A phase III multicentre randomised clinical trial comparing rituximab with CHOP given every 14 days and rituximab with CHOP given every 21 days for the treatment of patients with newly diagnosed diffuse large B cell non-Hodgkin's lymphoma – A trial developed by the National Cancer Research Institute Lymphoma Study Group and adopted by the National Cancer Research Network

Sub-protocol for the collection and storage of buccal DNA samples (funded by Cancer Research UK – The Translational Research in Clinical Trials Committee)

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1. Introduction

Around 60% of patients with Diffuse Large B-cell Lymphoma (DLBCL) are cured with standard therapy; the majority of the rest die of their disease. In order to optimize patient management it is important to be able to prospectively identify those who are at risk of a poor outcome who may benefit from alternative therapeutic approaches.

DLBCL is widely recognized as a highly heterogeneous disease. Tumours differ in their stage of differentiation and the presence of molecular and cytogenetic markers. In a proportion of cases there is an underlying indolent lymphoma that has transformed to a DLBCL. Some tumours arise at extranodal sites and other tumours may subsequently spread to non-lymphoid organs; involvement of the CNS is of particular clinical importance. Patients differ in their fitness, extent of disease and 'host response' to the tumour. Finally, there may be constitutional genetic factors affecting drug metabolism and efficacy, some of these are already known to be prognostically important, whilst others have yet to be fully validated.

For example the efficacy of CHOP itself may be affected by a range of polymorphisms in genes regulating drug metabolism and mode of action. The transformation of cyclophosphamide pro-drug is affected by differences in cytochrome P450 activity, a phenotype conferred by constitutional polymorphism in the CYP2B6 gene. Cyclophosphamide and doxorubicin are both subject to phase II detoxification via conjugation to glutathione, a process mediated by glutathione S-transferase P1 this may be affected by functional polymorphisms of GSTP1. Polymorphism of this gene has already been shown to be an independent prognostic marker after nitrogen mustard-based therapy for multiple myeloma. Plasma GSTP1 levels also predict disease-free survival in NHL patients treated with CHOP further implicating glutathione conjugation as an important determinant of therapeutic response.

The toxicity of nitrogen mustards, including cyclophopshamide, is mediated predominantly by the formation of N7 guanine monoadducts, which can undergo rearrangement to form an interstrand crosslink. N7 guanine monoadducts are repaired almost exclusively by the nucleotide excision DNA repair pathway. Specific polymorphisms in ERCC1 and ERCC2, components of nucleotide excision repair, are independent prognostic markers in small cell lung cancer, colorectal cancer and acute myeloid leukaemia. The cellular basis for this association is thought to be via nucleotide excision repair of so called "bulky" DNA lesions induced by chemotherapy agents, such as cyclophosphamide. As such, genetic variation in these genes may predict response to cyclophosphamide-based therapy for DLBCL. This trial provides an excellent opportunity to assess the role that constitutional genetics plays in determing response to R-CHOP and its effect on eventual outcome.

2. Rationale

Genetic variation is likely to play a significant role in determining the response and eventual outcome of R-CHOP chemotherapy in patients with DLBCL. This study will collect buccal smears from patients enrolled in the NCRI trial comparing RCHOP14 against RCHOP21 in patients with DLBCL. Constitutional DNA will be extracted from these smears and the data derived from this DNA will be matched from the clinical data collected as part of the trial to determine the effects of genetic variation on response and survival

3. Study Objectives

- To collect buccal smears from all patients enrolled in the main NCRI trial comparing RCHOP14 against RCHOP21 in patients with DLBCL.
- To extract DNA from these smears and determine the genetic variation in this DNA.
- To match the data on genetic variations with the clinical data from the trial to determine the effect of the genetic variation on response to RCHOP chemotherapy and survival.

4. Eligibility Criteria

1. All patients eligible for the main trial will be eligible for the sub-protocol
2. Patients who consent to take part in the sub-protocol for the collection of buccal smears

5. Trial Outline

1. All patients in the RCHOP14 vs RCHOP21 trial will be approached and this sub-protocol will be discussed with them. They will be provided with a patient information sheet to take away and read (appendix 1)
2. If they agree to participate in the sub-protocol they will be asked to sign a consent form for the sub-protocol (appendix 2).
3. Once they have given their consent patients will be asked to provide 2 buccal smears using the swabs provided.
4. Patients will be given instructions (appendix 3) about how to take the smears and a research nurse will be available to provide help it is needed.

5. The smear heads together with the top 2 copies of the signed consent form should be returned to EGU York using postage paid pre-addressed return enveloped:

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6. EGU will process the buccal samples and extract the DNA (see section 6)
7. This procedure will take no more than 5 minutes and will be carried out in out patient clinics or day care units and will be done during routine visits so no additional visits to hospital are required.

6. DNA extraction

Consititutional DNA will be prepared using standard techniques (specify)

7. Genetic studies

The prepared DNA will be used to investigate the role of constitutional genetic polymorphisms in determining the outcome of patients in the main RCHOP14 vs 21 study. There is evidence that single nucleotide polymorphism may affect the host response to the tumour, and the metabolism and efficacy of therapy.

As substantive data from the main RCHOP14 vs 21 trial will not be available for 4-5 years there is no need for work to start immediately on the genetic studies. Therefore DNA will be collected and archived. It is likely that during the course of the study new technology will become available which will allow genetic studies to be carried out more quickly.

8. Analaysis

The data from the genetic studies will be included in the assessment of prognosis. The number of polymorphisms is large and therefore the number of possible models is vast. A new prognostic model is being developed using Classification and Regression Tree Analysis (CART). CART is a flexible non-parametric technique that can handle both continuous and categorical data. It uses recursive partitioning to present results in the form of decision tress, and makes no assumptions about variable distributions. Using CART will make it possible to incorporate details of the pathways and interactions between genetic components.

Data from the genetic studies will be held at the matched with the clinical data Epidemiology and Genetics Unit at the University of York. These data will be matched with the clinical data from the main trial which is held at the Lymphoma Trials Office (LTO) at University College London. Data from these two sources will be matched by the unique patient trial number which is allocated when the patient is entered into the RCHOP14 vs 21 trial. The LTO will send the clinical data to the EGU but no patient identifiers will be included. All analysis will be carried out at EGU.