Hi Karl,

Our scientific advisory committee have a few questions regarding your application, the answers to which they hope will help them in their review and recommendations to our board of Trustees. Could you take a look at them please?

1. Could we be given a separate description of exactly what will happen to the Chronic Lyme Disease (CLD) cohort with all else excluded?
   So;

- how are they chosen? Patients are selected who tested positive for Lyme disease either by UK NHS/Irish testing, or by a Tezted Tickplex Plus test, or from clinical history (ECM rash).

- what treatment are they given (standardised or individualised protocols? Please name the antibiotic/drug combination) All patients will be initially treated with a 2-month standard antibiotic combination successfully used to treat patients in the Professor Jack Lambert's (JL) UCD clinic: cefuroxime 500mg bid, lymecycline 300mg od, and rifampicin 300mg od.

- when are blood and stool samples taken? Two cohorts of patients will be collected by JL at UCD with patients treated with long-term antibiotics. The first cohort of patients are currently being collected: 54 patients have enrolled into the current programme and blood will be used for the first part of the PhD study. Blood samples are taken at baseline (before treatment), straight after treatment and at 6 month follow up

- The cohort of patients will be enrolled to specifically investigate abnormalities in the gut microbiome. The second study (Cohort 2) will provide the stool samples for baseline assessment. We are not planning to assess the gut bacteria post antibiotics as we do not have the budget to run collect and analyse these samples. The gut microbiome will change with antibiotics and follow up long term studies on the microbiome post antibiotics will be possible if a budget is available. Long term follow up samples could be taken if the patients do well on treatments

  Bloods from the second set cohort will be also be sent to SoftCell for L-Form culturing once the PhD has started. L-form culturing ideally requires fresh blood samples

  As this is an in-kind contribution by Softcell we will need to discuss patient numbers with Softcell in the second cohort. This will depend on the findings of the earlier deep sequencing whole genome sequencing experiments on the 1st Lyme cohort. SoftCell have currently only committed to the 1st longitudinal sample set (outlined in (ii) below).

- when are PEM assessments made? As we do not have a budget for staff at UCD, PEM assessments will not be carried out on Lyme patients. If studies are successful in ME/CFS and Long Covid these methods could be applied to future chronic lyme studies,

  which tests are carried out on which samples?

  Lyme samples

  i) Lyme subjects in cohort 1

Measure blood markers of a leaky gut in plasma: 40 baseline and 40 post long-term antibiotics.
Measure markers of blood brain barrier breakdown: 40 baseline and 40 post long-term antibiotics.

ii)Selected samples at baseline, after antibiotics and at 6 month follow up from cohort 1 based on the data collected in (i) and the response to antibiotic treatment.

- 7 positive responders & 3 poor responders to antibiotics.
- 3 blood samples on each: baseline, straight after treatment and at 6 months follow up. 30 samples total.
- 30 blood samples: Whole genome pathogen sequencing on blood cells and 16sRNA sequencing Oxford. L-form cultures established by Softcell Biologicals.
- Raman characterisation of L-form cultures derived from Lyme patients, identification of pathogens inside blood cells using Raman spectroscopy

ii)Lyme subjects in cohort 2

Gut microbiome mapping will be carried out at baseline in this group of patients.

2. The deep total pathogen sequencing is to be carried out on only 10 CLD patients. Is this a sample size which will give robust results, or only indications for further research?

   Although numbers are small we hope the analysis of the data above will allow us to answer a number of key questions. Do we find a small number or a wide range of pathogens in the blood samples of CLD patients? Are these pathogen profiles unique of do they vary between individuals?

   Our hypothesis based on what we have seen in ME/CFS is that the total load of pathogens might be more important than any single pathogen. Is Borellia or other known lyme causing disease agents the key players in CLD ? Can we detect these pathogens in the samples and are they found together with other pathogens? Can they be cultured from the cryopreserved blood cells? Can 16S RNA sequencing (£60 per sample) detect the same pathogens as deep whole genome sequencing (£800 per sample)? Further research will be needed with a larger budget to follow up on our findings. More samples from UCD will be available and can be analysed in follow up studies.

3. What tangible benefits can we expect for Lyme patients from this study?

   1) Can we generate data that supports the use of long-term antibiotics in the treatment of CLD? Linking this with associated changes in disease biomarkers (i.e. leaky gut markers, pathogen load and type of pathogens present).
   2) If a dysbiotic gut is identified with evidence of a leaky gut this could provide data for intervention trials in CLD utilising Faecal Microbiota transplantation and Gut Floral Replacement therapy. This milder approach could be combined with specific antibiotic treatments based on the in vitro testing of antibiotics on pathogens cultured from individual patients.
   3) The deep whole genome sequencing/16S RNA sequencing has the potential to form the foundation for a diagnostic test for CLD disease.

There was also a general concern from a few members that Lyme disease is not central to the project, and has not been fundamental to the design. It was described as feeling as if it had patched onto another study on medical conditions thought conventionally to be non-infectious in nature.

All the conditions we will be examining in this study have a known or potential infectious element. Following feedback from Action for ME. The endometriosis samples have been removed from the study, which now focuses on ME/CFS, CLD and Long Covid.

This may be down to the nature of the application and how it was put together. But they are aware of many Lyme patients suffering with leaky gut issues, so they can see some possible links and benefits. If you are able to offer any reassurances that the Lyme cohort will be given equal staff time and effort, plus the answers to these questions and anything else that you think might be relevant, that would be helpful.

A lot of the work around the recruitment of the chronic lyme cohort has already been carried out by Jack Lamert at UCD with future collections planned to be used in the PhD study. This was carried out with existing funds in place at UCD. The PhD student will complete the project by recruiting the other ME/CFS and Long Covid patients in Oxford. All samples will go through the same assessments.

Further details on the PhD student.

Dr Inga Williams

Dr Williams applied for a PhD with us in January 2021 with a specific interest in pathologies associated with potential infectious agents. During her obstetrics and gynaecology training Inga carried out research into the causes of infections associated with the placenta with publications listed in her CV. Many of these appeared to be persistent infections transmitted from the mother. Now her family have grown up Inga is looking to take on a PhD programme and is interested in the underlying causes of ME/CFS, CLD and Long Covid, all of which potentially have an infectious element. Inga has attended lab meetings with my group since being accepted on the Oxford PhD programme, presenting papers at meetings and has recently started to help us with the statistical analysis on a gut microbiome data set. The PhD proposal is a combination of clinical, large data analysis and practical work involving established and new technology. I am confident with support from myself and the rest of my laboratory Inga will be able to cope with the challenges a PhD throws up and will develop strong practical skills in addition to expanding the clinical ones. I have been looking for a clinician interested in fatigue research for some time and I have finally found one.

Sorry if any of this seems disappointing or challenging, but we want to give you the best chance of success with your application so hopefully thi guidance helps.

Best wishes