November 2025

Research Update – Joseph's Goal

We are pleased to provide an update on progress. It's been a very active year in the lab and we've made exciting progress in several areas, including progressing gene therapy projects and developing new work. Our research strategy continues to focus on two main themes: We aim to better understand the NKH disease process, and to use this information to develop new treatments and evaluate how well they work.

Towards new treatments for NKH

We are taking several different approaches towards development of novel therapies, either to place a working copy of the affected gene into the liver or brain, or to use other approaches such as small molecules to counteract the effects of NKH. In principle, these could be used alone or in combination with new and existing treatments.

AAV gene therapy for GLDC

The aim of gene therapy is to provide a working copy of GLDC in the brain, using a gene therapy 'medicine' that contains the intact GLDC gene contained within a coat that can enter cells in the brain. In our mouse model of NKH we have shown that this is effective when delivered intra-venously (into the blood) or directly into the brain ventricles (the fluid filled compartments). Because the main problems in the NKH occur in the brain, we favour delivery to the brain andthis route has been used in clinical trials for other disorders. This also lowers the risk of harmful effects which have occasionally been found using intra-venous treatment with high-dose AAV in clinical trials for other disorders.

We have shown that we can effectively lower glycine and correct other biomarkers in the brain using this approach (published last year and supported by Joseph's Goal funding). We developed methods to analyse the epilepsy-sensitivity and other neurological abnormalities in the GLDC-deficient NKH mouse model, and we are testing whether these are corrected by the AAV gene therapy. With joint funding from MRC and Joseph's Goal we have expanded this project and the findings so far are positive.

The final preclinical work which needs (i) transfer of the vector manufacture to a process that can produce 'medicine-grade' (GMP) quality, (ii) confirm this vector works as expected in the NKH mouse, and (iii) a pivotal safety study conducted by an independent organization to meet regulatory requirements. Working closely with the UCL Translational Research Office (where we have an allocated project manager), we now have very well-developed plans for this next phase in the project. We were able to meet with the MHRA (the UK regulators) to ensure that the proposed work would support authorization for a clinical trial once the project is complete with satisfactory safety outcomes. We have applied for funding for these studies and our application has progressed to the final stage with a decision awaited – we are grateful for the support of Joseph's Goal in this application which enabled us to show evidence of additional funding.

Developmental Biology & Cancer Research & Teaching Department
UCL Great Ormond Street Institute of Child Health
30 Guilford Street, London WC1N 1EH
Tel: +44 (0)20 7905 2235
Email: ich.dbc.programme@ucl.ac.uk

Lentiviral gene therapy for gene therapy targeting the liver

This approach is particularly aimed at the liver, with insertion of the GLDC gene is inserted into the DNA, which means it won't become 'diluted' as the liver grows. We have just started an additional round of safety testing. We recruited a new research assistant in August who is helping with all the gene therapy projects.

Treatment for AMT-related NKH

The AAV gene therapy that we have developed initially aims to target GLDC as this is the gene that most commonly altered in NKH. However, as some children with NKH carry alterations of another gene, AMT, we also aim to develop an AMT-directed gene therapy. As we reported previously, we have generated the AAV-AMT vectors, tested them in cultured cells and obtained vector for mouse treatments. A key next step is to test the AMT vectors in a suitable AMT-deficient mouse model. We have made and obtained several mice strains with changes in the AMT gene. An important piece of work over the year has been to study these mouse models to work out which is best for testing therapies (work which has been supported by MRC and Joseph's Goal).

mRNA therapy for GLDC

We completed a pilot project to test an alternative method for delivering the GLDC gene to cells in the body. This uses a technology in which the RNA which encodes GLDC is used directly as a treatment by injection within a 'lipid nanoparticle' (LNP). This has been used in several applications including the COVID19 vaccines and in treatments for metabolic disease. We have been working with a company that specializes in LNP technology. We tested a number of different formulations of LNP with a 'reporter gene' to find out which works best in mice, and specifically in the NKH mice. This step was completed in the summer, and the next step is to use the best LNPs with GLDC RNA. We recently developed and submitted an application for further funding to expand the project.

Nutrients/metabolites for correction of folate one-carbon metabolism

We continue to build on our work in the mouse models, embryos and cell lines to identify nutrients/metabolites that have a protective effect. In addition to formate, methionine, folinic acid and choline (from previous work), we've identified additional possible protective approaches which are being tested in cells and mice.

Understanding NKH - experimental models of NKH

Alongside development of treatment, we aim to better understand the disease process. This is important to testing whether our therapies are working and may provide new targets for treatment. Among the genes whose alteration causes NKH, we have largely focussed on *GLDC* but as reported in our last report we've also been working on *AMT* using the new mouse models and in cell lines.

Studying NKH in experimental models of NKH (recent studies)

1. Analysis of altered metabolism, neurological function, and gene expression in NKH mouse models

In the last few year we have made progress putting together large datasets in several areas and two manuscripts are being finalised. This includes important new findings about the effects of metabolic changes in the NKH mice and cells, building on metabolome analysis (studying ~1,000 biochemicals) which was supported by Joseph's Goal. This has also highlighted new opportunities for possible treatments which we will expand on in our next report.

We have completed a major piece of work in which we aimed to develop a new mouse model (again with your support) which will be very useful in understanding the function of GLDC in different cell types in the body and how this relates to treatment.

2. Analysis of new AMT mouse models

We have started analysing these new mice in several different ways, including CT scanning, imaging and metabolite analysis. We're particularly focussing on similarities, and any differences, to the GLDC mice.

3. NKH cell and organoid models

We are continuing our work with human 'induced pluripotent stem (iPS) cells that carry mutations in GLDC and in AMT. These are being used in several different experiments including to make liver organoids and cells of the nervous system. We have identified key changes in the GLDC and AMT deficient cells – these studies provide a complement to the work in mouse.