[music plays]

[music plays]

[DR. SAWYERS:] The most accurate description of my work would be what's often called disease-focused research. So I pick one particular kind of cancer, try to understand what causes that cancer, and in doing so, gain insights into how it might be treated in a more targeted or rational way. This notion of disease-oriented research has actually transformed in the fifteen years or so that I've been doing it, to not just an aspiration of helping develop better drugs based on understanding the underlying causes of cancer, but the reality that you can actually do this. We have advances in DNA sequencing technology that allow us to sequence all of the DNA in someone's tumor. And not just one, but thousands and eventually millions of cancers will have their genomes sequenced. This allows us to assemble a complete dictionary of all the DNA mutations and other types of alterations that are present in cancer cells. Even when I was in my postdoctoral training, it never entered our minds that we could have this resource. We figured we would just have to discover the cause of each cancer one-by-one. But now, you can envision a look-up table, where you could just find the answer. So the question has moved, from not one of what causes cancer, to how does one use that information to develop new treatments for cancer.

I'm delighted to give the Holiday Lecture series talks because I'm hoping to inspire at least one student to follow in my footsteps and pursue a career as a physician-scientist. It's one of the most gratifying experiences I can imagine. And the time is ripe to apply this genomic knowledge to disease in a way that's never been possible before.

[applause]

[DR. SAWYERS:] Good morning everyone, I'm superexcited to be here. Like Dr. Walsh, I want to thank the Howard Hughes staff for inviting me to give this really exciting lecture. As you can see from the degree after my name, I'm a physician. I actually went to medical school first, got interested in cancer as a doctor and then have devoted the last 25 years to studying it in my laboratory. What I'm going to tell you about this morning is, in the first half, an introduction to what is cancer and what causes it, and in the second half, give you an example of how that knowledge has led to radically transforming new treatment for leukemia. And then tomorrow we're going to talk about how that leukemia example, which is just 10 years old, has completely changed the way the entire cancer community is thinking about how we will treat cancer in the future.

So let me get started by telling you something that I think you're all aware of and that is that cancer is incredibly common. I suspect everyone in the room has somehow been touched by cancer through a relative or a friend, and the statistics are that one out of two men and one out of three women will have cancer at some point in their lifetime. Cancer is a leading cause of death in the United States. It is equally important as heart disease. If you add those two forms of disease together, you account for half of all deaths in the United States. And it's an important problem worldwide. Just this year, a little over 7 million people will die from cancer around the world.

Now there are many forms of cancer, and we think of cancer, … we classify cancer based on the tissue or organ in which it arises, and what this chart shows you is which cancers most commonly lead to death. Lung cancer is by far the most common cause of death amongst the cancers, prostate in men and breast cancer in women are second, and colon and rectal cancer are third. We often refer to these as the big four, and they account for by far and away the largest proportion of cancer deaths, and we absolutely must understand the causes of these cancers in order to develop better treatments.

So this is an important table that demonstrates the major impact on public health that can happen when you understand the disease. So the blue bars show you the death rates from heart disease, stroke, which is a form of cardiovascular or cerebrovascular disease, and cancer back in 1950. And you can see the dramatic progress that has been made in heart disease and stroke in the second and third bars, 2003 and 2010, and that's 50 years of science leading, … such as understanding the important role of blood pressure and controlling blood pressure in preventing heart disease, and managing cholesterol levels in the blood, all science that was possible and then when implemented clinically led to this impact.

Now disappointingly, you see that the progress in cancer is not nearly so dramatic, although I'm a little bit optimistic that that drop between 2003 and 2010 is a real drop due to some science that I'm going to tell you about, which we hope will, during our lifetimes, will lead to a similar slide in which we look more like heart disease.

So what is cancer? Cancer is a proliferation of cells in our own body. We think of a cancer as a tumor. This tumor shown in green here is actually a benign tumor. It's not going to kill this particular patient. It begins with one cell that starts to divide abnormally, but it's respecting the boundaries of the tissue in which it's arising. We call a tumor like this a cancer if it starts to invade into the surrounding tissue as shown here, and if it invades far enough into and reaches a blood vessel or a lymphatic vessel, it can spread throughout the body, and we call that metastasis.

The top form technically is not a cancer, although many times if we can detect a tumor at that early stage, we'll still remove it, because we don't yet know or can't predict which ones will become a cancer and which ones won't.

So this slide demonstrates the concept of metastasis in a little more detail. So this is the example of a form of skin cancer called melanoma, which is caused by exposure to sunlight. It develops in the skin, and if caught early and detected, it can be removed surgically. But many times, these are missed, or are very difficult to detect and they can spread into the circulatory system and then spread throughout

the body to the brain, the lung, liver and other organs. And it's these metastases that are actually responsible for the death of the patient. The reason the patient dies of cancer is because these tumor cells in these distant organs interfere with the normal function of those organs, causing the body to no longer work properly.

So if we take a closer view of cancer and look at the cellular level, what exactly is happening? Well, normally our cells in many of our tissues, particularly the intestine, the lining of our mouths, also the skin, our blood, are undergoing a homeostasis. New cells are born and other cells die, and it's very important that that balance is kept proper. You can easily imagine if that gets out of balance, if you have an extra cell division, a tumor can start to develop, or if there is a problem in the machinery that causes the normal cells to die in the regulated fashion, cells will also build up. So that's the basic problem that we're trying to understand the molecular basis of.

So how do we treat cancer? For decades we've been treating cancer, as I think you all are well aware, by surgery. If a tumor is detected early enough, a surgeon can remove it. This is extremely effective. Obviously, we're removing the entire cancer. Some organs, it's a little challenging, for example, a brain tumor. As Chris will tell you it's difficult to remove a tumor from a part of the brain that's essential for the normal function of the patient.

The other modalities of treatment, which you're also familiar with, are radiation and chemotherapy. And these are two forms of therapy that very effectively kill cancer cells by damaging the DNA of the tumor cells. By damaging that DNA, when that cell reaches mitosis and needs to replicate its DNA, it will be unable to do so because of all the damage and the cell will die.

So why aren't these good enough? Well, surgery, even though by the surgeon's eye, as well as the best imaging technologies we have, … it looks like the tumor is localized, the cancer can come back because there can be microscopic invisible cancer deposits in other parts of the body that we miss. So for that reason, we often give radiation to get cells that are just outside of the surgical field. We deliver radiation through a beam that has to be focused in a specific area, but radiation has the problem, in addition to damaging the DNA of the tumor cells, it also damages the DNA of the normal cells, so you can imagine the consequences.

Similarly, chemotherapy, which is given either intravenously or by pills and circulates throughout the body, can damage, … can kill cancer cells throughout the patient, but also damages normal cells, particularly blood and bone marrow cells, and cells lining the gut that are dividing rapidly as well. So clearly, we need a new form of therapy if we want to make major progress, and I think that's the major point of that slide I showed you earlier, where the progress in heart disease has been so dramatic, but less so in cancer. And I think the reason for that, and one of the main reasons as a physician I decided to change my career and go into the laboratory 25 years ago, is 'cause I thought we needed new directions. And those new directions, as you can gather, are coming from genomics.

So when did we recognize that cancer was a genetic disease? I can tell you it was not obvious at the beginning. So I'm going to give you this example, which dates back more than 100 years ago, of a problem that a Long Island farmer in New York had with tumors in his crop of chickens on his farm. So

shown in the picture is a famous doctor, Dr. Payton Rous, who was working at the Rockefeller Institute in Manhattan, New York City, right across the street from where I work at Sloan-Kettering. This chicken farmer went to see Dr. Rous with one of his chickens and said I've got this problem of tumors spreading amongst my chickens. And Dr. Rous became very interested because, well, this must be an infectious cause of cancer.

So to prove that, what he did is he took the tumor from the chicken, ground it up, and passed that lysate through a very fine filter so that no cells could get through the filter. That filtrate he then injected into a chicken who did not have a tumor, and that chicken developed a cancer, a sarcoma. So that proved that there was some substance that was smaller than a cell that was capable of causing cancer. It took decades to get to the source of that substance, and that is a virus named after Dr. Rous called Rous sarcoma virus, and to give you an idea of how long it took to do this, he was awarded the Nobel Prize in 1960 for that work, and fortunately he was still around and able to go to Stockholm to receive it.

So advances in virology and in recombinant DNA technologies allowed other scientists to take apart the Rous sarcoma virus and figure out what was inside. It happens to be a form of virus called a retrovirus, an RNA virus. HIV is another example of this class of viruses. And when the anatomy of the inside of that virus was determined, there were four genes in that RNA genome. Three genes, named gag, pol, and env are genes that are well known to the virology community, classic viral proteins. But there was a fourth gene which was named v-SRC, SRC for sarcoma virus, v because it was in the virus, that had never been heard of before and had never been seen in another virus.

So where did the v-SRC come from? Further research that took place in the '70s found that in fact the v-SRC gene had come from the chicken genome itself, so it was a host gene, not a virus gene, that was somehow now part of this virus. Furthermore, you could remove these separate genes from the virus and prove that the ability of the virus to cause the tumors in a chicken absolutely required the SRC gene.

So what's going on? Well, in the chicken which has this normal c-SRC gene, cellular SRC, the SRC gene makes a protein that's very tightly regulated, but when it's now in the virus, v-SRC, it's no longer regulated and it's expressed inappropriately.

So how does a virus capture a cellular gene? Well, in some period of time, much earlier in the lifecycle of this virus, before this Long Island chicken farmer had this problem, the virus did not have the SRC gene. It inserted into a chicken genome next to the SRC gene, and then when it was excised to propagate the virus into the next cell, it was accidently captured by the virus, so now the virus had this passenger gene that was the tumor causing gene.

So the irony here of course is that the idea that cancer was caused by an infectious agent, which is true in this Long Island chicken coop, led us to the true discovery of the cause of cancer, which is, genes within our own bodies are host. And other animal tumor viruses' research led to the discovery of other such genes, and so a body of knowledge began to develop. And it led to the this idea of a word that I know you're familiar with called oncogene.

So *onco* is the Greek word for tumor, so this was the formal proof that it's possible to have a gene that can cause a tumor, therefore an oncogene. But when the gene, when the oncogene is present in the host, it's actually not causing cancer, so we call that a proto-oncogene.

So why would evolution allow such genes to exist in a normal host if they're just time bombs waiting to be activated and cause cancer? Well, of course, these proto-oncogenes actually serve extremely important normal functions in cells. Every protein listed in this cartoon which is the outside—the cell membrane, a protein called EGFR on the outside of the cell that senses signals or growth factors, and then propagates that signal down a sort of chain reaction to lead the cell to proliferate— every single protein listed on here is made from a proto-oncogene, and every single one has the potential to become an oncogene. I think you might recognize the one, *AKT*, over on the left, that's the gene you heard about from Chris that's involved in brain development and, when abnormal, brain disorders.

So now that we know that cancer is a genetic disease, let's think about it as a geneticist would think about it. So I suspect you all know about Mendel's pea plants. Is there any lesson we can learn from Mendel's pea plants? Well, here's the example of the tall pea plant versus the short pea plant. As you know, you can breed plants together. What happens when you breed a tall and a short plant together? You get genotypically a heterozygote, big T, little t. What does the plant look like? It's a tall plant, tall is a dominant trait over the short trait.

Can we think about this in terms of cancer? Well, we can actually do this experiment, it was done several decades ago. You can't mate cancer cells in the same way you mate pea plants, but you can fuse them together through some clever technology and create a single cell from two cells. So, show of hands, what do you think is going to happen when you run this experiment? Who thinks you're going to get a cancer cell? All right.

And who thinks you're going to get a normal cell? Anybody?

Clearly the cancer cell is the majority, and sometimes in fact you do get a cancer cell and in that case obviously the oncogene would be dominant. But a reasonable fraction of the time, depending on what your source of cancer cell, you actually get a normal cell. So now, through this experiment, we recognize for the first time that it's possible that there are recessive cancer genes. So we call these genes tumor suppressor genes, out of the logic that if they are disabled, because they're recessive, they must have a normal function of suppressing tumors. They also happen to be genes that are the cause of inherited cancers, cancers that run in families.

And I suspect several of you are familiar with these two genes at the bottom, *BRCA1* and *BRCA2*. These became very famous this summer when the actress Angelina Jolie, who happens to have a mutation in the germline in BRCA1, decided to have surgery to lower her risk, which is very high, of getting breast cancer or ovarian cancer. So knowing the names of these genes and the ability to screen for them has already had an impact on medical practice.

But the last two—RB which stands for retinoblastoma, and P53—actually play important roles in regulating a process that I know you're familiar with called the cell cycle where, whenever a cell

decides to divide, it has to go through this cycle in which it replicates its DNA, and then it undergoes mitosis. And both P53 and RB serve as important checkpoints in regulating how the cell goes through this process. If you lose either one of those, the cell cycle will proceed through more quickly.

So we now have this very tantalizing model in which some cancer genes are oncogenes that, when mutated, now function as accelerators, driving cell growth inappropriately, whereas others—the tumor suppressor genes—normally function as brakes, but when broken or disabled, the cell cycle can proceed more quickly.

So you can think of this genetically now on the left hand side, you've got two copies of a proto-oncogene. If one gets mutated, it's dominant, a mutated protein drives the cell cycle as a go signal, whereas in the right hand side, you have a tumor suppressor gene, one copy gets lost and there's no protein made, but the cell cycle is still okay, until you lose the second copy, and you have a mutant form of the protein or a missing form and the cell cycle proceeds inappropriately.

So I'd like to pause now and see if there are any questions about what I've told you about mutations and genetics as the cause of cancer. Over here?

[STUDENT:] I was wondering, like, your opinion on genetic testing to figure out your probability of having like breast cancer, ovarian cancer, and like I know a lot of people think it's really controversial to remove your breasts or remove your ovaries, I was wondering what you think about that.

[DR. SAWYERS:] The Angelina Jolie example is this *BRCA1* or *BRCA2* example, which is the most common gene in the population that increases risk of cancer, and it's one for which there is a medical intervention that can make a difference. So prophylactic mastectomy, or oophorectomy, which is the term for removing the ovaries, has been shown in clinical trials to make a difference. But obviously, that's a very important personal decision for each woman to make. And there are ways to follow such women who are at risk, using imaging technologies, more closely than you would someone in the general population in order to help make that decision. Anyone else? I think there's … right here in the middle.

[STUDENT:] How many of these people who have been diagnosed with cancer, typically how many of them have received false positives and have gone through with treatments that will kill their normal cells?

[DR. SAWYERS:] So that's an interesting question. So you're asking was the diagnosis of cancer incorrect? I don't think that's a very common problem. I think what we tend to do, perhaps, is diagnose cancer and jump very quickly to aggressive therapy in order to prevent what might be a relatively low risk of progression, by giving excess chemotherapy and radiation. That has happened in the treatment of breast cancer in particular. So there is a large body of work trying to use genetic analysis of the tumor itself, not just the fact that it's breast cancer, to distinguish between those tumors that have the greatest risk of having already metastasized versus those that have not, and those kinds of genetic tests are now in clinical practice, to spare women from chemotherapy that they may not have needed. How about you in the back?

[STUDENT:] Is there a mutation that causes cancer to not form a tumor?

[DR. SAWYERS:] Is there a mutation that would cause a cancer to not form a tumor? So that's a very interesting idea. I think what you're getting at … are there people who are at very low risk of cancer because maybe they have a defect in a gene that doesn't allow them to get cancer. We actually are interested in that question in very elderly people. People who are over 100 years old, there are genetic studies of their genomes underway to try to figure out why have they not gotten some of the diseases that most people would have gotten by that age. But I'm not aware of an example in which a specific gene has been identified for cancer. In the blue sweater.

[STUDENT:] What steps have been taken, what sort of machinery have been developed to detect those microscopic cancer cells that could be left over from chemotherapy?

[DR. SAWYERS:] I think your question is, can we improve our ability to find these microscopic deposits of cancer that might be present in patients that we can't see using a CAT scan or a MRI scan. It's a great question and there's a lot of research in that area. There are … just in the last five or six years, we've learned that you can actually find cancer cells, single cells circulating in the blood in a patient who has cancer. They're very rare, but we can fish them out using various tricks. What we don't yet know is if those are detectable in the blood, does that mean that patient is at risk for metastasis. It seems logical that they might be, but there's actually pretty good evidence that some of those patients have had just had surgery to remove their primary tumor, those circulating cells now disappear and they don't relapse. So I think we just have to do more research.

Okay, one last question on the bench here in the black sweater. Yeah.

[STUDENT:] I was wondering about single-nucleotides substitutions and how those are supposed to cause mutations that ultimately cause cancer. Do we have research that shows that single-nucleotide substitutions cause an oncogene rather than a suppressor gene or is it the other way or is there an equal opportunity?

[DR. SAWYERS:] Well, tomorrow I'm going to tell you about a very comprehensive analysis of all types of mutations and all forms of cancer, but the short answer to your question is that a single-nucleotide substitution in a proto-oncogene can cause cancer as a dominant cancer, but for a tumor suppressor gene, you need to disable both copies. Sometimes it's a simple point mutation that throws a stop codon into the protein, but the other copy has to be disabled as well. Oftentimes the gene is just lost.

I think I'll move on now. So let's go back to this cartoon of the cell cycle and the idea of an oncogene making a mutant protein and sending the go signal. Can we make a drug against the mutant protein to treat the cancer in a very rational targeted way? Well, I'm going to show you one of the compelling examples that this absolutely can be done, and that's a drug called Gleevec that I was involved in the development of, a little more than 10 years ago, that's used to treat a form of leukemia.

So what is leukemia? Well, leukemia is a cancer of the blood. It's too many white blood cells. We don't think of leukemia as a tumor in the classic sense because it's not a lump. We often call it a liquid

tumor. We diagnose leukemia by looking at the blood smear of a patient or looking at the bone marrow where the blood is made, and as you can see in these two slides, the patient on the left has a normal-looking blood smear, you see the red cells and then you see two white blood cells, whereas the patient on the right has chronic myeloid leukemia, too many of these white blood cells.

So what is the cause of this leukemia? Well, a little over 50 years ago, scientists in Philadelphia, who had become experts in developing chromosome spreads of cells growing in the lab, noticed an association that was very striking. Every time they looked at a chromosome analysis of a patient with CML, and lined up the chromosomes as shown here, here are the … you see chromosomes 1 through 22 lined up as pairs, and then this patient is a male, you see the X and the Y. Every time they looked at a CML patient, they noticed the chromosome 22, shown by the arrow, looks like it's too short. The bottom part of it has been knocked off.

So although they couldn't take it any further than that at that point, they sort of had to put it on a shelf like you heard from Chris earlier, because the technology wasn't available, it was a very powerful demonstration of a genetic alteration always found in this form of cancer.

So what's going on? Well, another 10 years later when it was possible to stain these chromosomes with different dyes and really recognize and tell the difference between chromosome 1, 2, 3, etc., with more precision, a reanalysis of patients with CML revealed that this short chromosome 22 was actually an exchange of genetic material from the tip of chromosome 22 to the tip of chromosome 9, and vice versa. We call that a reciprocal translocation, so instead of having a big deletion of DNA, there's actually very little DNA that's missing, it's just a swap. It's kind of mind-boggling that this happens. It turns out with next-generation sequencing, we now know that this actually does happen quite a bit more often than we ever thought. But when this particular combination happens, you get CML.

So what's actually going on? Well, there's a gene on chromosome 22 called *BCR*, there's a gene on chromosome 9 called *A-B-L*, or *ABL*. The reciprocal translocation occurs in the middle of both of those genes and creates a new gene, a fusion gene which creates a fusion protein which we call BCR-ABL.

So we know from studies that I was a part of during my postdoctoral training that when you introduce the *BCR-ABL* gene into the blood cell of a mouse, the mouse gets leukemia. That gene produces this protein, this protein must cause the leukemia; … what does this protein do?

So if you could run the video. This is the protein. I want you to watch it change. It's flipping back and forth from an active to an inactive conformation. Now we're looking at the skeleton, you see that loop, which is called the activation loop moving back and forth.

So what is this? Well, it happens to be a protein known as a kinase. Kinases have as their job removing phosphate from one ATP molecule and putting it on to a substrate. So if you run the next video, we'll see an animation of that.

So this is the BCR-ABL protein, ATP is going to come into the mix as a yellow molecule. It binds in what's known as a binding pocket. A phosphate is transferred to the substrate protein in purple. The

purple protein then undergoes a conformational change, a change in shape, and then it goes off and does its job. This is a very common mechanism by which signals are propagated from the outside of the cell all the way to the nucleus. So that switch, active to inactive, goes back and forth, ATP binds, transfer to substrate.

So I'm now going to show you how this works in a model. So … and after the lecture, in the hallway you can play with these models as well, so here's the BCR-ABL protein, and as another example of amazing technology, this was printed just a few weeks ago on a 3D printer. This is ATP. Some of you, if you look in your bag, will have a little molecule of ATP. It fits into this binding pocket, and phosphate is transferred.

So … So let's think about now making a drug. Since we know how BCR-ABL works—it needs this ATP. ATP has this binding pocket. Could you imagine making a chemical that could fit in that binding pocket and prevent ATP from binding? Well, this is exactly where the drug Gleevec came from. And scientists at a pharmaceutical company in Switzerland had this idea about 15 years ago. It was considered a crazy idea. No one thought it would ever be possible to make a drug that could fit into the ATP binding pocket, and if you did so, what are the chances that it would work, and wouldn't it be toxic because there are lots of other kinases in the cell as well. Nevertheless, they went ahead and proceeded, searched this library of different chemicals for the ability to fit into that pocket, and the one here, Gleevec, came out as a fit.

So let's now show the next animation in which we'll see how Gleevec fits into that pocket compared to ATP. So some of you might have a purple Gleevec in your bag. It's fitting into that pocket, very nicely. Now let's see what happens when ATP floats by and tries to get in. It can't fit.

So now … that's called competition, a competitive antagonist, but there's another really clever interesting property that Gleevec happens to also have. If you remember, the kinase has this movement, back and forth, of the activation loop.

Let's roll the next video, and you'll see when Gleevec binds, as we look at the skeleton view, the activation loop is in the closed or inactive conformation. The enzyme now wants to become activated in order to bind ATP. It can't. It bumps into that part of Gleevec, so it's locked into this inactive shape. And we can look at that in the model as well, Gleevec fits so tightly in there you actually have to take apart the kinase to get it to fit in. Once you fit it in, it's locked in there, so I would encourage you to play with this outside and you can see it for yourselves.

All right, this is really cool science, isn't it? So let's now go back to the clinic. So patients with chronic myeloid leukemia, before this drug was ever tested, would die within five years of their leukemia. It was an automatic death sentence. The only treatment we had was a form of treatment called bone marrow transplantation, where we give massive doses of chemotherapy and radiation to wipe out every leukemia cell in the body, which would also wipe out every normal bone marrow cell in the patient. The patient would die unless we did a transplant of new bone marrow from a donor who was immunologically matched. So this actually does work, but it's horribly toxic, and about 20% of patients

who can undergo that procedure actually die from the transplant, from the complications. So clearly we needed a better solution.

So I was one of the three doctors who first tested Gleevec in patients. So the way we decide a drug is working in leukemia is we measure the white blood cell count before we give the drug. In this case we're taking Gleevec as given as a pill, you swallow it once a day, turns out not to have any of the horrible side effects that people were worried about, and here's what happened.

So this is a chart that shows the blood counts of these patients. This is a logarithmic scale, so the normal blood count in you and me and others in the audience is in this shaded area called the normal range, somewhere between 4,000 and 8,000. Patients with CML of course have a higher level when they start, and you can see within about a couple of weeks, all of the patients on this trial had their blood counts come down to normal. This result was confirmed in many other trials, and within less than three years from when the first patients swallowed this pill, the drug was approved worldwide for the treatment of this leukemia because it was such a dramatic, exciting impact on the lives of these patients.

And so now if we look at the ways to treat cancer, we can envision a fourth modality which we now call targeted drug therapy, where by understanding the genetic cause of the cancer and making a drug against it, we can induce a remission without all of the side effects or complications that we went through earlier.

So is it a cure? Well, the answer is no. The patients have to keep taking the Gleevec. If they stop taking it, the disease will eventually come back. Some patients, even when they're still taking the drug, the leukemia comes back, and that's shown by the example of the patient in red.

So we first noticed this a little more than 10 years ago in the early clinical trials, and it now raised a very interesting new question—why would the cancer come back? Of course, the first question is well, was the patient still taking their Gleevec, or was the body somehow eliminating the Gleevec, and the blood levels weren't appropriate? We were able to rule that out. Blood levels were the same when they were responding and when they were relapsing.

So the next question is, well, we know that the leukemia is caused by this BCR-ABL protein; is BCR-ABL still inhibited? So a graduate student in my laboratory, not that much older than you, took on this project, and she developed a laboratory test in which she could measure whether BCR-ABL was on or off, and every single patient who relapsed, BCR-ABL was no long inhibited. It was inhibited when they were in remission, but it was back on when they had relapsed.

So, why? So we scratched our heads for a while trying to think of a reason this might happen, but then of course we recalled that doctors who treat infections had faced similar questions. I know, I suspect everyone in this room has taken an antibiotic at some point. We know about antibiotic resistance. An antibiotic resistance is caused by mutations in the bacteria that develop. Bacteria divide very rapidly, just like a cancer cell can divide rapidly, it can accumulate mutations due to errors in DNA replication, and if a wrong mistake happens, a red bacteria can become resistant to the antibiotic and grow out.

So is there a similar explanation for resistance to Gleevec? So, how did we ask that question? Using the old style of DNA sequencing, the Sanger sequencing technology, the student in my lab sequenced the *BCR-ABL* gene in these patients: when they started therapy, when they were responding, and when they relapsed. And what did she find?

In the first several patients who we saw in the clinic who relapsed, she found a mutation. This was a very exciting discovery, it was now a genetic cause of resistance. It was also very exciting because we knew the structure of the BCR-ABL protein, so we could know exactly what this mutation did. It turns out it's one of several mutations that map to the binding pocket where ATP binds. So this is exactly what happens. Shown on the left is Gleevec now binding in the ATP binding pocket and making contact with the amino acid threonine, and you can see how nice and smooth the fit is. The mutation leads to the creation of a new amino acid in that position called isoleucine that's bigger, creates a bit of a bump in the pocket, the drug can't fit, therefore there's resistance.

We sequenced a lot more patients, other laboratories as well, and over the next year, … so this is early 2001, 2002, … we realized the problem was more complicated. So here's a map of all the different kind of mutations that cropped up in patients who were developing resistance. So the ones in that sort of yellow-orange color are examples of these binding pocket mutations. They happen, but they're not the most common mechanism or mutation. The others in this red color have their property of changing the conformation or shape of BCR-ABL. And we were able to come upon that insight by working with structural biologists who were solving the structures of this protein.

So what do I mean by a conformational change mutation? What I mean if you recall back to the first video, BCR-ABL is a machine, it's moving back and forth between this inactive and active shape. What these mutations do is force the protein into the active conformation. If you also remember, I made the point that when Gleevec binds, it very cleverly locks the BCR-ABL protein in the inactive conformation. So if you have a mutation now that's making the protein stay in the active form all the time, Gleevec is not going to be able to bind.

So I actually presented slides like this at a cancer research meeting attended by thousands of scientists in about 2003, and a couple of days later, a scientist at a pharmaceutical company in New Jersey gave me a call and said, "Dr. Sawyers, I heard your talk, and I'm working on a project in our company and we have some interesting compounds that for reasons that are somewhat complicated, I think they actually would be worth testing against your resistant mutants."

So if you could roll the next video, so what I'm showing you on the left is the inactive conformation. On the right is the mutant form which creates this active conformation. Now in both forms, ATP, the yellow molecule can bind, no problem, but now when you try to fit Gleevec in, because of how nicely and tightly it fits into the inactive conformation, it can't fit into the mutant BCR-ABL.

So the drug that was sent to us, the one that worked the best is a drug that now goes by the name dasatinib. Let's see how it works in the next video if you could please roll that one.

So some of you, I think, have dasatinib in your bag. I think it's green. You can see dasatinib fits nicely in either pocket, and in both cases it prevents ATP from binding, so it accomplishes the mission. And when you go outside in the hall, you'll also see a red version of BCR-ABL. This is the active conformation and I'll show you, or I'll let you play with it yourself, it's very difficult to get Gleevec to fit into this shape. You can't fit it in this way. If I take it apart, I can't jam it into the pocket, it just doesn't work. But if you take dasatinib in green, … it fits in very nicely, and tightly, and prevents ATP from binding.

So, elegant science, another kinase inhibitor, is this going to be a safe drug to give to patients? Well, in the same type of clinical trial that I showed you with Gleevec, we did a clinical trial with dasatinib, and it worked in every single patient who had a mutation that was sensitive to dasatinib. This is not from the clinical trial, this is from our laboratory, but it shows that if you give increasing doses of dasatinib, you now block the growth not just of the wild-type BCR-ABL, the normal BCR-ABL, which is in the thick line, but all those different mutants impair their growth.

 So I want to close this section with a little bit of a step back on the history of this. So it took 41 years—1960 is when the scientist in Philadelphia first saw the Philadelphia chromosome—till the approval of Gleevec as by the Food and Drug Administration as a treatment for chronic myeloid leukemia. But it took that long because the technology wasn't ready to capitalize on that initial discovery that CML is a genetic disease caused by a certain mutation. We had to have the fields of molecular biology, DNA sequencing and chemistry catch up to that first observation. But the last line in this slide makes the point that once the technology's in place we can move much, much faster. So the student in my laboratory found that first resistance mutation in 2001. Dasatinib was approved by the FDA in 2006 as a treatment for chronic myeloid leukemia. And now patients with that disease are expected to live for decades. So radical transformation of a lethal disease to at least a chronic condition that can be easily managed. We now have three other drugs that are dasatinib, or Gleevec-like ABL kinase inhibitors that is available for these patients.

So the question that that experiment catalyzed amongst my community of scientists was, well, if this sort of poster child example exists, does it exists for other cancers? And in parallel with the progress in DNA sequencing technology that Chris told you about, it raised the question of should we do a large, big-science project and understand the mutations in all cancers, to capitalize on these opportunities. And I'll tell you about that tomorrow. So thank you.

[Applause]

So more questions? Right here.

[STUDENT:] Theoretically, would you have to be creating new drugs if the BCR-ABL mutated to resist the dasatinib, and is there a way to ensure that BCR-ABL won't countermutate again these new drugs or do you just have to keep going, creating new drugs?

[DR. SAWYERS:] I love that question. It's one that I struggle with and I think you absolutely nailed it. If you give one drug at a time, we know this I think from antibiotics, the selective pressure and the ability

of these cells to divide and mutate will lead to some escape mutant of some sort. But what we've now learned … some patients on dasatinib have relapsed and some of the time they have mutations that we never have seen in a patient who relapsed on Gleevec. And the reason is, because those mutant, dasatinib-resistant mutants are still sensitive to Gleevec. So I'll let you tell me the answer as to what we should do next. Perhaps we should give those two drugs together?

[STUDENT:] Yeah.

[DR. SAWYERS:] So this is the idea of giving combination therapy, targeted therapy. It's one that's catching on. The problem in terms of not moving fast enough is that we don't yet have enough of the right inhibitors of all the different targets, but tomorrow you'll hear about some more examples like that. In the front row.

[STUDENT:] I was wondering if you could talk for a minute about like the whole databank of drugs you use in order to find something that would target the active sites?

[DR. SAWYERS:] So how does … if you have a compelling idea like that, how do you find a chemical that's going to inhibit ATP binding? Well, with the knowledge that we have today, we could probably use a concept called rational drug design, where we know the structure in such precision that we could dream up the shape of chemicals that would fit in that pocket. But back then, we didn't have that knowledge, so we took a more empiric approach, we made libraries of chemicals, millions of chemicals in the library, and then screened them one-by-one in an enzyme assay. So how so you screen millions of compounds in a library? Another technology that's required is robotics to do these kinds of tests at scale, and that's what pharmaceutical companies are very good at. Next, back here.

[STUDENT:] I was wondering if you thought anything about dealing with angiogenesis and something that could work for all cancers instead of being specific to just one.

[DR. SAWYERS:] Yeah, that's a fantastic question. There are a number of drugs that target angiogenesis. In general, they have not been as successful when given as single agents. Many, however, work extremely well when given in combination with either chemotherapy or another targeted therapy. The one exception where they've worked extremely well as single agents is a form of cancer called kidney cancer. And kidney cancer happens to be a very vascular tumor, and for reasons that have to do with the genetic cause of kidney cancer, they attract blood vessels immediately as part of their ability to sustain their growth. But what you're hitting on is not just angiogenesis, but other strategies in which, could you develop drugs that have a much broader spectrum of activity. And at the end of my talk tomorrow I'll tell you about another strategy like that that's become very exciting in my field, and that is using the immune system to do that as well. Okay, in the, next to the camera, you in the … yeah.

[STUDENT:] How do environmental factors such as smoking and exposure to radiation increase your odds of getting cancer?

[DR. SAWYERS:] You're anticipating something I'm going to tell you tomorrow, but I'll just tell you. Smoking causes DNA mutations. And if you increase the number of DNA mutations in a cell, you're going to increase the probability that one of those mutations is going to occur in a cancer gene. UV light, sunlight, same point. Okay, over here in the tie.

[STUDENT:] Is the BCR-ATP protein embedded in the membranes of the cells or is it not? And if it is, then why not target the receptor instead of targeting the kinases that are part of like the phosphorylation chain.

[DR. SAWYERS:] So it turns out that BCR-ABL is not in the membrane, it's in the cytoplasm, and so is its … the proto-oncogene of BCR-ABL is a protein called ABL. It's also in the cytoplasm and sometimes in the nucleus. So it's different from some other examples of cancer genes that I'll tell you about tomorrow which are in the outer membrane and pass through the membrane in which you can target them either form the outside or the inside. Over here.

[STUDENT:] When the BCR-ABL protein is degraded within the cell, does Gleevec also degrade with it?

[DR. SAWYERS:] Well it turns out that BCR-ABL is not degraded when you block it with Gleevec. It just is inactivated, it's put in its off state and as long as the Gleevec is on board, ATP can't bind, so it just sits there. What eventually happens is that cell will then die, and so by default BCR-ABL is degraded because the cell dies. There are examples of other drugs against other targets in which the goal actually is to make the protein degrade, the oncoprotein disappear in that way and in general, that's a challenging chemistry trick, but when it's been accomplished it's quite effective. So in the glasses here.

[STUDENT:] These patients who received Gleevec, were they also receiving chemotherapy at the time and if they were not, do you believe like it would have helped if they were?

[DR. SAWYERS:] Yeah, that's a great question. It has to do with a number of issues about how you actually do a clinical trial as well. So these were patients who had failed other therapies. Ethically, when you test a new chemical or any drug for the first time in a human, you need to test it in patients who have exhausted other reasonable treatment options. So it turns out these patients had to take an oral form of chemotherapy in order to keep their blood count in the range that I showed you. If they weren't on that, it would be even higher, it would be off the charts of the graph and they would die from having too many white blood cells in their bloodstream. So they were on that drug, then we added the Gleevec to it, the blood counts dropped and once they dropped to that safe level, we removed the chemotherapy drug and the blood count stayed down when Gleevec was still onboard, so that proved that Gleevec was doing the action there. So, over here in the white sweater.

[STUDENT:] At what point can you start calling targeted drug therapy like this a cure?

[DR. SAWYERS:] The cure word, that's a very important and insightful question. We actually debate in my field as to what is the definition of cure. I think a cure is treatment, stop treatment, and wait five plus years. It depends. … I say five plus because it depends on the type of cancer. Some cancers can relapse very late. So far I would say none of these targeted therapies, certainly not that I've talked

about today, are cures. They're more like chronic maintenance therapy in the same way that you could treat high blood pressure with daily antihypertensives or diabetes with insulin daily or other forms of therapy. But I'm not ruling out the possibility that we could cure patients with combination therapy, and I think some of this new data with these immune therapies, patients could be cured because the immune system is constantly surveilling for the relapse of a tumor. Right here, in the … yeah, you in the black sweater.

[STUDENT:] Since you've identified the translocation that causes the BCR-ABL to be mutated, is it possible that you could create a drug that stops that particular transgression and then you wouldn't have to deal with the evolution of the protein?

[DR. SAWYERS:] Yeah, that's a great idea. Yeah, why even let the translocation happen in the first place? We don't understand enough about the science of why these translocations happen. In fact, until the last five years with the next-generation sequencing technology, we thought they only existed in blood tumors. And the reason I say that is because it's very easy to grow blood tumors in the lab and let them divide and you need a cell to undergo mitosis to get a chromosome analysis so you can see the translocation. But now that we can just sequence the whole genome and we're doing it in thousands of tumors, we see these translocations all over the place. And many of them exist and don't cause a problem, so I think we're kind of poised, and maybe this is something you want to work on, is to start putting all that together and come up with a theory of how they're happening in the first place. Thank you all.

[applause]

[music plays]