquine is a drug, and the brand name for this now is called Plaquenil. It's used to treat patients with lupus and rheumatoid arthritis. And so these patients with rheumatoid arthritis-- actually, if you treat them with Plaquenil, hydroxychloroquine, they get some relief.

So we ask the question, if you inhibit the lysosome with hydroxychloroquine-- by the way, it's also used for malaria prophylaxis and to treat malaria-- then can you actually increase the PD-L1 on these cells? And in fact, we saw that we could. Inhibiting the lysosome increased PD-L1, and very interestingly, sorry-- there we go. When we treat mice that bear tumors-- these are tumors that are [INAUDIBLE], so we have mice that have tumor derived from the same strain of mice, so therefore these are good models to study the immune system. And if you just treat these mice with-- sorry, I should say, just give them tumors and don't do anything, treat them with sort of salt-water, you can see that these-- this is the blue line-- saline placebo control, the mice-- pretty much all of them die at about 40 days after you implant them with tumors.

However, if you treat these mice with PD-1, which is the antibody that targets that PD-1 molecule, then you can see that they enjoy a prolongation of survival. And in fact, this increase in survival is not dissimilar to the treatment with chloroquine itself. So there's some improvement by about 10 days here. However, if you give these two in combination-- that's what this purple line is-- combo, chloroquine with anti-PD-1 antibody, there's a tremendous improvement in survival. In fact, the response rate doubles or triples. So this was very exciting to us, because this tells us that we may now be able to use hydroxychloroquine in patients.

And in fact, our data was replicated recently in melanoma, a different disease side by a group at Penn. Ravi Amaravadi is a colleague of mine who's been studying this for a while. And so he's been able to replicate this data at Penn. And so this tells us that this is not a fluke phenomenon or something that only happens in one tumor type, but this is probably real and important that we're going to move this forward. And so what we have on the docket in the very near future is to treat patients with hydroxychloroquine with one of these anti-PD-1 drugs and then perform a biopsy before and on treatment to see what the biology is of changes and see whether or not these patients will actually respond to therapy.

And if you remember before, I told you that only a small group of patients actually respond to this anti-PD-1 therapy. Well, it turns out that one of the ways that we stratify these patients is to look at their PD-L1 expression levels. And the hypothesis is that those at a high PD-L1 expression at baseline, they'll respond well to the drug. It's logical. Those that have zero expression probably won't respond. Again, logical. What about those people that are in between? Can we personalize therapy for those people that are in between, so that you're not giving chloroquine or any drug to somebody who doesn't need it but that you can really help those people.

So, in fact, that's what we're doing here. We're tailoring this trial. This is a personalized medicine trial, because we're tailoring it to people that have this intermediate what we call CPS, or combined positive score, of 1 to 19. So 0 means you don't have anything. Greater than 20 means it's high. But what about those people in between? That's what we're trying to focus this trial on, and this is now currently working its way through the mechanism of we're actually raising funds to support this trial, which will only be 55 patients. Just two patients a month is what we're thinking, so a very small pilot trial to figure out whether this makes sense, whether this works so that we can then move it into a bigger phase trial in the future.

But all of these trials require money. They require resources. And, of course, we want to make sure that these combinations are safe in people. We don't want to give drugs to people that may be potentially harmful. Now, we know that Plaquenil itself is used in millions of people in this country. We know the dosing. It's just an oral pill, very safe. We know PD-1 treatment by itself is very safe. We've done the trials on it. But what happens when you combine them? We don't quite know.

And this is a common problem to lots of trials, lots of drugs. So instead of just going out and doing a study with 1,000 patients or 100 patients or whatever the case might be, at the University of Pittsburgh, we refined and pushed forward this concept called the window-of-opportunity trials. And what this lets us do is treat patients with various drugs but in a very short window, just a couple of weeks. So you're not trying to cure people. You're just trying to figure out whether these drugs are safe and whether they actually do what they're supposed to do. If you're trying to block a particular molecule, does that molecule actually get blocked? If you're trying to prove that chloroquine increases PD-L1, then can you give that drug to the patient just for a couple of weeks and prove that the PD-L1 measurements went from point A to point B? OK? Very simple concept, but important because we need to do these tests in real life patients while being safe. So this is a very small window.

However, what we also know is even in these window trials, we have about a 10% to 20% exceptiona response rates in these people, which you didn't plan to have these-- some massive response, but it turns out that it works. So here's a guy who had a tumor here, delineated in pink. I hope you guys can see this. This was a big tumor in the front of his jaw. This is his lower jaw. We were going to make cuts all the way from here over to here and basically remove the front half of his jaw and do a reconstruction on this with my colleagues. And so this was a major operation. We put this chap-- he was from Eerie-- on one of our window trials, and when he came to the operating room, this is all he had.

He had a tiny little tumor. The tumor shrunk so much that by the time he got to the operating room, his wife said you know, doc, the first dose that he got, his pain went away. We were able to take a trip to New York. This was several years ago-- four years ago. Now he's alive and well with a massive tumor that shrank by about 90%. So what we did there was we grabbed a little bit of this tumor, and we sequenced it. And we found a molecule that was amplified in this particular patient.

And maybe this amplification event-- this particular gene that's altered might be causing good responses in these people. And so this is now moving forward with the clinical trial to evaluate this, and the company that is actually doing that, and we look forward to seeing the data from that. So again, another example of having precision medicine by seeing a phenomenon that we didn't expect but by keeping our eyes and ears open, getting those tissues, and doing the appropriate testing that needs to be done.

So with that, I'm going to end. Of course, a lot of this work was done by the people at laboratory, many of whom were supported very, very generously by philanthropic funds and collaborations with many of my colleagues, both here at Harvard, at University of Washington, University of Texas, all over the place, so a wonderful group of collaborators and colleagues. And in fact, the people in my lab currently who I need to acknowledge are Avani Vyas, Silvia Cruz-Rangel, who's a wonderful post-doc who joined us from Mexico, Anastasia Jacko, who's a local [INAUDIBLE] who's a recent graduate of the University of Pittsburgh, and Nayel Khan, who is one of our trainees in otolaryngology who took a year off in the laboratory to be able to do some of this very exciting work.