Fabrication Techniques for Mems-based Sensors: Clinical Perspective

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Lecture – 25

Hi, welcome to this particular module. This is one of the most important aspect in cancer when it comes to selecting a particular therapy. Treating cancer is not very difficult in my opinion; early diagnosis is more important right. And that is why that is where the novel devices that can early diagnose cancer becomes extremely important. But let us say if we diagnose a person suffering from cancer, what kind of treatment we can give; so that we can save the person's life.

Now, traditionally we are what we are using chemotherapy we all know right. And in one of the module I have shown you; how to design a device for rapid drug screening in case of chemotherapy. So, if you remember it was a patient centric platform, where you can take the cells from the patient you can load in the device with Matrigel. You can flow different drugs and depending on the efficacy of the drug we will be getting different electrical signatures right we have seen that.

Having said that chemotherapy is not so effective, this is what the statistics says and it has lot of side effects you may have observed a patient suffering from cancer if given chemotherapy goes to lot of side effects one being losing a lot of hairs right. And what not; immune system goes down, the weight decreases, appetite is different, the person is agitated right. So, what are the other techniques to treat cancer?

Now, there is a radiation therapy; radiation therapy has been used after chemotherapy and again there are lot of side effects. So, recently the research has been focused on developing immunotherapy, and we have in market few immunotherapy drugs. Now as the name suggests immuno therapy; that means, a therapy that is related to your immune system right. So, what is this therapy? Now again as an engineer or as an scientist or as a researcher whether your clinician or you are a student.

It is important to develop a patient centric platform. So, if there are 3 different immunotherapy drugs which drug would be more effective for that particular patient. So, how can we do that, and when exactly this immunotherapy is used right? So, today in

this module or set of modules, we will learn how to design a microfluidic chip that can be used to evaluate the efficacy of the immunotherapy drugs performance of the immunotherapy drugs.

So, let us see let us understand what is immunotherapy, and let us see one aspect of how our device can potentially solve a very important clinical problem. That is bridging the clinician not exactly clinician, but a patient and a drug a system that bridges a patient in a drug and helps the clinician to determine which drug to give to a patient let us see. So, if you see the screen we will be talking about if you see the screen we will be talking about a microfluidic chip for evaluating the efficacy of immunotherapy drugs right..

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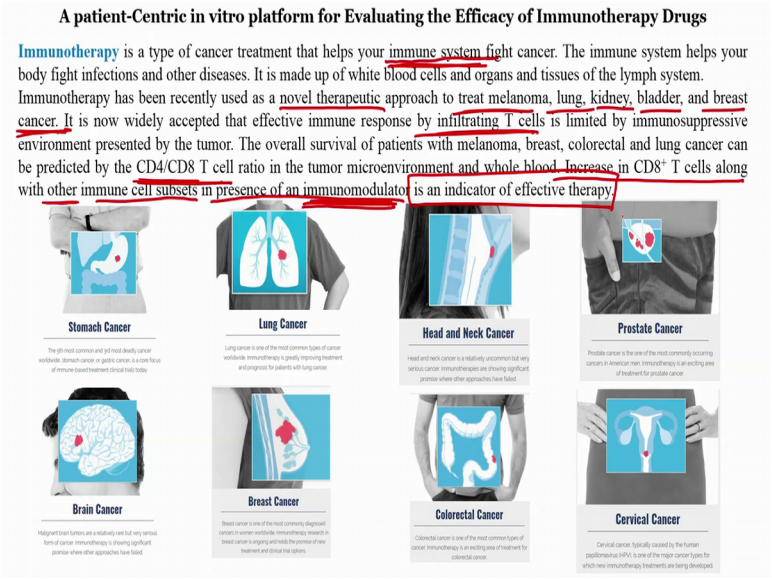

So, what we see on the screen, we will be talking about a patient centric invitro platform, for evaluating the efficacy of immunotherapy drugs.

Now, if you see several cancers right now. So, if you talk about stomach cancer stomach cancer a fifth most common and third most deadly cancer worldwide. Stomach cancer or gastric cancer is a core focus of immune based treatment clinical trials today. If we talk about lung cancer, lung cancer is the most common type of cancer worldwide, and immunotherapy is greatly improving treatment and prognosis for patients with lung cancer.

If we talk about head and neck right oral cancer head and neck is oral cancer is a part of head and neck cancer; is relatively uncommon, but serious cancer immunotherapies are showing significant promise with other approaches which have failed. So, immunotherapy is showing a promising result. When we talk we talk about prostate cancer it is one of the most commonly occurring cancer particularly for white people.

Immunotherapy is an exciting area of treatment for prostate cancer again immunotherapy can be used for prostate cancer. Brain cancer or you can say malignant brain tumors are rare, but very serious form of cancer, again immunotherapy is showing promising results. Breast cancer like we know is the second largest cause of death in women cancer related death in women.

Worldwide and also in India and immunotherapy is used for treating cancer. Colorectal cancer again one of the common type of cancer, cervical cancer typically caused by human HPV virus and it is one of the major cancer types for which immunotherapy treatments are being developed.

So, what exactly immunotherapy is? Immunotherapy is the type of cancer treatment that helps your immune system fight cancer right. First thing that we need to understand is that it is a therapy that helps our immune system to fight cancer. The immune system helps your body fight infections and other diseases and as we know the immune system is made up of what white blood cells, and organs and tissues of lymph system.

Immunotherapy has been recently used as a novel therapy approach to treat melanoma, lung, kidney, bladder and breast cancer. It is now widely accepted that effective immune response by infiltrating T cells; will study about T cells is limited by immunosuppressive environment present by the tumor. The overall survival of the patient with melanoma, breasts, colorectal and lung cancer; can be predicted by CD 4, CD 8 T cell ratio in tumor microenvironment and whole blood.

Increase in CD 8 T cells along with other immune cell subsets in presence of immunomodulator is an indicator of effective therapy. What does this sentence mean; increase in CD 8 T cells other with other immune cell subsets in presence of an immunomodulator is an indicator for effective therapy.

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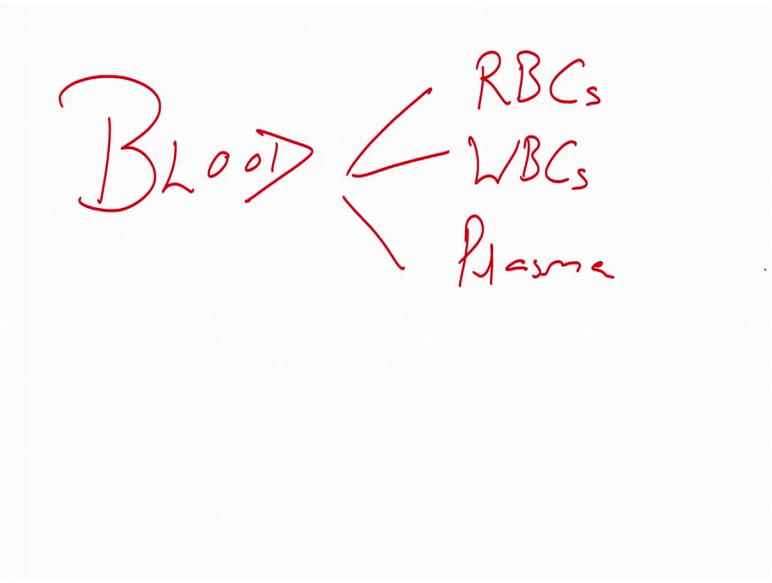

So let us see, we all know blood right just help you out RBCs red blood cells, white blood cells, plasma. This thing we know correct blood RBCs, white blood cells and plasma good.

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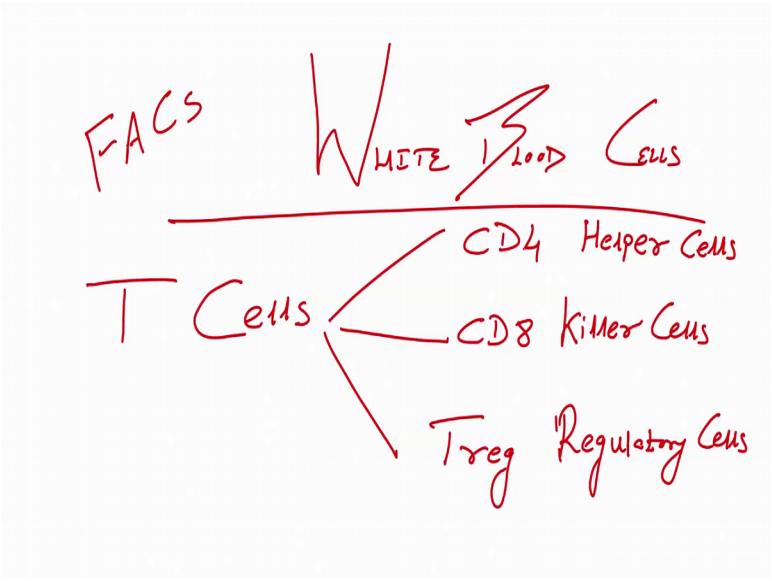

Now white blood cells; these are our immune cells, immune cells helps for keeping our immune system intact.

Now, there is something called Thymus cells or T cells ok. T cells if we divide it there is CD 4, CD 8, T reg. CD 4 is helper cells or CD 4 cells are helper cells, CD 8 cells are killer cells and T reg as it name suggests are regulatory cells. So, when a patient suffers from cancer he is given a immunomodulator or immunotherapy drug and the concentration or the ratio of CD 4 and CD 8 is measured, to see whether the immune system has been activated to kill cancer or not.

So, CD 8 level is higher compared to CD 4 or not, or comparative ratio. This can be found in the blood of the patient; CD 4, CD 8 can be found or from the blood of the patient, using flow cytometry analysis is also called FACs flow cytometry analysis all right. So now, we at least know that ok, white blood cells and then in that white blood cells we have T cells in T cells we have CD 4 cells we have CD 8 cells and we have T regulatory cells right.

So, what we will do with that; this is how to take this T cells right, T cells are thymus cells they can be obtained from blood like I said.

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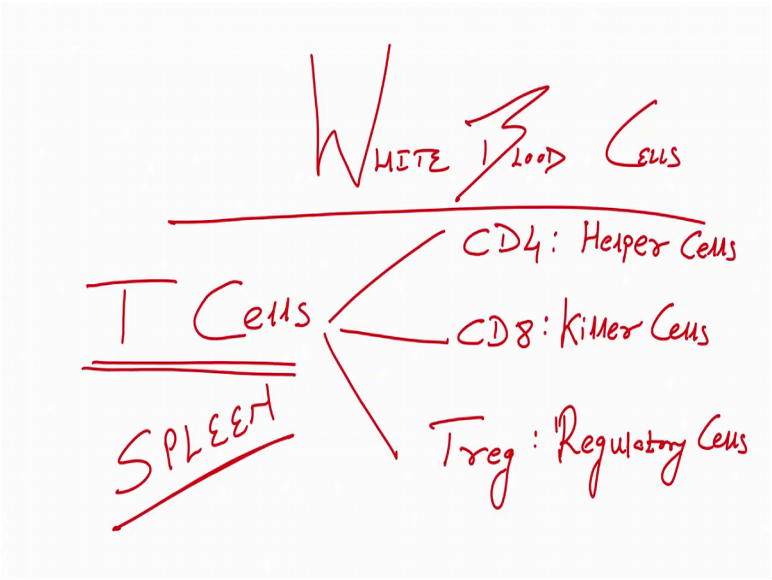

They can also we can also get T cells from SPLEEN ok, spleen of the mice.

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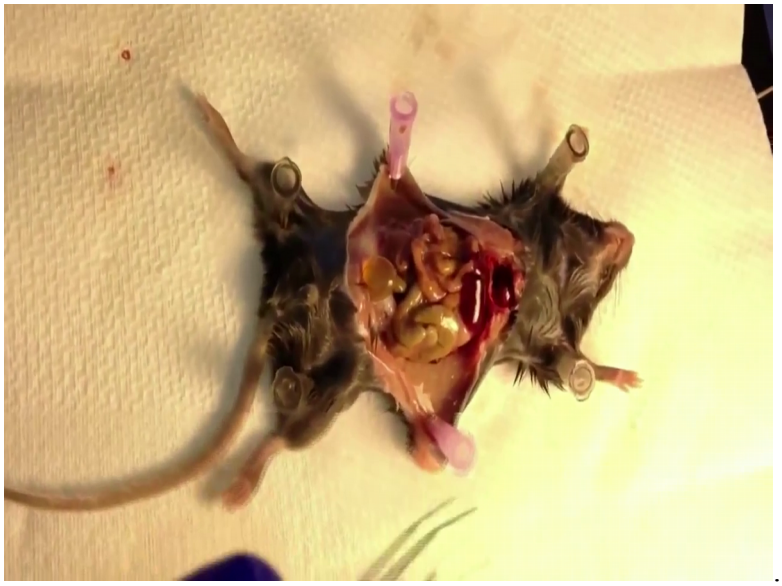

So, this is a video where it is shown how the mice is operated, actually how we can take this spleen from the mice. So, that spleen when you take it out when you look at the video first look at the video and see, how the spleen is taken from the mice.

So for today's extraction you are going to need 2 sets of forceps, and the small pair of scissors. So, he goes (Refer Time: 15:07) so first spray the mouse or pin the mouse to the board. And sprayed down 70 percent ethanol you give it a nice little wipe. So, this is so that the fur does not stick here as much. And then what we do is pull the skin up, and make an incision at the bottom. And then you will see this foamy layer here you what you want is the tip of your scissors to go through that, and then you are going to cut up. So, you are cutting basically the layer of film and also through the skin and you are going to cut all the way through the sternum ok.

So now I can make some incisions on the sides, and this is just to open it up to see what you are working with ok. And so, it is not necessary, but you can in this if you want to get a better look ok. So now, you see the general here that we want to work with. So, this is the liver here this is the intestines.

And so, what you want to do is kind of move this all to the left. And so, this exposes here the liver here is the spleen and in between and then here is the kidney. And so, it could be

a little bit difficult to tell between the 3, but the spleen should be in the middle and it should be long and kind of solid as opposed to the liver which is very squishy ok.

So now you kind of take the spleen out, and you pull the fibers off and you do this slowly. So, that the spleen does not move because even though it is pretty firm it can still rip ok.

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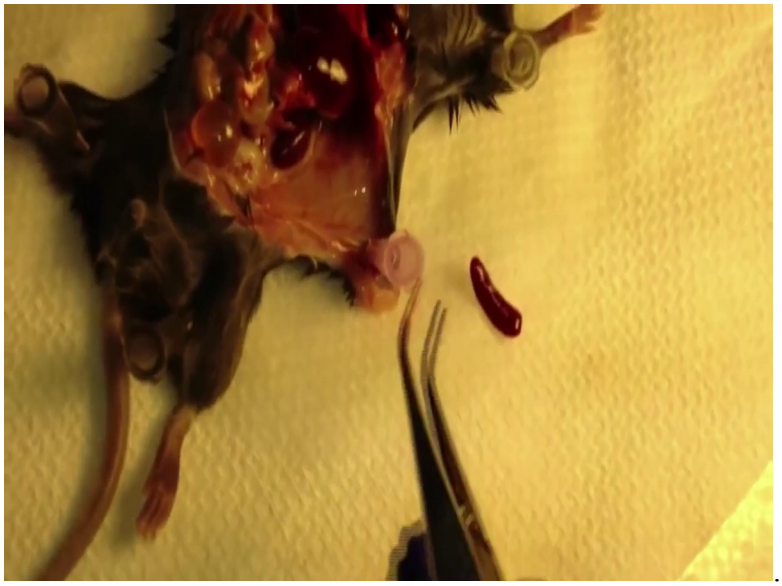

So now I have extracted the spleen and I am going to make sure that there is no remaining fat or fiber, someone is calling us. And so now, you can still see the kidneys here and liver here and say the spleen was right here. And now all you have to do is take that put into a 58 and a conical full of media. So this is complete media and just drop it in and that is it ok.

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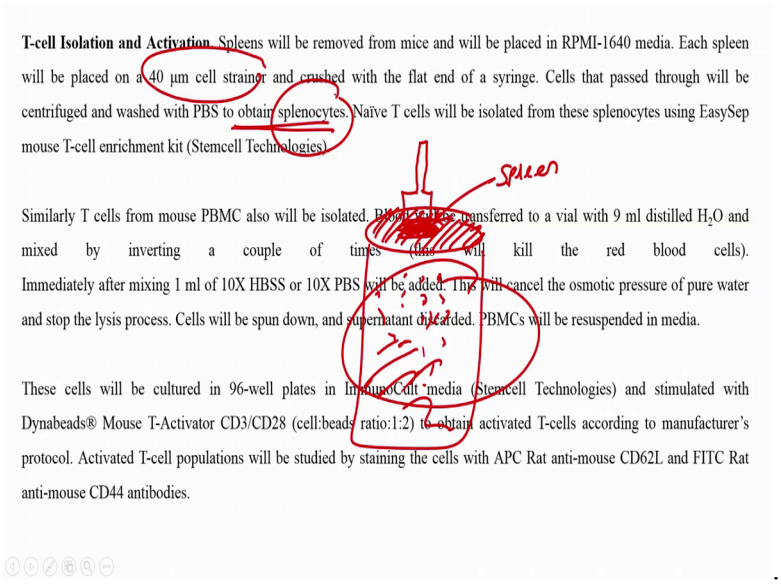

So, as you have seen in the video, the spleen that is taken out from the mice or from the mouse will be placed in our RPMI 1640 media. And further to extract T cells from spleens the each plane will be placed on a 40 micrometer cell strainer and crushed with a flat end of a syringe. Cells that pass with will centrifuge and washed with PBS to obtain splenocytes from spleen we get splenocytes.

What does it mean; if we take this kind of tube and we have filtered right, which is our 40-micron cell strainer. And you put a spleen on this, and you crush the spleen with syringe with a syringe, you crush the spleen this is spleen right. There is a media here and the cells will come out from the spleen, when you crush it these cells would be nothing but your splenocytes this spleen is obtained from the mice. Now, after you obtain splenocytes what you will do?

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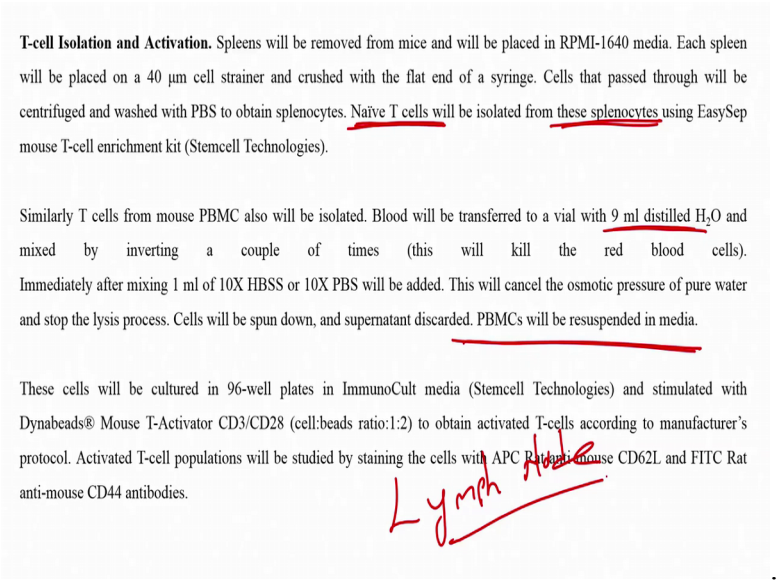

The native T cells sorry, naive T cells T cells which are not activated right naive. Naive T cells will be isolated from this splenocytes using easy step mouse T cell enrichment kit from stem cell technologies right. So, what we are doing after we get splenocytes we can extract T cells from this splenocytes with the help of easy sap mouse T cell enrichment kit. This is obtained from we can get it from stem cell technologies.

Similarly, T cells from mouse PBMC will also be isolated so we can get T cells. Now so from the spleen we have seen how we can extract T cells, we can also extract T cells from mouse PBMC in that case blood will be transferred to a vial with a 9 ml distilled H 2 O, and mixed by a inverting a couple of times. This will keep the red blood cells immediately after mixing 1 ml of 10 X HVSS or 10 X PBS will be added. This will cancel the osmotic pressure of pure water and stop the lysis process right; cells will be spun down and supernatant discarded.

PBMCs will be resuspended into the media. Now once you have T cells and what you do, once you have T cells what you do? You have to activate these T cells why because in our immune systems the T cells are activated. So, you have to study lymph node, how lymph node helps in our immune system. So, in immune systems these T cells are act in activated condition right, but what we get from cell from blood or from the spleen we get naive T cells.

So, once we get these T cells this T cells will be cultured, we have to culture these T cells in 96 well plate in immuno cult media, this is a media specially for culturing the T cells and stimulated with Dynabeads. So, Dynabeads are used to activate T cells. So, that we have the activated T cells along according to manufactures protocol and then we can study these activated T cells with either APC rat anti mouse or F FITC rat anti mouse CD 44 antibodies.

So do not get confused, this is a procedure to activate T cells. It is known by a cancer oncologist; it is extremely easy process if you follow the protocol right. We have we are learning this. So, to understand what kind of device we are using and how it will be used to, how will be used in immunotherapy. So, that is why we are learning that what are T cells, what are T cell isolation kits, from where T cells we can obtain, what are kind of T cells, when you understand T cells. We have seen CD 4, we have seen CD 8, we have seen T regulatory cells then what are naive cells when we obtain the cells from the blood or from the spleen it are naïve cells they are not activated.

So, to activate it because we are developing the invitro platform that is a platform that we are using in the laboratory right. So, how can we activate those T cells, that is what we are learning, but the idea is not to really go down into depth of understanding how the T cell forms know. We have to understand what kind of device I can use to understand immunotherapy, but to understand how we can design this device we have to go through the steps guys. So, do not get confused it is very easy. So, if you see the screen.

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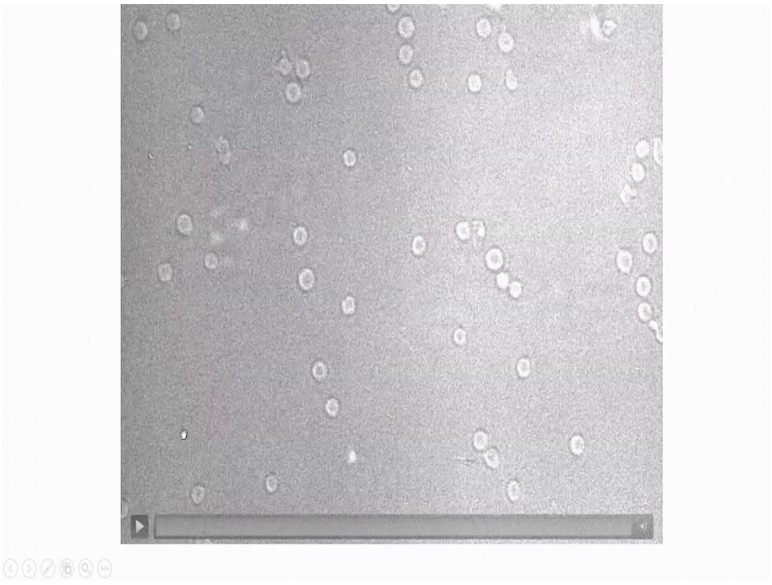

This is quickly I have taken a video of T cells these are T cells flowing onto microfluidic chip. And you can see right as the T cells are flowing T cells are about 4 microns in size right. So, T cells these are T cells and like I said about 4 micron in size ok.

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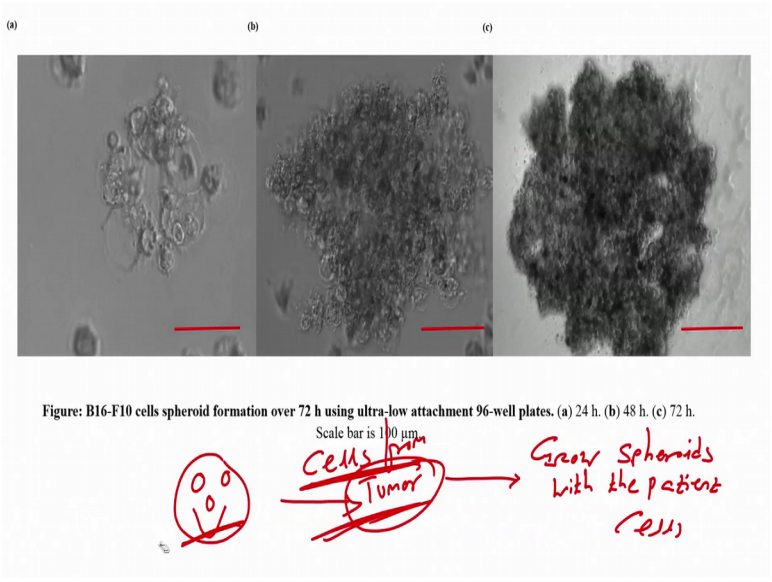

So, what is the idea? The idea is that let us say, a given patient we take the cells from tumor. And we grow spheroids right with the patient cells. We grow spheroids with the

help of patient cells. These cells are extracted from the patient right from the tumor region.

So, let us see how it looks like when you extract these cells which are cancer cells right, and you want to grow a spheroid in the laboratory then you have to load these cells in ultra-low attachment 96 well plates ok.

And this is the example of B 16 F 10 cells spheroid formation over 72 hours. First is 24 hours image you can see this 24 hours, in 24 hours it starts forming spheroid it is not ready. At 48 hours you can see it is the cells are growing and developing in the 96 well plate there is a media for providing nutrition.

Finally, around 72 hours we can see a spheroid formed using B 16 F 10 cells right. We will be loading the spheroid in our device and learn the efficacy or evaluate the efficacy of our drug. That is why we are understanding how does a spheroid looks like when it is formed from the cells, from the cells; how it is found how much time it is taken right. So, about 72 hours we can get a spheroid.

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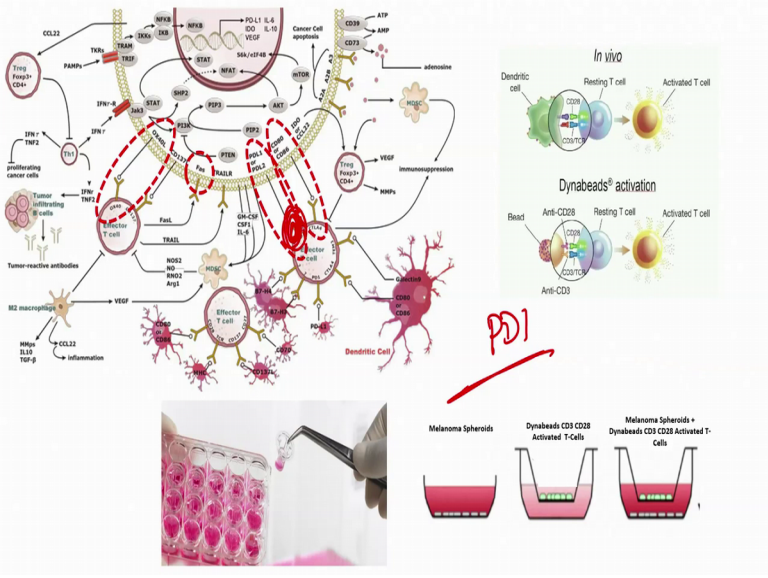

Now, let us this see this diagram it is very easy, very easy do not get too much tensed right. What we are interested is just immuno checkpoints, this is a cell, this whole thing from here to here this is our cell, everything this is cell ok. And there are several checkpoints so what do you, what I mean by that; I will give you an example.

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Suppose you go on so you can look at me if you can. So, I can show you in a correct example ok. So, suppose I am driving a car right on a toll road right and I should have a pass, that I can show it to the tollbooth that ok. This is my pass and let me go ok let me allow to go through that toll booth right. So, if I have this pass then the toll booth guy will allow me to pass on the toll road. Or if I get a ticket and I show that I have paid he will allow me to go past the toll road.

If I do not have it then what will happen? I will be I had to pay fine or I can be in jail for not following the rule right. So, in case of our body, every cell has to show the pass to the T cells. So, whenever T cells comes to let us say this is our cell this is T cell.

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Whenever T cell comes it checks ok, show me your pass; if it shows the pass it will go further and this cell can survive. If the cell does not have that pass, then these T cells will start prosecuting the cell and it will kill the cell ok.

So, when you talk about this pass these are the immuno checkpoints immuno checkpoints. You get example right you I hope you understood the example. Same thing like toll road you take this example it is very easy to remember. So now, what are these different passes are different immuno checkpoints that a cell can show to the T cell right.

So, if you see the screen there are certain immuno checkpoints starting from ox 40 L O X 4 0 L, then there is PDL 1, there is C D 80 right to name few CD 80 or CD 80 6 PDL1 or PDL2 right. There is a OX 40 L. So, when you have this immuno checkpoints the T cells would have another checkpoint, so they can see it can it can talk right. So, when we have PDL 1 T cell would have PD 1.

If you have CD 80 this will have CTLA 4 if you have OX 40 L, T cell will have OX 40 you see that is how it can interact. So, if we are targeting one of the immune checkpoint that is OX 40 L, then T cell would have OX 40. If we are if you are looking at PDL 1 T cell would have PD 1. If we are talking about CD 80 T cell would have CTL F 4. So, there are this lock and key mechanism available with T cell and the normal cells or

cancer cells, with which they communicate and they understand where everything is going well with the cell or not ok.

Now what we are interested; we are interested and in general the community immunotherapy community is interested, one of the aspect is if I stop this PDL 1 if I block PDL 1.

The T cell cannot find PDL 1 so PD 1 cannot interact with PDL 1 and T cell may start killing the cancer cell. Or if I block PD 1 on the cancer cell then even there is PDL 1 on my cell which is my cancer cell, if I block PD 1 on T cell, then since T cell cannot communicate with cancer cell; T cell may start killing the cancer cell right. Because it cannot talk it cannot communicate the immune checkpoint is absent, that is of our interest.

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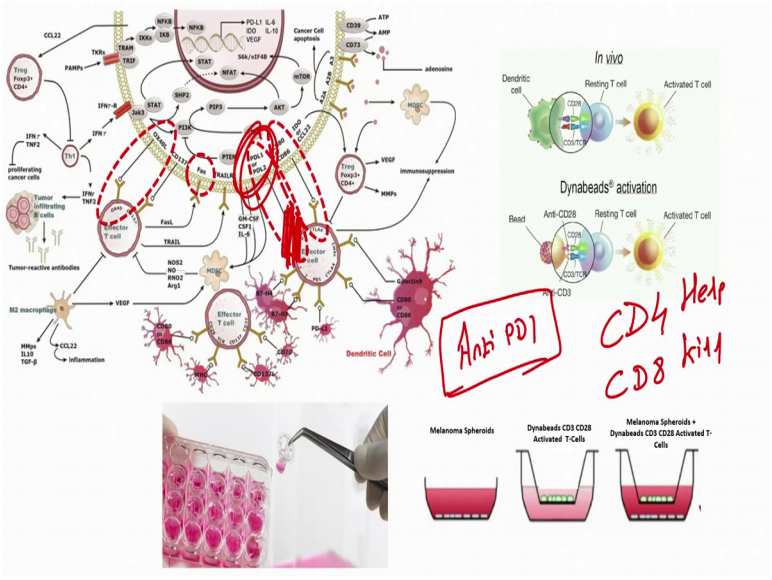

So, we will be learning immunotherapy drug called anti PD 1. Anti PD 1 means it will block PD 1 on T cell. So, if it blocks PD 1 on T cell, T cell cannot interact with PDL 1 right, what will happen to CD 4, T cell is CD 4 T cell is CD 8 right. What will happen to CD 4 and CD 8 ratio? If I use anti PD 1 if I block PD 1 on T cell what will happen to CD 4 CD 8 ratio. So, we had to study this particular property using our device using our device ok.

Now, having said that you see in vivo there is a dendritic cell CD 28 and CD TCR there is a resting T cell when there is this kind of communication between dendritic cell or dendritic cell and resting T, cell T cells gets activated in vivo means within body ok. So, if we want to perform similar mechanism we can use Dynabeads to activate these T cells how dynabead; dynabead will have anti CD 28 and anti CD 3.

So, this will again cause the resting T cell which is our naive cell to activate, this is just a mechanism that is a rule of dynabeads. Now let us come here, this image that you can see here is called Transwell, transwell t r a n s w e l l.

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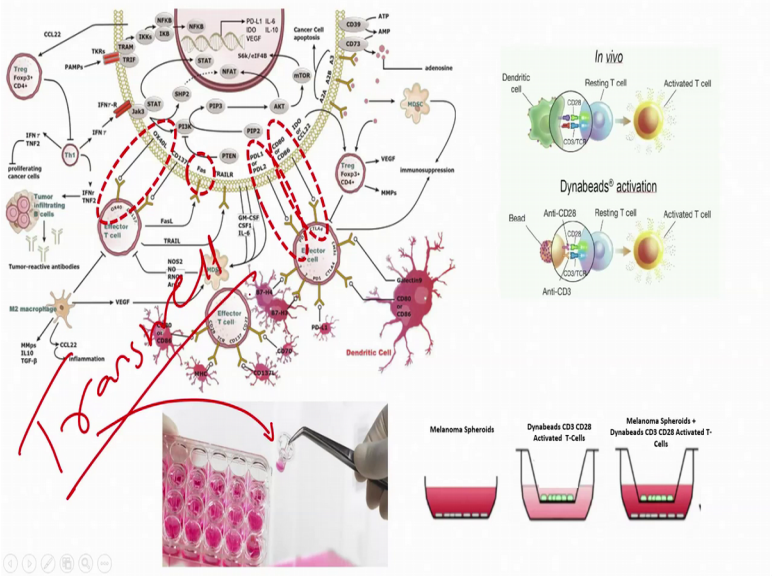

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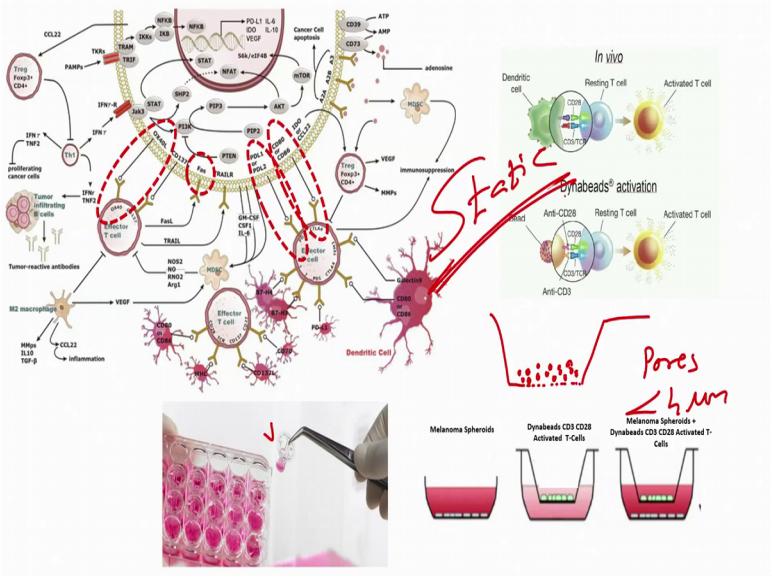

So, is a well with pores which are less than 4 microns, less than 4 microns. Now I told you the T cell size is about 4 microns. So, T cells cannot pass through this filter, through this well T cells cannot pass through it ok.

Now, what is the present study, present study is using static platform ok. In that what is the mechanism melanoma spheroids let us take an example of skin cancer ok.

So, melanoma spheroids which we have seen in the last slide how this spheroid looks like are placed into the Matrigel right this red one is Matrigel.

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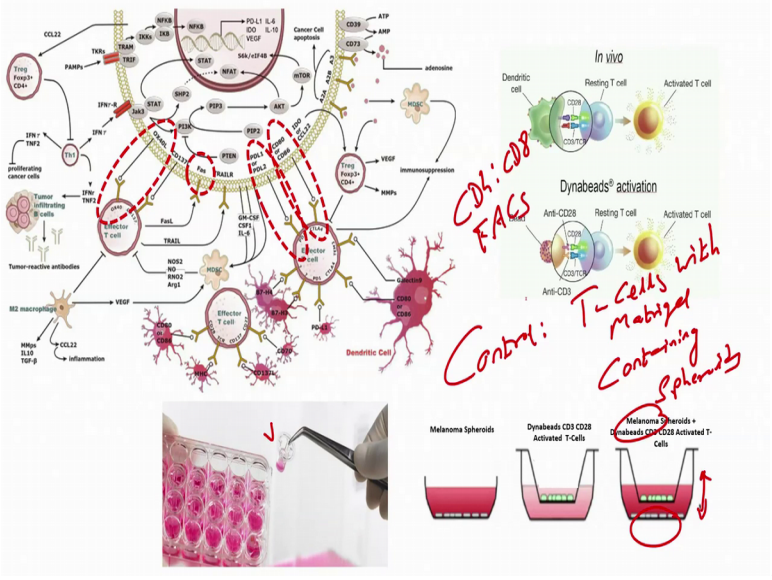

Now, you load the T cells and see the interaction between T cells and Matrigel T cells, Matrigel and of course your spheroids this will interact right this will interact. So, our control would be, T cells with Matrigel containing spheroids right. This spheroids is our melanoma spheroids melanoma spheroids, this is our control there is no treatment.

After 48 hours 24 to 48 hours we will see CD 4, CD 8 ratio using flow cytometry analysis ok. Next step would be you treat this T cell.

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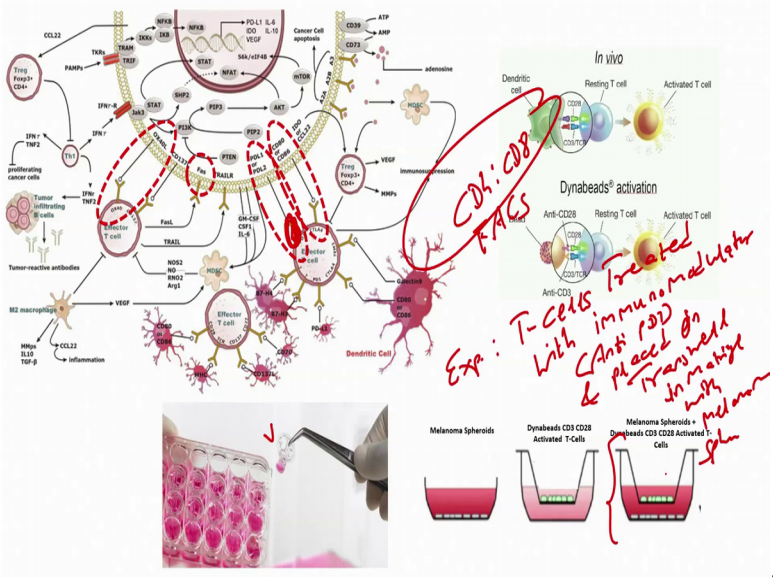

So, our experiment would be T cells treated with immunomodulator like anti PD 1 and placed on Transwell in Matrigel with Melanoma Spheroids.

Again wait 48 hours or 24 hours and see the CD 4 CD 8 ratio. Now when you treat T cells with anti PD 1, like I said we are blocking anti PD 1 on T cells and we want to see; what is the effect of blocking PD 1 on CD 4, CD 8 ratio. This is a static way of performing the experiments and you will see a certain signature in CD 4, CD 8 because you are treating the T cells or with an immune rapid drug. And you are allowing the T cells to directly communicate with spheroid right in a static manner.

The change in CD 4, CD 8 would be because of the release of Chemokines as well ok. So now, my point is our body static no. What is our body our body is dynamic when we when we take a drug this drug goes to a particular place and stays there no. It flows with the help of blood right it makes it in the blood and flows to the blood.

So, why to perform static experiments when our body is not static, that is a first question right that is a first question. So, we will see in the in the next module what we can do to create the dynamic platform, dynamic invitro platform to perform similar tests that we have just discussed and where exactly we are lacking all right.

So, understand immunotherapy once again whatever we have discussed. I will see you in the next module and we will discuss in detail, what kind of experiments and what kind of device we can design. That can evaluate the efficacy of a given immunotherapy drug, as well as it can act as a patient centric platform till then you take care I will see you in the next class bye.