

**(Mariya Moosajee) So, good afternoon ladies and gentlemen, I wanted to say thank you for inviting me to speak at this conference. The last two days of the scientific meeting have been inspirational and very informative.**

I think there is a real push that it is moving towards clinical translation and therapies. Today I am going to talk about clinical trial design for nonsense suppression therapy for USH2A Ushers disease.

So, to start with, I am just going to quickly go over what a nonsense mutation is. Now this is a single change in a letter in your genetic code within an instruction part of the gene that leads to the introduction of an abnormal stop signal. And these are quite common mutations, they can account for up to 70 % of human genetic disease.

Now, what happens in your cells normally is, you have protein making machinery, that reads the instruction parts of gene to create protein. When you have a nonsense mutation or an abnormal stop signal, when your protein making machinery hits that stop, it just stops and so you end up with shortened non-functional protein. This is what ultimately leads to the disease process.

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What we have done, we have identified a number of small molecule drugs, the one I am going to talk about today is Ataluren which has been commercialized by a company called PTC therapeutics. But when the drug Ataluren binds to the protein making machinery, it weakens its recognition of the abnormal stop signal and can overwrite it, leading to the production of normal full-length protein. And it is able to do that, so it generates around 20 to 25 % of normal USH2A or whichever protein that you are missing, and that can be enough in our patients to actually hold or slow the disease progression down.

Ataluren has a large body of evidence for various different conditions, multi systems and including retinitis pigmentosa and genes such as USH2A, USH1C and various others. It has approval for treatment in Europe and in the UK for Duchenne's muscular dystrophy, for patients which have nonsense mutations. And the drug itself is safe and tolerable to be used in children from two years onwards. It's a powder that is, that is dissolved in water and is drunk three times a day.

So far, there have only been minimal side effects, so transient diarrhea or nausea when you first start taking the drug, which subsides after about a week. But there have been no serious adverse effects or any serious ocular events and they have treated nearly over a thousand patients with 5 to 8 years follow-up now.

And currently there is a phase 2 clinical trial for aniridia, a different eye condition where children are born without

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the colored part of their eyes, their iris, and they develop cataracts, they have glaucoma. They can have wobbly eyes, nystagmus, they are born with poor vision. So, this trial is on the way and we hope to see the results of that in 2020. Now, we want to apply this drug to Ushers and in order to do that in a clinical trial setting we need to know what the outcome measures would be, so we can monitor a response to treatment.

So, what we started to do was a natural history study of our patients with USH2A. At Moorfields we selected 57 patients that had on average three clinical visits one year apart. So, we have many more patients, but these were ones with good data sets. And of those 57 - they were almost equally divided - a third had nonsense mutations. That is a depiction of a cohort generally in Usher syndrome Type II, USH2A, 30 % are due to nonsense mutations, and then a third were insertions and deletions and 17 were missense mutations.

The average age of that cohort was 40 years and it ranged between patients that were 15 to 66 years of age. The first thing we did is look at their visual acuity, their central visual acuity. And there is a graph behind me on the screens, there is a lot of blue dots and a lot of lines connecting it, but essentially, if you can see there are quite a few horizontal lines which means that over time their vision didn't change very much. So visual acuity isn't the best indicator of a treatment response over a minimal period of time.

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We then looked at another parameter, something called optical coherence tomography or OCT, it's a scan that most of you will have each time you go to see your clinician. And it is where we shine infrared light into the back of the eye, we take a cross section. We measured the area where you had intact light sensing cells, your photo receptors, and we measured that area, it is called the ellipse width zone length over a three year period. What we found was that on average across the whole range of patients we detected a 7 % reduction in that size of length of the ellipse widths zone, over a one-year period, but a 22 % change over three years.

So, we are detecting a change every year, but the issue that we have is that there is a measurement bias, an error that is introduced by people who are measuring that length. So, to be absolutely accurate, we felt that a one-year time point was on the carve of where you would detect a treatment response. But if you look at the graph as well, you can see that if we looked at patients younger than 30 years of age - The dots indicate a patient and the lines joining them are the change over time.

The younger patients have more steeper lines, showing a steeper decline, whereas the older patients that were 30 plus, their lines are much more gentle. There is not much change going on. So, if were to do a trial, the best cohort to detect the biggest change would be the younger patients.

Another imaging modality we looked at is called Fundus

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autofluorescence. Again this would be routinely undertaken in your clinic visits, where we shine a very bright flash of light into the eye. And in Usher syndrome we get this characteristic bright ring around your area of central vision. And we can draw a line around that ring. And that ring, the reason that it's bright, is because that supporting layer of your light sensing cells, called the retinal pigment epithelium it has a buildup of metabolite products that cause it to hyperfluoresce, and this is showing that the cells are present, but they are sick. They are not working very well, because they are burdened by this buildup of product, and so they autofluoresce, they shine brightly.

Now, over time, those cells that were under stress and are sick and are dying off, so that ring is encroaching into your area of central vision and is getting smaller over time. We measured it at one year and at three years, and we found that on average there was an 11 % reduction in that ring size over a one-year period and after three years a 32 % change. So, this is probably a better parameter than all of the others, but if we combine them all we are more likely to be able to gauge an effect.

So, then we come to the clinical trial design. So, we had patient discussion groups at Moorfields and some of the members that came to that are actually in the audience here. And one of the biggest things that they said was they didn't feel comfortable with a trial where you were treating a set of patients and giving the other set a placebo and not giving that other set of patients the opportunity to ever have the drug treatment.

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So, we decided to have a cross-over trial. One, because this is a rare disease and we need to maximize the number of patients who are on treatment. But we are giving everyone an opportunity to be on the drug, but also it will inform us about what happens after you stopped the drugs. By having a group that is on Ataluren and then having a small wash-out period, - the drug can be washed out of your body system after just a month - and then just following those patients on the placebo, we will be able to see if the change of decline, the rate of ring constriction or loss of light sensing cells remain stable or when they start to decline again. And we felt that we needed a two-year period to be absolutely safe, to be able to gage whether there was a change at all.

So, this is what we decided: Around 20 patients would be on Ataluren and 20 would be on a placebo, and then have a wash-out period. Then the patients that were on the placebo will then be given the drug, to see if that would slow down their degeneration and the other 20 patients would continue on a placebo and we would monitor the effect of the drug. For the outcome measure the trial, this drug has never been tested on a population with retinal disease, so working with the company it was felt that the primary outcome measure should be safety to ensure that there were no adverse effects. And as secondary outcome measures we would consider measuring the auto fluorescent ring size, using OCT to look on that ellipsoid zone. But we would also look at visual acuity, because even though those cells which were brightly fluorescing,

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were sick, they were still alive, and if we could provide protein to them, by mechanism of the drug action, then maybe they would start working better and maybe the vision would improve slightly. So we wanted to include that.

And then there were a number of other tests that we would include as exploratory parameters, like visual fields, color vision, adaptive optics. So, how can you get involved? The first step always is to establish your genetic diagnosis. If you don't know the gene that is causing your condition I urge you to seek out your clinicians to get genetic testing. If you do know the gene, then it is important to find out what type of mutation.

Because if you have a nonsense mutation then this therapy may benefit you in the future. And the fact that this drug works on a mutation, it doesn't matter what gene causes it, or what the name of your disease is, if you have a nonsense mutation, you may benefit from this. And if the trial is successful, we hope to move to a phase three clinical trial, where we involve patients with all different retinal disorders caused by nonsense mutations.

If there is a natural history study on the way, near where you live, please participate in that, especially if you are unsure about going into treatment trials. Because by allowing clinicians to study your disease, it gives us insides, it helps us to develop therapies and it helps us with the outcome measures with trials. And if any of you have any questions or you would like me to check your genetic mutation, then please feel free to contact me, using my

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e-mail address which is mariya.moosajee@moorfields.nhs.uk. And if you need that, I am sure that the Usher conference will circulate that. And so with that I would just like to thank my team and special credit to Dr. Adam Dubis, who really lead on the natural history study. Thank you very much. (applause)