Introduction to Proteogenomics

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Lecture – 40
Clinical Considerations for OMICS- II

Welcome to MOOC course on Introduction to Proteogenomics. In the last lecture, we had invited clinical scientist Dr. Sachin Jadhav who gave you very broad clinical perspective and thought provoking points about how various type of clinical requirements can now be met using the OMIC data sciences; however, there is still lot of gap and there is a need for good collaboration.

So, today's lecture Dr. Jadhav will talk to us about the proteogenomics in haematology and BMT, and how it will go into impact the patient care. He will also talk about the treatment planning of a patient based on the mutational profiling. He will further illustrate how tailored therapy for the patients are required and reason why it could not be resolved or achieved even till date. So, let us welcome Dr. Sachin Jadhav for his talk on clinical considerations for OMICS and how it could be helpful in treating diseases effectively.

Rational drug design, the tyrosine kinase which is constitutively activated.

Student: Sir, here is it due to phragmidium?

No.

Student: If this is pathogenic mutation.

It is a pathogenic mutation. This is the first pathogenic mutation that was identified philadelphia chromosome translocation 9 to 22. So, this mutation gives rise to an abnormal tyrosine kinase which is constantly active, and hence the cells multiply and hence leukaemia happens in those patients, right. So, now, this is the abnormal tyrosine kinase, and it has a certain pocket. And it was thought that if we can create a small molecule which will go and sit there, then this tyrosine kinase will not be able to work.

So, this is called as rational drug development rational drug designing. Until this point, all the medicines in our life from chloroquine for malaria, penicillin for you know as an antibiotic or chemotherapy were found by accident, were found by accident, most of the scientific discoveries are by accident, right. People would try hundred things and something kills the cell ok, let us use this. This is where first time rational drug design, because now you know the pathobiology and you know maybe if I can create something that will go inhibit it, right.

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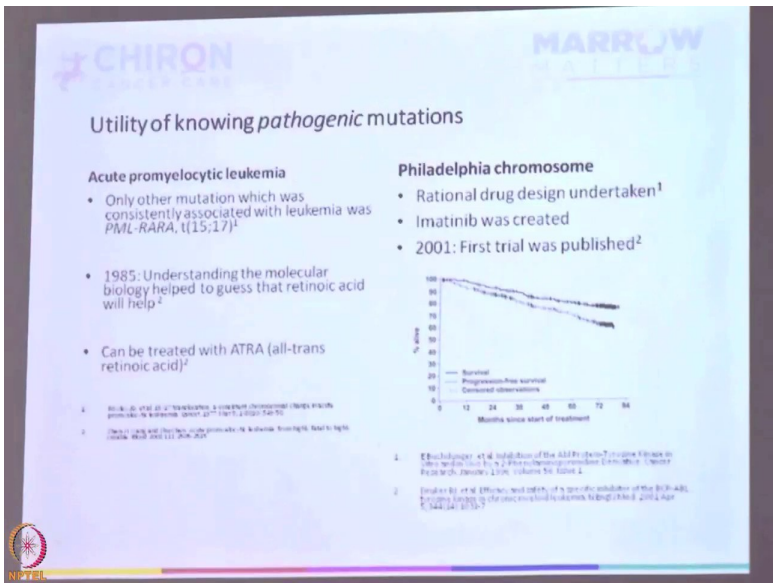

The first time this was done glivec that is imatinib was created and it change the world for CML patients. All of these patients with Chronic Myeloid Leukaemia all for cure, they had to undergo bone marrow transplant, they had to that was the only known treatment of the best treatment, there were other treatments but not good enough. After this medicine came this is one tablet, it is one tablet per day. This medicine turned CML - chronic myeloid leukaemia, the treatment became as simple as the treatment of diabetes.

You just take 1 tablet a day. You take one tablet a day and you can see the overall survival at 80 almost 70 percent overall survival at 5 years. No bone marrow transplant, just 1 tablet, because now this tablet goes and attacks that abnormal tyrosine kinase, and this is the targeted therapy that you mentioned, right.

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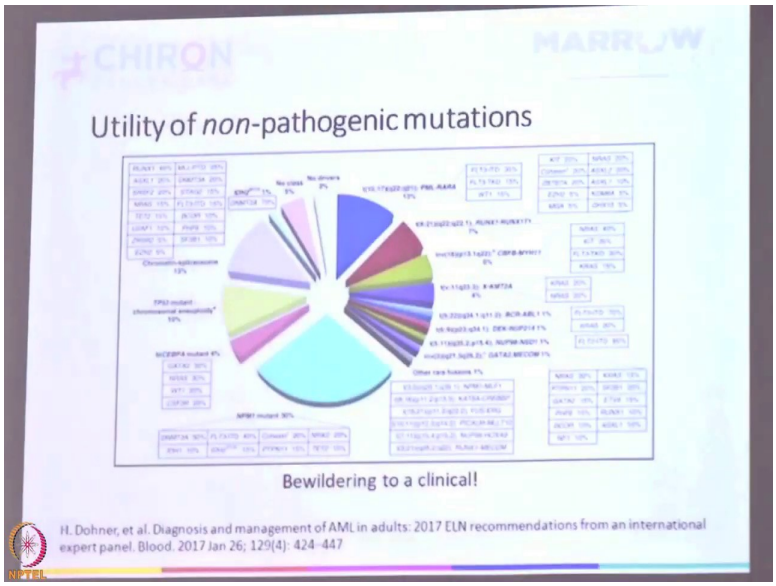

So, this is where now we are getting into rationale therapy. And then people started thinking well let us find more mutations more, targeted therapy, let us make life easy until this happened. Everybody started doing genomics on acute myeloid leukaemia, acute lymphoblastic leukaemia, and this is the current mutational profile of AML patients. So, if you take a 1000 patients with acute myeloid leukaemia and look for mutations, you will get npm, CBPA, ATP 53, you know DNMT everything every patient will have one or two mutations. And we really do not know, we do not know what to do with this, and then you have to follow them for 5 years and then you understand well.

If this subset with mutations, they have better prognosis, those have bad prognosis and then you think those who have bad prognosis, chemotherapy is not going to work. We have to do a bone marrow transplant etcetera, etcetera. But genomics has thrown so much information that we cannot deal with it; we do not know how to use this rationally to practice in a sensible manner.

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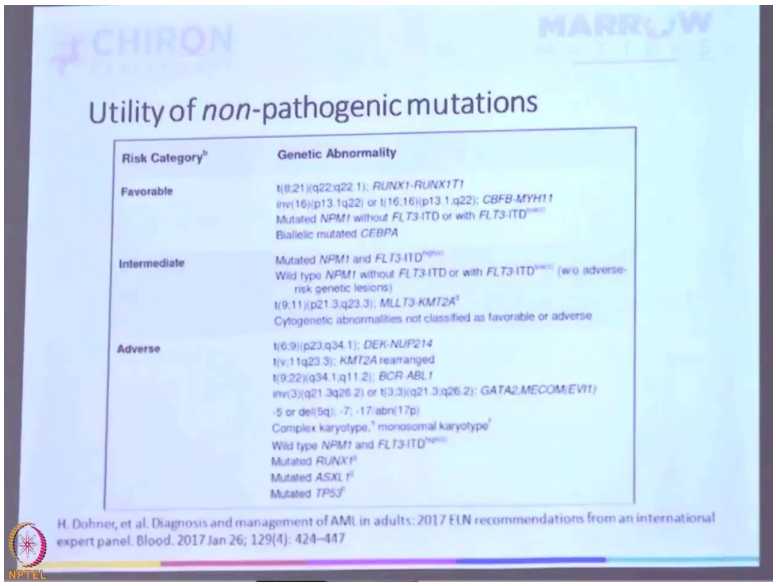

So, we have some guidelines. Now, remember these are non-pathogenic mutations these are just discovered. They are not necessarily driver mutations. They are just discovered, but they affect the behaviour of the cancer. Some mutations will worsen the outcome, others will make it better. So, whichever mutations make it better great favourable risk, just give chemotherapy bone marrow transplantation not required. Those who have bad mutations, you have to do bone marrow transplantation etcetera etcetera, so that is how we are using this information currently, right.

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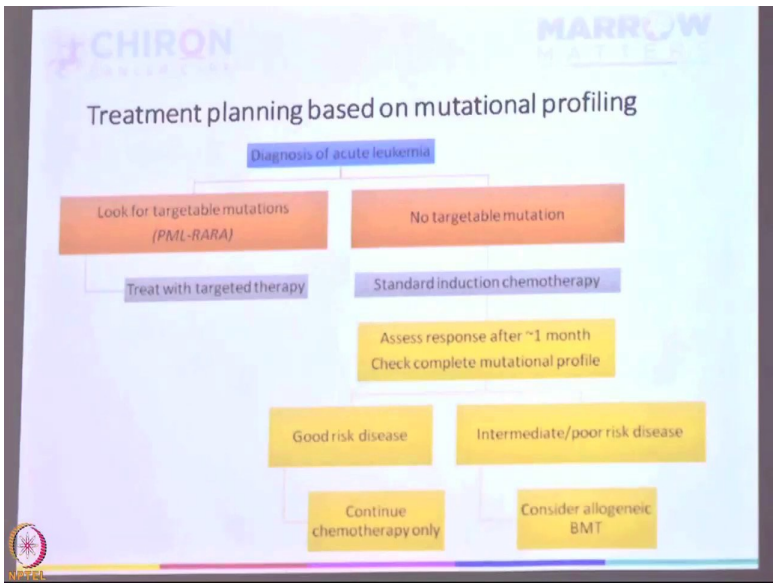

So, diagnosis of leukaemia, if there is a targetable mutation like PML-RARA or BCR ABL that is Philadelphia, treat will target itself, there is no targetable mutation. Give some chemo, look at the mutational profile at the end of the first month. If that person has some good mutations give further chemo and stop, if by chance that person does not have good mutations, then chemotherapy is not going to cure, you need to do a bone marrow transplant correct, so that is how what we are doing.

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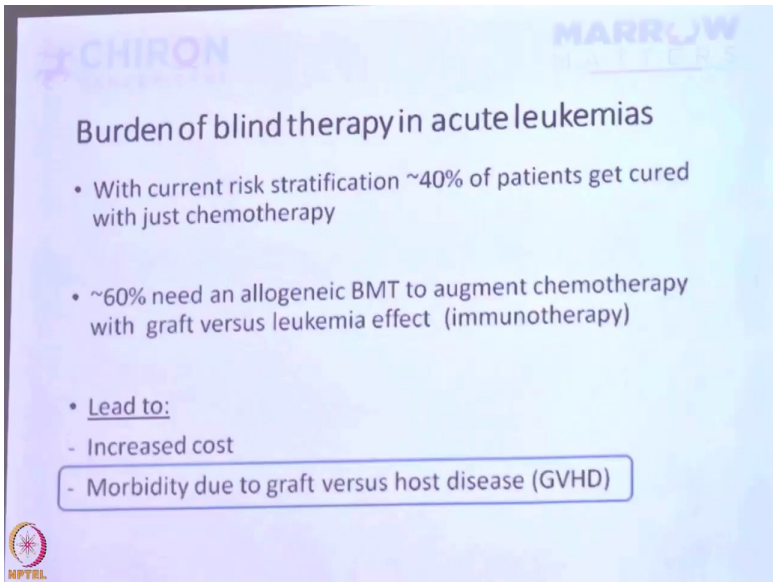

But then this is blind therapy to some extent. It is better than what we were doing even 5 years ago, because now at least we have some mutations which are guiding us, but it is largely blind right. And in this current risk stratification about 30-40 percent of patients unnecessarily get a bone marrow transplant that is the limitation of our understanding today.

We are not able to perfectly risk stratify patients and give perfectly tailored therapy. It was supposed you know 5 years ago, we were supposed to have tailored therapy for everybody it really has not happened. And we end up recommending transplant of 50-60 percent of acute myeloid leukaemias. And you know what is the biggest side effect of a bone marrow transplant?

Student: Graft versus.

GVHD; Graft Versus Host Disease, anything else.

Student: Rejection.

Rejection.

Student: Change of blood group of the patient

Blood group changes. Sure, if the patient and donor have different blood groups, anything else.

Student: We are matching donor in the process.

We get because even 50 percent matches, today we are getting good results, so almost everybody has a donor nowadays. We get side effect of bone marrow transplant?

Student: Immuno compromised.

There will be immunocompromise, they get infection, some patients die of infections about 20 percent of our patients die of infection. What else? Financial toxicity ok, financial toxicity, and that is why this is a problem. So, we need you guys to do a better job, so that we do not do this. We depend on you guys to tell us how to better categorize cancers, how to treat, what not to treat; what to do, what not to do. Because ultimately we are delivering healthcare based on what you publish. And it is not only this, but like you said there is a lot of morbidity.

So, 60 percent of our patients after transplant do well, 20 percent will die of chemo therapy of complications, 20 percent more will survive with bad complications. And I will show you a patient, right. And one of this is one of the worst complications if the patient is surviving with graft versus host disease in about 5 percent of patients is they lead a miserable life they are alive, but it is not a good life, right.

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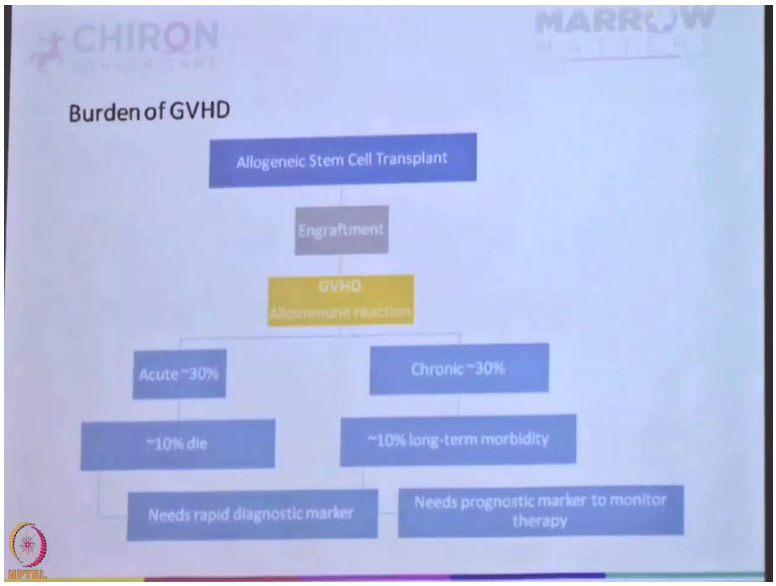

So, we do an allogeneic stem cell transplant. Allogeneic means we take a donor. We put the donors stem cells into the recipient. Before that we give chemotherapy and kill off the recipients native marrow, yeah. And then you put in the donor stem cells, the donor stem cells go in and they engraft, they create a new marrow. As they create a new marrow, the donor cells may attack the host and cause graft versus host disease, GVHD. This GVHD will happen in the first 100 days in 30 percent and 10 percent will die because of it right. Now, this is so bad in spite of the best immunosuppression patients will die.

So, we need a rapid diagnostic marker. If we wait until clinical symptoms develop, it is already too late. We need a serological marker which we can track right. It is like checking for temperature until the patient becomes septic. We need a serological protein marker which we can track before there are symptoms of GVHD, because after that 10 percent die, no matter what you do, how much ever money they have. Because when this happens families tell us, doctor, I will bring as much money as is required, do everything, you can be anybody in this world.

If you have bad GVHD, you are going to die; there is no medicine in this world which can stop you. If you survive, 30 percent will get chronic GVHD that means, they will have long term problems. And for these again 10 percent of them will have long term morbidity, they cannot go to work, they cannot go to school, they cannot play with their

friends, children cannot play with their friends, etcetera, they are restricted because of chronic GVHD.

So, this needs prognostic marker to monitor therapy, we are giving treatment, but we have to look at the patient after 3 months we need something which can give us an early indicator of response to treatment. So, you see the gaps in our knowledge, you see how much work has to be done. So, how can we go ahead? We need to understand graft versus host disease is an immunologic disease, it is a donor cells, immunity the new immunity attacking the host from inside. This is the new immunity is a new police which are supposed to help, but the police are attacking civilians, right.

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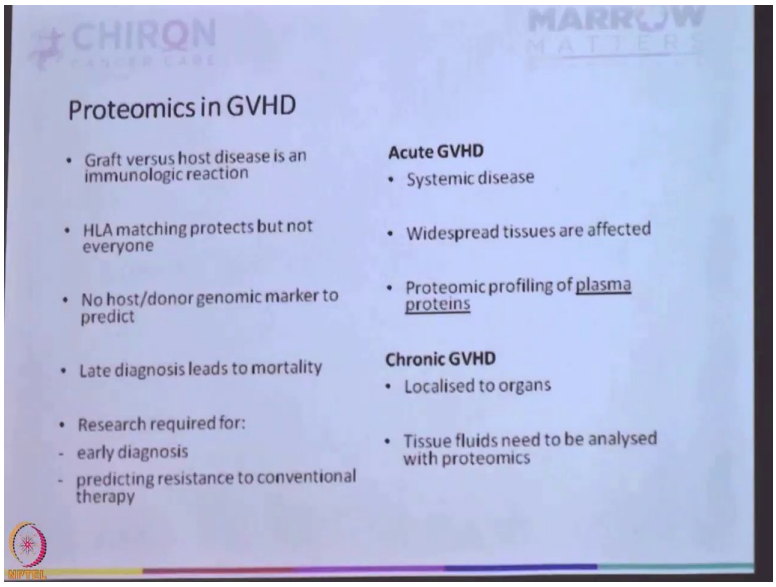

We try to do HLA matching like you said so that we decrease the risk of GVHD. We ensure that the patient and donor are as well matched as is possible. But still it does not really protect everyone. And there is absolutely no genomic marker which can help us predict, that yes this patient is at a higher risk or this donor recipient pair is at a higher risk of GVHD, there is no genomic marker late diagnosis like I said leads to mortality.

So, we have to find something by which; by way of by means of which we can do an early diagnosis right and predict resistant to conventional treatment. Currently we give medicines and you have to wait for 3 days. In those 3 days if the person is not responding he is gone. We need some early predictive marker for response to treatment, right. Acute GVHD is a systemic disease, skin is affected, gut is effected, liver is affected, there is

widespread tissue injury and hence plasma proteins can be looked at. Maybe in the blood will find some markers because this is a widespread systemic disease the entire body is practically affected, acute GVHD.

Chronic GVHD is localized dry mouth, dry eyes, skin lesions. So, we can look at the tears for a proteomic profile, because this is a localized disease, right. And hence tissue fluid tears saliva, because the GVHD is localized to that part of the body, it is not generalized tissue fluid needs to be analysed.

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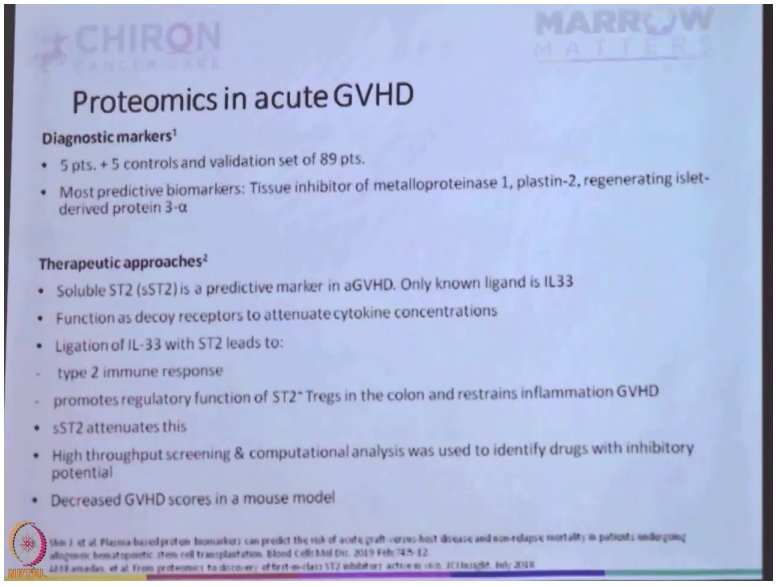

There is some work which has been going on for over 10 years now for over 10 years, and many people are claiming when we have a panel which can predict etcetera etcetera. Work is still going on this is one of this I am just giving you a flavour of what is happening in the world. This can also help us provide a therapeutic guidance.

So, for example, proteomics has shown that soluble ST2 receptor is elevated in chronic GVHD. So, if you can create an inhibitory drug maybe it will help and it has shown in this study which was conducted on a mouse model recently reported in I think July this year in JCI. So, maybe if we do good work we can figure out some treatment, you know policies, strategies etcetera. And, if in this paper at least in this study when they created an inhibitor to soluble ST2 receptor, it decreased GVHD in the mouse model.

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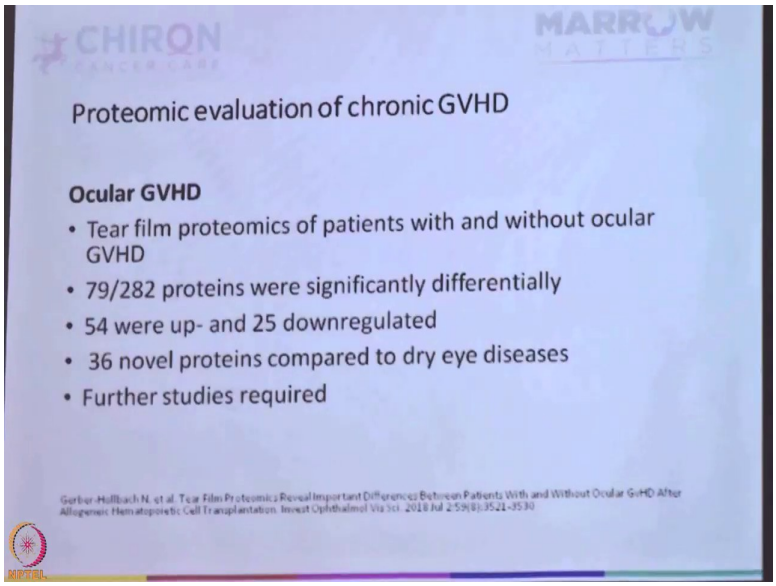

Same thing chronic GVHD ocular tear film was looked at some novel proteins were identified.

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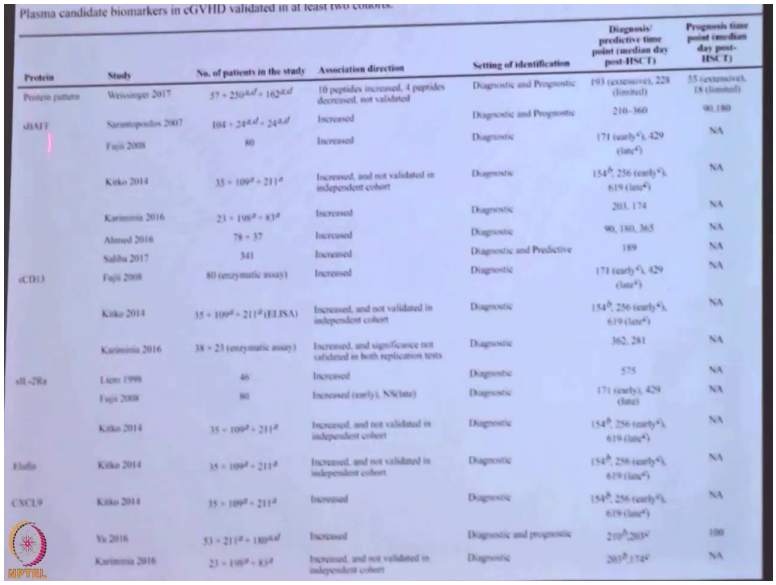

This is a review of various proteins which have been identified to be up regulated or down regulated.

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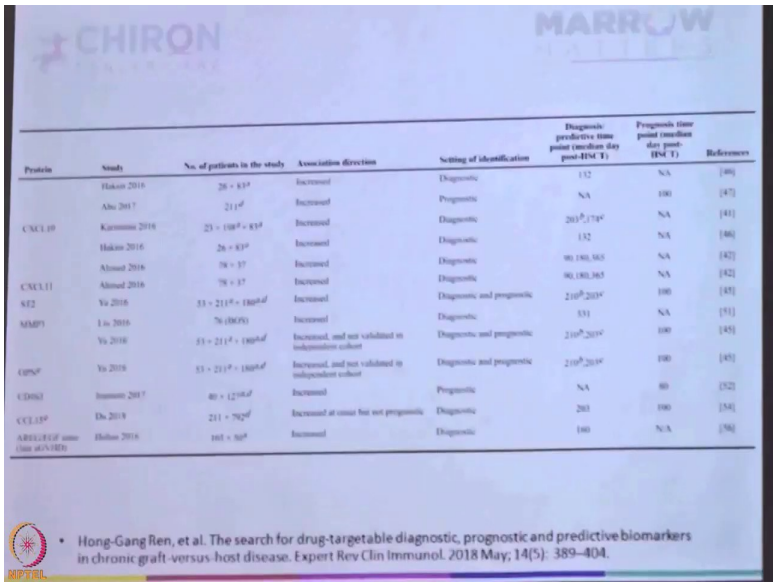

But you can see really nothing that is clinically relevant today. So, much more work needs to be done.

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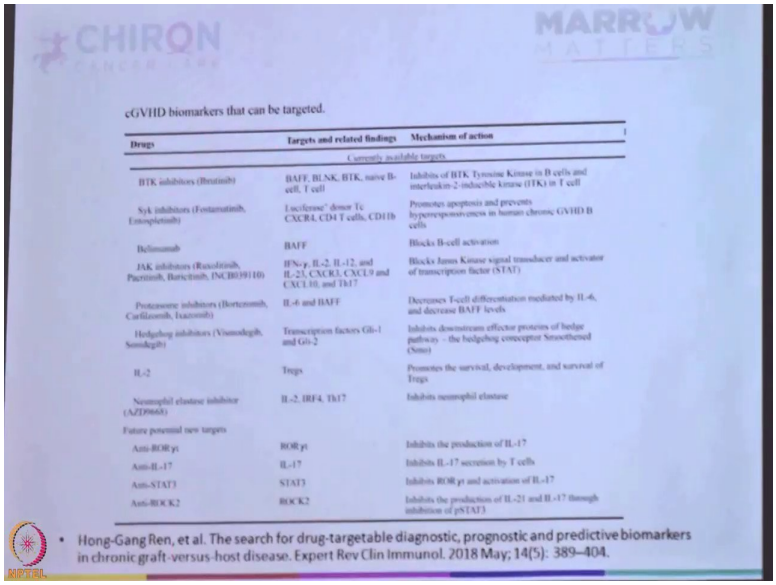

Maybe if something is clinically relevant we can target it with some targeted molecule, targeted medicine.

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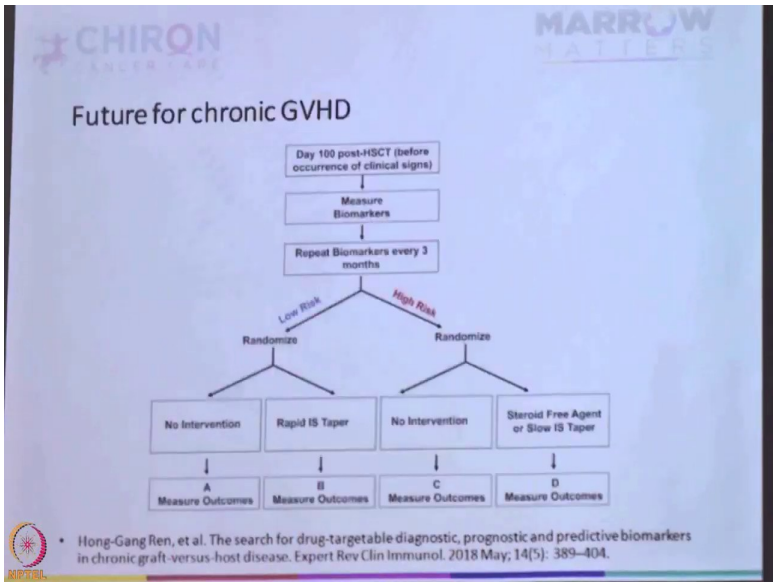

But well we have to see, maybe what we can do in the future, and hopefully the near future because we are transplanting patients. We are currently transplanting patients who need this today, but at least maybe, in the near future what we can do is we can look at the blood biomarker before clinical symptoms come up; yeah, in the early stage. And if that person's blood biomarker stays negative, we can decrease immunosuppression rapidly; we have to give immunosuppression to transplant patients, so that they do not have rejection and GVHD, but that leads to infection.

So, we can decrease immunosuppression rapidly, so that they do not get side effects of medicine. However, if they seem to get a high risk because the biomarker is going up, then we will we may actually intensify immunosuppression and prevent GVHD if we can find a biomarker. I will just show you two patients and then I end.

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This is the impact which could have been created. This is a boy he is now do you have you seen and you have not seen. So, this is a boy from north Karnataka. I think he is now 10 years old. He came to us with acute myeloid leukaemia. We did a pre emptive transplant him that is because he had an MLL mutation. So, acute myeloid leukaemia with MLL mutation is bad, you need to transplant them. So, we transplanted him. He went through lot of complications and now this is chronic GVHD. This is his skin those are his legs. It is not bad; he did managed to go to Thailand with his family.

But he is he is not normal, he cannot go to school, he cannot play with his friends, because he is on a immunosuppression, right. And he can anytime pick an infection he is on so much steroids that he can pick TB for all we know because his immunity is suppressed by our medicines. And these medicines were given to treat GVHD and they can have a side effect of immune suppression.

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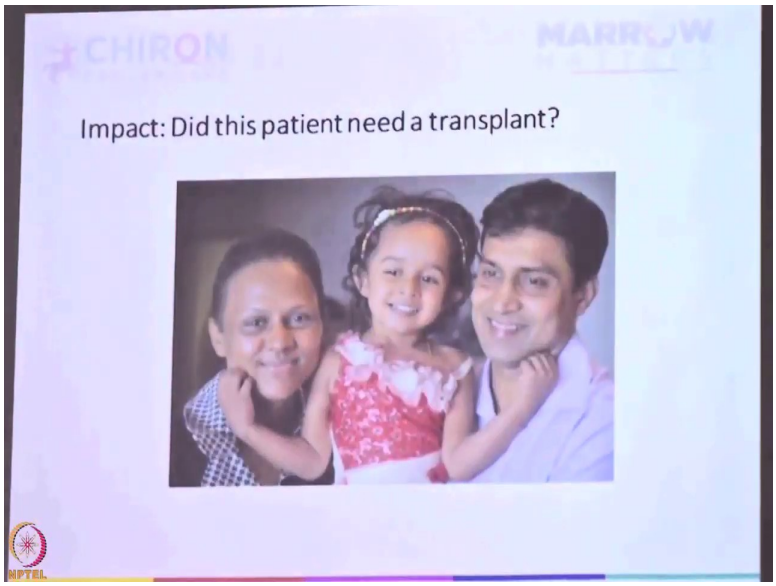

So, we need to do better in this that is another patient she is from Srilanka, and again AML she had intermediate risk, so not really bad high risk, but intermediate risk. And we felt it was best to again treat her with a prophylactic transplant before she gets a disease relapse because she still has intermediate not good risk.

She has some minor lung GVHD. So, she has some coughing phlegm, but not bad very active works in a bank in Srilanka leading a near normal life, but then what about the financial toxicity they had to borrow money. So, if we could have a better understanding of whom to transplant and whom not to transplant maybe we should not have transplanted her, right.

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That is our team right, those are the doctors and then you have the nurses and that is the administrative team, we have clinical pharmacists everybody working together those that is the nurse educator BMT coordinator etcetera, etcetera. But, what about this? Thank you.

Student: When we have matching donor, what are the chances of developing GVHD?

GVHD, so 40 percent of patients will develop GVHD, I mean it is between 30 to 50 percent in different series, let us say 40 percent will develop. Now, out of this 40 percent 10 to 15 percent will die because of this. We are able to avoid 60 percent from getting GVHD, but 40 will still get.

Student: The matching does not help?

It does not help, because there are many more minor antigens. We are matching 10 HLA antigens HLA a b c dr dq and now dp as well, so 12 HLA antigens, right. But there are lots of minor antigens which are different and it still causes. So, the reason GVHD developed is much it is different. Before transplant, we have to give chemotherapy. Chemotherapy causes tissue injury and reveals neoantigens. And the upcoming new donor cells in the body will look at these new antigens and then neo antigens and mount the immune response. So, it is not just the HLA, but various other antigens which are different between the recipient and the donor.

Student: I have a suggestion why cannot we use a patient's own stem cells.

Yeah.

Student: I mean we can induce pleuripotency, it is a kind of synthetic but will reduce the chances of graft rejection.

Step one good idea let us do it ok, no problem, that is called as an autologous transplant, autologous, your own stem cells and that is done, that is done in some patients. There are reasons why we do autologous transparent in some and allogeneic in others. The reason we do allogeneic transplant; that means, you take another normal human being his or her stem cells and inject into the patient is you are trying to create a new immunity. One of the concepts of cancer it is a failure of immune surveillance is that right malignancy is a failure of immune surveillance.

So, this is a patient who had has bad cancer. His own immunity failed to prevent it and it is so bad that if it comes back it again cannot fight. This is a terrorist I mean cancer is like a terrorist correct, immunity is like the police. If while we are sitting here, imagine after this talk you opened your phone and you saw that 5000 terrorists were discovered in Mumbai. The first thing that you will you will think is what were the police doing, how did 5000 terrorists come in. And even if we kill these terrorists what is the guarantee that this police will not be able to stop the next attack, because these fellows are useless.

So, there are some cancers that is what I said high risk for relapse, where you are scared that this person's immunity cannot fight the cancer if it relapses that is where you change the immunity by giving somebody else's stem cells and thus new RBCs, new WBCs new platelets and new WBCs means new police.

Student: What are the chances of rejection?

So, rejection today in today's transplant practice is less than 5 percent, because we have good HLA matching less than 5 percent risk of rejection.

Student: is between immunosuppression?

No, for about anywhere between 9 to 12 months, after that we take them off immunosuppression. They go back to normal life one of our patients was a BSF soldier,

we took him off immunosuppression, he had to go back to the border and he said I do not want to go back to the border, right. So, they lead a healthy I mean 60 percent of patients lead a healthy normal life.

Student: In an allogeneic transplant cannot we keep the T cell population of the donor stem cells in check?

Fantastic question, so that can be done and we do that. So, we use a medicine called cyclophosphamide which is given on day 3, day 4 of transplant to cut off the alloreactive T cell population which is coming up on day 3, 4. T-cells can also be reduced by putting some other medicines in the stem cell bag or given them as injections, but they do not uniformly help. And when you are cutting the T-cells you may also be killing the good t cells which will give immunity to the recipient. So, it is not yet a perfect science, but that is a very good question.

Student: There are lot of talk about finally, percentage of.

Yeah.

Student: Dendritic cells towards the cancer and then get him them back to attacking cancer cells how far is that successful it is.

So, that is that is another form of immunotherapy allogenic BMT is immunotherapy, because you are changing the immunity, there is another form of immunotherapy where either you use dendritic cells or you use what is called as car T-cells.

Student: Ok.

Chimeric antigen receptor t cells. It is US FDA approved for certain cancers, but we have to look at the long term. So, less than 50 patients in the world have received it, and currently one patient's therapy cost 3 crore rupees, yeah.

Student: Sir, what I am trying to ask is while applying chemotherapy, what you do to preserve immune cells, these cells destroyed nah, cancer cell immune cell as well as normal cells, now what technique do you apply to preserve that immune cells?

You cannot do anything. So, chemotherapy is like carpet bombing.

Student: Sir.

Yeah like I said there are 5000 terrorists were found in Mumbai, get a plane from above drop a bomb, terrorists may die normal people will die. So, cancer cells will die normal bone marrow will die in all of our patients the bone marrow actually gets completely killed. So, WBCs go down to 0, let us go down to 0, there is no way as of now we do not know.

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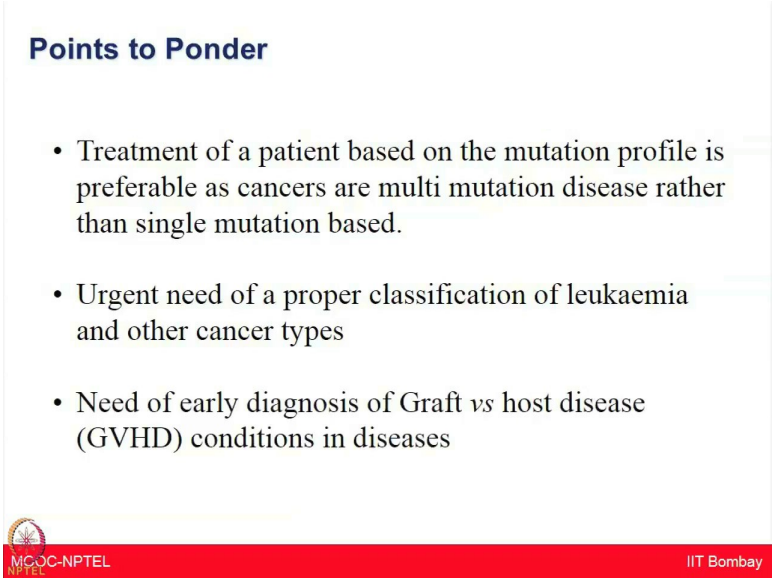

In conclusions, today you have learned why there is an urgent need of a proper classification of disease like leukaemia or in fact it is true for any cancer type. Many diseases like leukaemia which required organ transplant mostly leads to the drafts versus host disease also known as GVHD, blood group even may change and many their immune system based complications. Those rare number of patients which live longer than most others, they live diseased and painful life.

In India, these cases are also economically toxic as most of the Indian population cannot afford these kind of treatments. We also heard the need of early diagnosis of GVHD conditions in such diseases. Also we learnt about how proteomics genomics or proteogenomics could help clinicians and patients to fight diseases like leukaemia better and effectively.

The next lecture is going to again shift gears bring back the proteogenomics, how to integrate genomics and proteomics information, and utilize the combined power of proteogenomics for various diseases especially in context of cancer. And we will have another noted speaker Dr. David Fenyo who will deliver the talk.

Thank you.