So, when I first came to town, the Red Sox hadn't won for 42 years.

Now it's been 86 years, so the whole town all of a sudden changes its psychology. A time for great celebration, at least for you Red Sox fans. At the, after last lecture I had an interesting conversation with one of you. One of you came up to me and said very politely, Professor Weinberg, do you have some lecture notes that you have written out what the important points of the lecture are that you could give me or that you could give us?

And I said, you know, I, I'm not really interested in doing that. And the reason I'm not really interested in doing that is that the main function of this course is to enable you to listen to, when somebody is talking about conceptually complex things and to distil what's being said and to figure out in your own mind what's important and what is just another piece of drivel coming out of my mouth or Eric Lander's mouth. In fact, speaking for him, if I will allow, can allow myself, and myself, in the end we don't really care that much whether you know the difference between DNA and RNA or proteins and phospholipids. What we're really interested in doing is to use this course as a vehicle for pushing you to get your brains functioning even better than they already are.

And, therefore, if you learn in this course how to, to think about very complex subjects and figure out what's really going on then this course will have more than paid for itself in terms of the energy you put into it. So, that's part of the reason why we don't provide you with lecture outlines. We want you to figure out what's important on your own. Being able to do so will itself be a major triumph. At the end of our discussion last time we talked about the cell cycle, the fact that it has, has four major active phases. G1, S, G2 and mitosis.

We talked about mitosis. And we talked about the fact that when cells emerge from mitosis, from M phase in the absence of growth stimulatory factors then they go into G zero.

And if they're provided with growth stimulatory factors then they'll go back into the active cell cycle. And this G zero phase we talked about is a quiescence phase, it's a resting phase.

In fact, there are two kinds of quiescent cells in our bodies.

Those that go into quiescence, non-growth reversibly, and are able to reemerge back into the active cell cycle, and those cells which have irreversibly retreated from the active cell cycle.

So, for example, there are many cells in our brain where no matter what you do to them they will never be able to go back into the active cell cycle. And they are, in that sense, considered to be post-mitotic. Post-mitotic meaning that they will never again grow. It's really not clear which of the post-mitotic cells, which tissues in the body harbor, harbor post-mitotic cells. We used to think that most of the differentiated cells in our body are post-mitotic. And when I say differentiated, a topic we will not get into for the moment, what I mean is that different cells in different

parts of the body have become differentiated by becoming very specialized to become neurons or becoming liver cells or to becoming skin cells and so forth. And it is probably the case that there are many kinds of differentiated cells in the body which once they differentiate will no longer enter into the active cell cycle. Now, getting back to this.

As I also mentioned, if we look at a Petri dish then and we put cells like fibroblast, connective tissue cells in the bottom of the Petri dish, as I told you last time, if you provide those cells with medium which contains all the requisite nutrients, these cells will sit here happily for an extended period of time but will not proliferate. However, if you add to their serum, add their medium, you add serum to this medium. Serum usually comes from cows, so therefore it's called bovine serum.

Then the serum, in addition to the nutrients present in the, in the medium will indeed provoke these cells to proliferate, and there are agents in the serum, in fact there are agents which are called growth factors which are contained within the serum which are responsible for inducing these, these cells to begin to proliferate.

Well, it's instructive just for a moment to step back and ask ourselves what actually is serum? How do you get serum?

And the way you get serum is you take blood and you allow it to clot.

And when blood clots the platelets in the, in the blood, platelets are small cellular fragments, they have an intact plasma membrane but they're just, they're very tiny, they don't have a nucleus, and these A, A nuclear fragments, these platelets, when blood is induced to clot the platelets aggregate with one another and you form a big clump, and that, a clot settles to the bottom of the test-tube. But in the context of wounding, let's say you make a cut on your skin, what happens is that blood rushes into the site of, of the, the cut or the wound, clotting occurs in order to create coagulation. And why is there coagulation? In order to staunch the bleeding. In order to prevent there to be further hemorrhage, further loss of blood. But at the same time, as the platelets are aggregating to help form the structure of the clot that, that creates a physical barrier to prevent further bleeding, simultaneously the platelets are releasing a lot of growth factors into the medium around them.

Why are they doing that? Because what's happening simultaneous with stopping the bleeding is that the platelets are, are releasing growth factors which are used in order to begin to reconstruct and heal the site of wounding. Consequently, what happens is that the platelets release growth factors.

These growth factors stimulate cells right around the sides of the wound which are still viable and have not been destroyed to begin to proliferate in order to reconstruct and intact tissue.

One of the most important of these, of these, of these factors that they release is platelet-derived growth factor.

Remember GF is just growth factor. And platelet-derived growth factor, or as it's called in the trade PDGF, I mentioned it briefly last time, is a very potent mitogen. A mitogen is a growth stimulatory agent. It's an important mitogen for fibroblasts.

Fibroblasts, as you recall, are the connective tissue, the connective tissue cells that are found throughout the body.

And, therefore, if you were to, for example, add platelet-derived growth factor, PDGF to those fibroblasts that were sitting there in G zero, PDGF will stimulate the fibroblasts to begin to enter into the active cell cycle, to exit from G zero, to enter into G1, and therefore to complete a, a full cell cycle.

And, by the way, recall what I said before, that after the cells leave the active, leave G zero and move throughout the active cell cycle and they have a lot of growth factors, they'll go all the way around the active growth cycle to mitosis.

And when they emerge from mitosis, once again they'll ask themselves the question whether it's a good idea to continue to be in the active cell cycle or whether they should exit into G zero, perhaps doing so reversibly. Interestingly, if you look at the way that the cell cycle is organized then what you see is the following, if I can draw the cell cycle again. Here's G1.

Here's S phase. And here's G2. And right at, at a distinct point toward the end of G1 is something called the restriction point, which is going to be very interesting shortly. And what happens is after cells emerge from G, from mitosis and they move throughout the, the most of G1 they're continually assessing their extracellular environment to determine whether or not there are enough growth factors around to justify their continuing the rest of the cell cycle. And ultimately they'll reach this restriction point, or as it's sometimes called the R point, and here they will make the final go versus no-go decision.

So, if there have historically been enough growth factors from the beginning of G1 all the way to the R point then cells will commit themselves essentially irreversibly to going through the entire rest of the cell cycle, through M. Conversely, if cells reach up to this R point and they calculate that there are enough, there are not enough mitogenic growth factors to justify proliferation then they'll jump out of the active cell cycle and go back to G zero. What that means, in effect, is as follows. Once the cells have passed through the R point and they're over here and they are committed to complete the rest of the cell cycle then you can take growth factors out of their medium and they don't care because they only want to receive these stimulatory signals.

They only care about in this window of time. Hereafter, they're committed essentially irreversibly to go through the rest of the cell cycle. There are, as it turns out, also growth inhibitory factors. So, here we've been talking about

mitogens, the growth inhibitory factors. So, an important growth inhibitory factor is, for example, TGF beta.

And TGF beta works exactly opposite to PDGF because it is a single which is present in extracellular space and tells the cell it should stop proliferating. And, therefore, TGF beta, if it's present in large amounts in this part of the cell cycle, if the cell experiences it in large amounts, that will influence the cell not to move through the restriction point.

Conversely, if it's absent then obviously PDGF can have the, the undiluted tensions of the cell. And, therefore, what I'm trying to convey by this is to tell you that cell balances both its growth stimulatory and growth inhibitory signals that it's receiving from extracellular space, these decisions are weighed, and finally down here the cell with make the, the binary decision to go ahead or not to go ahead, depending on historically how many of these factors its recruited in this specific window of time.

Recall, as we said last time, that once a growth factor like PDGF goes to the plasma membrane it encounters a receptor on the surface which let's say we call the PDGF receptor. And I'll just draw it like this for the moment. It's a transmembrane protein.

The extracellular domain is on the outside. And I'm drawing two copies of the PDGF receptor here for reasons that will become apparent in a moment. And what happens is that PDGF which, for example, can be itself a dimer-, it can be a dimeric growth factor.

So, it has two distinct subunits in it. They're both essentially equivalent to one another but it is dimeric. And this dimeric structure, PDGF, allows it to bind to two growth factor receptors simultaneously. Well, why is that interesting?

It's interesting for the following reason.

These transmembrane PDGF receptors, like the ones I've indicated right here, they're anchored in the plasma membrane because there's a portion of their sequence right in here in the transmembrane domain, I'm indicating it here in the orange, which contains highly hydrophobic amino acids. And those hydrophobic amino acids obviously love to be in this hydrophobic environment of the lipid bilayer and their, by they don't, they have no effect at all on whether the PDGF receptors can move, can traverse in the plane of the plasma membrane.

So, the PDGF receptors can move across the face of the plasma membrane. These ones can move to the right or the left, but they're not going to come in or out because they're anchored in this lipid bilayer by this stretch of hydrophobic amino acids.

Now, what happens interestingly when PDGF, the dimeric receptor binds to two of these PDGF receptor

molecules, which, as I told you, have lateral freedom to translate laterally on the plane of a plasma membrane, what happens is it will bind two of these receptors. And, in so doing, it will pull the two receptor molecules next to one another.

Previously, they were just floating around in the plane of the plasma membrane having bound their ligand, recall that PDGF is considered a ligand for the PDGF receptor, having, it will cause these two receptor molecules to now become, become pulled very close to one another. So, I'll redraw it now like this.

Now these two receptor molecules look like this, they're right, they're cheek by jowl, they're right next to one another, and this has some interesting consequences. Of great interest here is the affect this has on the ability of the PDGF receptor to emit signals into the cytoplasm. Because recall that the end game here is always how does the, how does the intracellular part of the cell know that this, there's been an encounter with the growth factor in the extracellular space?

And this signal-emitting power to PDGF receptor comes from the fact that once these two domains are brought together, each of these two domains is able to modify the other and change the other, i.e., subunit A modifies subunit B, subunit B modifies subunit A. And how is this modification achieved?

For the follow, it is achieved in the following way.

That the, this domain, which I've been calling, I've just been writing like this, as a rectangle, is actually a catalytic agent. It's actually a tyrosine kinase.

So, it's an enzyme. And a tyrosine kinase is an enzyme that takes the gamma phosphate from ATP, the high energy phosphate from APT, ATP and transfers it to tyrosine amino acids that are present on substrates. So, if here is an amino acid sequence in the single letter, letter code, and if we admit that Y is the, is the code for tyrosine then if here's a protein that it functions as a substrate for a tyrosine kinase, a tyrosine kinase will add a phosphate group to the side chain of the tyrosine, which I'm not drawing here, but tyrosine has a hydroxyl group in its side chain and, therefore, it will phosphorilate this tyrosine. That is to say will tetraphosphate group do it? It will phosphorilate this tyrosine.

So, these two rectangles are, in fact, tyrosine kinases. And what happens, after the two subunits of the receptor have been brought together, is thereafter, what one finds is that each of these receptor subunits becomes multiply phosphorilated.

And each of these lollipops that I'm indicating here are sites where there, a tyrosine residue has become phosphorilated.

In fact, there's a tail of the PDGF receptor that extends even further to the cytoplasm which also acquires a number of different phosphates on it. And, again, I'd remind us that this phosphorylation is really what's often called transphosphorylation because each receptor molecule phosphorilates the tyrosine residues on the other. Obviously, when these two receptor molecules are far apart in the plain of the plasma membrane, this transphosphorylation cannot occur.

But once the two tyrosine kinase residues, once the two tyrosine kinases have been brought together, pulled together by the dimerization of the receptor, now this cross-phosphorylation, on each phosphorylating the other can occur, and soon the receptors are highly phosphorilated. All of these phosphate groups, to repeat myself, being attached to tyrosine residues in their cytoplasmic domains. And this, in turn, creates interesting docking sites for a variety of other cytoplasmic signaling proteins. And we'll talk about some today and next time, but what I want to leave you with is the following impression.

That after this phosphorylation actually occurs there are a number of molecules in the cytoplasm, signaling molecules that have affinity for binding these phosphotyrosines.

When I say phosphotyrosine, obviously, I'm referring to the phosphorilated form of tyrosine that's been created by a tyrosine kinase enzyme. So, here's one molecule that can bind. Here's molecule A combined to one of these phosphates, another one combined to this phosphate specifically, and each of these molecules, once they're attracted to this phosphorilated receptor, can then emit downstream signals, send a variety of signals into the cell that ultimately end up in persuading the cell to proliferate.

And so these effects here of growth factors in the G1 phase of the cell cycle are mediated by this transmembrane signaling, by the activation of these, of this PDGF receptor, for example, and by the resulting a release of downstream signals into the cell which pursued the cell to proliferate or not to proliferate.

To be sure the, when platelets clot, when platelets aggregate and they release PDGF, they also release other kinds of growth factors. For instance, there's another growth factor that's called IGF1, insulin-like growth factor, and that has its own receptor on the surface of cells. And there are, on a cell, hundreds to thousands of these PDGF receptors, there are IGF receptors, there are EGF receptors.

And a cell often will require several distinct kinds of growth factor activations in order to proliferate. So, this is only a minor part of the entire exposure that a cell experiences in the G1 phase of the cell cycle. To elaborate on a point that I made last time, an important biological distinction between normal cells and cancer cells is the fact that cancer cells require relatively little growth factors or in the medium in order to proliferate. Normal cells have a very strong requirement for growth factors in their medium. And, therefore, what we can already imagine is the following kind of scenario. That cancer cells have someone deregulated this signaling pathway. Somehow they

have become independent of the stimulation that is normally required, usually required for cells to proliferate. And, in fact, we know of several different ways by which cancer cells can acquire this independence.

One of the most important ways, it's, it a really interesting one, is here's a cancer cell, which we'll talk about very shortly.

And what you find in certain kinds of cancer cells is that the cancer cells themselves release growth factors into the medium.

So, there are certain kinds of cancer cells that will release, let's say, a growth factor that's like EGF into the medium around it, around themselves. Well, you'll say that's kind of amusing. But so what. The important part here is that these same cancer cells have receptors for TG, for EGF on their surface. So, they're producing a growth factor and they can also respond to the same growth factor.

And, therefore, this EGF, once it's released, can swim over here, activate the receptor and pursued the cell to start proliferating. This is, if you will, a positive feedback loop. But note here importantly that this, the growth of this cell is not being controlled by growth factors coming from cells elsewhere in the tissue or the body. Here we're not talking about different cells talking to one another. Here we're talking about a monologue where this cell is talking to itself. This is sometimes called autocrine signaling and refers to the fact that certain kinds of cancer cells are able to make growth factors to which they can respond.

In fact, in normal tissues it's rare for a single cell type in a normal tissue to be able to make a growth factor and to be able to respond to the same growth factor. Why can it normally not respond to that growth factor? Because it won't make the receptor for the growth factor. For example, epithelial cells like the cells in your skin or the cells lining the gut, they can release PDGF, but they don't have a PDGF receptor on their surface. And, therefore, even though they release copious amounts of PDGF, that will not result in this auto stimulatory proliferation and, therefore, you don't have this decontrolled self-proliferation that you see often in cancer cells, this autocrine loop. In many kinds of cancer cells you have another alteration of this growth factor signaling pathway. And here what we see is the following. Instead of there being a small number of growth factor receptors on the cell surface, now there are ten or twenty or fifty times more than are normally present on the cell surface.

In other words, this growth, the growth factor receptors are what is called overexpressed.

And, therefore, a delicate balance is disrupted because normally, let's say, a cell with have on its surface 500 EGF receptors, but in many kinds of cancers probably 30% or 40% of all carcinomas, carcinomas are the tumors from epithelial tissues, you'll find overexpressed EGF receptor. Well, why is that interesting? It's interesting for the following reason. I told you before that the activation of a receptor depends on its ligand to persuading two

receptor subunits to come together and start firing, as we just discussed.

But if now all of a sudden the cell contains large amounts of this growth being expressed, of the growth factor receptor being expressed on the surface, ten or a hundred times more than normal, then these growth factor receptors are going to be rather densely packed on the cell surface. And now they may just come together because they happen to bump into each other very frequently.

They don't need the growth factor to pull them together just because there are so many of them. So, there's random interactions, random bumping together And these two growth factor receptors may just bump together and thereby send signals into the cell persuading the cell that there's been some kind of growth factor in the extracellular domain that's been encountered when, in fact, all that's happened is that there are so many of these growth factor receptors around that they're constantly bumping into each other, and while they've collided with one another they can activate signaling and release growth stimulatory signals into the cell.

There's another kind of, of alteration of growth factor receptors that's also seen in many kinds of human tumors.

For example, in, in glioblastomas, which is a brain tumor. And a glioblastoma has the following kind of, of receptor on the surface.

It has truncated forms of the EGF receptor on the surface where a lot of the ectodomain is simply not present. So, here's the normal EGF receptor, here is a truncated EGF receptor where a lot of the extracellular domain, which I'm calling the ectodomain, has simply been lopped off. How has it be lopped off?

Well, there's been a mutation it the gene which has, in effect, deleted segments in coding the N-terminus of the receptor protein, which normally sticks its head out of the cell. And now you have these truncated receptors.

And such truncated EGF receptors are able to fire constitutively.

Constitutively implies that these receptors are able to fire in a fashion that is no longer regulated by physiologic signals.

It's a high steady rate. So now these receptor molecules, these truncated receptor molecules flood the cell with growth stimulatory signal and, for reasons that aren't really clear to this day, these two, these receptor, truncated receptor molecules can dimerize, they can come together even if there's no extracellular ligand present, even if there's no growth factor in the extracellular space. And we now realize, for example, that there are a variety of structurally altered receptors that fire constitutively into a cell in many kinds of human tumors. And in each case the cancer cell is deluded into thinking that some growth factor has been encountered out here when, in fact, there's not at all. Once again, what we see is a situation in which the cell, the cancer cell is being deluded into

thinking there's growth factors present, extracellular space.

None has been present at all. There have been a variety of drugs developed against, for example, lung cancer.

And there are a variety of different kinds of lung cancers.

One is called non-small cell lung carcinoma. We don't have to deal with the subsets of lung cancers. And it turned out, it turned out that one of these drugs, it's called Iressa, had very mixed effects on patients.

In about 90% of these, of the class of lung cancers, patients that were treated, the drug Iressa, used over the last several years, had almost no effect on the tumor treatment and the patients continued to, to proceed to their death. It had, it really had no effect. But in 10%, in fact, there was some dramatic responses and tumors shrunk down.

Now, normally a 10% response rate would be enough to cause a drug company to abandon all further development of the drug because it's just too low a response and who wants to take a drug where the chances of having a good response are as low as 10%?

It's just not a good situation. But then some geneticists here in Boston, one group at the MGH and another over at the Dana Farber, began to look at the lung cancer cells that responded, i.e., from tumors of patients that responded and shrank in response to the drug and the lung, and the lung cancer cells of patients who didn't. It turns out that Iressa is an inhibitor of the tyrosine kinase of the EGF receptor.

That's how it was designed. In other words, this drug, it's a low molecular weight drug and it goes into the tyrosine kinase domain, that rectangular thing I showed you before very schematically, and it shuts down the firing of the receptor.

That was the motivation behind creating this drug.

So, Iressa shuts down the EGF receptor and 10% of lung cancer patients, their tumor shrank, the other 90% didn't, weren't effected at all. And what these two groups of geneticists found over the last three or four months is that the patients whose tumors responded had tumor cells where the EGF receptor was mutated and therefore firing in a constitutively active fashion.

That is to say there were actually structural alterations in the receptor. This is a massive structural alteration of the receptor here, this truncation.

But, in fact, in certain patients the 10% of patients that responded, there were much more subtle changes in the cytoplasmic domain of the protein which allowed these receptors to constitutively dimerize, once again, in a ligand

independent fashion.

So, these subtle mutations mimic, in effect, the consequences of deleting or truncating the extracellular domain in that in both cases one gets a ligand independent receptor. In those cases where these, where the patients had a mutant EGF receptor, structurally altered EGF receptor that was firing constitutively, there were dramatic responses to the Iressa drug.

In the 90% of patients where there was no effective response to the drug, the EGF receptor was wild-type, it was present in a wild-type configuration. It might have been slightly overexpressed but it wasn't, but it continued to, to function essentially as a normal EGF receptor. And this represents a major advance in cancer therapy because it suggests that one has to begin to understand what subsets of patients one should treat with a drug which can, on its own, have quite toxic effects on the patient. And, from now on, to state the obvious, when one gets lung cancer patients one will check quickly using various reactions, like the PCR reaction, to see whether or not their cancer cells have a mutated EGF receptor.

And, if they do, they will be candidates for Iressa treatment with the expectation that 60%, 80% or even 100% of them will have tumors that respond. And if they don't have a mutated EGF receptor then they will not be subjected to a treatment by this drug. This is the beginning of a new era of cancer drug treatment. It's called rational drug design, or rational treatment, where you don't just lump all the patients with a certain disease together and say let's give them all this drug and throw things up in the air and see what happens.

Here one begins to do a genetic diagnosis of the genomes of the patient's cancer cells in order to determine whether or not they have certain mutated genes. In this case we're referring to one of these growth factor receptors. By the way, we're talking about lung cancer today, right? If you are smoking now, I always ask the class how many people are smoking, and nobody has the, has the moral fortitude to raise their hands.

But if you are smoking now and you started at this age and you continue.

And, by the way, if you start at your age and you continue smoke, stopping smoking is actually a bit more difficult, quite a bit more difficult than stopping heroine. That's pretty interesting, right? So, if you continue to smoke now you will be healthy for a pretty long period of time, probably another 20 or 30 years.

And for you that sounds like forever, but when you get to be about 40 or 50 things are going to start falling apart.

Soon you won't be able to be very athletic, soon your lungs are going to be able, are going to degrade, and by the time you reach your fifties, sixties or seventies what's going to happen is you will, on average, have a six to eight year shortened life expectancy.

Now you say six to eight years is not that much, but it really is. You know, when you get to be 70 and you think you're going to die next year or you're going to die in six or eight years it makes a big difference. Six to eight years is an enormous difference in life expectancy. 20% of all people who died last year in this country, 20% of all deaths came from cigarette smoking. Imagine that. And when you die from cigarette smokes, smoking sometimes you get lung cancer. There probably were, I think, 600,000 people who died from smoking last year.

Six hundred thousand. There were 55,000 American soldiers who died in Vietnam in the whole war, there were 220,000 American soldiers who died in all of World War II, and last year in this, and there were 3,000, or 2, 00 people who died in the World Trade Center. All right? Got all those numbers?

So, last year 600, 00 people died premature deaths because they were smoking. How many people died last year from smoking marijuana? Maybe two or three, I don't know. [APPLAUSE] Am I urging you to do any kind of smoking?

I'm not saying marijuana smoking is good for you, but I just want you to get these things in mind, the perspective.

If you smoke, you know, in many countries, including this one, there isn't much tension by, given by the government to, dissuading people from smoking, and here's the reason why. If you smoke, and you going to get, get sick eventually, eventually the country is always going to have to pay for your medical costs, right? Sooner or later we all have to pay for the costs of people who get sick. It's all shared in one way or another.

But it's not such a big problem for a government like the American government. Because if you smoke you're going to die early enough that you won't draw on social security.

And, therefore, the government actually saves money by your smoking, because by the time they add up how much they get on the tobacco tax and how much they earn by your not living long enough to draw a pension, it's much better, it's much more money than how much it's going to cost to take care of you while you're dying from emphysema or bladder cancer or lung cancer or heart disease.

Many more people die from heart attacks due to smoking than die from lung cancer, in fact. So, think about this.

Think about this. If you smoke it's probably a good time to stop because if you continue at your age, especially if you're women, which is women, for some reason women have a harder time stopping than men, they can't say why. It's probably some physiological thing.

If you, if you continue now at your age, it will be almost impossible to stop. If you live with smokers ask them to leave. [LAUGHTER] If you live at home with your parents and they smoke tell them it's time for them to leave. Throw them out of the house.

Smoke, second-hand smoking killed probably between 60, 00 and 80,000 people last year in this country. Second-hand smoke.

And, by the way, if you want to see an interesting phenomenon, go to a veterinary hospital because there they with great frequently, frequency treat dogs who have lung cancer. And why do they have lung cancer? Not in 99% of the cases, in 100% of the cases these dogs live with owners who smoke.

An average tobacco smoker goes through six or eight dogs in his or her lifetime. [LAUGHTER] It's true. It's absolutely true.

So if you, if you think the dogs, if that's a fact for the, for the, for the dog owners, think about what's happening to the inside of your lungs. And so I'm going to take back what I said before. Before I told you that the most important thing for you to do in this course is to learn how to think clearly and to assess and to distil conceptually complex material, there's actually one more thing that's even more important to get out of this course, if you do, and that is to stop smoking. If you do that, if you do that it'll be vastly more important for the rest of your life than anything you learn here.

So, write that down, vastly more important. You may think it's glamorous, you may think it's exciting, but keep in mind, people who stop smoking have vastly greater effects on reducing the morbidity and the mortality in this country than anything that cancer researchers can do. Keep that in mind.

And if you start smoking now and you think that somehow the cancer researchers are going to be able to come up with some miracle cure by this time you start coughing and start spitting up blood, don't be so certain. They may not be able to save you, to pull your fat out of the fire. So, I don't know whether I, I gave this message in a very subtle way or I hit you over the head with it. [LAUGHTER] But think, think about that. Now, now we're going to focus on lung cancer, we're going to focus on cancer because it's one of the consequences of cigarette smoking, but it's a disease we want to talk about both this time and next time, and we want to relate it here to the cell cycle and, and how the growth of cell proliferation occurs.

I told you last time that a human tumor is roughly-speaking about, a human body roughly carries three times ten to the thirteenth cells.

So, that's quite a few cells. That's how many cells there are in the human body, plus or minus. A human tumor of one, let's say one cubic centimeter is roughly ten to the ninth cells.

So, a tiny tumor this way already has a billion cells in it.

And what I want to say is that those billion cells, or if the tumor grows larger to ten to a hundred billion cells, it's still

not that much compared with the overall size of the body.

But tumors of that size can kill you. And one, an interesting and important thing to realize about all the cancer cells in that tumor mass is that they all descend from a single progenitor.

In other words, if we imagine a situation where here are a whole bunch of normal cells and here's the boundary between normalcy up here and malignancy, malignancy obviously refers to cancer, we could imagine where there are many cells which independently cross the boundary from one to the other and become the progenitors of a vast tumor mass. But that's not what happens.

What happens, in fact, is that only one cell gets converted or becomes, as one says here, it becomes transformed from a normal cell into a cancer cell. And this transformation causes this one cell to become the progenitor, the ancestor of all the cells in the tumor mass. So, one important realization we have about looking at different tumors is that cancers are monoclonal growths, i.e., they form clonal populations. They're monoclonal in the sense that they all are genetically derived from a single common ancestor rather than being polyclonal. What else can we say about cancer cells or the cells in a tumor? If you take cells out of Petri dish, out of an animal or a human and put them on a Petri dish, excuse me, and you put them there, then what you'll see is following interesting behavior. If you put normal cells in a Petri dish they'll grow across the bottom of the Petri dish until they cover the entire bottom of the Petri dish.

So, you can put a hundred cells in and they'll continue to proliferate.

Let's look at the Petri dish from top down, so you might have a small number of cells here and here, and normal cells will continue to proliferate until they begin to touch one another, and then they'll stop growing. And this stopping of growing is, is the phenomenon that's called contact inhibition.

So, a normal cell will indicate contact inhibition.

And to state, to state the obvious, this phenomenon or this behavior of contact inhibition creates what's called a cell monolayer because if the cell stopped growing once they touch each other they're not going to be two or three or four layers of cells in the Petri dish.

So, here we're looking at this Petri dish in cross-section and there's a monolayer of normal cells here.

If you put a cancer cell in the Petri dish, or let's put here a cancer cell, we'll seed it amidst normal cells, what will happen is that the cancer cell lacks, has lost contact inhibition, and the cancer cell will continue to proliferate even after it's touched its neighbors. So, it has lost cancer, it has lost contact inhibition and will start growing on top of, the cancer cells will start growing on top of each other because they don't mind growing in spite of their having

intimate contact with neighboring cells. And, in fact, what you can do is the following experiment. You can put cells in a Petri dish like this, a whole bunch of normal cells in a Petri dish, and then you can put into them some kind of transforming influence, which we'll talk about very shortly, i.e., you can influence some of these cells to become transformed. And how we do so we'll tell, we'll hold in abeyance just for a moment. So, we'll have this cell. We'll do this cell to become transformed and this cell to become transformed.

And what will happen is that those cells will begin to form a very thick clump of cells, these blue ones, the ones that are transformed. Whereas, all the other cells will grow until they form a monolayer at which point they'll stop proliferating.

So, the cancer cells keep piling up and the transformed cells, transformed by one or another agent, we won't talk about that yet, continue to proliferate long after the normal cells have stopped proliferating. The normal cells having stopped proliferating because they're contact inhibited.

And, therefore, they'll form this clump of cells which we'll call a focus. And if you hold the Petri dish up to the light and you look at it and there are some transformed cells present, you can see the foci, they're very thick. Whereas, the thin monolayer of cells will just look pretty transparent.

But this focus will look highly opaque by virtue of the multiple layers of cells that are involved in it.

Now, in fact, we can begin to ask the question of how and why cells like this become transformed and exhibit this behavior.

In fact, until the 1980s there wasn't really a clear understanding about how that happened. I've already given you some clues because I've already told you the fact that certain cancer cells carry mutant genes. What kind of mutant genes?

Well, I gave you, in anticipation of discussion, the fact that certain cancer cells carry mutant genes that specify mutant growth factor receptors. And these mutant growth factor receptors, as I indicated, begin to push the cell the proliferate. And so that already anticipates a conclusion we're about to make, which is that the reason why cancer cells behave abnormally is that they carry mutant genes. Now, let's talk about the nature of these mutant genes because, if you look at these mutant genes, invariably they are the consequences of what we call somatic mutations.

By that I mean, I mean the following. Let's say we all start out with a really good set of genes. And, thank the good Lord, we all do.

But as we go through life through accidents or through intent we can damage these genes. We can muck them up

in different ways. And these genes, this damage may occur to cells in the skin, they may occur to cells in your belly, they may occur to cells in the brain, and these are called somatic mutations in contrast to the germline mutations that affect one's offspring. Because, as you must realize by now, the only way you can have mutations that affect your descendents is if those mutations strike in the gonads and affect the genomes of either sperm or egg.

But the mutations occurring everywhere else in the body outside of the gonads, because they occur in, I think I, this is somatic, excuse me, because they occur in the soma, the soma is defined as the entirety of the body outside of the gonads, outside of the germi, these somatic mutations might affect the tissues around them but they will not be transmitted from one organismic generation to the next. And, accordingly, we begin to imagine, in fact, correctly we begin to construct this model that one of the most important mechanisms of creating a cancer cell is to damage its genome. So, I'll tell you a story now, which I'm only going to finish on Monday. We have, let's imagine, a 55-year-old, this is a true story, 55-year-old man who's been smoking since he or she, he was 15 years old. So, he's been smoking for 40 years.

And during those periods of 40 years, by the way, I'm not saying whether I'm for or against smoking, you understand that. During that period of 40 years this person has been introducing large amounts of tobacco smoke compounds into his lungs. Now, it turns out that these compounds, this tobacco smoke compounds are carcinogens.

You know carcinogen means it causes cancer. But it happens also to be the case that a lot of carcinogens, cancer-causing compounds are also mutagens. That is to say they can mutate DNA.

So, here we have a scenario that we're going to set up for next time.

55-year-old man. Smokes for 40 years. Dumps a lot of carcinogens into his lungs. The carcinogens which are highly, which induce mutations very potently are passed from his lungs into his blood and there go from the blood into the kidneys.

And from the kidneys they are excreted into the urine and then they sit around in the bladder for a while. And let's imagine now that the urine of this man has all of these highly mutation-active carcinogens in his urine, which, in principle, can begin now to strike out and attack the genomes of the cells lining the urinary bladder. In other words, by, by smoking cigarettes you can actually mutate the genomes, somatic mutation, the genomes of cells lining the bladder of the, the urinary bladder, or, of course, to state the obvious, you can also mutate the genomes of the cells lining the alveoli in the lungs. That's why you get lung cancer.

And a consequence of this can be, with serious probability, that now some of these cells become mutated and

that, in turn, will lead to a life-threatening tumor. So, on this very cheerful note and your having heard two major take-home lessons, have a great weekend. See you on Monday. Enjoy the parade tomorrow.