**QNS: What is your latest research and how does it relate to gene editing?**
I do a lot of things related to gene editing. Gene editing is a bad word, the techniques we have at the moment are not precise but there are ways to make them more. I work on human embryonic stem cells and other human cell lines. Roger Foo, our principal investigator is a cardiologist so we do a lot of research related to the heart. An example of what we do is making reporter lines – basically tagging an endogenous gene of interest at the 3` end where the protein-coding ends, add a fluorescent tag to create a reporter. When we take stem cells and apply the differentiation protocol, we can see when the cells turn on the genes by observing the colour. We can then gauge the efficiency of differentiation, or engage in fluorescent-activated cell sorting to enrich for specific well-differentiated cells. We made several different reporter lines using Cas9 editing. We use the most well characterized SPCas9. We have also done knock-outs in cell lines, using Cas9 – to see if deletion of genes of interests will need to phenotype differences.

Other things, we do a lot of sequencing of disease and human samples. We have an ongoing project to sequence a selection of heart tissues from people who died healthy, as well as people who died from cardiac issues. We have been able to pin down on potential small nucleotide polymorphism, a list of candidates which are enriched in the diseased people. These SNPs are not in the protein-coding gene, they are elsewhere, thus there are no obvious phenotypes. We think that these SNPs are actually enriched in enhancer regions, which would affect the expression of other genes. We will make wild-type and mutant stem cells, differentiate them into cardiomyocytes to see if there are any phenotypic differences. Some of these SNPs have been associated in genome wide studies to a cardiac phenotype, there's only a link but no causation. We are making these SNP changes using both normal Cas9 cutting and then homology directed repair with repair template and also with base editing, testing different versions of the Cas9 base editors.

Other projects use genome-wide CRISPR screens looking for various things. We have cell-line models, which we try to model a disease in a dish. If we have a robust enough model, then we can apply CRISPR screen on them.

I do other things with deadCas9, like imaging the chromatin, just trying to see what its activity and location.

**QNS: You mostly use gene editing as a technique to modify things for your studies not as a therapeutic tool. What is your opinion about the shortcomings of using gene editing when you are using it? If through some means gene editing can be extended to a therapy, what do you think are the risks?**
There's definitely potential there, there [are] thousands of people and companies working on these things and some of these ideas will be translated to the clinic eventually. As I said, gene editing as a term sounds too precise because there are errors. There's been several papers suggesting that there are loads of errors but actually those are wrong.

When you design everything really well - you get the perfect guideRNA and use the best available Cas9 which has been improved in specificity and all that, it can work very precisely.
The thing is that you work on the reference genome. The reference genome is not yours or mine. It is based on one dude in America, that's the reference genome on the public database. Obviously, there have been a lot of SNPs that have been characterized and these have been annotated in the public databases. But, none of these databases are fully comprehensive of all the SNPs of everybody in the world.

There's always a possibility that even if you get a 'perfect' guide, maybe patient A unfortunately carries a mutation in whatever gene that makes the sequence now match to your guideRNA, and now you have an off-target. Unless you sequence every single patient before you do that. Even if you do that, you probably will have sequence if whoever has the money to do gene editing therapies on themselves probably have the money to sequence themselves.

But then, it becomes a question like – are the SNPs the same in every single [person] you've got from the sample? For example, if you're sampling the blood, will the SNPs be the same as the muscle/tissue you're targeting? This is the fact that, there's always going to be a risk but then again for any drug taken, there's people [who] are scared of gene editing because all these terms of cancer and everything, you poke around with the genome, it will make you a mutant.
Like I said, there are going to be risks, there's no way that all the risk can be taken out of the picture for any potential therapies. And the question becomes like what is the acceptable risk?
I can't claim to be the top expert for any of this, there's a lot of people who work on putting gene therapy into clinics.

Early this year, there was a conference here, where they had several people, mainly from the US, who are working on implementing the actual therapies. The current ones they are implementing are not with Cas9, they are other gene therapy trials. But a lot of these people are working on Cas9 for future projects.

And the consensus is, there is going to be some inherent risk. There will always be some, is just that, somewhere along the lines FDA or EMA, all these regulators will need to set the level – what is the level of off-targeted risks that is allowed for therapies.

**QNS: Follow up from the question of therapeutic techniques, at the start you were saying that all genome, if you can be very specific on the guideRNA and DNA sequence. My question is, even if you can be very specific, you may not know the effects of your target because we do not know the full effect of the gene sequence, being specific doesn't mean there will be a specific outcome.**
There's very little that we actually know of how things work in the genome. In the research programme, it's very easy to knockdown the gene and see the effect. If you see an effect on the dish, great that's the effect on the dish (its observable).

If you knock down that gene in the body, how do you [know] that the interactions, if there were some other interactions that you have not modelled in the dish.

There are specific targets, so if you know somebody who has a specific disease where this a faulty misfolded protein or something like that, for example the Integrin that keeps your skin attached. Basically those proteins are mutated, they don't work or are very weak, and that's one of the diseases that people have successfully treated in gene therapy

Basically, there are diseases that you know a target specifically. So if you can target the gene in the correct cells then you're likely to have the correct outcome. But if people are talking about, "I want to cure cancer", that's a very big statement." I want to make my baby smarter, where do I start?" Now, we don't even know what makes people smart. People have lists of genes but do you have to edit everything? Do you have to turn everything on hundred times, what does that do?

We don't know enough to go poke around a lot because it's likely the we are going to mess with things that are unexpected. That being said, there are diseases that are very clear targets that have been characterized – there is that one gene that gone awry in these cells. So if you can fix that one gene in the cells, that is a very good thing to start with.

Going beyond that, then you'll be going towards sci-fi, not science. Sci-fi is nice but we are not there yet.

**QNS: A lot of the things you do are about DNA, but for Cas13 it is for targeting RNA for RNA editing. So what is your take on RNA editing – do you foresee any future or risks?**
There will definitely be applications for that, I do not work on the RNA editing so much but there are a couple of papers that came just in May, finding new Cas13 strains – Cas13d and Cas13rx.

They allude to specific cases, where for the diseases there are problems at the RNA stage – they have intron and exon skipping. So, you end up getting RNA, for example with Tau protein. You can have one copy with 4 exons, another copy with 3 exons – if there is a wrong ratio then you'll end up with the disease. So you can target one of the exons and that will neutralize the ratio error.

They were talking about the intron-exon problem is actually [found in] 15% of RNA diseases. So that's definitely something that this Cas13 will be good to target. The other options like with the ADAR or APOBEC, you can definitely see how you edit the actual RNA molecule.

My own question is why is that better than editing the DNA? Is there a reason why it would be better?

**ANS: The reason other people,  and we also agree, is that some diseases you cannot totally knock out the DNA, it is caused by over-expression. Editing of RNA can reduce the**

**expression to the acceptable or basal level. So it will be better. Another reason is that, people are concerning about Cas9 gene editing that will change the genome permanently. But, changing RNA is just transient, so people feel safer.**

**ANS: At the same time, we recognise the problem of this temporal and transient editing. You cannot fix the problem in one shot.**
I mean it's very good for the drug company if you have to keep taking the treatment every year instead of fixing it one time. Which actually, may get more likely that these treatments will get translated by these drug companies. The first ever gene therapy that was approved has stopped getting marketed because it's so expensive. This was a treatment approved in 2012, it has only ever been administered to one patient and cost a million dollars – it was just too expensive. There were also other issues with that particular treatment.

It was the first ever therapy to be approved and it's great because that means that gene therapy can be approved – so there are other things in the pipeline now, which hopefully will do better than that one.

One other problem with that therapy is you have one treatment and you pay one million but then nothing after that, it becomes – how many people can afford that? Maybe more people could afford if they can pay 10 thousand every year or something like that.

Getting back to the RNA question, you could argue that if you edit RNA it is transient and it won't be permanent but then, the other argument is you have to keep going back to get the treatment. There is two sides to that.

Obviously for a lot of proteins, you don't want to completely kill them. You just want to adjust their levels. A lot of base editors have been shown that they can be used in animal models and all that and that suggest that we would be able to use them. Whether it's better to base edit the DNA or RNA… is another thing.

**QNS: But RNA is like 98% of the genome so it's all the non-coding regions – it's a huge market out there for RNA unlike for DNA because more and more research are going into RNA**

There's a lot of work happening but when you look at research – research doesn't necessarily translate into market for drugs. If you target the DNA with the base editor and you change that base in the noncoding RNA so that RNA will be changed.

I might be biased because I work on DNA a lot. On the other hand, it's definitely true that there will be applications for RNA editing. Whether [eventually] will there be more applications for RNA or DNA – these things are very far still. People are working on them, there are Cas9 trials going on that have been approved. There's at least one such trial.

**QNS: We conducted an online survey, part of the question was, what is your definite of enhancement in the context of genome editing?**

Enhancements in genome editing, you mean enhancement of methods of genome editing or enhancement of people?

**QNS: Those will be the public opinion, we actually gathered their majors, like if they study life science or something else. Now, we want to see what is your definition, from a scientist perspective, what's your definition of enhancement?**

If you just say define enhancement, then that to me is something that you're trying to change an embryo/a child to [be] faster, be a better runner or have 6 fingers to play the piano better , it's not that you're curing a disease. That's from Gattaca. But then again, all of these things – there's no way we can do anything like that yet because we don't know the genes – what should we change? What can we change? To make these kind of ideas of what people have about what would be a better person, the ideal human, it's very eugenics you know. You go down a very dark path when you start this conversation.

**QNS: For genome editing, are you for it just in the therapeutic sense? Or also for enhancement?**

I don't see an issue with trying to fix a way like cystic fibrosis of things like that. You don't want anyone to have cystic fibrosis, it's not a nice thing to have. So, the question is – should anyone who carries these genes have prenatal screening and discard any embryo that has that? Or try to have gene editing on those embryos and fix the gene. This are difficult questions.

At the moment, it's not society. Society in general, Singapore or anywhere is not there yet. Prenatal screening is happen[ing] already, people screen for some diseases; prenatal you screen when the embryo is growing in the womb. If you have a Down's [syndrome] baby, are you going to abort?

I can tell from personal experience. Because my son is 6 months old and we had this personal screening. We didn't want to know the result. Not everyone does that. We have a couple of friends, their son was born one month later than us and they specifically wanted to know the results of the screening.

What would you do? If you know, are you going to do something about it? And if the tools are there to change the cystic fibrosis, which parent wouldn't want to? Which parent would want their child to have that? So I would expect that this will eventually become widely available for people to be able to do this type of treatment. But, that changes a lot of things for you to do any of these, you can't do things the old-fashioned way, you have to do IVF to do anything like that. It makes human procreation different.

**QNS: What will be your opinion on the outlook of gene therapy, especially the types that edit the gene, like in an adult you edit the genome to treat some disease. Do you think it is something achievable in our lifetime?**

Using Cas9, muscular dystrophy is something you don't want to have as usually people die before they are 30. Basically, the dystrophin gene is mutated, that's what hold your muscle fibres and what makes them work. [There is] a group in Texas, what they have done is they found the dystrophin gene is really long. If you have the mutation, there's a premature stop and the 3` end is not there. Even though the protein is made, it is not functional as it cannot attach to the other end of the structure. If you use Cas9 and you cut away one of the exons in the middle, or you mutate it so that the exon actually gets skipped out. Now, the frame is there again, the complete protein is there but you're just missing a little bit in the middle. It is not 100% functional like the real thing will be but it works. So they have published a couple of papers on this. They targeted a specific splicing (the acceptor), they target the acceptor and that deletes the acceptor one exon ahead and now it splices the next one. Now, you get rid of the one with the mutation that destroyed the rest of the gene. They have done this in mouse models and shown that it can restore the function. I'm not sure if they have done this in clinical trials but they are in the progress. They even have a small spin-off company from it. They are in collaboration with the Muscular Dystrophy Association in America, and they have patients who currently have muscular dystrophy. They have taken cells from them and [made] IPS cells. They've done the treatment on these IPS cells and shown that they can make functioning muscle that has some level of functionality of dystrophin. These kind of things, like any scientific work takes a long time to translate into the clinic. But within our lifetime, even mine, some of these treatments will definitely be available.

[45:07] Even here in GIS, we have groups who have programmes that aim to bring gene editing to the clinic. The work I do will not reach the clinic as I build models and play around with the tools but I'm not making anything that will go to the patient. Maybe you'll find out something with my tools that will eventually go to the patient. But, I am not building anything myself that will reach the patients themselves directly. But, there is definitely stuff out there that will reach the clinic. You can give the standard answer of 5 to 10 years but within our lifetime yes.

**QNS: What do you think will be the biggest obstacle in terms of translating science into clinic? Will it be something like achieving specificity, delivery of the editor or any other factors?**

The specificity like you mentioned, it will never be 100%. There will always be a possibility that there will be some issues with the patient. But, I think there has to be some level of risk that has to be accepted when it gets translated into clinic.

**Delivery methods**, there are already a lot of methods that are being delivered and used. That one gene therapy that was first to be approved was using adeno-associated virus, which is one of the things that people are also looking to use gene editing with Cas proteins – not Cas9 because it's too large most of the time.

There are these viable methods also like using nanoparticles or things like that. What's going to be the biggest hurdle – I don't know, my guess is going to be as good as yours really. All these things have to be solved before they can bring them into the clinic. But, people have been working on these things and there are avenues that people are actively pursuing.

**QNS: Through our survey, we identified some myths and misconceptions that people frequently have. Could you debunk them for us?**
**QNS: Gene therapy is an experimental technique that introduces genetic materials into cells to enhance the characteristics of an individual. A lot of people said it's true, about 20% got it correct.**
I mean yes, gene therapy could, I mean this kind of thing would fall under gene therapy but it's not gene therapy. And per se, therapy means that you are trying to cure or fix something, it's not like you will make someone prettier. Gene therapy doesn't necessarily mean you will introduce new genetic material. You're not going to necessarily introduce a gene in there. Although in a lot of cases, you will introduce the correct functioning gene in hopes that you will fix the problem with the gene that doesn't work. Sometimes you're introducing, for example Cas9 to try to delete something and you're not introducing anything new. Those already account that the statement is false.
[50:22] Gene therapy is to cure or fix a problem not to do eugenics.

**QNS: Gene functions are modular and changes are predictable**
That is a very naïve belief because they are not. In simple model systems, when you turn on gene A [or] gene B, there will be a cascade. But then, once you link that one pathway into all the thousands of other pathways in a cell and inside an organism. You can't be a 100% sure what you have in a dish or yeast model. That doesn't all translate directly. For other genes, yes we know what they are supposedly doing, we know what their main function is. But for many of these genes, there are interacting partners. If you have certain transcription factors in the development they do one thing, but once you grown up now they do a different thing. If you're thinking that "oh I'm targeting this gene and I know that in a development it directs the cells that way", if you target it at a different cell type, it might be doing totally different things. So, this statement is false.

**QNS: Current genome editing techniques are able to make changes to all genetic information in the cell.**
No, this is wrong, they cannot.

**QNS: This might be one of the reasons why people are afraid of genetic engineering.**
All these Cas proteins, most of them have limitations. You have to have 20 base pairs plus the 3 for Cas9, like spCas9 to work. But, it means that if you don't have the 3 bases that make the PAM sequence, if they are not in the region you are wanting to look out then there's nothing you can do. You can't target that place if you don't have the PAM. Even if you have the PAM but you don't have a guideRNA that is good – that's my first starting point when somebody suggested we do this. There's usually something there, like some guide but maybe these guides are really

bad – they are not specific at all. As I said before, if we have the best guide ever, like the perfect guide, then we can target stuff and we don't have to worry about off-targets. If you don't have a perfect guide that is very specific, you can't do anything in that region. If you use a guide there that will cut in 20 other places in the genome, that's not really anything of precision. For some research application, that's fine. You just want to see if it cuts this place, you don't care about any others. Any kind of therapy or whatever is obviously not possible. Obviously there is lots and lots of different Cas proteins are being discovered all the time. You guys know already there are already different Cas13 with different specificity and all that. The area and the specificity that are possible to target, there's more and more available because you can use a different Cas9 with a different sequence requirement so if one doesn't work maybe the other one will. You will be able to try to find other targets or other guides.

[new clip 00:02] This statement kind of suggests that you can change/target any sequence and you can do all the changes in that place, which again is not possible. Even within all the regions you can target with Cas9, some regions are very difficult to make any changes with the homologous directed repair because this is very inefficient. Depends on the cells you are working on, some cells don't have the necessary protein components to do any homologous recombination. You may be able to get around that with base editors. But then again, with the base editors you are limited by your guide. You need to have the guide at the correct spot, and even within the guide sequence it is only those few bases within those 20 that the editor will efficiently target. So again, if you don't have a good guide at the spot you want to target, that's only thing you can do. That's one other limitation we have in our study, currently we have some candidates that we simply cannot target as there is no guide in that region that will be specific enough for us to use, so we cannot do anything about it. Obviously we try some other things, if the base editor does not work we try it with homolog directed repair. But then again, you need a guide that works, if you don't have a guide that works in this space, there's very little you can do with the current techniques. More and more Cas proteins are being discovered and they will bring about new avenues. I am sure for quite awhile still this statement is false.

**QNS: DNA bases in the gene cannot be changed or altered in our body.**
This statement says "in our body". They are being altered and changed by random mutation all the time but in a directed manner by Cas proteins or other proteins, nobody has done it yet. Because we are not there yet. It has been shown to be done in animal models so there is not real reason why it cannot be done in human cells also. But again, like all these limitations that I have rambled on for awhile, not all bases or targets can be edited. But, definitely bases, certain targets will be possible to targets. Other things can be targeted. Everybody carries different mutations. If you spend too much time on sentosa on the beach, you will have lots of mutation on your skin cells and eventually that won't be good.

**QNS: Do you think public engagement is necessary to navigate the ethics of human genome editing?**
You cannot expect the general public to have deep in-depth discussion of all these specificity of how these techniques work. Maybe someone will have an opinion on what is the acceptable

level of risk but, somebody needs to do all the math to give you the percentages of risk. Maybe someone will tell you they are happy with 5% risk of getting green hair of whatever. The point is, yes you need to engage the public in all of these. One, at least within A*STAR, most of the funding comes from the taxpayer, so we are working for the public. That's one aspect and it's an important aspect. On the other hand, researchers will want to push the boundaries with all the research but then, not everything should be necessarily done. Again, I personally have my doubts about doing gene editing to get enhancements and all that stuff. I am fairly certain that people in the general public also have these doubts. This is not discussed and correctly addressed by the regulators, so the regulators need to engage all parties – the scientists that are doing the work and also the people who will be eventually affected when these things get to the clinic. You cannot expect the public to have deep discussion on the technical stuff but you have to get their opinion on what is acceptable or what avenues are acceptable. I work a lot on stem cells and people have strong opinions about embryonic stem cells also. Everybody is entitled to their opinion. My opinion is that in research, the embryonic stem cells that are used in research come from embryos that are discarded from IVF treatment. So if these embryos are not used for research to derive cells out of it will be thrown in the bin and burned in an incinerator. To me, personally it's not an ethical issue because this embryo was never going to become a person. And the cells I have on my dish are not [people], they are a cell line. But, you have to appreciate that they did arise from an embryo and it's not something to take lightly. Other people have strong opinions towards this kind of research also. And I am sure people have strong opinions about enhancements or even just treatments. Especially if it's done on potentially embryos or the next generation.

**QNS: Explain gene editing to the general public**

Gene editing is a technique or methodology that attempts to modify the DNA sequence within cells. Be it cells in a dish or in an animal model, and possibly in the future, in the clinic to, in most cases, enable a change in protein expression. This could try to fix a broken gene. Or in research purposes, just to see the function of a gene or model a disease in a dish.

**QNS: Explain RNA editing to the general public**

RNA is the [produced] molecule in cells, by editing the RNA you are not changing the underlying genome of the cell but you can still change the phenotype of the cell. So you can change the functioning of the cell by editing the RNA but when you do that you are not changing the underlying DNA so the change won't necessarily be permanent. The outcomes and the applications will be similar as to gene editing.