Drug Delivery Principles and Engineering
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Lecture – 58
Cancer Vaccine Immunotherapy

Hello everyone, welcome to another lecture of Drug Delivery Engineering and Principles. We have been discussing about Cancer Vaccines in the last class. So, just let us do a quick recap before we discuss it further. So, cancer vaccines as the name suggests are vaccines that will be used against cancer.

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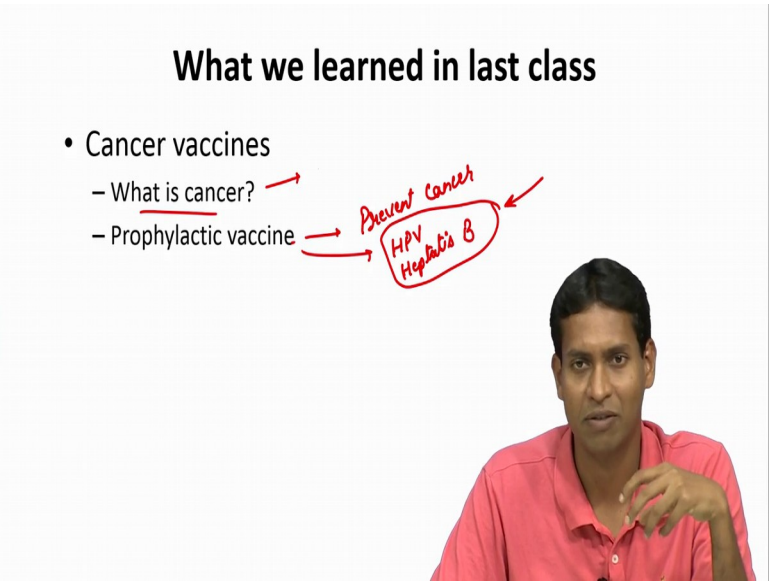

And so, we discussed what is cancer? Cancer is something that results in growth of cells which are not supposed to be there due to some mutation. And there are prophylactic vaccines for this which means these prevent cancer from developing.

Not very high in incidence these cancer vaccines are basically targeting pathogens like HPV and Hepatitis B that is causing that is a byproduct causes the cancer to be developed. So, these are viruses that are trying to replicate in a body and during this process of infection they are also causing induction of tumors. So, if we give vaccines against these viruses we will not get tumors.

So, there was whole prophylactic vaccine. Then, towards the end we started talking about some autologous cancer vaccines of how it's actually difficult to get the antigen to be presented to the immune system cancer has several strategies and how we would like to overcome those challenges by giving some adjuvants with the tumor lysates. So, there was autologous vaccine and then we discussed few other things with that.

In today's class we are going to look at more allogenetics allogeneic vaccines, which are more relevant and more available for use. We will give some examples and we will talk about a research paper which is working on a different strategy for cancer vaccines. so, let us get into that.

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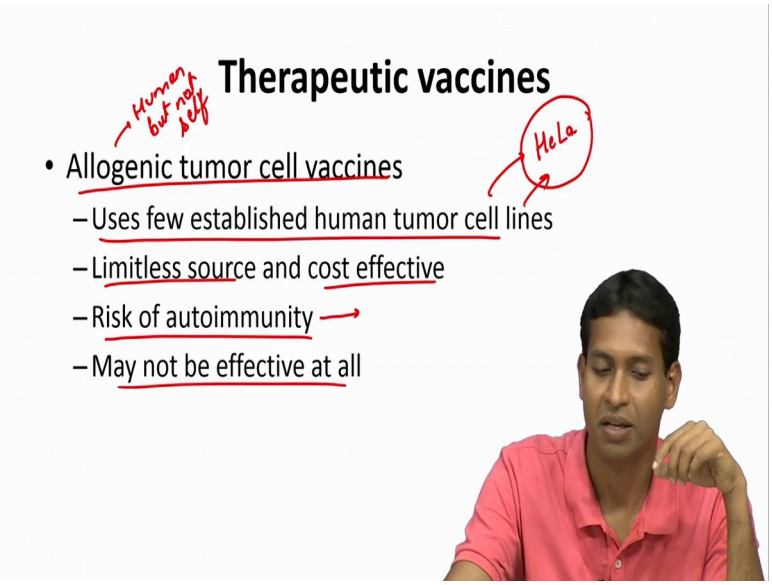

So, what about allogeneic tumor cell vaccines. So, in this case what I am saying is we are using somebody else had a tumor we have cells from those already. So, those are allogeneic is still human, but not self. So, not from my own body, but let us say from some other patient that had the cancer and what this does is, it uses a few established human tumor cell lines. So, maybe at the time when a patient came we were able to isolate some of the cells, we were able to use them in cell culture and we have now established them as tumor cell lines. Some of the; one of the biggest example for that is hela cells. So, which is cervical cancer there was isolated from one of the patient.

And so, this was used quite widely in cell culture. So, something like that, so, you can develop cell lines from a tumor and because now those tumors are also harboring those

mutations you can then use them as antigen. So, there is a limitless source. So, you can expand these cells to whatever amount you want they are fairly cost effective because hospitals are directly not involved. So, there is no surgery and there is no risk all of those processes are not there.

There is still a risk of autoimmunity because again these are human cells. So, even though they are allogeneic pretty much 99.99 percent of the proteins are same as us. And so, you can still induce autoimmune reactions if you first of all put the whole protein, whole proteome, the whole lysate into a body. And then the other problem is they may not be effective at all. So, maybe the tumor that I got is very different from the tumor that is present in these cell lines. So, maybe it is not the same mutation this mutation does not exist in these cell lines. So, if that is the case and we will not be effective at all, because you are training the immune system against an antigen which is not present in my body.

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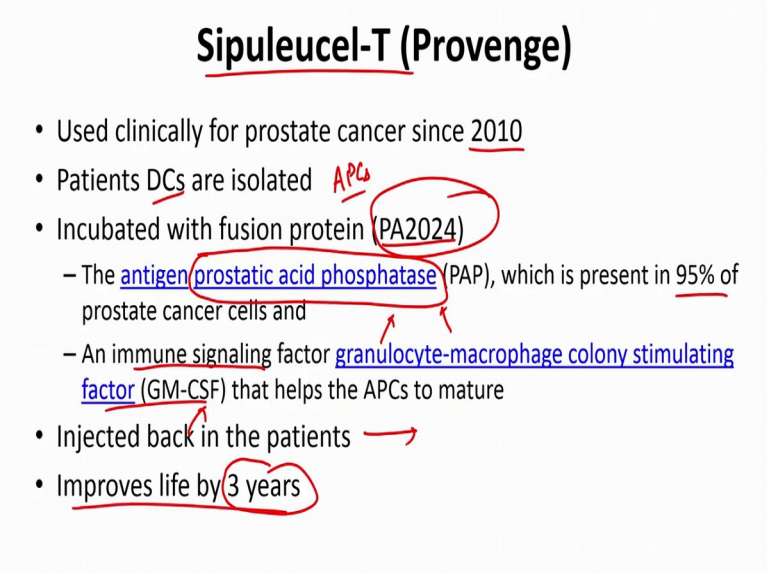

So, those are some of the challenges there, here is an example, so, this is a product that is actually out in the market from provenge. So, this was used clinically for prostate cancer since 2010. And in this what is done, is patient dendritic cells are isolated. So, these are APCs. From the patient itself, that you are isolating from the blood. Then you incubate with a fusion protein which has been named as PA2024. And this is nothing but, this is an antigen which is the prosthetic acid phosphatase. And this particular protein was

found to be present in almost 95 percent of the prostate cancer cells. So, it was observed that almost all the patients that are coming in or at least 95 percent of the patients are coming in have this particular antigen this is what gets mutated and leads to prostate cancer.

So, what they have done, is they have made a fusion protein that contains this protein and then it also contains an immune signaling factor which is the GM-CSF. So, this GM-CSF actually signals these APCs to get mature, as well as to come to the site. So, they have made a fusion protein some out of these two proteins. So, now, what they are saying is, whoever whatever APC is taking up this protein has the antigen as well as is getting mature through this GM-CSF.

And once they have done this they have activated these DCs in vitro, they inject it back in the patient. So, the whole cocktail is just taking and put it back in the patient and then now since these DCs are active, they can go and signal to the rest of the immune system the leukocytes the B cells in the T cells. To then, mount effective response against this particular fusion protein which contains the antigen that x is expected to be there in 95 percent of the cases.

So, it its all good in theory, it does work well as well. But, the improvement we get is about 3 years which is actually quite significant the patient life gets extended by 3 years, the patient can now do more work or whatever their aspirations were. But, still we would like this 3 years to be much higher than then this number, we would like this to be something that is not even dependent on the cancer. We would like the patient to live a healthy life after that which is not the case with this particular vaccine at this point, but it does help in prevention of some of the symptoms. ok.

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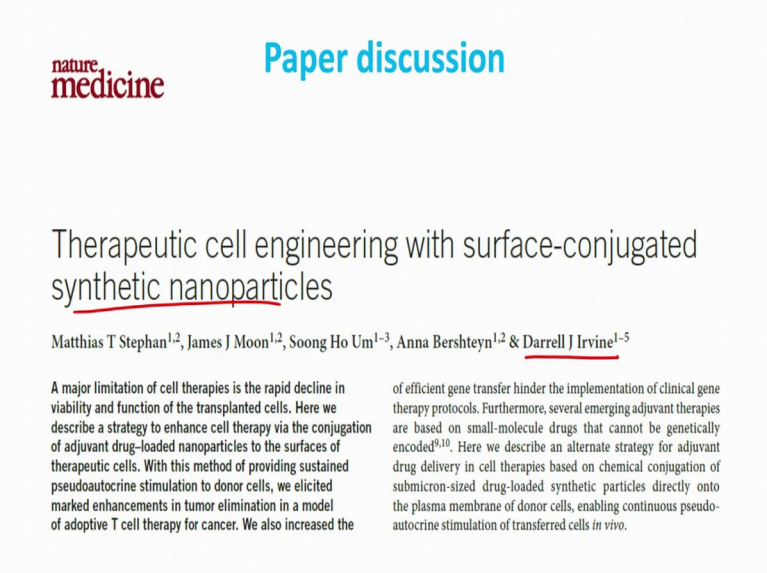

So, we will now discuss a paper, this is a paper that was published in nature medicine by Darrell Irvine's group. And this uses some material approaches to enhance the immune response against cancers, in particular synthetic nano particles here. So, let us see what these authors did.

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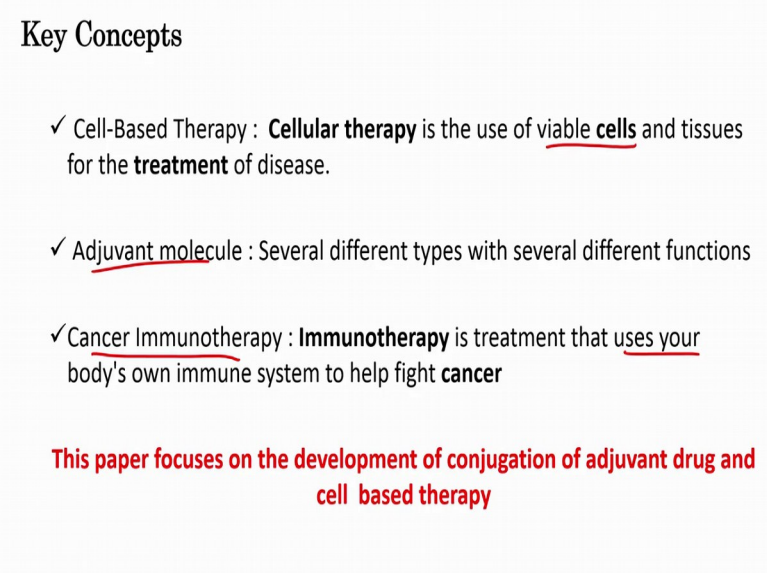

So, one of the key concepts from the paper were they were using cell based therapy. So, which means that they were using some viable cells and tissues for treatment of a disease. They were using adjuvant molecules. So, again as I said there are several types

and different functions of these adjuvant molecules that they were using. And the major goal was to do cancer immunotherapy which is to basically use your own immune cells to help fight cancer. And this paper focuses on the development of conjugation of adjuvant and drug based cell therapies.

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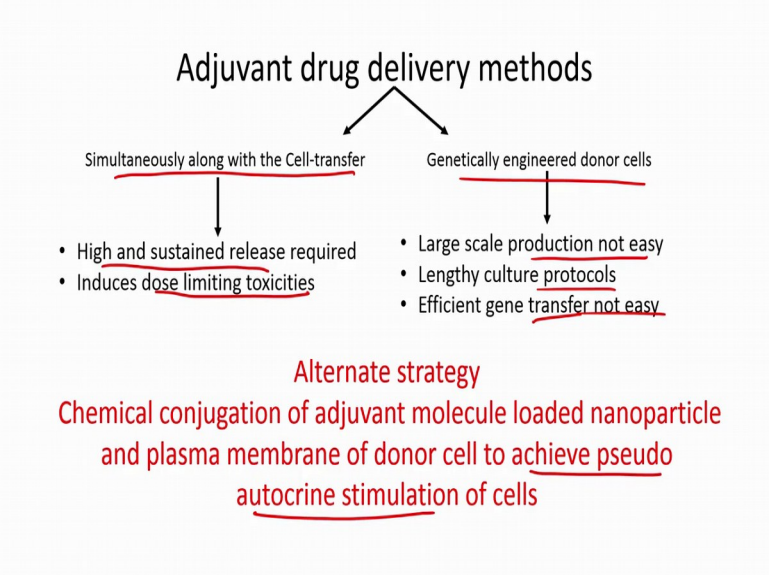

So, let us see what they did. So, first of all let us talk about their drug delivery methods for adjuvant. Then what they have done is they have simultaneously along with the cell transferred these or they have taken the genetically engineered donor cells. So, these are different approaches here. So, if you are using adjuvants that are used with cell transfer, you have a very high and sustained release is required. And that induces those limiting toxicities and if you are using genetically engineered donor cells, then first of all the large scale production is not easy, there is a lengthy culture protocols and then the gene transfer may not be very efficient.

So, what they are saying is instead of using these two strategies they are looking at alternate strategy which is chemical conjugation of the adjuvant onto a particle and loaded onto a plasma membrane of the donor cell. So, this is the ultimate goal is to achieve a pseudo autocrine stimulation of the cells. And will discuss this in detail in next few slides ok.

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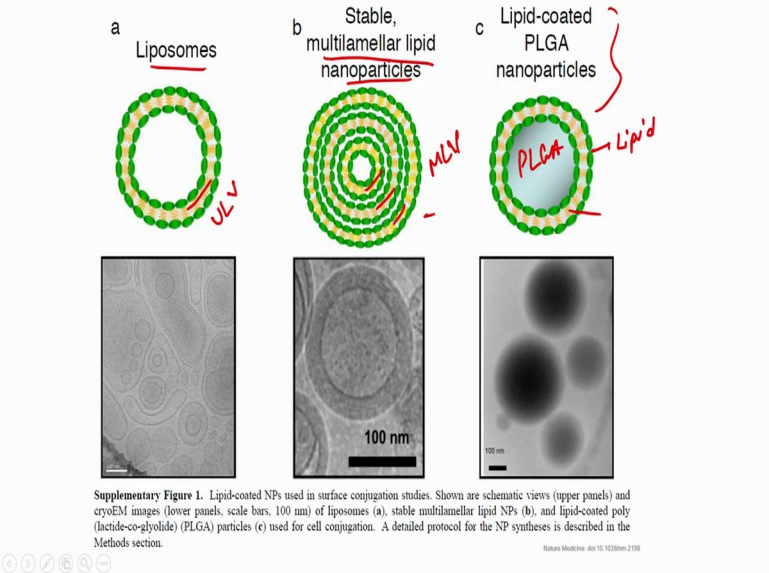

Supplementary Figure 1. Lipid-coated NPs used in surface conjugation studies. Shown are schematic views (upper panels) and cryoEM images (lower panels, scale bars, 100 nm) of liposomes (a), stable multilamellar lipid NPs (b), and lipid-coated poly (lactide-co-glycolide) (PLGA) particles (c) used for cell conjugation. A detailed protocol for the NP syntheses is described in the Methods section.

So, first what they started is they used few particulate system, one was liposome one was multilamellar liposome and another was a lipid coated PLGA particle. And this is just pictorial cartoon of the three system. So, here you have PLGA, which is coated by lipid. Here you have multi lamellar vesicles and so as you can see there are several lipid bi-layers three are shown, but this could be many. And then you have a unilamellar liposomes which only has one vesicle. And here is just some pictures that they are showing for that as you can see here under transmission electron microscope.

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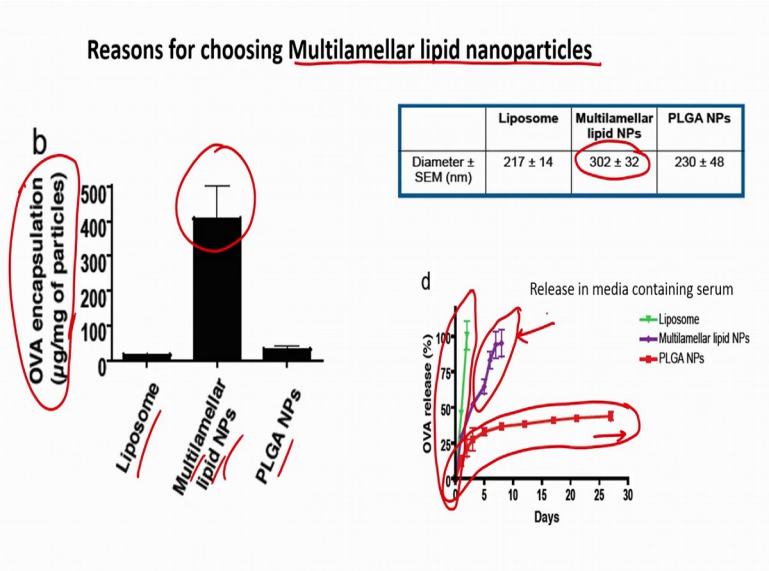

So, then they went ahead and honed in and decided to use multilamellar lipid nanoparticles and the major reason for that was they tried to use OVA for encapsulation which was acting as their antigen in their model. And what they found that they could get much higher amount of OVA, per milligram of their particle compared to the compared to the other two systems.

So, the multi lamellar lipid nanoparticles they were able to encapsulate more that is why they decided to go with this particular system. They then measured the diameter and they found the diameter to be fairly similar. So, that was not causing this change in this overloading. And then they also looked at how long you can release this. So, what they found is they are if they are using PLG nanoparticles they have a lot more control and they are getting much higher times for the release.

If they are just using liposome it gets released out in 1 or 2 days fairly rapidly; however, the multilamellar vesicles are able to give them the release up to a week which is what they were targeting in this particular application. So, combined with this and this, they decided to go with multilamellar lipid nanoparticles.

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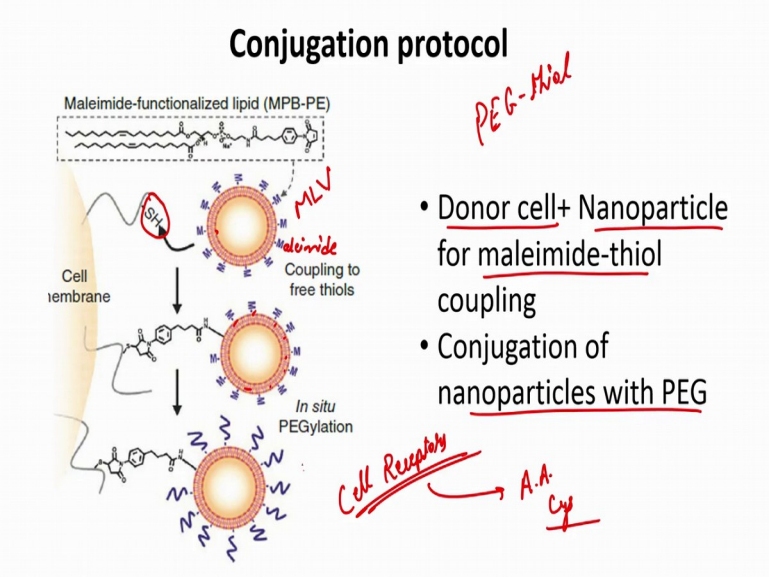

And so, what was the conjugation protocol for the cell. So, what they were using? So, they took donor cells and these nanoparticles were then modified with maleimide thiol chemistry. So, and then they have also conjugated nanoparticles with PEG. So, here is

what it looks like. So, you have these multilamellar vesicles that have maleimide group displayed on their surface right so right here.

And once they have these maleimide groups and they incubated with cells what will happen is these maleimide group will go and bind to the surface thiols on the cell. So, cell surface contains several cell receptors and proteins. And all proteins will contain amino acids. And we know that there are amino acids such as cysteine that contains thiol. So, these thiols are then available for reaction with this maleimide.

So, all they did is and this maleimide thiol chemistry is extremely efficient. So, all they did and it is very compatible at the neutral pH. So, what they did is they reacted this and then whatever is the leftover maleimide that was present. They went ahead and used a PEG thiol to passivate rest of these maleimide. So, this we put in an excess and that caused the passivation of rest of the surface. So, it would not react anymore with new thiols.

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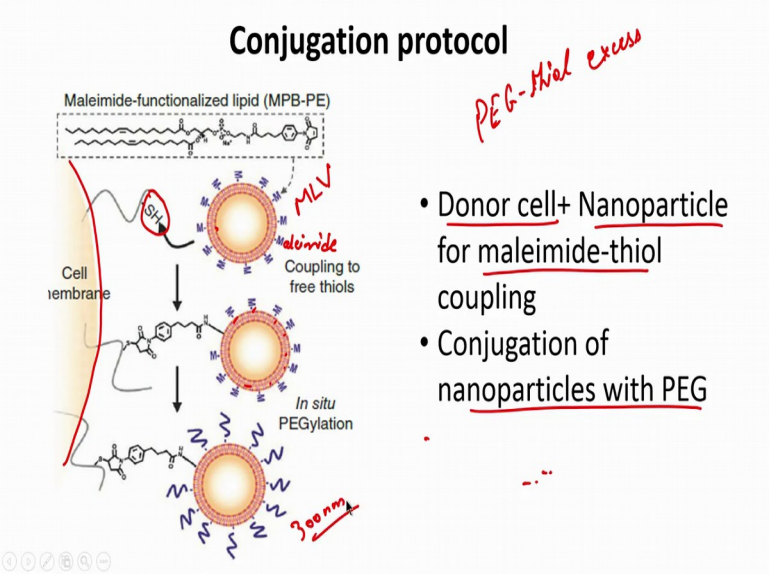

And so, now, you have a system where you have; where you have a cell membrane which is now conjugated to these and nano particles which we said, were about 300 nanometer in diameter.

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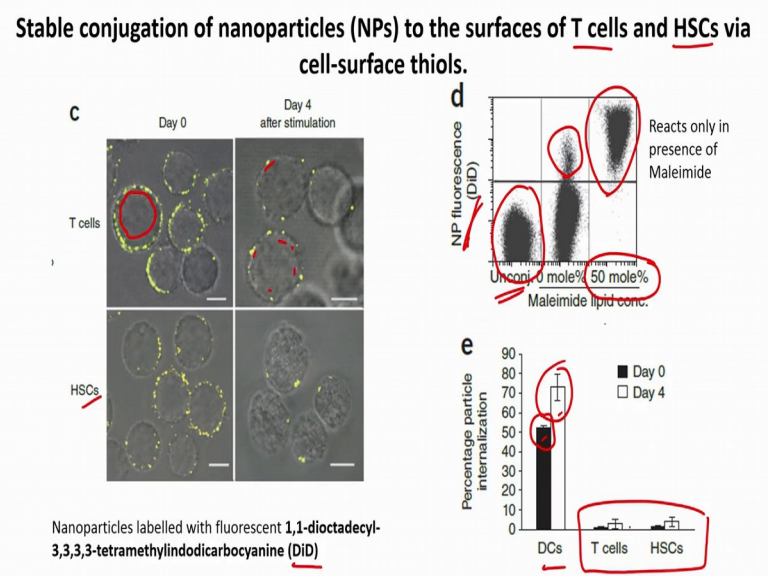

Then they got stable conjugation of these nanoparticle surfaces on both the T cells as well as the hematopoietic stem cells via these cell surface thiols. So, here are some image, so, here you can see a T cells and these stem cells and what you can see is a day 0 at the times conjugation you can see. So, all this green yellow signal that you are seeing is particles and the light microscopy shows you these rounded cells. And so, you can see quite a lot of the surface is actually loaded with these particles both on the T cells as well as on the stem cells. And even after several divisions you see that even though the amount has reduced there is still quite a bit of particles that is still present on the surface. So, they got fairly stable conjugation of these nanoparticles onto the surface of the t cells and these stem cells.

And this is for the proof of that. So, what they have done is they have taken these nanoparticles which were labeled by a dye called DID. This is a dye that binds to the vesicle directly insert itself into these lipid membranes. And so, what they see if they use the cells which were not conjugated, they get a certain population if they then make the particles with no maleimide, they get some adsorption of this particle on these surfaces, but not much. But if they do put maleimide at a certain concentration on the surface of these particles you see almost all of your cells are now fluorescing for these particles. So, that means, all of them had got conjugated for these particles.

And then they looked at what happens whether the cell is going to take up these particles and internalize them. So, that is not what they wanted. And so, what they found was; if they do it on dendritic cells pretty much quite a bit amount by day 4 gets internalized. In fact, even immediately gets internalized into these dendritic cells. However, this T cells and in the stem cells do not really take these particles up and they keep them on the surface only even after 4 day of incubation. So, that is why they went ahead with further studies on T cells and these stem cells.

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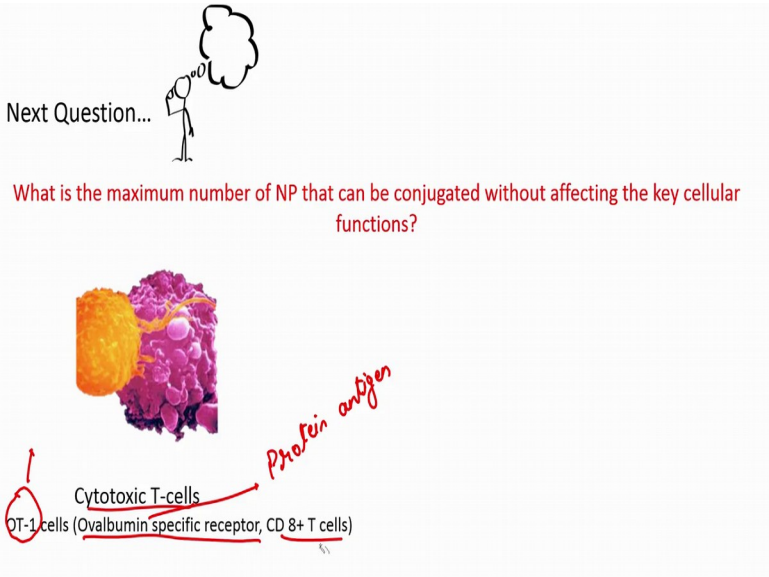

So, I guess the next question was, what is the maximum number of nanoparticle that can be conjugated? Without affecting the cell function. So obviously, you are taking these cells from the patient itself or that is the idea. In this case they are using it on a mouse system. So, you are taking this from the mouse system, but what if it is impairing the function of these T cells. That is the last thing you want if you want to treat a cancer to further impair the current immune response that is being generated by the mouse itself. So, that is what they tried to test now.

So in this case, they are using cytotoxic T cells. These cytotoxic T cells derived from a mouse which is called OT1. And this is a mouse in which all their CD8 positive T cells have a receptor that is specific to ovalbumin. So, ovalbumin is a protein antigen and in this particular example what they are using is they are using a mutated mouse, in which

all the T cells or the CD 8 positive T cells have a ovalbumin specific receptor. So, they will be able to bind to ovalbumin and get activated.

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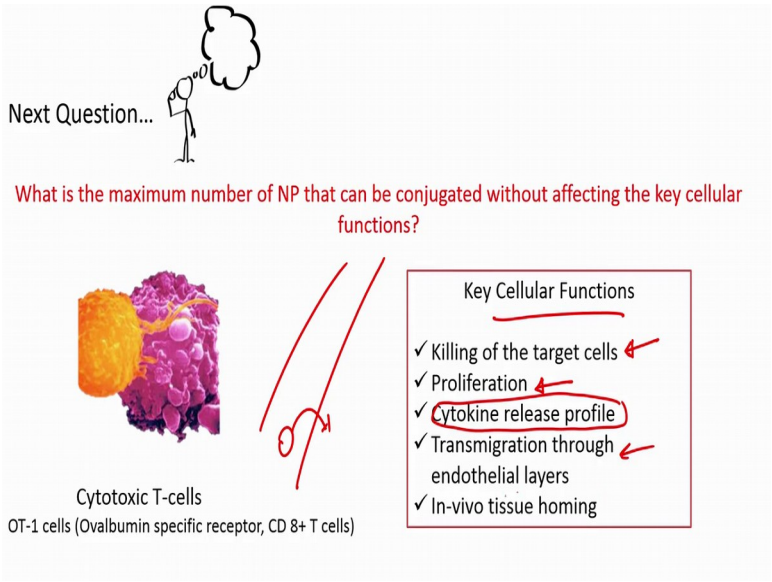

And few of the functions they are now testing for these T cells is to see first of all, whether its ability to kill target cell is same as before the conjugation whether it can proliferate. So, these are some of the major key functions of the T cell whether the cytokine release profile is any different before and after conjugation of the particle. One other important function that these cells do, is migrate through the endothelial layer. So, when they are flowing in the blood vessel, they need to come out when there is inflammation remember the rolling adhesion and so, they want to see if the transmigration can still happen. And then finally, whether they can home into the tissue where these T cells are required. So, these are all the functions they are looking to test.

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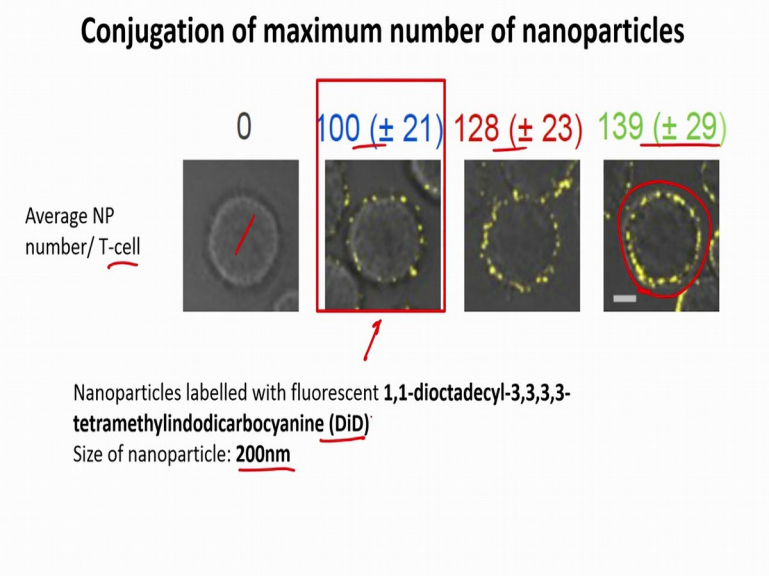

So, first thing they try to answer, is how much is the maximum number of nanoparticles you can conjugate. And so; obviously, this is if you do not do any conjugation you get a T cell without any particles and then they have used different amounts of their particles to T cell. So, the 100 particles per T cell. And they get a certain conjugation and similarly the rest of them. And again in this case the particle sizes that were using was about 200 nanometer also labeled with the DID dye. That is being shown here as yellow.

So then, they decided that they do not want to affect the function too much maybe if they do a coating which is quite extensive, it might cause some aberration in the T cell function. So, then they went with a coating which is basically giving about 100 nanoparticles per T cell.

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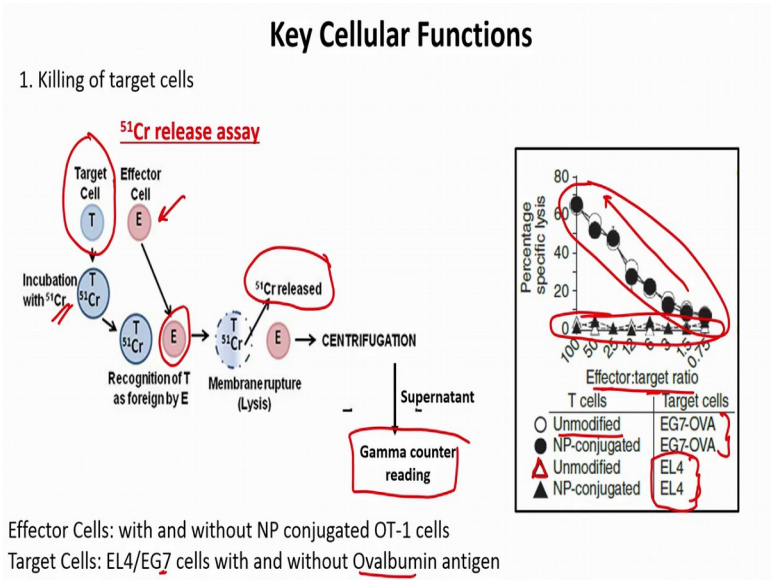

Then they looked at whether it can kill the target cell. So, in this case what they have done is they have taken the target cell. They have they have cultured the target cell in presence of a radioactive molecule chromium. And then they have went ahead and used these cytotoxic cells in this case represented as E and if these cells can kill it then it will cause release of this radioactive compound which you can then assess by gamma counter reading.

And what they found here is that if you use the T cells that are unmodified, you get quite a bit killing as you can see here. So, this is at various ratios. So, T cells to the target cell, in this case the target cell is expressing your ovalbumin, so, EL4 is expressing So, there used two target cells one is EL4 and another is EG7.

So, the EG7 is expressing ovalbumin EL4 is not expressing ovalbumin. So, you would expect if this is specific then the EL4 do not lyse at all whereas, the EG7 to go ahead and lyse. So, the triangles are EG4. So, what do you see that the triangles are right at the base there is no lysis at all. Whether you have used T cells with or without particles, but in case of EG7 there is no difference in the killing. So, as you increase the ratio of the T cells with the EG7. You see the killing is increasing almost 60 percent of them are lasing in this given amount of time. And there is no difference with the conjugation of particles. So, at least in terms of killing the cells these particles on the surfaces of T cell does not impair its function.

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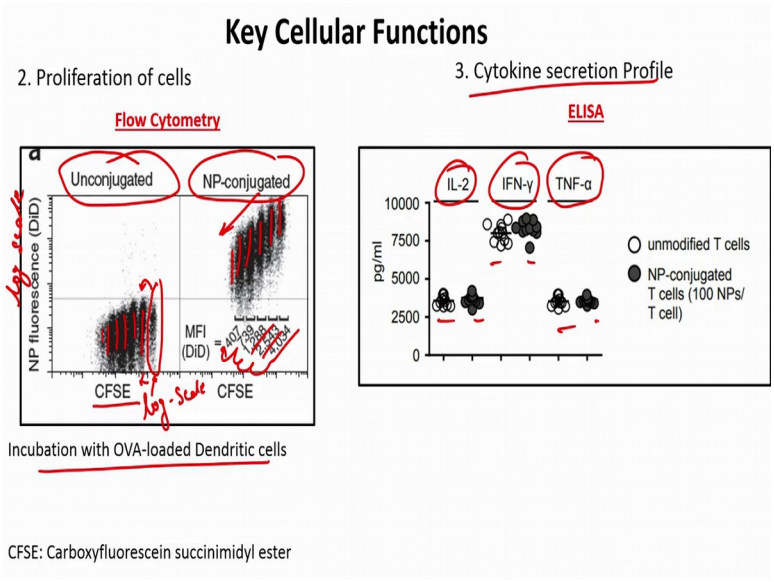

Then they looked at proliferation of cells. So, in this case they are looking at flow cytometry. So, once these T cells get activated they start proliferating. So, now, they have used this dye CFSE. That labels a DNA and since it has label the DNA what will happen is, every time the cell multiplies the intensity of the dye will decrease. So, this is a log scale and what you are seeing in this also is a log scale. So, what you are seeing here is: let us say this is the labeling that you achieve initially, but then when the cell starts dividing its CFSE content per cell will decrease. So, that is why you see a shift on the x axis. And then this is going to continue as the cells divide further and further.

So, they see that a certain division pattern is seen if the particles are not conjugated versus the particles are conjugated. So, you can clearly see and now this time this nanoparticle is also getting diluted. So, you see a decrease in the nanoparticle fluorescence as well. And you can see these patterns are extremely similar, indicating that and you can actually see these numbers of 4000 become 25 12. So, subsequent 2 times reduction is seen and that shows that the proliferation is also not affected.

So, to get this activation they are incubated with genetic cells. Next thing they saw was what about the cytokine being produced, the number of cytokines, amount of cytokines being different with the modified versus the unmodified. And again they tested for three different cytokines, this is IL2 IFN gamma and TNF alpha. And what they find is their amount is not changed whether the T cells is conjugated with the particle or not.

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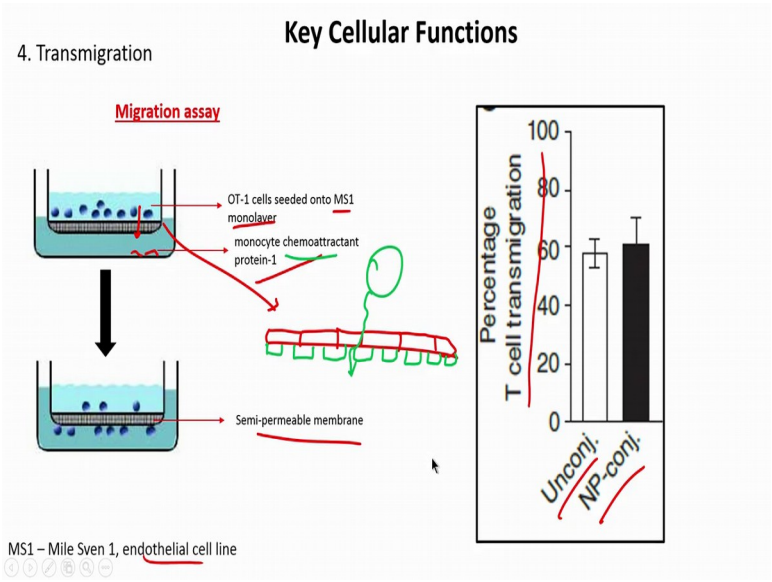

Then they looked at transmigration. So, what they did is they used the semi permeable membrane. And they put all these OT-1 T cells as monolayer at the base, and they have also put a chemoattractant to cause the T cells which are in this chamber to go in. So, this is a semi permeable membrane it has very small pore size. So, only if these T cells can transmigrate they will be able to come down. And that is what they see is and that the percentage of the T cells that are transmigrating, is same in both the un-conjugated and conjugated almost 50 percent or 60 percent of them are transmigrated in the given amount of time.

They have also used M S 1 monolayer which is an endothelial cell line in here. So, if I zoom into this layer what you will find is there is an endothelial layer which is sitting on a top of a porous membrane, which is obviously, a smaller than the cells itself. So, maybe there is a porous membrane, on which these endothelial cells are cultured. So, these T cells are able to migrate right through and able to reach their target through this chemoattractant. So, even this function they found that at least at 100 nanoparticles per T cell is not getting affected.

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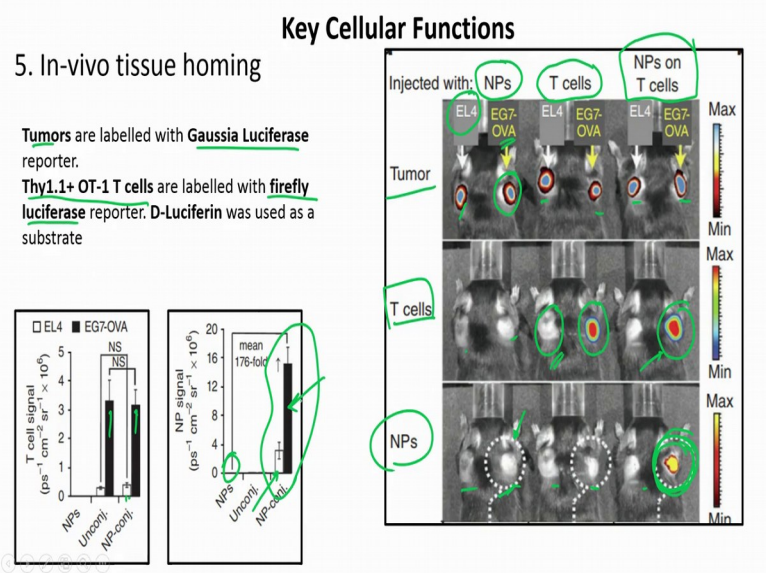

Then they looked at in vivo data. Then they see whether they can home into the tissues. So, in this case what they used, they use a tumor cells which were genetically modified to express a Luciferase gene. Luciferase gene is used for production of light when a substrate is given and this light can then be detected to figure out whether; where these cells are and where the signal is coming from. And then they have used these OT-1 T cells which are again labeled with a different type of luciferin and what they see here is this.

So, if they have a tumor which is EL4, does not have ova then they get a tumor. And then on the other side of the mouse they generate another tumor, which is ova containing tumor. And then they have injected them with either just the nanoparticles or just the T cells or T cells that are combined with these nanoparticles as we just seen.

So obviously, if you look at the tumor signal, you find that the tumor is present where it should be in, all the mice. If you look at the T cell signal; obviously in this case they have not injected any T cells you do not see any signal at all. In the case where they have injected T cells you see that these T cells actually home into your tissue that is expressing over, but not in the tumor that is not expressing ova. So that means, the T cells without nano particle binding to them is able to perform its function. And then they saw the same result when they have conjugated the nanoparticles on the T cell surface,

thereby indicating that these particles are not affecting the in vivo tissue homing capability.

And then when they look for the nanoparticle signal they do not find it anywhere except for the graoup, where the nanoparticles are conjugated with T cells. So, remember even though these nanoparticles are small and you have good capability to be able to go and do the EPR effect. As we have discussed in the past is not anywhere close to as efficient as it is. So, if they really home into that they will find some nanoparticles here, but by conjugation to these T cells they have been able to increase the signal by a huge amount.

And this is again further quantified here, where they are seeing the signal for the T cells. So, again it is not significant between the conjugated un conjugated whereas, the EL4 does not show anything. And then the similarly nanoparticle you see quite a bit enhancement. So, as I was saying this is because of the EPR effect, that you are seeing, but then, actually no you do not really see anything because the EPR effect in this particular case. But, here you see quite a bit of enhancement in the signal where the antigen is present.

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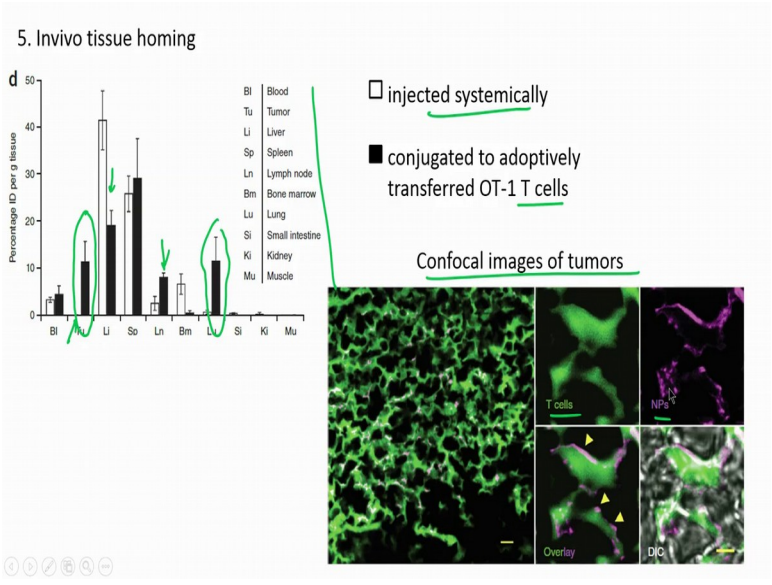

And this is further, so, that what they have done is now they have quantified what happens if you do particle systemically versus conjugated on these T cells and then giving systemically. And these are various organs that are listed here. And what they find is in tumor region you actually see quite a bit of particle when you conjugated it to

yourselves whereas, very little almost undetectable when you are just injecting particle by itself. And obviously, it has changed other patterns. So now, you are also going a bit to the lungs. So, your accumulation in the liver has decreased and some other lymph nodes have also increased because these T cells will also go to lymph nodes.

And these are confocal images. So, they are clearly showing that this is confocal images of the tumor regions they are sectioned it and imaged it. And what they are seeing is these T cells are colocalizing with your nanoparticle signal. So, this is actually specifically through the T cells that these particles are going.

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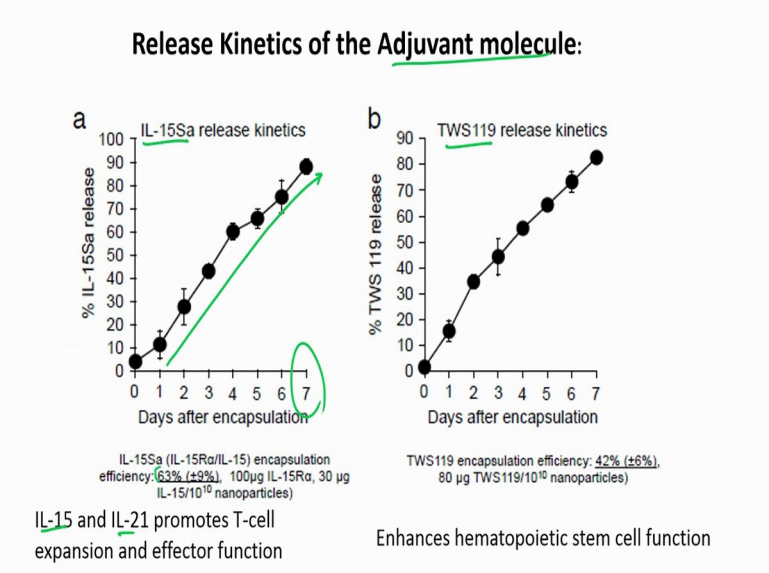

Then what they have done is then they have encapsulated two adjuvant molecules in this particle; one is what they have labeled is IL15 Sa this IL 15 Sa is a combination which is a IL-15 then IL-21 and promote T cell expansion an effector function. So, if the T cells have access to this particular interleukin it gets activated much better it expands it proliferates much better and so, there as was shown before they see quite a good release over a period of 7 days with these particles. And then this TWS119 is something that enhances hematopoietic stem cell function, so, that is another part that they have done in this paper that we will talk about in the moment.

And then capsulation efficiency they got was also good about 63 and 42 percent respectively. So, this was something that was acceptable.

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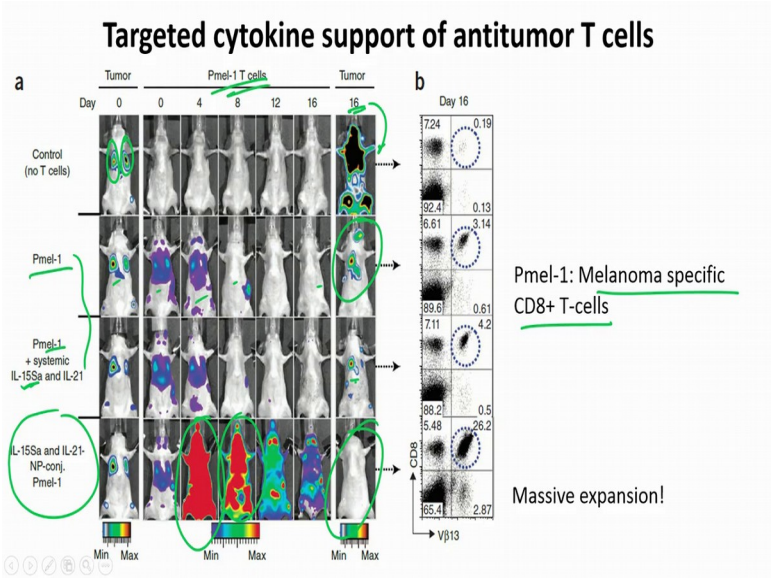

Then they have targeted the cytokine and seen what happens with the tumor function. So, here they have used Pmel-1 cells which are Melanoma specific CD8 T cells. And what you see is if you give the tumors and you do not give this, what do you find is quite a bit of tumors at day 0 and then by day 16 the body is completely spread with these tumor cells. If you give Pmel-1 cells and you see that initially the tumor was there, but then these Pmel-1 cells have also expanded and but at the end of it you still find some tumors. If you give Pmel-1 T cells, but only give IL-15 systemically instead of conjugating to the particles. You see a very similar response as you saw here some reduction maybe and if you do the conjugation you actually see a clear mouse.

So, the tumor has completely disappeared and by day sixteen. So, it is fairly impressive. And you see massive expansion here, look at the signal of T cells this is all over the place in they are expanded by quite a huge margin.

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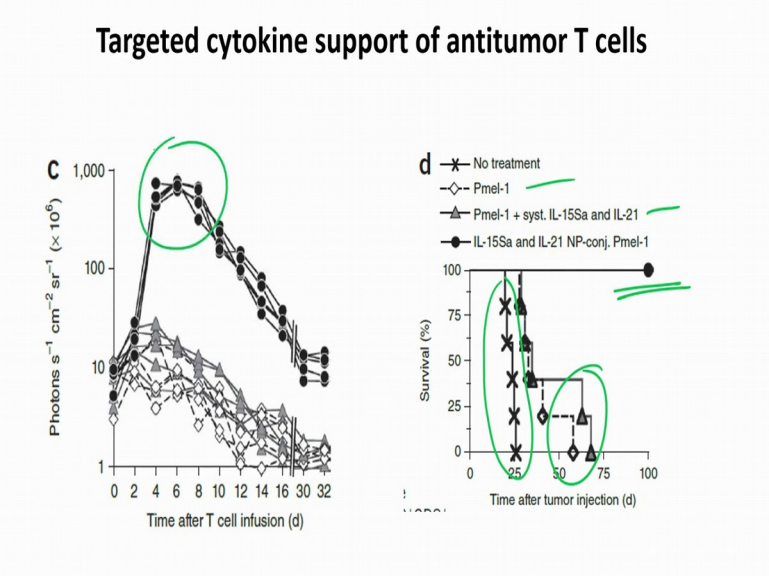

The next thing they saw is what happens to the survival of the animal. So, again similar to what we see these animals died without any treatment even if you give some treatment with just T cells or separate cytokines you get some benefit, but not much. But if you give this treatment which is conjugated with the cytokine along with the T cell you get a 100 percent survival. And this is just showing the signal of the tumor so or signal of the T cells here and you can see that they have expanded to massive amounts and then after once the tumor is disappeared, they start to go back down again.

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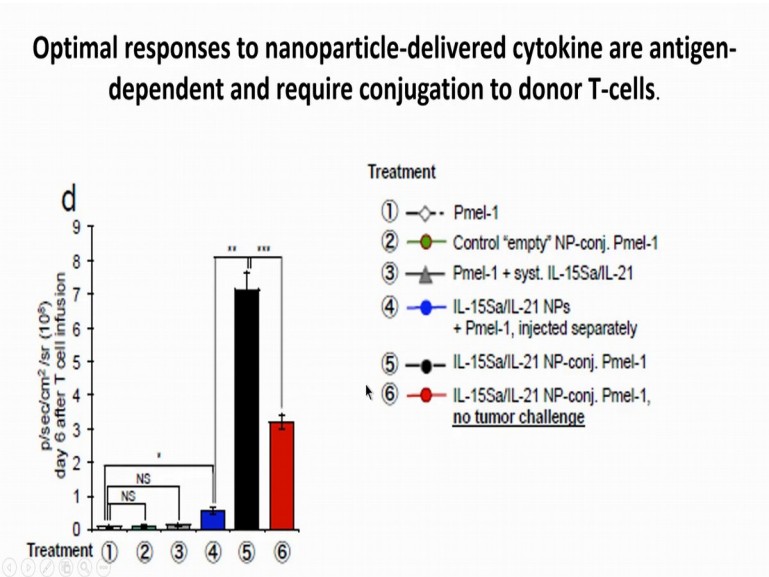

And this is further showing more characterizations optimal response with the nano particle require conjugation to the donor T cells. So, if they do not conjugate it just like in the previous case, they do not really see much signal that is being present.

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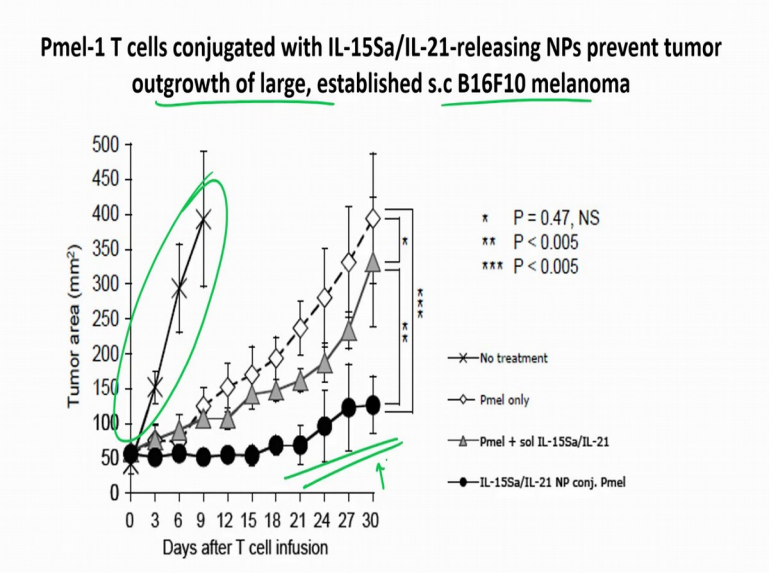

And then one thing that they saw is then they actually let the tumors grow big. So, they have large melanoma tumors which were done subcutaneously. And then they have given the treatment and what they find is these have become extremely large if they give no treatment. Whereas, if they give treatment even in those large tumors it is beneficial. Which is actually quite significant as I said these tumors accumulate mutations and the larger it becomes the more stronger the tumor becomes. So, even in those large tumors which sometimes have seen in the clinical settings also this therapy could be beneficial.

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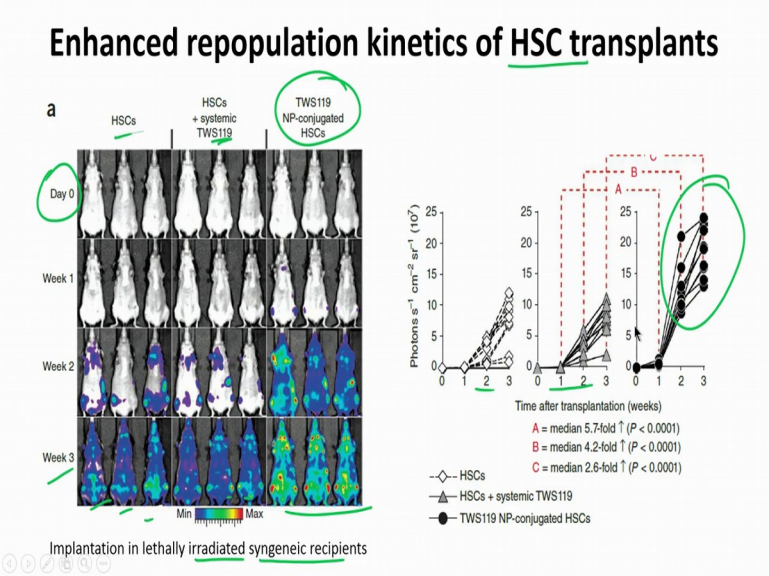

And then the same thing they did with these stem cells, so, here is what they have done. So, at day 0 if they give stem cells, this is implanted into irradiated syngeneic recipient which means that they do not have their own cells anymore because they have been irradiated and killed. And what they find is just like in their tumor data if they do after week 3 what they find is if they only give the stem cells with certain signal for the stem cell.

If they give stem cells with some of the drug and the stem cells have expanded a bit. But if they give it with conjugated to the stem cells itself they have a massive amplification of these stem cells. And these are again just some more quantification showing that the expansion is quite a bit as you can see here compared to the other two groups.

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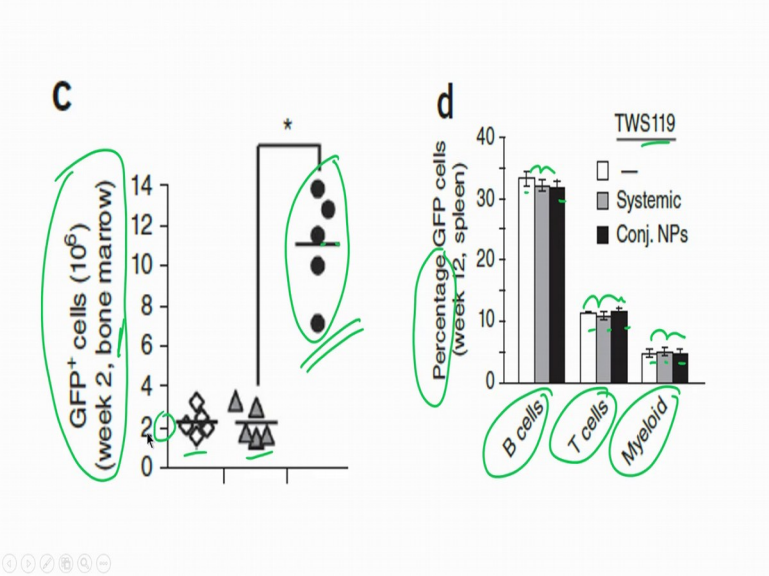

And then finally, they have shown that, these stem cells with GFP positive that they have transplanted. So, they show that in various groups they have lot more stem cells further quantification in bone marrow, that means, that more and more of this graft of your stem cell has been accepted by the body and then they looked at what about the differentiation of them.

So, even though the numbers are higher is there a tendency to form a certain types of immune cells from these stem cells. So, three of the major cells they differentiated. It is myeloid B cells and T cells and then, they found that among the three groups the percentage of their differentiation is not different is that that all the numbers have increased. So, this is percentage, so that is why you see all of them equal. Which suggests that these cells even though they conjugated they still retain their ability to differentiate into all types of cells and actually maintain that ratio.

But just a numbers of all of them is very high, compared to what you get with just the stem cell transplanted or stem cell transplanted the systemic drug the TWS119. So, here you are getting about 2 million cells after 2 weeks, but when you look at when you have conjugated it you are almost getting 11 million cells after 2 weeks so, 5 6 times amplification. Okay. We will stop here and we will continue further in the next class.

Thank you.