Organic Chemistry in Biology and Drug Development
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Lecture - 57
Cancer and Chemotherapy

Welcome to this session on the Chemistry of Organic Chemistry in Biology and Drug Development. We are covering the second part of this course and we have already discussed the general concepts of drug design principles, high throughput screening and combinatorial chemistry- basically how to arrive at a hit very quickly. Then we started discussing about different kinds of drugs. We discussed the chemistry of neurotransmitters, the chemistry of antimicrobial agents and the antiviral compounds.

And now, today we will be discussing the chemistry and biology of a disease which is yet to be conquered and that is what is known as cancer.

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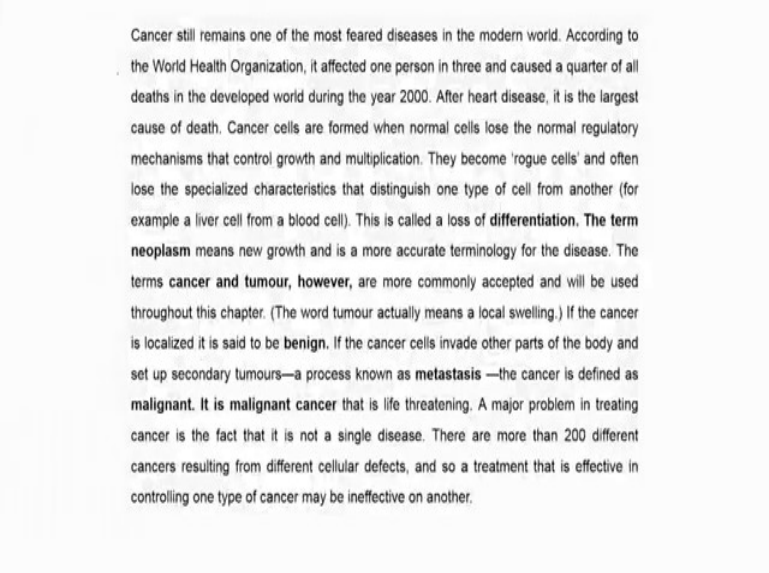

How can we design drugs which are aimed towards eradicating cancer? Cancer still remains one of the most feared diseases in the modern world. It is known for a long time, but the actual cure or the proper cure is still not in our hand. According to the World Health Organization, it affected one person in three in 2000. So, after that almost 20 years have gone. Cancer is on the rise. It is not on the downward trend. Basically, heart disease is the one which is the major cause of death followed by cancer.

Now, the problem with treating the treating cancer is the migration of the cells from one place to another. There is a process called as metastasis where the cells are formed on liver or heart and they remain at that position.

Liver cells will never migrate to other. But in cancer, there is an uncontrollable growth somewhere and finally it migrates from one place through the bloodstream to any other organ or tissue. So, that is the major problem and that is called the malignancy.

There are other types of growth is called tumor. If the tumor stays there is no metastasis. That is called a benign tumor. So, a tumor becomes malignant when it has the capacity of migration from one place to another.

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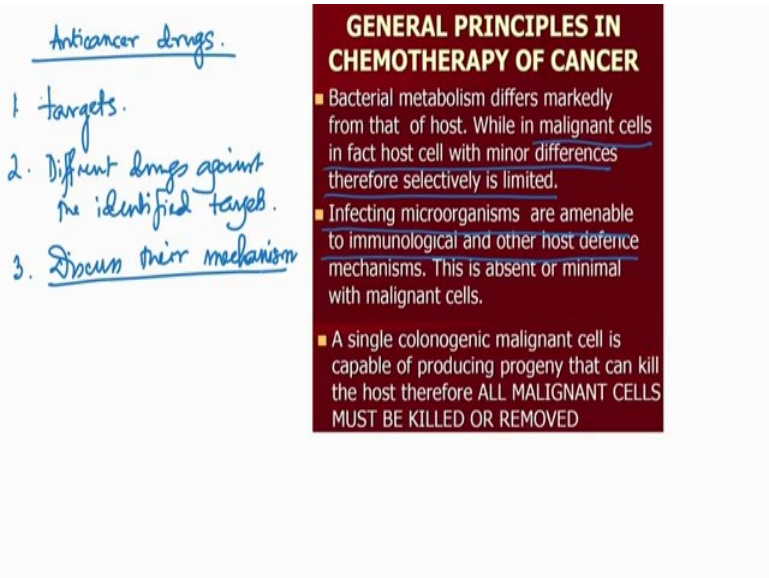

Why cannot we get a cure for cancer? The problem lies with the fact that the cancer cells belong to the host itself. It is caused by some organism coming from outside that happens in case of bacterial infection or viral infection or fungal infection. So, you can use different chemicals to kill those and there is a distinction between bacterial cell life and versus the host.

The problem with cancer is that it belongs to the host itself. While the malignant cells, have some minor differences with the normal cells. There are some differences and on the basis of that one can try to develop anti-cancer drug. There are only minor differences or there may be some differences which are yet to be discovered. That is of

course, we must mention. There must be sub differences due to which some cells become cancerous *i.e.* the uncontrollable growth.

If we take antibiotics few cells of the bacterial origin will remain in my body and now the remaining cells are taken care by the immune system in our body. In case of viral infection, it is the unity which is very important to eradicate the virus. But in cancer somehow this immunity does not work. The cancer cells evade the immunity.

It belongs to the host even it is always developed against foreign organism. The immune system usually works against foreign organisms. Even in the case of host if some cells become unruly (*i.e.* the rogue cells) there immune defense can work. But in case of cancer, the cancerous cells they somehow evade the immunity.

If one has to develop a holistic approach to treat cancer then possibly it is a immune system. Immune system is a very general strategy. The cancers could be in different varieties different forms because it can be head and neck cancer, it could be cancer of the liver, it could be cancer of the pancreas. Each cancer has their own differences, their own identity. So, the same drug cannot work for all the types of cancer. Some drugs work for breast cancer, some drugs work for prostate cancer. If you want to have a holistic approach then possibly it is the immunity which has to be understood properly and then exploit that to kill the cancer.

We will first discuss about the targets for developing anti-cancer drugs. We know targets are the most important. The first aspect of drug discovery program is that you want to know the target. It could be a protein, it could be nucleic acid or it could be a small molecule.

Now, after identifying the targets we will discuss that different drugs against the identified targets and then discuss their chemistry. The chemistry means basically their mechanism of action, how do they work.

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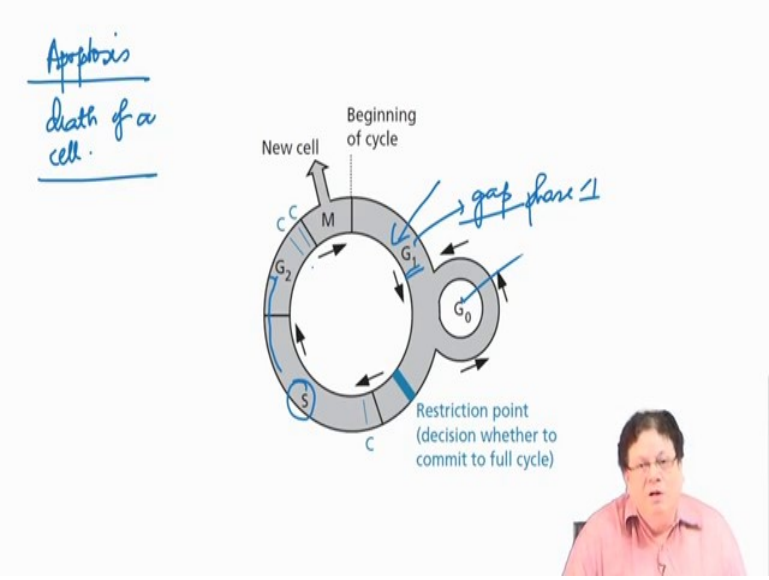

I mentioned that cancer cells have an uncontrollable growth whereas the normal cells have a definite life cycle pattern. It does not grow uncontrollably. When it is required the normal cells will grow. When it is not required either it goes into a phase which is called a resting phase. If the DNA is not proper they are marked to be destroyed and that is called programmed cell death or also called apoptosis.

All cells are not apoptotic. When the body decides that this cell cannot do the function properly it tries to repair that. If it is not repairable it is usually programmed to death. On the other hand, if the signal comes that it need any growth any further right now the cells go into G_0 phase or a resting phase. But if there is this mitosis i.e. cell division takes place, so before cell division all these important molecules like DNA has to be replicated and then the different proteins have to be expressed. All these have to be done before the mitosis.

So, it all starts with this G_1 phase and then it goes to the S phase. This is G_1 means the gap 1 phase- it is now preparing the cell for synthesis of the DNA. Synthesis of the DNA means the replication of the DNA that is required. Then after the synthesis is done then there is again a gap- a gap phase 2. So, it prepares the cell for the mitosis *i.e.* for the cell division. So, the first one prepares the cell for the synthesis and the second one prepares the cells for mitosis. After the mitosis they divide and one copy leaves with the parent cell and the one with the daughter cells.

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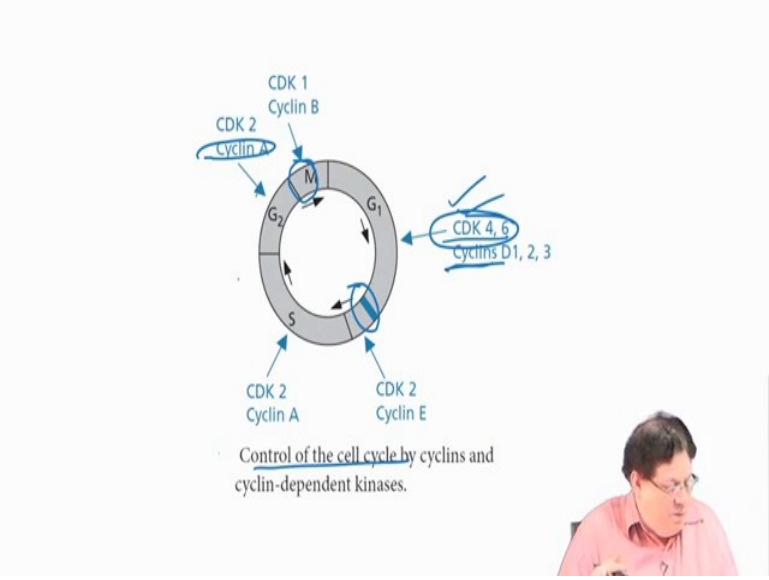

Now, there are safety points, like the checkpoints. If something is processed like the making of a car, see you have to make all the parts and then assemble them and finally, put the tires audit is ready to go. Similarly, in case of cells, you have to make all the parts, but the question is whether all the parts have been made properly or not. So, there are checkpoints. There are proteins which will see that everything is working or everything has been assembled in a right fashion.

So, these are the proteins like CDK. They are called CD kinase. They are collectively called cyclins but they are basically kinase. Kinase does the phosphorylation. Serine OH or threonine OH are the common phosphorylation sites. Now, this cyclins check that whether everything is there or cell is well prepared. Then it push the cell towards the synthesis phase. There are other cyclins, like cyclin A. It checks whether the DNA has the correct information. If it is not correct it will again throw. If it can correct then it is it is ok otherwise it will push it into the apoptotic cycle. Once everything is correct then you have this mitosis- the cell division takes place.

In cancer cells, somehow this process does not take place. It just goes via the G_1 phase, S phase, G_2 phase and the mitosis the M phase. All the time it is growing. Basically it is a breakdown of the program of cell cycle that causes cancer.

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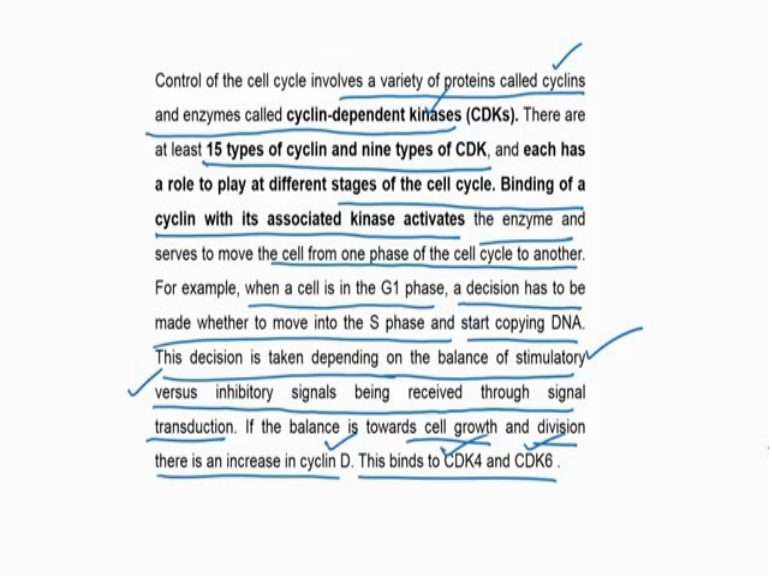

We are talking about a life cycle that grows all the time. What causes this life cycle to break down. That is the major point between the difference of a cancer cell and the normal cell.

Control of cell cycle involves a variety of proteins called cyclins and enzymes called cyclin dependent kinases. Cyclin and cyclin dependent kinases both are proteins. When they are bound with each other then it can show the phosphorylation power. There are 15 types of cyclin, 9 types of CDK. Each has a role to play at different stages of cell cycle binding of a cyclin with associated kinase. When it binds the enzyme it activates the enzyme. Enzyme is the kinase enzyme, the protein is the cyclins. This enzyme cyclin complex basically serves to move the cell from one phase to another phase.

When a cell is in the G_1 phase a decision has to be made whether to move to the S phase and start copying the DNA. This decision is taken depending on the balance of stimulatory versus inhibitory signals being received through signal transduction.

The point is that when a cell goes from G_1 to S phase then that decision has to be taken. Now, the decision depends on whether there is a need for growth or there is a need for inhibition of growth. So, it depends on the various signal processing. Ultimately the balance means the overall direction that whether to move forward or stop there. Depending on the balance of stimulatory it will say that you have to grow. The inhibitory signals says that do not grow. So it is a balance between the two. If the balance is

towards cell growth then cell division will take place and a particular type cyclin D will be increased. This binds to the kinase 4, CDK 4 and CDK 6. So, it is basically the importance of this kinases.

Adrug which binds to the kinase or binds to cyclin and do not allow the binding of the cyclin dependent kinase then that will a good way of stopping the growth of the cancer cell.

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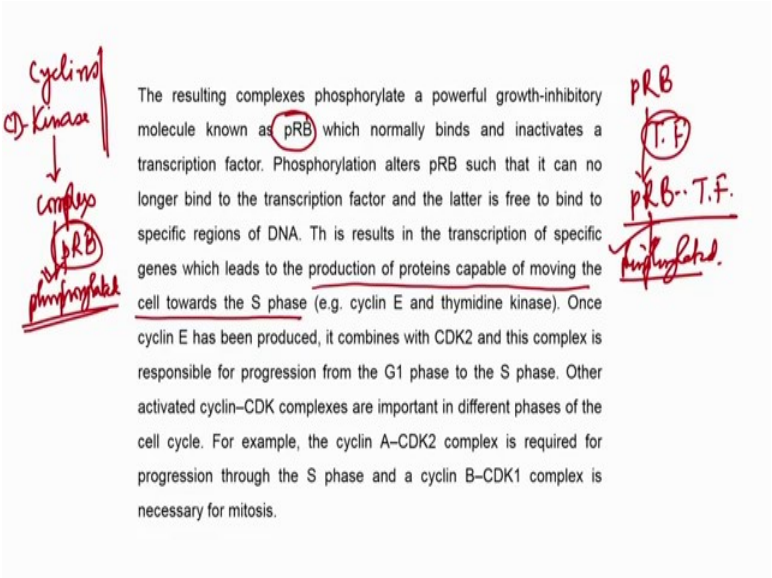

The resulting complex *i.e.* the cyclin and cyclin dependent kinase phosphorylate, a powerful growth inhibitory molecule normally binds and inactivates a transcription factor. The transcription factors are one which actually starts the process of the transcription- from where the transcription has to take place. There are some promoter sites where the transcription factor binds and then the system knows that the DNA has to be copied.

The kinase and the cyclins form a complex which phosphorylates a powerful growth inhibitory molecule pRB. This phosphorylated pRB binds and inactivates the transcription factor which is necessary for the transcription. If the pRB is phosphorylated the growth will be inhibited.

However, phosphorylation alters pRB such that it no longer bind to the transcription factor.

So, pRB usually binds to the transcription factor and in that bound form transcription factor is not active. So, it cannot promote expression of the gene. It falls off from the transcription factor. So, the transcription factor is now free to bind to the promoter region and once it binds to the promoter region there will be activation of the genes. Then that will ultimately lead to production of proteins and these proteins are capable of moving the cell towards the S phase. So, we are talking about the cell going from the G_1 to G_2 phase, so in that case pRB protein is very important.

Phosphorylation means it cannot bind to the transcription factor and dephosphorylation means now it is bound to the transcription factor. So, it is the nature's way of controlling the growth of a cell. Similarly, there are other checkpoints like when the G_2 to S phase, then S phase to the G_1, and S phase to the G_2 phase that is also will be checked and basically G_1 and G_2 are the ones which are called preparatory stages where this balance and checks are made. Then the cell is pushed forward for the M phase that is the mitotic phase.

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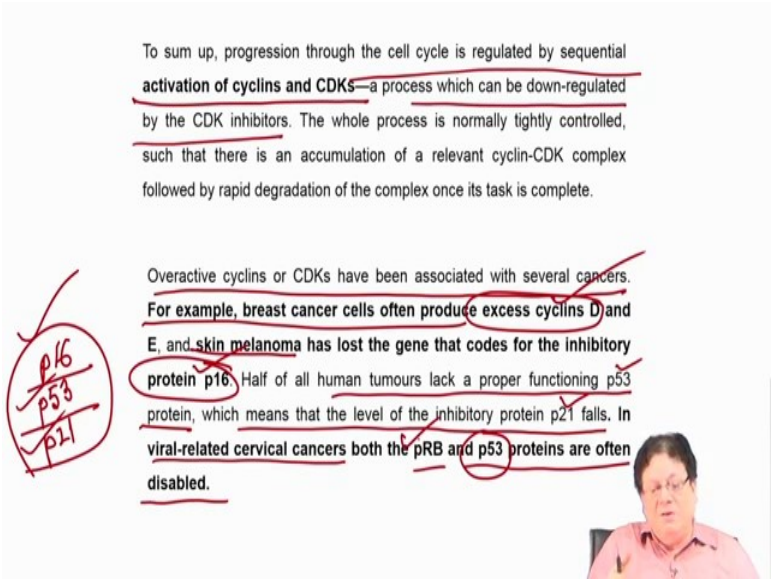

Now you can tell that this kinases are important targets for discovery of anticancer agents because that is a way to control the growth of a cell. The progression through the cell cycle is regulated by sequential activation of cyclins and CDKs. This process can be down regulated by the CDK inhibitors. When the cell moves further towards the mitotic

stage is called downstream processing. So, it will be down regulated by the CDK inhibitors.

The whole process is normally tightly controlled such that there is an accumulation of relevant cyclin CDK complex followed by rapid degradation of the complex. Once its task is complete it is tightly controlled. This complex will be formed, phosphorylation will be done and then once the job is done then again the complex will be broken.

Overactive cyclins or CDKs kinases means that the cell is going very fast. For breast cancer, cells often produce excess of cyclins D and E. Skin melanoma, another type of cancer of the skin has lost the gene that codes for the inhibitory protein. There are some inhibitory proteins which are named as p16 or p53. All these are very important. This p16 is the gene which codes for the protein p16 that is an inhibitory protein.

In case of breast cancer you see excess of cyclins. Cyclins are more over expressed in case of breast cancer. In skin melanoma, the inhibitory proteins of the corresponding gene is missing. Half of the human tumors lack of a proper functioning of p53 protein. There are many inhibitory proteins p16. Inhibitory means which inhibits the growth. p16, p53 is the original one and then there is p21. These proteins actually control the growth. Actually they have an inhibitory effect of the growth.

There is a lack of proper functioning of p53 protein. There is also the level of p21 and in skin melanoma p16 protein is lost. There are different types of cancer. There are these viral related cancers- both pRB and p53 proteins are often disabled. The virus disables this proteins which safeguards of our body. Our body has the natural defense mechanism via these p16, p53 p21, pRB. If they are malfunctioning that will be growing very fast. If there is excess cyclins it will grow fast. Cyclin activity mode means more growth.

So, we have some idea regarding the targets. Cyclins could be a target, the kinase could be a target. So, you have to see all these and that is some differences in the normal cell. You can design molecules which will activate or deactivate some of these.

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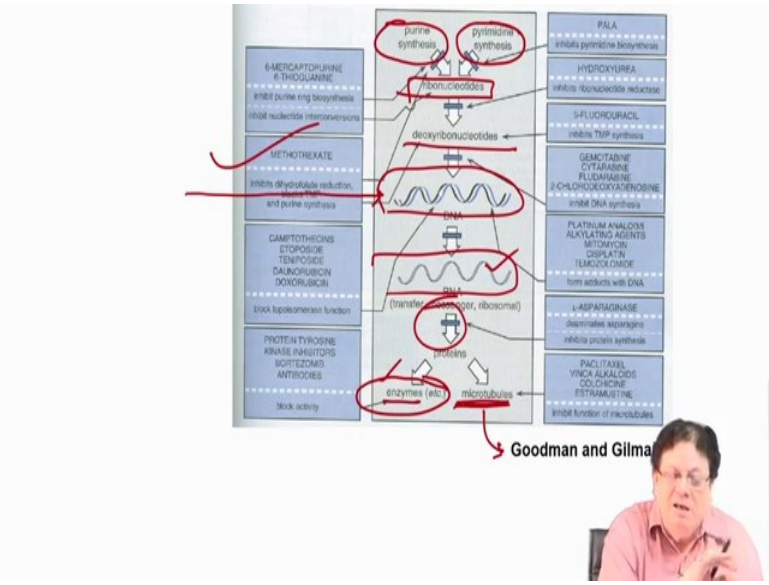

In the S phase, there is the synthesis. Now, before the mitosis this is your mitotic stage. You have to drug molecules which are needed for a cell to perform its activity.

The cell has to get this pyrimidine and the purine bases. So, they have to be biosynthesized and then you assemble sugar plus this pyridine or purine bases. Then you you get the ribo nucleotides coming from ribose. Then ribonucleotide is reduced to deoxyribonucleotide because you know that the 2 prime is missing in the DNA. But that actually comes from the ribonucleotides. Then the deoxyribonucleotides basically form the double stranded DNA and from the DNA you get the messenger RNA. Finally, the messenger RNA gives the proteins which are enzymes and they also produce other proteins. These are called microtubules which are extremely necessary to segregate the cell into daughter cells in mitotic process.

For the cell to complete the S phase and then go from N to the G_2 you can stop any of these. If a cell is in the resting phase you can target the cell by targeting any of these processes. So, everything is a target. You can target this enzyme ribonucleotide reductase because the deoxyribonucleotides will not be produced. You can directly target the DNA so that the process of transcription does not take place or replication does not take place. Then you can target the messenger RNA also. If you use antisense compounds which binds to a portion of the RNA the translation process will be stopped. So, you can stop the translation process.

At the enzyme level, you can also target different enzymes which are vital for the growth of the cell. Also microtubules can be targeted because it does the cell division. So, these are the different targets and on both sides you have different kinds of drugs.

The best target is to destroy a cell because DNA is basically the commander in chief of this cell cycle. All information is coming from the commander. If you kill the commander in chief the whole battalion will fall.

The major target of anticancer drug development is the nucleic acids and followed by other different processes.

Methotrexate, an anticancer drug inhibits the dihydrofolate reductase which is important for making the thymine from uracil. So, that is one of the earliest anticancer drug that was developed.

First we will go by the DNA as the target.

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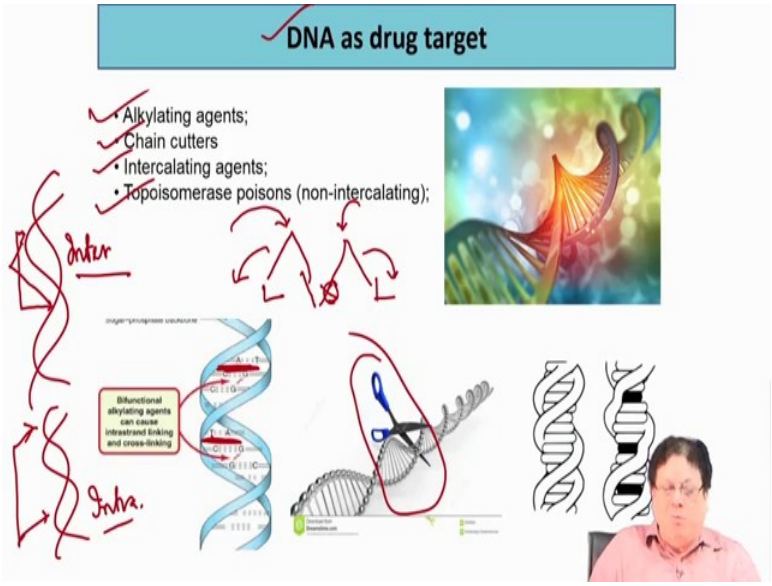

You can target the nucleic acids by many ways. One is by using alkylating agents. What are alkylating agents? Alkylating agents are nothing, but like if you have a phenol if you add methyl iodide the phenol will form OMe. If you add say again methyl iodide or dimethyl sulfate or even diazomethane to a nitrogen amine that will form the N-alkylation. There are different alkylation- N-alkylation, C-alkylation, O-alkylation, S-alkylation all these are possible.

In DNA, the most nucleophilic part is the bases. We have nitrogen bases which can act as the nucleophiles. Guanine is imidazole ring fused with pyrimidine ring and that imidazole nitrogen is called N-7 nitrogen which is the most nucleophilic part. Alkylating agent should have a good leaving group. A nucleophile comes, attacks and these leaves. If you have a bisalkylating agent, so one nucleophile comes from here, another nucleophile comes from here and both leaves.

In DNA, there are different types of cross linking. This cross linking is between two strands. Basically after cross linking it will become something like this. So, that will be bend at that point. But the most important point is that as there is a cross links these two strands cannot be separated.

Other type of alkylation can happen in the same strand. This is called intra strand cross linking. The whole thing becomes a kind of an oval shape at that point and that also fall out of these replication. Transcription stops because you have a deformed DNA in this case and there is a knot which you cannot release. So, that is alkylating agent.

Chain cutters, that is even more direct that you have an agent which cuts the double strand DNA into two pieces.

DNA is double stranded and you have these bases. There are certain planar aromatic compounds which can go inside. There is lot of space between these base pairs and then another flat aromatic ring can go inside and then sit there and stabilize the DNA. It is called intercalation. If there is intercalation it will require lot of forces. There will be change of shape because the DNA has to change a little bit to accommodate that intercalating agent. It gets a stabilized because it is called pi stacking. The base pairs have a π cloud and then you have an planer aromatic kind of system and then another base pair. There is always pi stacking which stabilizes the DNA.

Another way of stopping the transcription is topoisomerase inhibition. When the DNA unwinds there is this supercoiling in front. So, topoisomerase takes care of that supercoiling. But if you can inhibit the topoisomerase then also your transcription will stop. So, these are the 4 classes of agents that will target the replication and the transcription process.

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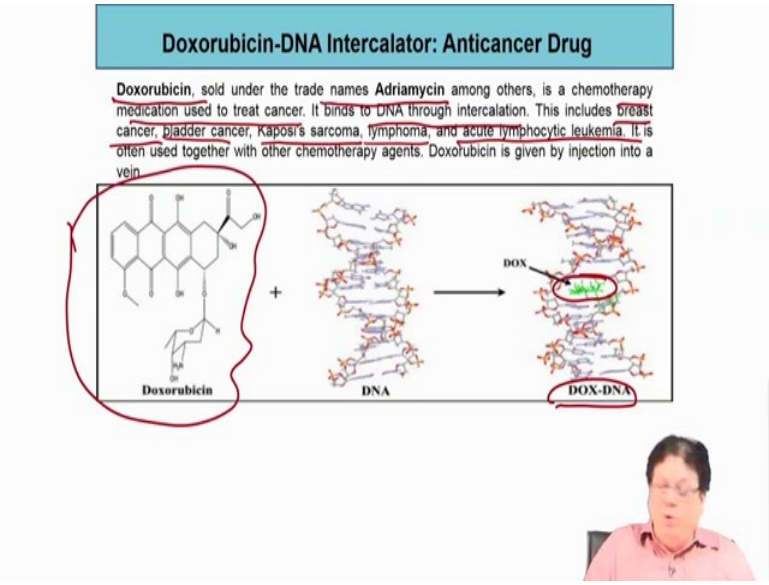

The first which works via intercalation is called doxorubicin. Doxorubicin has this aromatic ring. It is an anthraquinone based compound called anthracyclines. They are planar. So, they go inside the double stranded DNA, so this is DOX-DNA. When it is inside DNA it cannot replicate or transcribe properly. Trade name of doxorubicin is Adriamycin. It is a chemotherapeutic medicine to treat cancer. It binds to DNA through intercalation.

Basically weak forces play part here. No covalent forces are working. It is used for many cancers like breast cancer, bladder cancer, Kaposi sarcoma (a kind of skin cancer), lymphoma, leukemia. As there are different types of cancer only one single reagent cannot cure the cancer. So, you have to give a cocktail of different anti-cancer drugs. Whenever doxorubicin is given at the same time other anti-cancer drugs are also given which work on a different principle. Also the cancer cells become resistant to the drug which is used earlier by efflux mechanism. I told you that the some of the bacterial cells also have that efflux mechanism that it pumps out the entering drug. So, these cancer cells also have very efficient efflux mechanisms and then can remove the drug very quickly.

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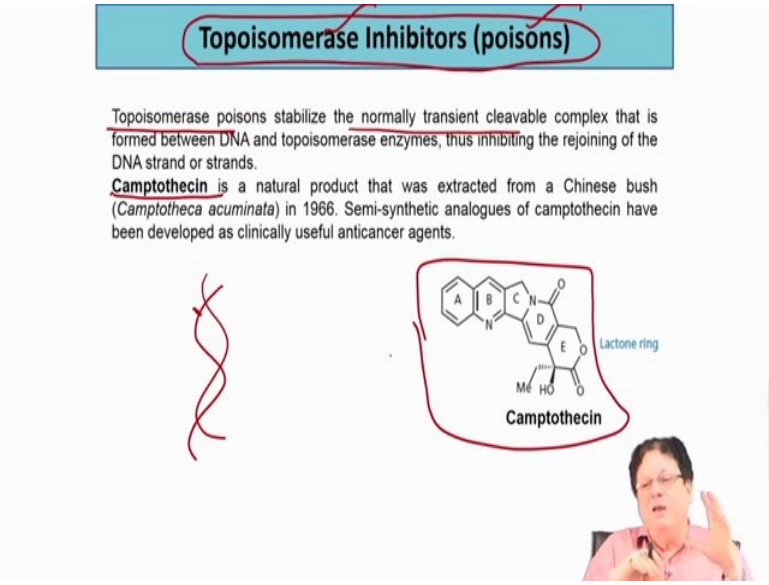

You can also get anti-cancer drug by inhibiting topoisomerase and a very important natural product acting as topoisomerase inhibitor is called camptothecin.

During transcription there is supercoiling ahead of the replication fork. So, to reduce the supercoiling the topoisomerase forms a nick at some point and then it turn over the other strand.

In this case, topoisomerase forms the nick and then this nick is basically stabilized by the topoisomerase. Topoisomerase and the nick DNA complex is very transitory. It has to be transitory because as the replication fork moves it has to again join it back. Camptothecin molecule or this type of topoisomerase inhibition molecules stabilizes that nick DNA topoisomerase complex. It is usually sometimes called poison. It is a topoisomerase poison. Truly, it is not an inhibition. Actually, the topoisomerase first does the job of nicking forms a transient complex and the transient complex again is broken. The nick is again repaired but here that repairing process cannot take place because the transient complex is stabilized.

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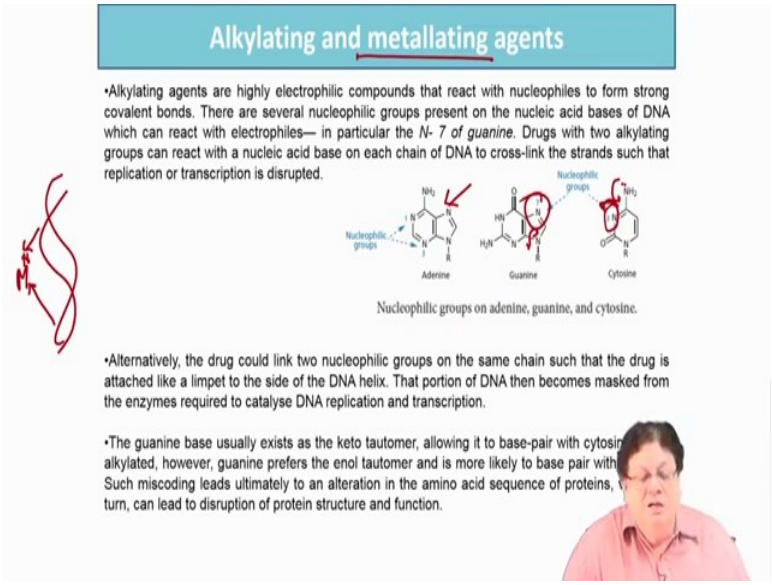

People have made different analogs of camptothecin and those are also used as drugs. Camptothecin has a quinolone an isoindole moiety. It is also quite planar. It can stabilize the nick DNA topoisomerase complex.

Now, let us come to the alkylating agents. It also says metallating agents because a metal ion can be stabilized by nucleophiles which can be good ligating agents to metals. This is also possible that a metal ion is bridged between the two bases of different strands and that also forms double metalation. Double metalation is nothing but double alkylation.

N-7 nitrogen is most susceptible to the N alkylation and also N-3 nitrogen lone pair also increases the nucleophilicity. Let us see what the structures of these alkylating agents are.

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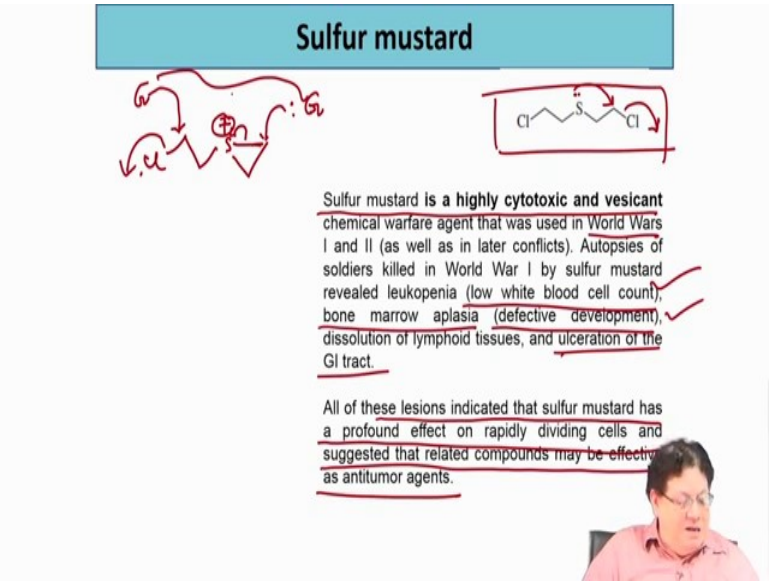

There is a history behind discovery of this alkylating agent. There is a compound which is called sulfur mustard because it is yellowish in color and looks like a mustard. Mustard also has yellow color. Sulfur mustard is very interesting because it is a double alkylating agent. The alkylation proceeds via neighboring group participation and the sulfur first attacks the carbon, then the chlorine is lost and that forms an episulfide, like your epoxide. So it is an episulfide and then you have this CH_2Cl. Now, suppose the G with the nitrogen attacks there and the sulfur opens up and the process is repeated again.

This sulfur mustard is a highly cytotoxic agent and vesicant. Vesicant means which causes blister and it was used in World War I as well as World War II and recently in the Gulf also this has been used in Syria and parts of Iraq. During World War I and II, this was used as a chemical warfare agent. It was found that the persons who have been exposed to this sulfur mustard develop Leukopenia. Leukopenia is a low white blood cell count and with aplasia (defective development of bone marrow) and also there is ulceration.

All these things have a profound effect on rapidly dividing cells suggesting that these compounds might be effective as anti-tumor agent. So, this is basically direct fallout of the World War I and then mustards came into the market after the World War II. Apart from producing the blisters, it stops the rapidly dividing cells from dividing. The rapidly dividing cells are cancer cells. So, it will have effect on the cancer cells.

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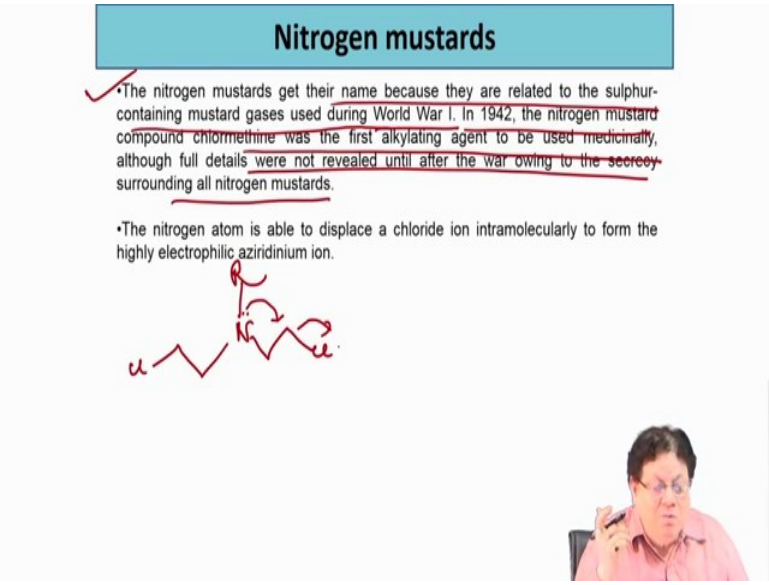

However, the sulfur mustard is extremely unstable. The nitrogen mustard was resorted. They nitrogen mustard are related to sulphur containing mustard gases which was used in World War I that I already said. Nitrogen mustard gives you some ways to manipulate the structure because sulphur is bivalent. So, you cannot attach anything on the sulphur, except the two alkylating arms. But if you have a nitrogen then apart from these two chloroethyl handles *i.e.* alkylating agents you have NH here. So, you can put an R group here. R group can give you the stability.

Instead of the episulfide you will get aziridinium ion as the transiently formed reactive species. But here also this anchimeric assistance is taking place.

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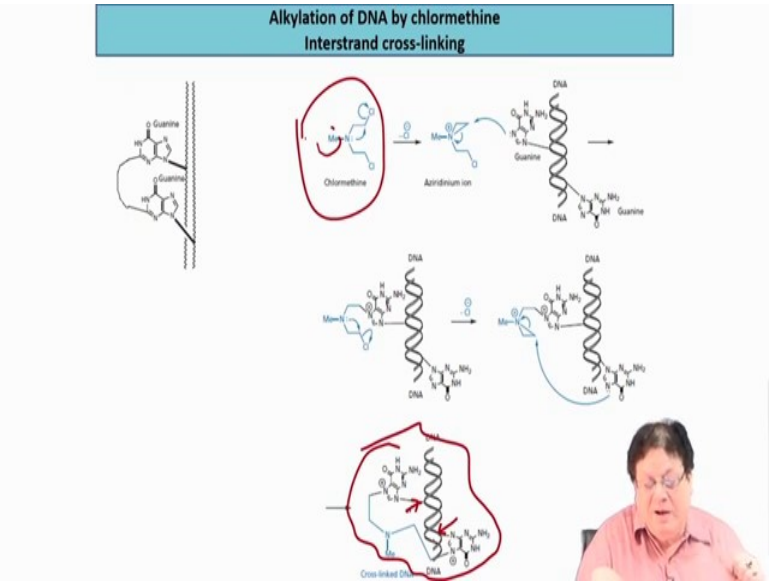

Now, depending on the R you have different alkylating agents. The first alkylating agent which is used as the drug was this chloromethane. Chloromethane is nothing, but you have a methyl here. The guanine is attacking here and then another guanine is attacking the other aziridinium ion. You have an intermolecular cross link.

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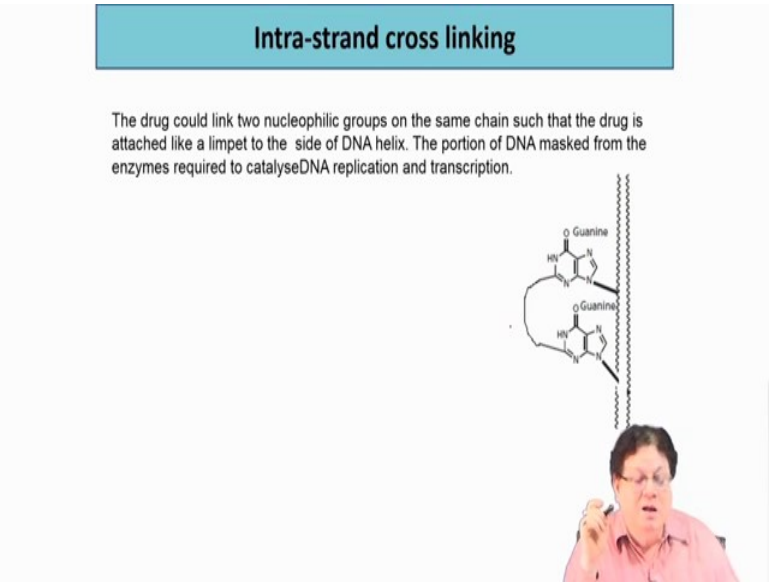

You can have intermolecular also like this, guanine from the same strands are attacking with that nitrogen mustard. Basically, this will stop the transcription process.

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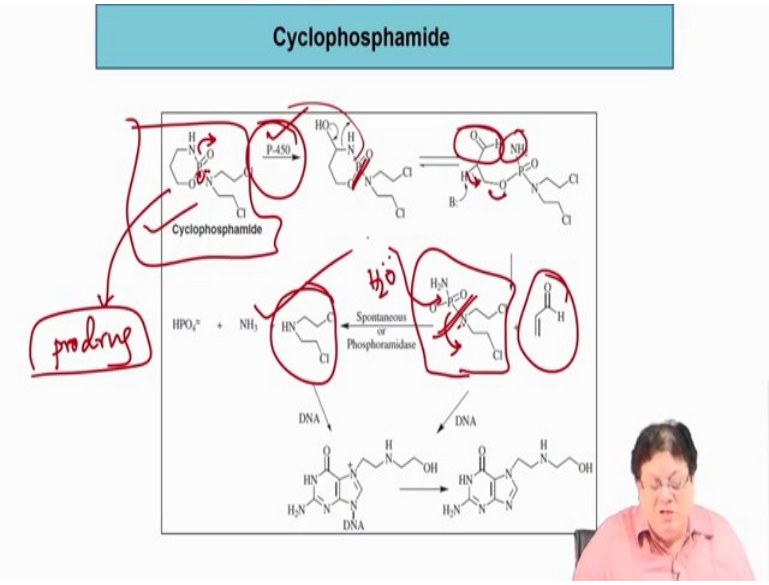

However, these molecules were very toxic because these small molecules can react with other protein molecules inside the body before entering a cancer cell. They are not the very good anti-cancer agents because of their toxicity. They can destroy the cell very rapidly, but they are not very specific. Ultimately research gives rise to the drug cyclophosphamide.

In Cyclophosphamide, the nitrogen lone pair is delocalized with the phosphorous oxygen double bond. So, the nitrogen lone pair cannot form the aziridinium ion because the nitrogen is lone pair is locked because it is towards the phosphorus oxygen atom which is a more electron withdrawing group. It is not a drug. So, you have to activate it. Activate means ultimately you have to break this nitrogen phosphorous bond.

When it goes through the liver oxidation takes place (specifically hydroxylation) in presence of a mono oxygenase enzyme P-450. Mono oxygenase does hydroxylation by delivering one oxygen atom. So, hydroxylation takes place at this carbon and then produces an aminal. Aminal means like acetal, but here one of the oxygen is replaced by a nitrogen. This is also not stable. So, this becomes an aldehyde, the amine is free. Once it becomes aldehyde the hydrogen is lost here. So you get acrylaldehyde. Still the nitrogen is locked with the phosphate. After that, there is a hydrolysis. This is spontaneous. You can hydrolyze this molecule, water comes and then breaks this. So, basically it releases the nitrogen mustard in the actual form where the nitrogen is NH.

It can alkylate the DNA and sometimes when it becomes too reactive water can also displace the chlorine. So, there could be mono alkylation, there could be bisalkylation also. But this is the way. In mono alkylation also it is difficult for the DNA to replicate because the DNA will be miss copied, they may not realize that this is a guanine, ok. So, there may be miss copying possible. So, this is the mechanism. So that means, cyclophosphamide is what is called a pro drug. Pro drug means it is not the actual drug, but in the biological system it is activated and the actual drug is nothing, but this compound and that is released. So, that is all about the nitrogen mustard.

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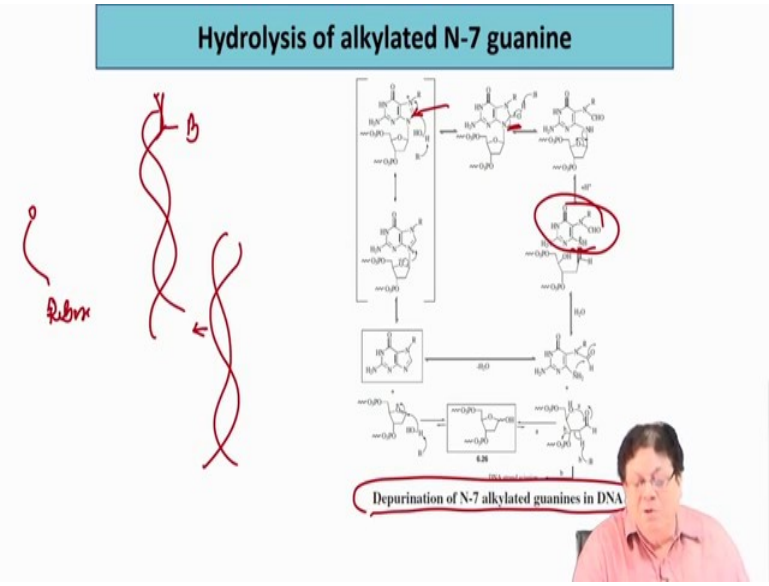

When you add the alkylating agent the guanine is the specifically is more preferentially alkylated and as a result there will be bisalkylation because it is a bisalkylating agent and this can be intermolecular or intramolecular bisalkylation. Both cause the stoppage of the replication and the transcription process. Another thing that can happen -the DNA can lose the base.

This type of alkylation takes place but in one of the DNA the base may not be there. So, it is basically only deoxyribose and the phosphate. No base is attached at the site. This is what is called abasic site. Abasic site means the base is not there; that means, there is deep urination or what if you say that the guanine has left. How does it left? You have an alkylating agent, dimethyl sulfate followed by hydrolysis. So, that takes care of the first the base goes out and then the phosphodiester bond breaks.

But that breakage is carried out by piperidine. Here the base is not present means piperidine is not present. After the alkylation, N plus water comes and attacks this carbon, and ultimately results in the hydrolysis of the base. If you look at the mechanism plus charge water comes there so that the electron goes here. If this is an aminal this will become an aldehyde and that will become a nitrogen NH. Then NH opens up the sugar and imine is hydrolyzed.

So, basically the base goes out. One of the bases, usually guanine comes out. If the base is missing the polymerase can now interpret that it could be any base. So it can produce different base which is not required in the mRNA. That also causes mutation.

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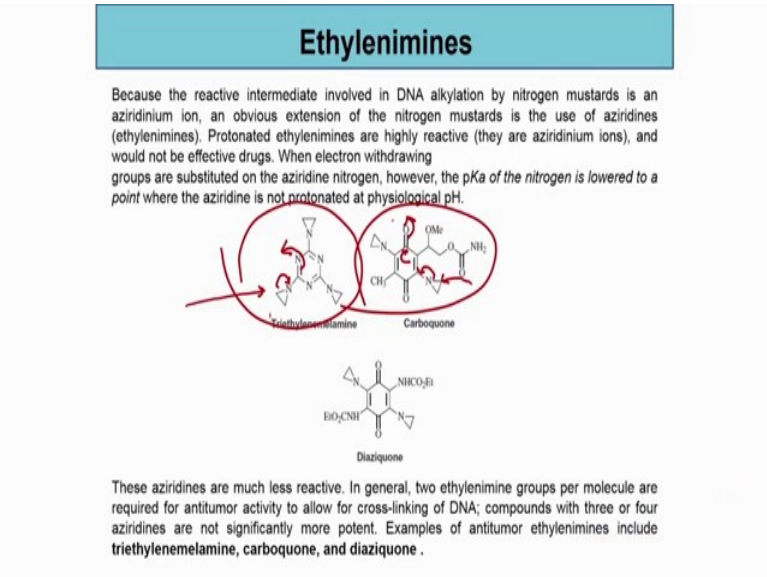

This is all about this all alkylating agents. Now, they have made different types of stable alkylating agents which are quite interesting. You can start with aziridine because these are not are aziridine ion. Aziridine ion will be more reactive. So, you can have a simple molecule like this. This is a tri-alkylating agent. If the nucleophile attacks here that goes there. Because of this amine nitrogen, the bond breakage becomes very fast. So, it is a tri-alkylating agent. This is a bisalkylating agent.

Here the driving force is that the nucleophile attacks here, that goes there and ultimately that becomes O minus. So, different types of molecules are possible. All are working by alkylation and but these are more stable than what we have said earlier. Cyclophosphamide is quite stable because the nitrogen lone pair has been locked in a

product form to start with. So, that is all about the bisalkylating agents. In the next session, we will talk about the other kinds of drugs.