Pre-natal Gene Therapy for Thalassaemia: Project Update

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These are exciting times for thalassaemia gene therapy with a number of clinical trials either ongoing or soon to start in both the USA and Europe. All these trials will employ what is known as an "*ex vivo*" procedure for genetically correcting the bone marrow blood stem ("parent") cells of the patient. The *ex vivo* procedure involves (i) harvesting blood stem cells from the thalassaemic person's bone marrow, (ii) infecting them with a modified virus (known as a "lentivirus") gene medicine that contains a normal functioning copy of the β-globin (or α-globin) gene, (iii) treating the patient with chemotherapy to destroy their diseased bone marrow blood stem cells and (iv) returning the now genetically corrected bone marrow blood stem cells back to the patient. This procedure is very similar to that of a bone marrow transplant only the patient's own bone marrow blood stem cells are used and not those from a donor. Therefore, the major problems of finding a suitable donor, donor bone marrow graft rejection or donor graft verses host disease are avoided. This *ex vivo* bone marrow blood stem cell gene correction procedure has proved successful in treating other inherited blood disorders such as severe combined immune deficiencies and so it is thought to hold a great deal of promise for treating thalassaemia as well. Indeed, a partial successful treatment of a person with transfusion-dependent thalassaemia intermedia in the clinical trial based in Paris using exactly this approach is a testament to this possibility (Cavazzana-Calvo M et al., 2010).

Despite its promise the *ex vivo* procedure possesses a number of properties, which stand to severely limit its scope in terms of reaching all affected peoples in the world that would need to be treated. This arises primarily from the requirement for an extended hospital admission, sophisticated hospital and laboratory facilities as well as the added high expense of the relatively large dose of globin gene lentivirus gene medicine. The vast majority of people with thalassaemia are born in the developing world where unfortunately there is a major shortage of basic medical care. As a result most born with thalassaemia are not even diagnosed with their condition let alone treated. It is therefore difficult to envisage how a sophisticated and expensive *ex vivo* gene therapy procedure will have a major impact in regions such as India, Bangladesh, Southeast Asia and even Southern China, where hundreds of thousands potentially await treatment. This leads us to ask, is there a way to try and reach more who need to be treated with thalassaemia gene therapy?

Pre-natal thalassaemia gene therapy

Regular readers of Thalassaemia Matters will know that UKTS has for a number of years supported a research project in the laboratories of Dr Michael Antoniou (King's College London) and Dr Simon Waddington (University College London) to try and develop an

alternative procedure for thalassaemia gene therapy that is complementary but distinctly different from the standard *ex vivo* approach. The idea here is to try and treat the affected child before it is born and is known as “pre-natal” gene therapy (Mattar CN et al., 2011).

The concept behind a pre-natal approach to thalassaemia gene therapy is, in principle, quite straightforward. The blood stem cells during our life as a foetus in the womb are located not in the bone marrow but in the liver. However, around the time of birth the blood stem cells in the foetal liver migrate to the bone marrow, where they then stay for the rest of our lives. So the idea is to try and genetically correct the blood stem cells whilst they are concentrated in the foetal liver. These now genetically corrected blood stem cells will then migrate to the bone marrow at birth providing a life-long cure (**see Figure 1**).

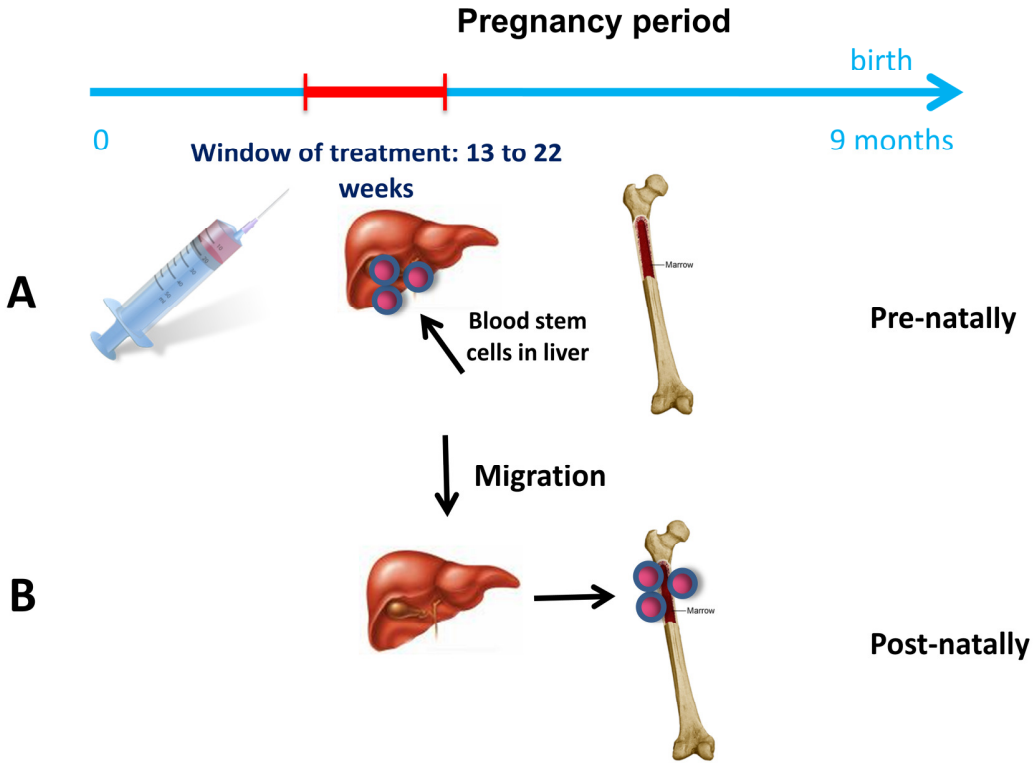

Figure 1. Development of *pre-natal* gene and cell therapy for thalassaemia
Concept:
A. Globin lentiviral therapy gene medicine correction of blood stem cells **pre-natally in foetal liver**
B. Migration of corrected blood stem cells to **bone marrow post-natally**

The advantages of a pre-natal gene therapy approach over an *ex vivo* procedure are numerous and include, (i) prevents the birth of an affected individual (ii) direct delivery of the

globin gene lentivirus gene medicine to the foetus, (iii) delivery procedure can be done by adapting already established foetal surgery methods on an outpatient, perhaps even a field hospital basis avoiding the need for sophisticated and expensive hospital facilities and therefore applicable in all regions of the world, (iv) lower dose of gene medicine required that increases safety margins and decreases costs, and (v) avoids moral dilemmas associated with pregnancy termination as a thalassaemia control measure especially in Islamic and Catholic regions of the world where on perfectly acceptable religious and cultural grounds this is not an option. **It is foreseeable that a successful application of a pre-natal gene therapy approach on a global scale could within a generation significantly reduce the number of affected births with thalassaemia.**

Nevertheless, it is obvious that the pre-natal approach to thalassaemia gene therapy cannot be used to treat someone who is already born with this condition. It is therefore important that *ex vivo* and pre-natal gene therapy approaches as well as other therapeutic options be researched side by side to meet all clinical needs.

Pre-natal gene therapy: project objectives and progress

The principal objective of our UKTS-funded project was to obtain proof-of-principle that we could successfully deliver the globin lentivirus gene medicine to blood stem cells in the foetal liver of mice as a model test system. If successful, we would be able to detect the presence and function of the gene medicine in the bone marrow and circulating blood cells of animals after birth. In addition, a second important objective of the project was to improve on the basic "GLOBE" globin lentivirus gene medicine design we have developed to date (Miccio A et al., 2008) to obtain a higher, more reproducible level of globin therapy gene function in order to minimise the therapeutic dose required. It was also ultimately hoped that we could test the pre-natal gene therapy approach in β-thalassaemic mice. The experiments were conducted by two PhD students in Dr Antoniou's laboratory, namely Sonia Ferreira and Vincent Kao, who worked closely with Dr Waddington.

Various lentivirus gene medicine test designs were in the first experiments injected into normal mouse foetuses between 14 and 16 days of pregnancy via a blood vessel linked with the placenta. [Note: this is very similar as to how we envisage applying this pre-natal gene therapy delivery approach in humans]. The injected and non-injected control mice then developed normally and were born after the normal mouse pregnancy period of 21 days. Mice were analysed for the presence and function of the test lentivirus gene medicines at 3-8 months after birth. Very encouragingly we were able to detect the presence of the test gene medicines, including those containing the therapeutic globin gene, in both bone marrow and circulating blood cells at levels, which in some cases at least, we would predict to have a therapeutic effect if reproduced in thalassaemic patients.

In addition, we readily detected therapy gene function that again was stable out to the 8-month duration of the experiment. This included β-globin therapy gene expression in bone marrow and peripheral red blood cells, which again in some animals was at a potentially therapeutic level.

In addition, we were able to make significant improvements in the globin lentivirus gene therapy medicine design. The latest results show that one design increases therapy gene function by approximately 4 – fold compared to the basic GLOBE design, which we have previously reported (Miccio A et al., 2008).

All the above experiments were conducted in normal rather than thalassaemic mice. Unfortunately, due to unforeseen technical problems we were unable to obtain results in a thalassaemic model test system animals during this first phase of the project.

Nevertheless, the presence of the lentivirus gene medicines in peripheral blood and especially bone marrow cells of mice out to 8 months after birth is a clear sign of successful infection of blood stem cells pre-natally in the foetal liver. These results are therefore very encouraging.

In summary, we were able to successfully meet the primary objective of this first phase of the project; that is, obtain proof-of-principle that blood stem cells in foetal liver can be readily infected with lentivirus gene medicines, which then persist after birth following migration to bone marrow. Furthermore, we were able to achieve a degree of success in our secondary objective, which was to advance globin lentivirus gene medicine design for more efficient and efficacious therapy gene function. Therefore, with the continued support from UKTS we look forward to embarking with greater confidence on experiments in thalassaemic mouse model test systems to demonstrate cure of this condition via a pre-natal delivery approach.

Status of Clinical Trials for Haemoglobinopathies

The GLOBE lentivirus gene medicine developed with support from UKTS to Dr Antoniou is currently undergoing final safety evaluation required by regulatory authorities in Italy. Prof Giuliana Ferrari, who jointly developed the GLOBE gene medicine with Dr Antoniou (Miccio A et al., 2008), anticipates that clinical trials with thalassaemia patients using GLOBE will begin at the gene therapy centre in Milan sometime next year.

Prof Michel Sadelain (USA) will soon commence a thalassaemia gene therapy clinical trial with his version of the globin lentivirus gene medicine (“Thalagen”). Prof George Stamatoyannopoulos (USA) plans to use the same Thalagen gene medicine in a thalassaemia trial in Thessaloniki (Greece).

Prof Punam Malik (USA) has a proposal before the American regulators for a gene therapy clinical trial for sickle cell disease. This will use her version of a globin lentivirus gene medicine in an *ex vivo* procedure as in the case for thalassaemia.

At the American Society for Gene and Cell Therapy conference held in Philadelphia, USA in May of this year, it was announced that last October another thalassaemia patient had been treated in the gene therapy trial led by Prof Philip Leboulch and Prof Marina Cavazzana-Calvo in Paris. It is early days for this patient but to date the level of the globin lentivirus gene medicine detected in their blood is very low.

References

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