Thanksgivings. We're talking today about rational medicine, and really what we're talking about is an understanding of the molecular biology of disease has actually helped to revolutionize the new science of therapeutic medicine. And here, more often than not, the discussions are focused around cancer. And so, I will therefore talk about an interesting story, vis-Ã -vis the modern treatment of cancer, and how our understanding of the molecular biology of the disease really helps in developing radically new kinds of therapies. By way of background, let's just mention that most of the chemotherapeutics that we use today to treat cancer were developed over the last 40-50 years at a time when the molecular and biochemical defects inside cancer cells were totally obscure. And therefore, to the extent that one developed chemotherapeutics, they were developed simply empirically, trial and error.

For example, some of the most effective chemotherapeutics against childhood leukemia are alkylating agents, which attach methyl and ethyl groups to target molecules inside cells. And their utility in cancer was first discerned because of an explosion in a container.

I think it was in a ship off Naples in World War II where a bin of alkylating agents was dispersed. Many people were exposed to it, and these people, as a consequence of that, came down with what's called leucopoenia. Poenia generally means a depression, in this case, a depression of their white blood cells.

Such alkylating agents had actually been used during the First World War in gas warfare because during the First World War, one used so-called mustard gases, which was a very effective way, even more effective than artillery in killing vast numbers of enemy soldiers. And, somebody noticed this leucopoenia in 1946-47 as a consequence of inadvertent exposure to these alkylating agents, which became dispersed as a gas.

And about five years later, somebody made the logical leap that if these agents were able to suppress normal white blood concentrations that perhaps they might also be effective against what seemed to be ostensibly a related problem, which is the problem of leukemia. And keep in mind that when we talk about leukemia, the suffix -emia refers to blood generally, and leuk- once again refers to white blood, i.e. an excess of white blood cells in the blood. And so, through this accidental discovery, one began to develop alkylating agents that turned out to be extremely successful in treating, and often curing, childhood leukemias, most notably acute lymphocytic leukemia, which turns out to be very sensitive to this and other related agents. So, this is a very common form of childhood leukemia, which is now actually cured in 60 or 70% of the children who were treated, which would have been unheard of half a century ago. But I return to what I said before, which is that this kind of treatment was developed in the face of total ignorance concerning the nature of the disease, the molecular defects that were present in the disease, and that were responsible for the runaway, can you still hear me OK, were responsible for the runaway proliferation of the cancer cells. So having said that, I want to go to a different kind of leukemia, and this is called chronic myelogenous leukemia, to give you an indication of the path of discovery that led from its original description to the development of rather successful treatments.

So, chronic myelogenous leukemia, I mentioned the prefix myelo- last time or the time before referring to bone marrow, and this is a leukemia of cells coming from the bone marrow from the myeloid cells in the bone marrow,

which are the precursors of things like macrophages and granulocytes. So, these are cells which are playing an important role in the immune response, and during this chronic myelogenous leukemia disease, which is called CML, there could be a period of three or four years where individuals develop large numbers of these cells in their blood stream. And after a period of about three or four years, all of a sudden there is an eruption into what's called blast crisis. And you may recall I mentioned the word blast also on one occasion earlier. This all fits together in a nice puzzle. Blast refers to primitive, embryonic-like cells, and all of a sudden there is an eruption of primitive, embryonic-like cells, less differentiated like these macrophages and granulocytes, which until this point had been present in vastly excessive numbers in the blood. There's blast crisis.

This leads to acute myelogenous leukemia, and death ensues usually within a year or two, or that's been traditionally the case. No one really had any idea about the possible causative mechanisms of the disease, and that allows me to use another word which you might one day come across if you should stay in biomedical research. And that is the etiologic agents.

When we talk about etiologic agents, we talk about the agents which are causally responsible for inducing a disease. These can be external agents, or they could even be internal agents, molecules inside cells which are responsible for the creation of the disease. And the key discovery was made in 1960 when individuals were looking at the chromosomal makeup of the CML cells.

The chromosomal makeup, I'll use another word just so we could expand our vocabulary this morning, the chromosomal makeup is often called the karyotype, that is to say the constellation of chromosomes that one can see at mitosis under the microscope.

Keep in mind, as we've said before, that during the interphase of the cell cycle, chromosomes are essentially invisible, but during the metaphase of mitosis they become condensed, and on that occasion, individuals noticed a 9-22 translocation.

So here is chromosome nine normally. Here's chromosome 22. And as you may know, the numbering system with human chromosomes goes from the largest number one all the way down to the smallest.

So, this is the smallest, with the exception of the Y chromosome. And what they notice was instead of seeing this regular chromosomal array, they noticed instead what looked very much like a structure of this sort here, i.e.

a translocation.

And this translocation resulted in a swapping of sequences between these two chromosomes. Note, by the way, this is reciprocal, i.e. in the sense that nine donates something to 22, and 22 donates something to nine.

However, the segments that are swapped are not necessarily of equal size. So, it turns out here that in this case, chromosome nine has actually gained a lot more than chromosome 22 gained as a consequence of this exchange of genetic segments.

And this 9-22 translocation made the smallest chromosome even smaller.

So, this was already the smallest chromosome as I mentioned besides the smallest autosome, the smallest non-sex chromosome.

Now it got even smaller because it lost some of its bulk as a consequence of this chromosomal translocation.

And because this discovery was made in Philadelphia, it became known as the Philadelphia chromosome. This is now about 40 years ago, or as it's sometimes called, PH-1 for reasons, I don't know why it's called PH-1 except for Philadelphia.

And, as investigators began to look at other cases of chronic myelogenous leukemia, they discovered that this translocation was present at the Philadelphia chromosome most importantly was identifiable in virtually all cases, more than 95% of the cases of chronic myelogenous leukemia.

And moreover, this chromosome was present as well in the more differentiated macrophages and granulocytes that were present and circulating in the blood of the CML patients. And that began to suggest the notion that there was a stem cell of some sort, oligopotential stem cell that created various kinds of more differentiated white blood cells that had sustained this chromosomal translocation because that's what it is, a translocalization, a translocation, that all the cells of these patients had sustained this chromosomal translocation. And that began to suggest the notion that somehow as a consequence of a random genetic accident happening in these people's blood, this particular chromosome was repeatedly identified. And it was with great likelihood causally or etiologically important in the genesis of the disease.

But that in itself led nowhere. One could simply talk about its association until work from a totally unrelated area, which is to say the study of retroviruses discovered Abelson murine leukemia virus. And Abelson was named after the fellow, Herb Abelson, who first discovered it at NIH and undertook its molecular characterization here in our own cancer center, and Abelson discovered that this virus which he studied carried the ends of the murine leukemia virus, which was a parental virus. It was as hybrid virus.

And into the middle of it, Abelson leukemia virus has acquired a cellular proto-oncogene, which it had activated into an oncogene. And therefore, here we have a situation where a cellular gene like sarc in the case of Rous Sarcoma Virus has been activated. This became called ABL for obvious reasons.

And this gene, it turned out, was critically important in understanding hwo the chromosomal translocation led to cancer.

In fact, if one infected mice with a retrovirus carrying this genome, this is just to indicate the fact that the repeat ends, the long terminal repeat ends of this provirus, they occur twice at the ends of this retrovirus. If one infected a mouse with the Abelson virus, out came a disease which was superficially similar at least to chronic myelogenous leukemia.

And that began a search, then, for the chromosomal localization of the Abel proto-oncogene.

And what was discovered subsequently, fascinatingly enough, was that the Abel proto-oncogene was right at the break point between two chromosomes, nine and 22. And what happened as a consequence of this translocation, and the resulting fusion of this chromosome with this chromosome was the creation of a fuse gene, a hybrid gene that now carried the reading frames of two previously unconnected gene, one on chromosome nine, and one on chromosome 22. Here's the normal, Abel protein.

It's called C Abel, meaning the cellular or the normal form of Abel.

And you see it up here. It's shown in a very schematic way.

And here's a second protein which is encoded on the other chromosome.

So, Abel is encoded here, and the other gene, which is called BCR is encoded here, and as a consequence of the translocation, Abel is encoded here. BCR is encoded here. As a consequence of the translocation, one now has not only the fusion of chromosomal segments. But one has the fusion of the reading frames of two previously unlinked genes.

And here, one creates as a consequence of these fusions, any one of a series of three quite distinct fusion proteins, which do not naturally preexist in the normal cell.

And there shown here is P-1 85, P-2 10, and P-2 30. These translocations allow different parts of a second gene called BCR.

BCR refers to breakpoint cluster region. The area of the point of fusion is called the breakpoint between the two genes.

So, the point where each gene is cut and fused with the other is called the breakpoint. And it turns out that within the region of the chromosome where BCR maps, there's actually three sites at which the fusion can occur. If you look carefully at this diagram, you see that there's differing extents of the BCR protein, which can be contributed to

the fusion protein.

And, what this says, in effect, is the following, that here, let's just refer to this diagram right up here.

Notice, by the way, in all three of these, that the Abel protein is present at the C terminal end of the protein. The BCR is present at the end terminal end. So, here's the BCR gene.

Here's the Abel gene down here. And what investigators found is that there could be a break at this part of the BCR gene, at this part of the BCR gene, or at this part of the BCR gene, resulting always in the fusion of Abel, to one, or two, or three different kinds of BCR proteins. And, breakpoint cluster region signified the fact that there was a whole cluster of sites in the previously existing BCR gene to which this fusion could take place, resulting, if the break occurred here, the breakpoint occurred here, and BCR to get the longer one.

Here you get the medium-sized one; here you'd get the shortest one.

And, interestingly enough, as one explored virtually, other kinds of different leukemias, one could see different of these fusion proteins that were produced. Here's chronic, myelogenous leukemia, which I talked to you about before. Here is acute lymphocytic leukemia, and here's chronic and neutrophylic leukemia, three different kinds of leukemia. We don't have to worry about the details of these diseases, aside from the fact to say that the structure of this fusion protein encourages the outgrowth of different kinds of stem cells in the bone marrow, which in turn create three different kinds of diseases. Most importantly for our discussion was an attempt to understand the nature of the resulting fusion protein, which as a consequence of this fusion caused by the chromosomal translocation now clearly acquired biological powers that did not preexist in either of the two parental proteins.

These various notations here indicate a whole series of different functions which are associated with the Abel protein, and alternatively with the BCR protein. And we don't need to get into them, except to say that each one of these different names here allows the protein on its own to associate with other proteins and do activated downstream signaling cascade.

What's most important about our discussion is the realization that this SH-1 domain, indicated here, SH-1 refers to the sarcomology domain, equals sarcomology, equals a tyrosine kinase. And therefore, what one has here is a protein, which is much more elaborate than sarc, has vastly more signaling capabilities, by virtue of the fact that these different domains that are indicated here allow the resulting fusion protein to grab hold of a whole bunch of different signally partners so that it can send out a diverse array of downstream activating signals. If one examined the structure of the SH-1 domain, it had a tyrosine kinase activity very much like sarc, and most importantly, if one introduced this fusion protein into a retrovirus, now instead of Abel, one could make a BCR Abel fusion protein.

One could put this into a retrovirus as before, just like up here.

One could infect mice with it, and now get out of a disease which was indistinguishable, in essence, from chronic myelogenous leukemia in humans. If one put a subtle point mutation in the tyrosine kinase domain, all of the able protein, here is the tyrosine kinase domain, SH-1, up here. Here, we see the tyrosine kinase domains represented in the three different fusion proteins. Keep in mind SH-1 is always the tyrosine kinase domain.

If one put a subtle, inactivating point mutation in the tyrosine kinase domain, that immediately wiped out all biological powers of creating leukemias on the part of this retrovirus here, or any one of the other closely related kinds of fusion proteins. And therefore, that indicated that the tyrosine kinase domain indicated right here was really critical to creating the tumor, and that any effects on its tyrosine kinase signaling ability would, in the end, result in the collapse of the tumor, or the inability of the resulting retrovirus to actually create cancer.

And so, now one had, really for the first time, a clear demonstration of how a commonly occurring human cancer, chronic myelogenous leukemia, is unfortunately not so rare, could arise as a consequence of some random, chromosomal translocation event. You might ask, why does one always get this particular kind of translocation? Well, the answer is, we don't really know. It would almost seem as if there's a homing device which causes this fragment and this fragment to target each other and to exchange one another. It's probably not the case.

What probably happens is that chromosomal translocations take place rather randomly within the bone marrow, and on rare occasion there is a chromosomal translocation that creates exactly this kind of fusion. And this kind of fusion, in turn, is what's responsible for creating this fusion protein, and this fusion protein in turn creates the outgrowth of this CML, the chronic myelogenous leukemia disease. So what that means is really that a randomly occurring chromosomal translocation on rare occasion hits a genetic jackpot, and the cell which happens to have acquired this kind of chromosomal translocation now begins to proliferate wildly, creating first chronic myelogenous leukemia, and then subsequently erupting into a subsequent acute phase where there are seemingly additional genetic alterations beyond this chromosomal translocation that conspire with the initially present chromosomal translocation to create a very aggressive disease which rapidly leads to the death of the leukemia patient. That offered, in principle, an attractive way of beginning to develop an anti-cancer therapeutic because what one might imagine was that one could develop a tyrosine kinase inhibitor.

Now keep in mind that tyrosine kinases are a class of enzymes which attach phosphate groups onto the tyrosine residues of various substrate proteins. And keep in mind as well the fact that we drew a series of growth factor receptors which have tyrosine kinase domains in them. And I'm drawing the tyrosine kinase domains here like this, that when these growth factor receptors become activated, they attach phosphate groups onto the tails of one another. And I'll draw those phosphate groups like this, i.e. the binding of ligand or let's say epidermal growth

factor ligand or plate ligand causes the two receptors, which are normally mobilized in the plasma membrane to come together to transphosphorylate one another, and having done so, to acquire actively signaling powers, because once these phosphates become attached, they now represent sites where other molecules can anchor themselves and send out downstream signals. In fact, there are altogether 90 different tyrosine kinases encoded in the human genome.

And so, to the extent that these tyrosine kinases become hyperactivated in various kinds of human cancers, this represents in principle a very attractive way of developing an anti-cancer therapeutic. But let's think about the problems that are inherent in such a compound. First of all, if one wants to develop an anti-cancer therapeutic, it must be reasonably specific for the Abelson tyrosine kinase, and not the 89 kinds of tyrosine kinases that also coexist in the human genome, and are active, and apparently responsible for normal cell metabolism in a whole variety of normal cell types.

So, one has to begin to think about the issue of cell activity.

How can one possibly make a low molecular weight compound, which is selectively able to inactivate the Abelson tyrosine kinase as indicated here, the SH-1 group, but doesn't disturb a whole variety of other tyrosine kinases that are responsible for other normal physiological mechanisms.

Well, you'll say that's pretty easy. We have 90 different genes.

Each of the 90 different genes makes a distinct protein, and these proteins should be very different. And therefore, if one can, in fact, if one does the three-dimensional structure of these proteins, all the tyrosine kinases look quite similar.

They have a biload structure. Here is the active site of the enzyme. That is to say, in here is the catalytic cleft, the site where the actual catalysis takes place, the site where the gamma phosphate of ATP is taken from the ATP and attached to a substrate protein to the hydroxyl of a tyrosine of a protein that's about to be phosphorylated. So, you just make a low moleculoid chemical that's specific for the tyrosine kinase domain of the Abel protein. And when I draw this biload structure, this biload structure is carried here within the SH-1 domain right here. So, this has a biload structure. It's obviously not indicated here in this very schematic drawing.

The problem with that is the following.

All of the SH-1 domains, all of the tyrosine kinase domains are evolutionarily closely related to one another.

They're all derived from the precursors of the tyrosine kinase domain that probably existed maybe 600 or 700 million years ago, and has as a consequence of gene duplication been diversified to make 90 different tyrosine

kinases. And if you look under x-ray crystallography at the three dimensional structure of all these tyrosine kinases, they all pretty much look like this, i.e. they all have rather similar catalytic clefts because they diverge from a common ancestral protein, and they retain this three dimensional configuration because this three dimensional configuration seems to be important for the retention of their function.

You could imagine, conversely, that if there were some descendants of the ancestral tyrosine kinase domain that some of them became mutant and lost its three dimensional structure.

Those descendant kinases would lose their ability to phosphorylate tyrosines on substrate proteins, and therefore would be eliminated from the gene pool because they would be defective.

And that explains the strong conservatism in the structure of these 90 different enzymes. They all look very similar to one another, and that creates a great difficulty for the drug developer because a low molecular weight drug, which one would like to develop, that fits in here. So, here I'll draw a low molecular weight drug that interacts in a stereo-specific fashion with the amino acid residues that are aligning this pocket, this catalytic cleft, might bind and nicely inactivate the tyrosine kinase domain of Abel. But at the same time, it might also bind and inactivate a whole series of other tyrosine kinases, and that in turn could lead to therapeutic disaster.

For instance, if you had a non-selective agent, you could treat a chronic myelogenous leukemia patient with a low molecular weight inhibitor, a low molecular weight compound, which would get into this pocket of the BCR Abel protein.

But it might similarly get into the catalytic cleft of the EGF receptor.

And if it shot down the EGF receptor, it might cause a fatal diarrhea because after all, the EGF receptor, I will tell you, is needed to maintain the structure of the epithelial lining of the colon. And so, you might kill the patient simply because you had deprived the cells in that person's colon of their ability to maintain themselves. There are a whole series of growth factor receptors that are required for hematapoeisis that we discussed last time. And there, once again, if you had a nonselective compound, which got into the domain of one of the growth factor receptors that is responsible for hematapoeisis, you might shut down the entire bone marrow, and once again kill the patient. I'm just giving you those as overly dramatic examples of the fact that cell activity is an extremely important consideration in developing such a drug. The other thing is affinity for the target, for the catalytic cleft that is being targeted.

What do I mean by affinity? If you look at those response curves of various compounds, what you see is the following.

You can draw out a line that looks like this, a graph that looks like this, where here we have log of drug

concentration.

And here is 10-4, here, let's do the other one, 10-8 molar, 10-7 molar, 10-6, 10-5, 10-4.

And this is molar drug concentration. And here is the percentage of inhibition. Let's say, for example, we were able to take the BCR Abel protein and study it in a test tube. And let's say we were interested in studying how well its tyrosine kinase activity responded to an applied drug that we developed against it.

So, here's the percentage of inhibition of tyrosine kinase activity of BCR Abel protein. Now, I might be able to develop a drug whose dose response curve would look like this.

And you'll say, well, that's terrific.

That's a drug which shuts down BCR Abel. We haven't even dealt with the issue of cell activity, but let's look at where one begins to see a dose response right here, 10-5 molar. And if you calculate back as to how much of the drug you need to deliver in order to shut down the BCR Abel protein in a patient, the size of the pill they'd have to get would probably be this big everyday.

So, what you need to do is you need to be an acceptable range of drug concentrations is down in this area here. And therefore, only until you get a drug which has a dose response curve that looks like this, which is two or three orders of magnitude more potent where it's able to shut down the kinase activity already at 10-7 in a, this is called a submicromolar concentration.

Micromolar is 10-6. Here already at a tenth of a micromolar, 10-7 molar, we're already getting a shutdown of the enzyme function. And if one can do that, then one might in principle be able to develop a pill that's this big and give that to the patient rather than a pill that's a size of a football. And by the way, if you have to make lots of a very complex, organic molecule through organic synthesis that has an affinity of this, it's also very expensive.

Obviously, if you can make a compound that's a hundredfold more potent and requires a hundredfold less material to deliver to the patient body, then you might have some success in treating the patient. Here's another issue.

So, we've talked about cell activity. We've talked about potency or affinity, affinity for the substrate or potency. So, this would be an acceptable drug.

It works already at molar concentration where the inflection point of this curve is. This is an unacceptable drug at 10-5. We can also talk about pharmacokinetics. I want to give you a feeling for how complex drug development is, and why it so rarely succeeds. By the way, you know how much it costs to develop a drug that's useful in the clinic

these days and test it on people? Anybody have any idea?

How much? Yeah. It's pretty close to $1 billion, between $900 million and $1 billion. That's a lot of money. That's more money than you and I are going to earn together, all of us maybe, in a lifetime. OK, anyhow, pharmacokinetics, well what's pharmacokinetics? Glad I asked that question.

How long does the drug stay inside of you after you take it if you're a cancer patient? What happens if the drug is excreted by the kidneys within minutes of its being taken, let's say, either by injection or orally?

So here we can imagine, let's talk about drug concentration.

I'll use the word drug concentration in blood.

And here's time. And here's what some drugs look like when you give them, let's say, orally. Here's what they look like.

So, let's say here's the effective drug concentration: effective concentration. And we know the effective concentration from doing measurements like this.

We just measure it, work that out. So, let's say we develop a drug which is able to hit the BCR Abel protein. What are the kinetics with which the drug becomes soluble in the blood stream?

And it might look like this, where I'm drawing here now, this is one hour. This is two hours. This is three hours, four hours.

Is that long enough?

Well, the fact of the matter is, if you're going to try to kill a cancer cell, and that's what the name of this game is, you want to have it around for a while because it turns out, as one learned, the continued viability of the CML cancer cells of the leukemia cells was dependent on the continued firing by the BCR Abel kinase protein. In fact, as one learned, if one shut off firing by the tyrosine kinase molecule in a chronic myelogenous leukemia cell, the cells would implode.

They would undergo apitosis. So, this began to reveal that in fact the BCR Abel protein is not only responsible for forcing these cells to proliferate, but it also independently provides them with anti-apoptotic signal. It keeps them from falling over the cliff into apitosis. It keeps them from killing themselves, and that's obviously critical for the ability of this tumor to proliferate, for the number of cells to expand in the body of a patient. It turns out that if you provide these cancer cells with an effective way of shutting down their BCR Abel protein for 30 or 40 or 50 minutes, not much happens to them. You need to deprive them of the drug for a very long period of time, well, 15-

20 hours, and therefore you need pharmacokinetics that look like this. It needs to be present for an extended period of time, or even better, let me re-draw that, even better look like this.

It stays in the blood for an extended period of time.

Some drugs stay in the circulation for a long time.

Other drugs stay in the circulation for a very short period of time.

There's another problem which we haven't even begun to talk about, and that is the metabolism of the drug. It turns out that many drugs that you give a patient are rapidly converted by the enzymes and the liver which are normally responsible for detoxifying chemicals that come into our body. And therefore, many of the drugs that come into our bodies are with greater or lesser speed altered into something else, detoxified, and therefore rendered innocuous. Now you'll say, well, you can figure that out too, but here's an additional fly in the ointment. Because we are a polymorphic population, because we humans are genetically heterogeneous, one from the other, some of us metabolize a given drug much more rapidly than others do.

And here, we have a situation where potentially, most of us might metabolize a drug very quickly, in which case the physicians would want to give us a very high dose of the drug so that we have enough of the drug around for a long enough period of time to do some effect.

So let's say that 97% of us are able to metabolize the drug very quickly, and as a consequence, we're given a very high dosage in order to have some effective dosage reaching the tumor to compensate for the fact that much of this drug is rapidly eliminated by metabolism in the liver. It's inter-converted into another chemically innocuous compound. Well, you'll say, that's good.

We'll just take a large dose of that compound, but let's think about the other 3% in the population who metabolize this compound very slowly. Like the other 97%, these individuals will be given a high dose of the drug because experience shows that in general, most human beings metabolize a drug very quickly. These individuals metabolize the drug very slowly, and what's going to happen to them?

Well, they might croak. Why? Because that drug is going to be around in potent biologically active form for an extended period of time in their bodies, and might have in them a lethal outcome. So therefore, we have to deal with the effects of variability in drug metabolism, variability in metabolism because it turns out that different people metabolize the drug differently and that variability in drug metabolism is vastly greater if you compare the way we metabolize drugs to the way that laboratory mice metabolize drugs. Well, you'll say, why should we care about how laboratory mice metabolize this or that drug?

Why is it important? The fact is, the first tryouts of a candidate drug are tried out in laboratory mice where laboratory mice are given a tumor, and they're injected with the drug to see whether the tumor begins to shrink. But if it's the case, if the laboratory mice metabolize a drug in a vastly different way than do humans, then the outcome of working with laboratory mice might be enormously misleading. And these are just some of the problems that bedevil the development of a drug.

In any case, around 1994, at a company which was a precursor of Novartis, it was called Ciba-Geigy in Basel, Switzerland. They developed a highly specific and potent anti-Abel, low molecular weight compound, which came to be called Leveck.

Or in Europe it's called Gleveck. It's also pronounced Leveck, but it's spelled differently. In fact, it was one of the other difficulties of developing this drug was the following.

The higher ups in the drug company who were paying for this research wanted on repeated occasion to scrub this entire drug development program. Why? Because the number of cases of chronic myelogenous leukemia overall worldwide is relatively small.

How many are in this country every year? I don't know, 10 or 15,000. So, the question was, economically speaking, would the relatively small number of cases of this disease justify their investing $1 billion in the development of the drug. Maybe it would take them a generation to get any payback from their initial investment.

And so, they tried time after time, time and again, to shut down this development program because it didn't seem to have any clear, long-term economic benefit. Of course, now we're not talking about biology. We're talking about economics, and rational economics.

This is not avarice on their part. A drug company like that cannot go on spending $1 billion here and $1 billion there without at one point or another leading to a major financial hemorrhage.

So, Gleveck turned out to be highly specific for the Abel kinase, and as it turned out, for two other kinds of kinases as well.

Another kind of kinase is against a tyrosine kinase receptor called KIT, this is a receptor tyrosine kinase, and another receptor tyrosine kinase called the PDGF receptor, which we've also encountered in passing earlier. These two other growth factor receptors, KIT and the PDGF receptor also have tyrosine kinase domains.

They therefore follow this overall structural plan here, and it turns out by evolutionary quirk that the structures of their tyrosine kinase domains are actually similar in certain ways to the tyrosine kinase domain of Abel, and therefore of BCR Abel.

So, in fact, they didn't actually have a totally specific drug which would attack only one out of the 90-tyrosine kinases encoded in their genome. It attacked three of the 90-tyrosine kinases, the Abel, the KITT, and the PDGF receptor. And this might, on its own, have already proven to be the death nail for the protein, except they began to try it out for patients, and they saw some remarkable responses. It turned out that the great majority of CML patients who were treated with Gleveck at therapeutic concentrations ended up having a rapid remission of their chronic myelogenous leukemia disease, which ultimately resulted in their being outwardly free of the disease. This is your question of the day.

So, Gleveck goes into the catalytic cleft of the Abel tyrosine kinase.

It blocks the ATP binding site because keep in mind that these enzymes need to grab the gamma phosphate off of ATP and transfer it to a protein substrate, and it does so because it hydrogen bonds to the amino acids which are lining this catalytic cleft.

In other words, this catalytic cleft up here is obviously made of amino acids, and there are hydrogen bonds which Gleveck can form with the amino acid resides that you're lining on both sides of the cleft. I should have brought you a picture of that. And, a similar kind of hydrogen bonding can occur with the amino acids that are aligning the catalytic clefts of the PDGF receptor and KIT, and that hydrogen bonding can occur already at concentrations that are submicromolar, less than 10-6 molar, 10-7, even sometimes 10-8 molar under certain conditions. So, it's a high affinity binding, and it's relatively specific. Only three out of the 90 different kinases are bound. We can do the following kind of experiment. If I were to add Gleveck to cells with BCR Abel function, this is the response that BCR Abel would show. Here is the response that the EGF receptor would show.

So, if I dose the patient at this concentration of drug, Gleveck will shut down the BCR Abel protein.

But it won't shut down the EGF receptor, which requires vastly higher concentrations of drug in order to shut down its tyrosine kinase domain. And right here, we can see what we call selectivity. The fact that this enzyme responds at very log drug concentration, this enzyme EGF receptor and its tyrosine kinase, it's a growth factor receptor once again, requires a vastly higher concentration drug in order to elicit an outcome. So, what happened to the chronic myelogenous leukemia patients. The great majority of them between 70-80% had a miraculous collapse of their disease.

In most cases, this disease could be monitored microscopically. One could look for the immature myeloid cells in their blood and see where they were previously present in vast numbers. They were microscopically now indetectable (sic). However, in those patients where the disease seemed to collapse, one could still use the PCR test to demonstrate there were residual cancer cells in their blood.

How could one do that? Well, let's imagine that here is the PCR Abel fusion protein. So, here's PCR, and here's Abel over here. You can make PCR primers, one of which is specific for a PCR sequence, and the other of which is specific for an Abel sequence, and the only time that you'll get a PCR product is if these two sequences exist on the same messenger RNA molecule that's reverse transcribed into a CDNA.

You could even do this genomic DNA as well, and so one can specifically detect using this PCR test the presence of cells which have this chromosomal translocation. If one of the PCR primers is against BCR and the other is Abel, and the distances between these two primers is not too far away, not more than, let's say, kilobase, so you get rather efficient PCR amplification.

So, it turned out that the great majority of patients who were cytologically cured, cytology means a cytological analysis represents what you see through in microscopes.

So these patients, if you looked at a smear of their blood, cytologically they were cured. But if you used PCR analysis, which is far more sensitive, one could detect residual cancer cells that might be present in one out of 105 or one out of 106 cells moving around in circulation, which are almost invisible if you're looking through a very complex mixture of cells through the light microscope. And so, what happened was that patients began to relapse, and after a period of several years, a number of patients began to show a restoration of their CML condition.

In fact, in one recent European study, indicates that between 10-12% of the CML patients who were treated with Gleveck relapsed every year.

What do I mean by relapse? I mean they show a resurgence of their disease. The disease comes back to life, and they once again have the disease. And interestingly enough, if one now looks at their cancer cells, what do you see?

In virtually every case you see some alteration in the BCR Abel protein. In the great majority of instances, you see point mutations that affect amino acid residues lining the cavity here, lining the cavity of the Abel kinase protein.

Those amino acid substitutions do not compromise the tyrosine kinase activity of this enzyme. But they do prevent Gleveck from binding, and as a consequence, now you begin to have patients whose tumors are no longer responsive to Gleveck. And what's happened now is one has developed a new generation of compounds which binds into this pocket even in the presence of these amino acid substitutions to retreat these patients. See you on Wednesday.