Biophotonics
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Lecture 36
Introduction to Photodynamic Therapy (PDT)

Hello, and welcome, we will continue our discussion on Biophotonics and today I have for you a very-very exciting and very new and very promising new field of Light-Activated Therapy. Remember that one of the two major goals of biophotonics is cure, curing of human ailments. Thus far we have seen how light could be used for imaging, imaging beyond diffraction limit, imaging at a very-very small scale, imaging just few cells or intracellular components.

At the same time, we have seen that how light could be used for sensing, sensing or detection, we are able to detect single molecule single viruses or different types of pathogens or early detection of cancer using optical biosensors. So, light has thus far as I have told you, we have seen is being used to both image as well as to detect and since diseases, pathogens, things that cause diseases etc.

This time in this particular module, we are trying to see if we could use light for curing any such disease that we have either imaged or we have sensed or detected and that could be there could be a plethora of different types of diseases both communicable as well as non-communicable something that has been caused by an external agent like a pathogen or something that has triggered in your own body.

Say for example, some kind of gene mutation have taken place as a result it has, it has caused cancer or some sort of an auto immune disease. So, we are going to discuss this new topic and this very interesting exciting topic of light activated therapy, photo therapy and in this particular case, we are going to discuss about photodynamic therapy, how to utilise light to cure certain specific diseases. So, let us get on to it. What exactly is Photodynamic therapy?

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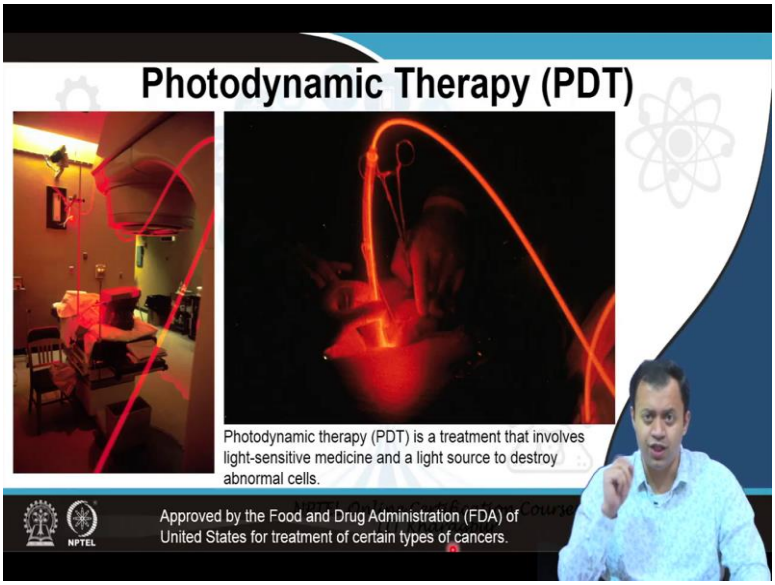

So, photodynamic therapy is a treatment that involves very-very simply very-very simply, PDT Photodynamic Therapy is a treatment that involves light sensitive medicine and a light source to destroy abnormal cells.

You produce or you inject into the body some sort of a medicine that is sensitive to light, you excite that specific medicine with light with specific bunch of photons with specific wavelength, with specific energy of photon, that light activates the medicine and then the medicines perform some sort of a function a biochemical reaction, say for example, upon activation, by light this medicine, this drug, this chemical agent performs certain chemical reactions biochemical reaction and that in turn destroy abnormal cells.

So, these are two of the major free picture that I could get, usually the light source is laser and you are delivering it into the human body this is an actually a keyhole type surgery, person's body, a certain section of the person's body has been opened up, you could see the scalpel and scissors and thereby they are inserting a tube which is basically a optical fibre and that is containing the red laser light and that red laser light is used to damage, destroy annihilate cells and this therapy, this photodynamic therapy is approved by the Food and Drug Administration, FDA of the United States.

This is one of the largest, Drug Administration company, Drug Administration agency not company. It is not a private company. It is a federal government's branch; federal government's agency of the United States and it is actually a very big deal to get FDA

approval. Several of the vaccines that you are seeing these days going around for Coronavirus detection, coronavirus prevention as well as several other types of vaccines and drugs, they need to get FDA approval if they want to sell their product in United States or territories that are under the jurisdiction of United States.

And it is usually the case that they are one of the stringent agencies and if you are able to get it passed through them, it is easier for another agency to approve as well. Not always the case, not always the case. There are exceptions. But this is a bona fide, the point that I am making is this is a bona fide approved therapy for treatment of cancers, you know about chemotherapy.

I told you in the previous classes, the introductory classes that I will be taking phototherapy or light activated therapy and this is the one which it is, this is photodynamic therapy approved by one of the highest agencies of the United States government and we are seeing more and more use of PDT photodynamic therapy in treatment of cancer. So, what exactly does PDT do? What is PDT in a more detailed way? Let us try to understand that.

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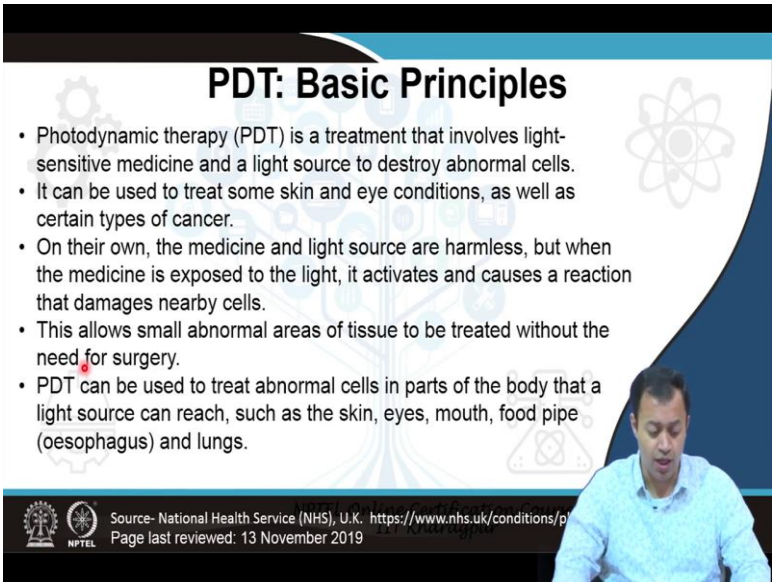

So, as I said photodynamic therapy is a treatment that involves light sensitive medicine and a light source that destroys abnormal cells very easily and it is used to treat some amount of skin and eye condition as well as certain types of cancers. It is though heavily used for the treatment of cancer, you will see that it is being used for other such conditions as well, including it has found applications in ophthalmology, dentistry, as well as dermatology.

On their own, the medicine and light sources are harmless. But when the medicine is exposed to light it activates and causes a reaction that damages nearby cells. Again, the medicine is light sensitive, the medicine will only work when it has been excited by a specific wavelength of light or group of specific wavelengths of light.

Maybe one maybe more than one, but there is a specificity of it, by itself the laser is harmless, the medicine is harmless you combine them to the medicine then starts producing some kind of a biochemical reaction with the surrounding area and that this surrounding area, this biochemical reaction that this medicine causes, ultimately destroys the surrounding areas, which happens to be either the cancer cell, the tumour, the malignancy or something else that where the medicine has been injected. It allows small, normal areas of tissues to be treated without the need of surgery and this is the most important part.

It does not require surgery and it has been used to treat abnormal cells in different parts of the body such as skin, eye, mouth, food pipe oesophagus and lungs and this is also approved by the National Health Service of the United Kingdom. This is the governmental health service. NHS I myself am a beneficiary of NHS it is a fantastic service and NHS is the government of United Kingdom's, government of Great Britain and United Kingdom's health service, almost free medical health service almost and it also utilises it.

So, I am not telling you something that is coming up in the future. Yes, it is coming up in the future, but certain section of it is already regularised, already optimised, already approved by various Health Organisation various health approval agencies all over the world and one of the major important advantages of phototherapy light induced therapy is that the tissue can be treated the disease can be treated without the need for surgery.

Now the medical students listening here will give you a whole plethora of reason why several times surgery is not that beneficial you are opening up a tissue and you are producing some sort of incision though here as well like in the previous slide I told you that incision could be done, but on several cases, PDT can avoid surgery altogether.

PDT can avoid surgery altogether and there are several areas where cancer may form which is an operable like say there is a tumour somewhere in the middle of your brain it is very-very difficult to take it apart take up the cranium, take apart the skull and taking your scalpels and scissors and whatnot inside the brain and taking that tumour out without damaging the other parts of the brain and thereby risking permanent paralysis or even death.

So, there are several areas where cancer can form which is simply inoperable which cannot be touched by surgery. PDT has the potential, mark my word has the potential to go into those areas light could be shined either externally or by making some kind of a keyhole not exactly going to that, but the light can be you know made to penetrate through the tissue and thereby activating the medicine that it has, that had that has attached to the tumour and then causing the biochemical reaction, the biochemical reaction in turn destroy the tumour.

So, that is the overall very rudimentary very basic principle without getting into too much medical jargons. I mean when I was doing my own study my own research on PDT, I had to take a mini course on cancer and believe you me that is incredibly rich as well as complex field and hats off to those medical students and scientists to spend their lifetime studying cancer and oncology and becoming specialist in oncology, this is truly a fascinating and very-very complicated subject, we the people who are staying outside have, are hardly scratching the surface, what is carcinoma, what is melanoma, these are very basic things.

But then how complicated it goes and how heterogeneously the cancer spreads all of those things are quite complex and quite fascinating and I thank all of the oncologist whosoever is listening for your work. So, let us move forward.

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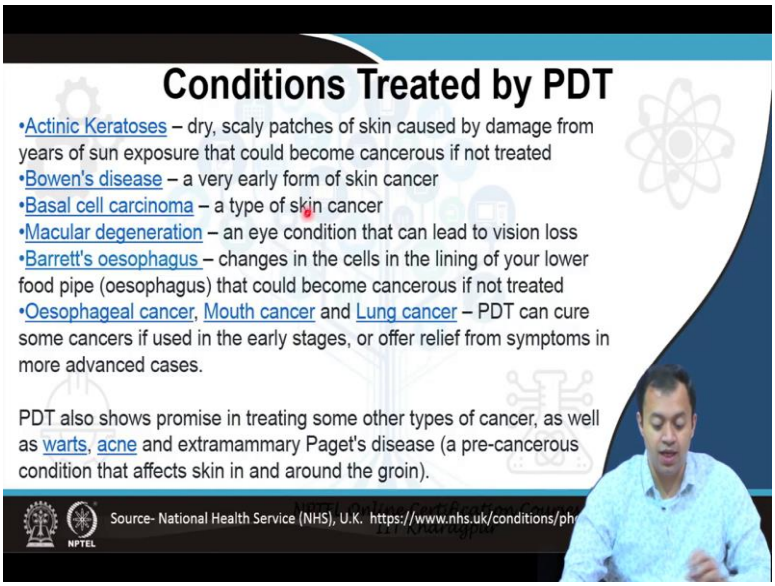

What are the different specific conditions that are treated by photodynamic therapy, well you can read several of them I am particularly interested in Macular degeneration, Macular is a specific part of your, usually central part of your retina and as a person becomes old this macular region simply degenerate, a part of your retina simply degenerate resulting in a

severe amount of vision loss, you do not usually get completely blind, but partial blindness can be caused.

So, and we do not know what is the main reason triggering macular degeneration, you must have heard several old people, after age reaching a certain age they are losing their eyesight, not necessarily always because of cataract or because of some kind of eye injury there is something significantly called macular degeneration, it is a part of your retina simply degenerate, a part of your retina simply refuses to take light and there is a degeneration forming and because of that your vision is partially lost.

Then there are other types of various types of cancer, Basal cell carcinoma, you have Barrett's oesophagus where there is a change of cell lining in your lower food pipe, mouth cancer, oral cancer as I said technically oral cancer is an Indian cancer, oral cancer is mostly prevalent in Indian subcontinent area, simply because we chew tobacco, this is our lifestyle we chew tobacco rather than smoke tobacco.

In Europe and North America, those people who smoke tobacco, they develop the risk of having lung cancer, but chewing tobacco beetle nut or different types of those pan masala etc, causes mouth cancer, oral cancer per say, mouth cancer is the overall set oral cancer is a subset and they can be treated using photodynamic therapy without the requirement of doing a full-fledged surgery and without doing a specific chemotherapy, without doing specific chemotherapy phototherapy, this is phototherapy light is being sent to destroy the abnormal cancer cells.

In chemotherapy, a drug is specifically target to destroy highly replicating highly dividing, highly multiplying cancer cells. Here it is the light that is being generated, the light is causing some kind of a biochemical reaction that is in turn destroying it has also been used for warts acne and other extra mammary Paget's disease these are several precancerous conditions, various skin conditions and my source here is National Health Service United Kingdom.

I am not randomly taking these names out and saying that PDT could be source, these are actual things, these are actual diseases specific, specific diseases that have been treated that is already been treated and the complete procedure exists for light-based therapy for photodynamic therapy of all of these and you will look most of them take care of the skin cancer part. Skin cancer to the best of my knowledge is a little less prevalent in India, we mostly have mouth cancer, breast cancer etc, but they are the one of the first to utilise PDT.

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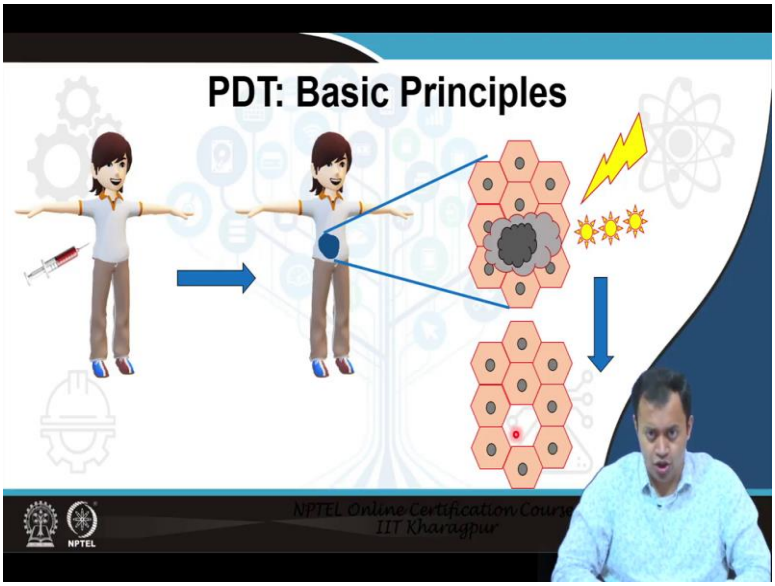

So, how you, what are the procedures, what exactly you do in photo dynamic therapy, what exactly is done, the patient comes and the patient is injected a specific drug, this drug is the PDT drug, this drug is the one which gets activated, this is the medicine, this is the drug that will only get activated upon exposure to light, this drug will only get activated upon exposure to light, so after the drug has been injected into the patient's body, the patient need to wait for something around 24 to 72 hours, it depends on the drug and that drug gets accumulated in the tumorous region, the drug gets accumulated a specifically at the area which is supposedly abnormal which is showing metastasis, which has formed a tumour which we need to treat.

So, the drug specifically gets accumulated at the cancerous growth at the growth which is causing problem which is, which is the basically the disease forming. So, this drug after 24 to 48 and 72 hours get accumulated at a specific location within your body where you have the tumour, then we induce light into that particular area.

So, this is the zoomed in version, this is the zoomed in version of the area, this is the zoomed in tissue, these are the normal tissue, this is the overall tumour that has formed, this is the overall tumour that has formed, this is the cancerous growth, this is the metastasizing part and you shine light onto it.

The photons create some sort of a biochemical reaction of that drug, which is attaching itself selectively by this time you know how we can attach specific bio molecules to specific location, remember we discussed this in the previous class, we do mostly antibody antigen complexes these are very specific, there are other processes, aptamers are coming up very

strongly, some sort of electrostatic procedures or some kind of thiolation, these are the way in which a specific drug could be attached to very-very specific location of a particular region of a cell or a tissue.

So, your drug has mostly been accumulating in the cancerous growth, you shine light, the light creates some sort of a biochemical reaction the light induces a biochemical reaction onto the tissue and the tissue therefore gets destroyed the tissue therefore gets and annihilated that tissue therefore gets burnt, dissolved, vaporised or simply annihilated leaving the nearby cells which the drug has not accumulated into with minimal damage, please put an underline over the term minimal damage, minimal damage, does not mean that there is no damage, minimal damage.

So, this is a very-very localised therapy, this is very-very localised therapy ideally only the cancer cell is destroyed, only the cancerous growth, only the group of cancerous cells only the cancerous tissue is destroyed, leaving nearby areas, nearby surrounding areas healthy or untouched or minimally damaged, that is the overall basic principle of your photo dynamic therapy.

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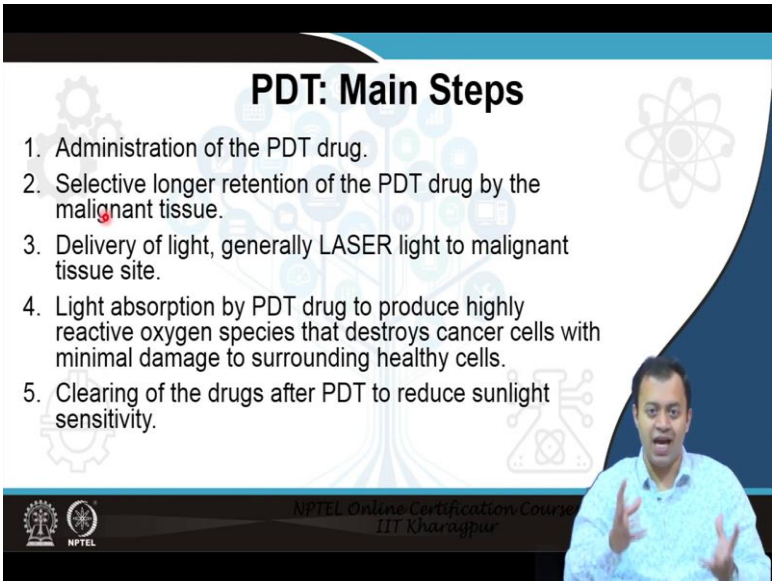

Now, again, I am just repeating myself the whatever I showed in the cartoon in previous step, this is something which I have written down because many of you in the feedback asked for notes I have previously just given you the cartoon and have spoken to you about it, but many of you are complaining that you need detailed description detailed notes, otherwise you are having difficulty preparing for exam and that is alright.

So, I have written what I previously, in the previous slide I just I just mentioned that you administered the PDT drug, there is a longer retention of the PDT drug by the malignant tissue, malignant tissue is the cancerous tissue, the cancerous growth, delivery of light generally laser light into malignant tissue site, either by entering through a fibre or if it is on a skin area, you can externally shine light on to it, if it is some sort of an orifice, if it is reachable through orifice, you just insert or if it is somewhere else, you make some sort of a keyhole surgery and then insert the light.

Remember, if this is the tissue light has to reach displace, it does not mean the fibre has to reach this place, the fibre can be few millimetres a few centimetres away as long as the light just like your torch light, just like your torch light you switch it on and the light emanates out of it.

So, even if your fibre is quite far away from it, if you have made sufficiently high intense light and you have ensured that this light is not scattered or not too much absorbed by the surrounding tissue, you have chosen a specific wavelength, a specific energy specific wavelength specific intensity, it can penetrate through or some amount, a substantial amount of it can penetrate to some amount will always be absorbed or scattered, it can go through and activate the medicine that is in that cancerous growth area, thereby causing a chemical reaction and destroying it.

So, the fibre does not need to be directly in contact with the tumour, the fibre does not need to be directly in contact with the tumour, the fibre could be far away, it could be like a lamp, a source of light, it could be like a lamp like a light bulb, like torch light, something of that sort, that as long as somehow the light the photon the energy is able to reach the tumour site, which contains the medicine.

That is the overall thing and what is the biochemical reaction, so for all these slides I have been harping about a biochemical reaction is forming a biochemical reaction is forming, what is the biochemical reaction, it creates highly reactive oxygen species. The biochemical reaction is formation of ROSS Reactive Oxygen Species oxygenated singlet or triplet oxygen states, singlet or triplet oxygen molecules, these molecules are highly reactive, chemistry students call them radicals.

You should know irrespective of whether you are a student of chemistry or not, you should know what radicals are, free radicals are, you should know I am not going to define what free

radicals are, they are oxygen radicals, they are reactive oxygen species, they are highly, highly reactive oxygen molecules and they basically oxidises the surrounding area, the surrounding area being the tumour in which the medicine is present.

So, you have a medicine which is attached to the tumour, you are shining light onto the medicine, the medicine is creating oxygen species, this oxygen species is oxidising the surrounding area, what does oxidation means? Burning. Oxidation simply means burning, it is burning its surrounding areas the surrounding areas are the tumour cells and since it is a very very localised state, since a particular dose have been optimised, a particular amount of drug is present there.

It is producing a specific amount of oxygen species, depending on how big or how small your tumour is based on that, based on that you generate, you destroy the localised cell, you destroy the tumour cell without harming any other cell in the nearby area, basically you optimise, you calculate how much amount of drug can produce how much amount of oxygenated species, this much amount of oxygen species is required to destroy, these many number of cells.

You make a calculation of how many cells have actually shown malignancy, how many, how much metastasis is there, what is the size, how much cancer has spread, how many cells you need to destroy. That is basically the overall thing.

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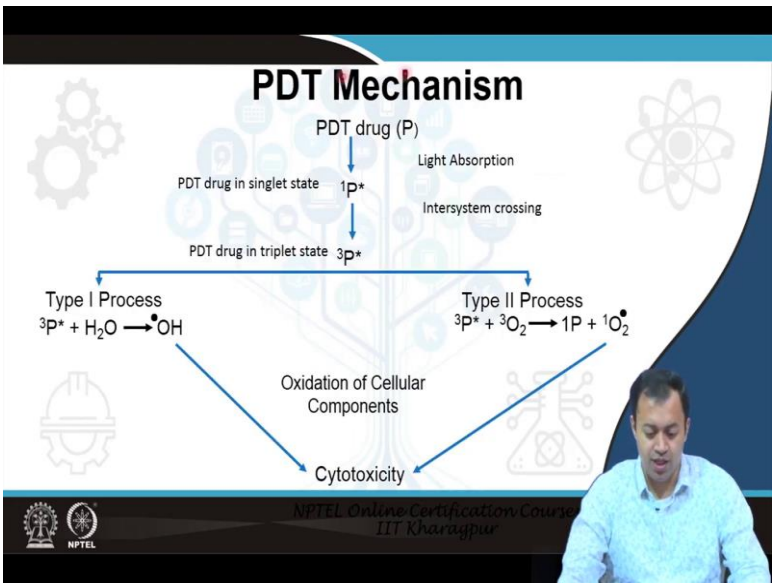

And from a chemical reaction point of view this is the overall chemical reaction now I tried to figure out but these are the usual notations used the PDT drug, which is considered P after light absorption goes to a singlet state. Remember we discussed singlet and triplet state while discussing fluorescence and phosphorescence in Jablonski diagram in I think lecture number five or lecture number six.

Where we have used the notation S1 and T1 singlet and triplet state, S naught is the ground state paired S1 is paired excited state and T1 is the unpaired triplet state. I do not know why but the pharmacy people instead of writing S1 and T1, maybe it overlaps with something else they denote they use singlet state as 1P star and the triplet state as 3P star.

So, this is the drug when it becomes S1, they put a star which probably shows excitement and one stand for singlet single is 1 and triplet stands for 3. I kind of like this one more than S1 and T1, but it is the same thing, so you should not get confused, this is the singlet state, this 1P star is equal to S1 and 3P star is equal to T1, this is paired, this is unpaired, paired excited, unpaired excited.

This shows phosphorescence, this shows what again I forgot you have to tell me what it is shows. So, I am not going to tell you singlet and triplet once again. So, the PDT drug upon light absorption becomes singlet state, excited singlet state it goes into intersystem crossing and it forms the triplet state.

This triplet state reacts with either the hydrogen or the oxygen already presents these are the two most common thing anywhere in biological cells, biological tissue biological matter, some amount of moisture, some amount of humidity, some amount of water content will always be there if we are talking about biology of this specific planet. Yes, there are exobiology which deals with biology of extra other planets. So, no, I am not making fun of you.

So, you will find oxygen and hydrogen common in most biological matter. These triplet states react with this oxygen or hydrogen produced highly reactive this dot chemistry student knows are radicals produces highly reactive radicals reactive oxygen species, they basically oxidise or burn the surrounding tissue area, oxidation of cellular components they oxidised certain areas of the cell and you will see that not entire cell needs to be destroyed, destroy some of its major components destroy the nucleus, destroy the mitochondria destroy the cell wall or the cell membrane, the plasma membrane, the cell will undergo destruction.

You do not have to completely any late every single part of the cell. The major important part destroys the nucleus the cell will die, destroy the mitochondria the energy part has been removed. So, certain parts the oxidation of cellular components certain components are burned by this this reactive oxygen species, resulting in cell death, resulting in cell death.

So, these are the free radicals, these are the free radicals that will impinge upon certain cellular components mitochondria cell membrane, et cetera, destroyed and the destruction of one such vital cell organelles will result in destruction of the entire cell, even if the cell is tumorous, even if the cell has undergone metastases, even if the cell is showing mutation, even if the cell is cancerous and what not, you destroy certain part of it and it simply falls out.

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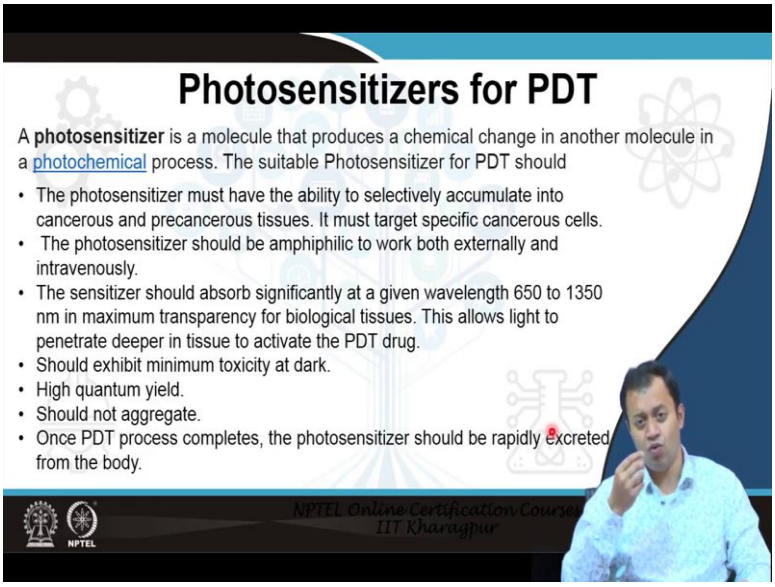

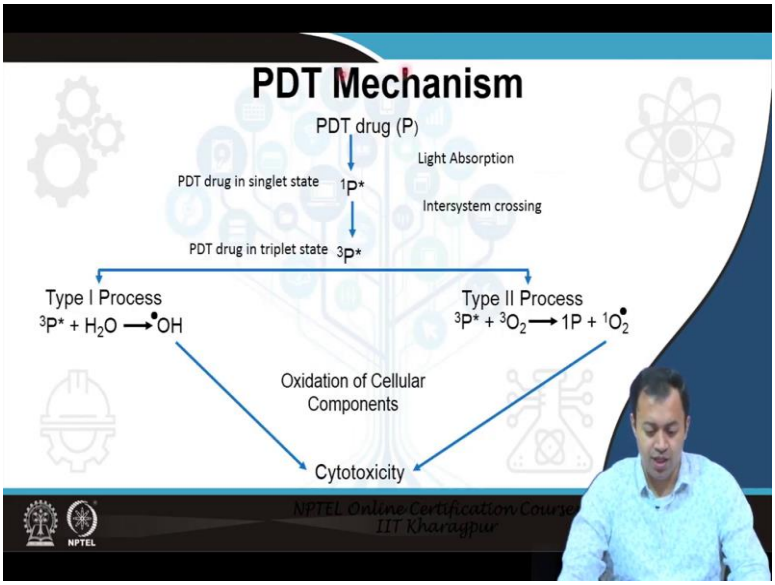

So, what is this drug, this light induced medicine PDT drug, this will only go into these different states upon light absorption, they are called photosensitizer they are called photosensitizer. Photosensitizer is a molecule or drug that produces chemical change in another molecule in a photochemical process, the suitable photosensitized, PTT should be.

So, this is this is a light activated or light sensitive molecule, which can undergo. So, PDT is the overall molecule, a part of PDT is photosensitizer just like you remember chromophore, we were discussing when we were discussing our I, not the entire molecule needs to be excited by light, you have fluorophore, you have chromophore So, photosynthesize. Photosensitizer is that part of the PDT drug that activates that gets excited by light and that activates that brings in that brings in the overall change in its singlet and triplet states.

Just like your chromophore, remember from the discussion we had about vision, how we see the chromophore, cis-retinal, et cetera, how it is getting excited by light and a chain reaction is forming is the same thing the photosensitizer is the part, is the specific part of the brain PDT drug that produces a chemical change that induces a chemical change, i.e. it induces the entire molecule to go from excited singlet state to excited triplet state and then this triplet state, then this triplet states react with oxygen or water producing radicals or producing reactive oxygen species.

And here is the drug development part, here is the most important part that this photosensitizer must have the ability to selectively accumulated into cancerous and precancerous tissue, the efficiency of this drug depends on its selectivity, it cannot simply spread all over your body and destroy everything that it has touched or it has got in touch by shining light, it has to be very-very, very selective.

So, think about how everything is coming into full circle, you do a spectroscopy first, you do a spectroscopy first and figure out what are the molecular changes between a normal cell and a cancerous cell, what molecules have changed, it has to undergo some sort of a molecular change, some sort of mutation has taken place, thereby either the protein has misfolded or there is in the DNA base pair something has changed.

Instead of AT AT, you have ATCG, adenine, thymine, cytosine and guanine some sort of base pair changes has happened resulting in some kind of molecular change from a normal cell to an abnormal cell to a cancerous cell. You understand the abnormality, from that

abnormality you develop a drug that can only attach to with that abnormality region and that is a huge area of active research.

Not everything that we have, not every PDT drug is selective, the efficiency and overall the cost also depends on how selectivity is, so that it can accumulate only in the cancerous and precancerous tissue, it must target specific cancer cell and this is the point that I figured out cancer does not spread homogeneously, it does not spread.

So, even if it is one area where the cancer is spreading, say for example, mouth cancer, oral cancer that is spreading, it is spreading at a different state, the same tissue, different areas of the same tissue will show, can show different stages of cancer, somewhere in the same oral tissue, 1 centimetre by 1-centimetre area, say 1 millimetre by 1-millimetre area, you see different spread of cancer.

So, it has to selectively accumulate to those areas, where cancer is presented at some amount of precancerous first early stage of cancer has been present and that is, that is the one of the most difficult part how to target that. Because the earlier precancerous you become the difference between normal cell and a precancerous cell is very less. Maybe you are able to detect it using some kind of an optical bio sensor.

You detect it, figure it out and then you target it using some kind of molecule. Do you see how everything is connecting together? The photosensitizer should be sensitive there should be amphiphilic, so that it has a hydrophobic part it can go inside cells, as well as it should be water soluble, partially water soluble, so that it can spread throughout the body, it should absorb significantly at wavelengths, these are the wavelengths that has maximum transparency for biological tissue.

I think if I have not mentioned it earlier, this is the window of transparency for biological tissue, biological tissue mostly starts absorbing or scattering at longer wavelength mid infrared, I have mentioned that and this allows more amount of photons to simply penetrate and it should exhibit minimum toxicity as dark, at dark meaning you have not shown the light.

If you have not shown the light, the drug should not work. That is the point, that is the point what is the point of having photo dynamic therapy if your drug does not require light and simply start killing or oxidising anything that it comes in touch with. High quantum yield

should not aggregate and most importantly, once the process is finished, i.e. when you have oxidised the metastasis tissue when you have destroyed it, the photosensitizer whatever is remaining of the drug should be rapidly excreted from the body.

So, you have a drug that got accumulated into the tumour, the tumour destroyed, whatever residual is left should be excreted out of either renally discharged or fecally discharged, it should be simply removed from your body, that is the type of material that we are looking for.

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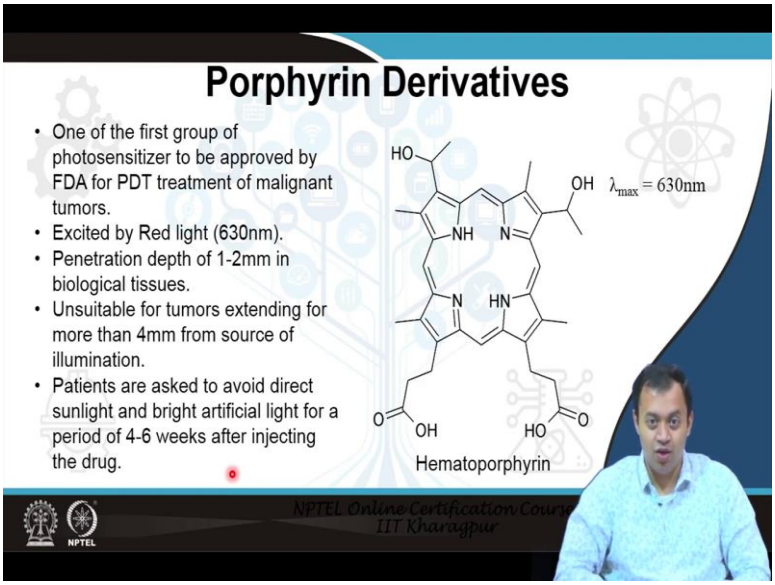

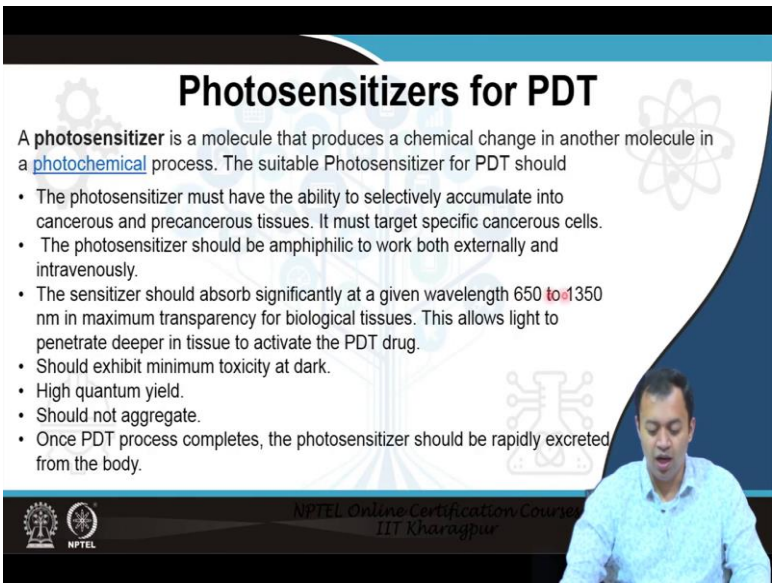

One of the earliest such a drug is this porphyrin, hematoporphyrin this is the drug. If any pharmacy students watching this, I want to give a shout out to you as well you guys are also

awesome. They spend their lifetime developing these sorts of drugs, which have these either some sort of a functionalized group which gets attached to specific tumour sites, think how difficult the processes and how much accuracy they require, this should only be attaching to a specific area of the tumour cell without affecting others.

This is one such example hematoporphyrin, porphyrin is a very common drug used in pharmacy I think most medical students must have heard, most life science students should have heard of porphyrin I have heard of porphyrin. So, one of the first group of photosensitizers that was approved by FDA, it gets excited by 630 nanometre light. So, this was your window of opportunity. 630 is obviously falling below it. So, it is obviously since it is the first generation, so efficiency is less.

Only 1 to 2-millimetre penetration depth and the problem with porphyrin derivatives the earliest the generation one of these kinds of PDT drugs, where unsuitable for tumours which are more than 5 millimetres from the source of illumination and patients were asked to avoid direct sunlight and bright artificial light for a period of 4 to 6 weeks. After you have been treated you have to stay at home in darkness for 4 to 6 weeks. Otherwise, the residue that is remaining into areas which are non-cancerous, could be oxidised as well.

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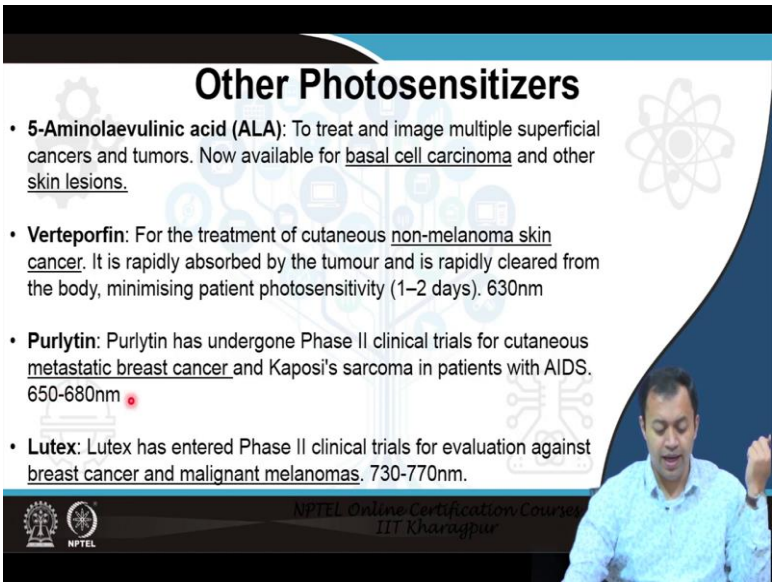

Since then, life has moved further you have developed all of these different types of drugs. This is obviously not an exhaustive list. Lutex is coming up very-very strongly. This follows in that range 530 to 770 nanometre it is already proven against breast cancer and malignant melanoma, melanoma skin cancer, a type of skin cancer, oncologist will go into my throat for

simplifying, oversimplifying it, non-melanoma skin cancers are verteporfin I cannot pronounce them properly. So, these are basically the area where they absorb light and thereby produces reactive oxygen species.

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So, this brings me to the end of the topic today I simply gave you a brief introduction of what PDT is the principles, the mechanism and a little bit of photosensitizer is the photosensitive molecule, which forms part of the overall PDT drug, the PDT drug, basically upon activation by light produces reactive oxygen species, the reactive oxygen species oxidises i.e. burns that nearby surrounding areas.

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So, please visit the National Health Service of United Kingdom, they have a fantastic website describing the various different human ailments in a very-very simplistic term. So, more than going into Wikipedia. I go into NHS website if I have to understand disease and since they are the governmental website of United Kingdom, they have mostly authenticated data.

Wikipedia can be unauthenticated. Anyone can edit it, not National Health Service; National Health Services is the standard version, if I have to look for something medically relevant and standard, either is National Institute of Health NIH United States or NHS which is United Kingdom. So, thank you. We will continue with the discussion further. Thank you.