An analysis of Relative Telomere Length (RTL) during chemotherapy in patients with advanced gastro-oesophageal adenocarcinoma

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PROTOCOL APPROVAL SIGNATURE PAGE

An analysis of Relative Telomere Length (RTL) during chemotherapy in patients with advanced gastro-oesophageal adenocarcinoma.

This trial will be performed according to the Research Governance Framework for Health and Community Care (Second edition; 2006) and World Medical Association Declaration of Helsinki Ethical Principals for Medical Research Involving Human Subjects 1964 (as amended)

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TABLE OF CONTENTS

1. STUDY OBJECTIVES

The primary objective of this study is:

(a) To analyse Relative Telomere Length (RTL) in blood samples taken from patients during chemotherapy for advanced gastro-oesophageal adenocarcinoma. This will allow us to determine if the baseline (pre-treatment) RTL correlates with tumour response i.e. to determine if the baseline RTL predicts tumours that are sensitive or resistant to chemotherapy.

The secondary objective of this study is:

(a) To analyse changes in markers associated with cellular senescence in blood samples taken from patients during chemotherapy for advanced gastro-oesophageal adenocarcinoma. These may include, but are not limited to, cathelicidin-related antimicrobial protein (CRAMP), EF-1a, stathmin, and chitinase 3-like protein 3.

The tertiary (exploratory) objectives of this study are:

(a) To analyse biomarkers of drug-induced changes in cellular function (e.g. apopotosis markers) in blood samples taken from patients during chemotherapy for advanced gastro-oesophageal adenocarcinoma.

(b) To analyse changes in senescence-associated biomarkers (e.g. micro-RNAs, immuno-profiling) in blood samples taken from patients during chemotherapy for advanced gastro-oesophageal adenocarcinoma.

(c) to analyse if any changes in RTL, or in genes involved in regulation of RTL, in blood samples taken from patients during chemotherapy for advanced gastro-oesophageal adenocarcinoma give an early indication of outcome (objective response, overall survival).

2. INTRODUCTION

Progress in understanding the cellular and molecular biology of normal and neoplastic tissues, together with rapid technological advances enabling multi-platformed genomic and proteomic analysis, will help identify specific pathways that are involved in cancer development and progression, and identify potential novel targets for therapy. Most cancers have a high degree of genetic and epigenetic heterogeneity between individuals, and analyses of genes or proteins involved in cancer development, progression, and in response to therapy, are likely to be biologically relevant and have an increasing potential role in the clinical management of cancer patients.

There has been a considerable impetus to utilise biomarkers detected in the blood of cancer patients in the study of malignant disease in relation to diagnosis, tumour classification, prediction of treatment response, and prediction of prognosis. Radiological imaging of tumours is an essential part of the practice of oncology, with a crucial role in screening programmes and in the diagnosis and staging of established disease. Furthermore, the assessment of tumour size by imaging, usually with computed tomography (CT) is a key component in determining tumour response in clinical practice. However, the development of a blood biomarker to monitor treatment response would be advantageous in terms of ease of repeated analysis and use of resources compared to anatomical imaging. In addition, such biomarkers might give an earlier indication of potential response to treatment rather than the

time lag, which is necessary to observe changes in tumour size. In patients with malignancy this may avoid administering toxic treatments with little prospect of benefit and hence start alternative treatments earlier.

Biomarkers are defined as characteristics that are objectively measured and evaluated as an indicator of normal biological processes, pathological processes, or pharmacological responses to a therapeutic intervention[1]. The ideal biomarker has high sensitivity and specificity for diagnosis; its level should correlate with disease stage and response to treatment; and it should be easily and reproducibly measured. Unfortunately, the biomarkers currently available for use in the management of solid tumours do not fulfil all these criteria and, therefore, are not presently recommended for screening of the general population, although studies examining the utility of biomarkers in population screening are currently ongoing[2-4]. There has been considerable interest in the application of genomic or proteomic techniques in developing novel biomarkers. However, gene or transcript profiling may provide an incomplete picture as mRNA expression is poorly correlated with levels of protein expression. RNA analyses cannot detect important post-translational modifications of proteins such as proteolytic processing, phosphorylation, or glycosylation, all of which are important in determining protein function[5, 6]. The extent of these modifications means that from the 30,000 – 40,000 genes now thought to comprise the human genome, several hundred thousand to million protein forms are proposed, leading to a much higher complexity than from RNA transcripts alone. Consequently, the development of a robust, reproducible, biomarker that could be easily applicable for monitoring response to treatment in a multi-centre setting remains an elusive goal in cancer medicine.

Telomeres are capping end structures of eukaryotic chromosomes essential for protecting chromosomal integrity. Each telomere is composed of a non-coding sequence consisting of $(TTAGGG)_n$ repeats, in complex with specific proteins (7, 8). Normally, telomeres shorten with each cell division until a critical length is reached and the cell enters cell cycle arrest[9]. Permanent cell growth requires telomere maintenance, and certain human cell populations, along with most malignant cells, possess activity of telomerase for telomere elongation[10]. Despite telomerase activity, the majority of tumour cells have shorter telomeres than the corresponding normal tissue and there is a relationship between short telomeres and genetic instability[11]. Thus, telomere maintenance is important for tumour cell growth and survival. Telomere length (TL) is determined by the balance between positive and negative factors impacting telomere homeostasis. In the last decade, TL has emerged as a promising clinical marker for risk and prognosis prediction in patients with malignant disorders. Tumour TL, as well as TL in healthy tissues such as peripheral blood, may carry valuable information for future treatment strategies[12]. There is an association between short telomeres in peripheral blood and increased risks for head and neck, bladder, lung, and renal cell cancers[13]. Telomere length was also shorter in buccal cells from patients with bladder cancer compared with controls[14]. These data implicate that individuals with shorter telomeres may be at higher risk of developing different forms of cancer. In an initial study on blood cell telomere length in spontaneous breast cancers, no difference was seen compared with controls[15]. Another report in sister sets has indicated an association between breast cancer risk and shorter telomeres in high-risk breast cancer families[16].

Most recently, blood telomere length as a possible biological marker for breast cancer risk and prognosis has been evaluated using real-time PCR. Relative telomere length (RTL) was measured in peripheral blood cells of 265 newly diagnosed unselected breast cancer patients and 446 female controls[17]. The patient group displayed significantly longer telomeres compared with controls ($P < 0.001$). Age-adjusted odds ratios for breast cancer risk increased with increasing telomere length, with a maximal odds ratio of 5.17 [95% confidence interval (95% CI), 3.09–8.64] for the quartile with the longest telomeres. Furthermore, RTL carried prognostic information

for patients with advanced disease. Node-positive patients with short telomeres (≤ median) showed an increased survival compared with node-positive patients with long telomeres (P = 0.001). For patients with ages <50 years with tumours >16 mm (median tumour diameter), short telomeres were associated with a significantly better outcome than longer telomeres (P = 0.006). Cox regression analysis showed that long RTL was a significant independent negative prognostic factor (hazards ratio, 2.92; 95% CI, 1.33–6.39; P = 0.007). These results suggest that blood RTL may serve as a prognostic indicator in breast cancer patients with advanced disease. Consequently, we propose to prospectively analyse changes in the relative telomere length in blood samples taken from patients during chemotherapy for advanced gastro-oesophageal adenocarcinoma in a multi-centre setting. This will allow us to determine if the baseline (pre-treatment) RTL correlates with tumour response i.e. to determine if the baseline RTL predicts tumours that are sensitive or resistant to chemotherapy, and if changes in RTL during chemotherapy give an early indication of outcome (objective response, overall survival).

In addition, we will also evaluate other biomarkers of cellular senescence including secreted proteins and micro-RNAs. A panel of senescence biomarkers have been identified from proteomic analysis of peptides secreted by bone marrow cells of 4th generation telomerase null mTR$^{-/-}$ mice including cathelicidin-related antimicrobial protein (CRAMP), EF-1a, stathmin and chitinase 3-like protein 3 [18]. These markers were also found to increase in senescent human fibroblasts and could be detected in plasma of mice and humans. The authors showed that combining ELISA detection of CRAMP and an enzyme assay of chitinase activity in human plasma effectively discriminated between young and old individuals. In addition, there was a significant increase in the expression of these biomarkers in the blood plasma of patients with chronic diseases associated with increased rates of cell turnover and telomere shortening including myelodysplastic syndromes (MDS). Analysis of a blinded, unrelated test set of plasma samples confirmed the discriminatory power of the biomarker combination.

Micro-RNAs are short non-coding molecules ranging in size from 19-22 nucleotides are highly conserved and regulate protein expression through interactions with the 3' untranslated region (UTR) of mRNA. The binding of a miRNA to the 3'UTR causes inhibition of translation through steric hindrance or degradation of the mRNA, depending on the degree of complimentarity between the two sequences. The ability of miRNAs to regulate a variety of target genes allows them to induce changes in multiple pathways and processes such as development, apoptosis, proliferation and differentiation. MiRNAs could therefore facilitate the complex cellular changes required to establish the senescent phenotype. We have recently identified a panel of senescence-associated miRNAs (SA-miRNAs)[19]. We will profile SA-miRNA expression in plasma samples[20, 21] as markers of senescence.

Cytokeratin 18 (CK18) is a major component of the intermediate filament of simple epithelial cells and epithelial-derived tumours, and makes up approximately 5% of the total cell protein (22). It undergoes cleavage by caspases 3, 7 and 9 during apoptosis into proteolytic fragments[23 - 25]. The monoclonal antibody, M30, recognises a neo-epitope of CK18 (CK18-Asp396 cleavage product) exposed after caspase-mediated cleavage during apoptosis, but not intact CK18[26]. A further ELISA (M65) uses two monoclonal antibodies specific for epitopes on CK18 to measure total (both caspase-cleaved and un-cleaved) soluble CK18. The two ELISAs can be used in conjunction to calculate the relative proportion of caspase-cleaved CK18 to total CK18 in plasma[27].

CK18-Asp 396 may be applicable as a pharmacodynamic biomarker in phase I clinical trials of novel anti-cancer therapies. Our group has previously demonstrated that disease stabilisation was associated with CK18-Asp 396 plasma levels in patients with advanced solid tumours treated in a phase 1 clinical trial of the novel

hydroxamate histone deacetylase inhibitor, belinostat[28]. We have also shown that plasma levels of CK18 at baseline in patients with gastrointestinal adenocarcinomas are significantly higher in patients with progressive disease compared to patients with partial response/stable disease, and that peak plasma levels of CK18 observed during treatment are associated with treatment response[29]. Consequently, analysis of CK-18 will be used in this study as a marker of drug-induced cell death by apoptosis.

The combination of biomarkers ranging from telomere length to analysis of genes involved in regulation of RTL and to secreted proteins, immune-profiling and micro-RNAs associated with cell senescence and apoptosis provides a matrix of assays covering key biological processes known to be important in the balance between driving and restraining tumour progression. Determining the clinical utility of these biomarkers requires testing in appropriate clinical trial settings.

Gastro-oesophageal adenocarcinoma has a poor prognosis, accounting for 10,000 deaths per year in the UK. Combination chemotherapy results in a significant survival advantage in patients with advanced gastric cancer when compared with best supportive care in randomised clinical trials[30 - 32]. High response rates may be obtained in these tumours by the use of protracted venous infusional 5FU, epirubicin and cisplatin - the ECF regimen[33]. In an initial study with this regimen, an overall response rate of 71% and a complete response rate of 12% were observed[33]. These encouraging results were confirmed in two subsequent studies, with overall response rates of around 60% and with complete responses occurring in around 10% of patients[34, 35]. In a multi-centre randomised study, ECF resulted in a significantly better response rate (45%) and median survival (8.9 months), with significantly less toxicity compared to the FAMTX regimen[36]. Recently, a randomised trial in the UK has compared four regimens in advanced gastric cancer in a 2 x 2 design comparing infusional 5-fluorouracil with the oral fluoropyrimidine pro-drug capecitabine, and comparing oxaliplatin with cisplatin[37]. Capecitabine was equivalent to infusional 5-fluorouracil but with the added convenience of oral therapy thereby removing the requirement for insertion of central venous catheters. Consequently, capecitabine has now replaced infusional 5-fluorouracil in combination chemotherapy regimens in the UK. The combination of epirubicin, oxaliplatin, and capecitabine gave a further improvement in overall survival, and it is likely that oxaliplatin will be increasingly used in the UK in the future as the standard comparator in clinical trials. Nevertheless, the response rates with these regimens, and the overall survival, remain disappointing with significant toxicity in patients who do not respond to treatment. Therefore, the development of a prognostic biomarker to aid patient selection for treatment would be advantageous for this patient population.

3. STUDY DESIGN

This will be a multi-centre, open, non-randomised study. Eligible patients will be those with advanced gastric or oesophageal adenocarcinoma who are about to undergo chemotherapy with either the ECX/ECF (epirubicin, cisplatin and capecitabine/5-FU) or EOX/EOF (epirubicin, oxaliplatin and capecitabine/5-FU) regimens. Additionally, patients randomised in the NCRN REAL-3 study to receive EOX + panitumumab will also be eligible.

Prior to starting therapy, 20 mL of venous blood will be taken, on one occasion, for laboratory analysis. Further samples of 20 mL of venous blood will be taken on day 1 of each subsequent chemotherapy cycle and at the time of any subsequent documented disease progression. Where possible (depending on patient follow-up arrangements at participating Cancer Centres and Cancer Units), 20 mL of venous blood will also be collected at each subsequent follow-up visit after completion of chemotherapy until there is documented disease progression. Patient treatment,

supportive care, and disease assessment will be unaffected by participation in this study. Buffy coats will be prepared and plasma will be frozen, and then samples will be transferred to the Translational Pharmacology Laboratory at the Wolfson Wohl Cancer Research Centre, University of Glasgow. Buffy coat samples will then be transferred for analysis of RTL to the Department of Medical Biosciences, Umea University, Sweden. Analysis of the other biomarkers of telomere-associated genes, cellular senescence, and of biomarkers of drug-induced changes in cellular function, will be performed by the Translational Pharmacology Laboratory at the Wolfson Wohl Cancer Research Centre, University of Glasgow.

4. ELIGIBILITY CRITERIA

4.1. Inclusion Criteria

1. Histologically or cytologically confirmed locally advanced or metastatic gastric, gastro-oesophageal junction, or oesophageal adenocarcinoma.
2. Patients who are due to start chemotherapy with either the ECX/ECF (epirubicin, cisplatin and capecitabine/5-FU) or EOX/EOF (epirubicin, oxaliplatin and capecitabine/5-FU) regimens. Additionally, patients randomised in the NCRN REAL-3 study to receive EOX + panitumumab will also be eligible.
3. Written informed consent.
4. At least one lesion which is uni-dimensionally measurable by RECIST[38]
5. Age >18 years.
6. Able to comply with study protocol.

4.2. Exclusion Criteria

1. Any evidence of any medical or psychiatric disorders that would be a contra-indication to venesection.
2. Women who are pregnant or lactating
3. Patients who have had systemic anti-cancer therapy or radiotherapy within the previous 6 weeks.
4. Life expectancy < 3 months.

5. PATIENT REGISTRATION

To register a patient on to the study, contact the Cancer Research UK Clinical Trials Unit, Glasgow. Registration to the study can be done by either telephone or fax on the following numbers:

Telephone Number: 0141 301 7950
Fax Number: 0141 301 7196

Patient eligibility criteria will be checked and, if eligible, a 3-digit Trial Number will be allocated at this point.

6. PATIENT ASSESSMENTS

Patients' pre-treatment evaluation, treatment and assessments during treatment, will be unaffected by participation in this study and will be the standard management for these patients.

7. SAMPLE COLLECTION

All patients will give written informed consent. Patients should be registered with the Cancer Research UK Clinical Trials Unit, Beatson West of Scotland Cancer Centre, Glasgow, at the time that patients give informed consent for blood sampling. Eligibility will be checked at this time point also. 20 mL of venous blood will be taken prior to starting chemotherapy and on day 1 of each subsequent chemotherapy cycle and at the time of any subsequent documented disease progression. Where possible (depending on patient follow-up arrangements at participating Cancer Centres and Cancer Units), 20 mL of venous blood will also be collected at each subsequent follow-up visit after completion of chemotherapy until there is documented disease progression.

Blood samples will be labelled with the patient's study number and the date on which the sample was taken. Buffy coats will be prepared at each collaborating centre and buffy coats and plasma will be stored at -70^0 C and transferred to the Translational Pharmacology Laboratory at the Wolfson Wohl Cancer Research Centre, University of Glasgow, at regular intervals (depending on storage capacity at site) . Subsequently, buffy coat samples will be transferred for analysis of RTL to the Department of Medical Biosciences, Umea University, Sweden. Analysis of the other biomarkers of telomere-associated genes, cellular senescence, and of biomarkers of drug-induced changes in cellular function, will be performed by the Translational Pharmacology Laboratory at the Wolfson Wohl Cancer Research Centre, University of Glasgow.

8. BLOOD SAMPLE PREPARATION AND ANALYSES

8.1. Relative Telomere Length

Buffy coat samples will be analysed for RTL at the Department of Medical Biosciences, Umea University, Sweden or by the Translational Pharmacology Laboratory, University of Glasgow, using a quantitative real-time PCR method as previously described (12, 17). Analysis of genes involved in regulation of telomere length will be performed by the Translational Pharmacology Laboratory using a targeted sequencing strategy.

8.2. Biomarkers of Cellular Senescence

i) Senescence-associated protein/miRNA biomarkers. Plasma samples will be analysed for circulating senescence-associated biomarkers at the Wolfson Wohl Cancer Research Centre, University of Glasgow, UK using methods as previously described[20, 21]

8.3. Biomarkers of Apoptosis/Cellular Function

Blood samples will be collected and processed using a method which has been validated in our laboratory[28, 29]. Samples assayed for apopotosis-associated biomarkers which may include caspase-cleaved CK18.

9. TRIAL MANAGEMENT AND DATA COLLECTION

9.1. Data Collection

All patients will be registered at the Cancer Research UK Clinical Trials Unit, Beatson West of Scotland Cancer Centre, Glasgow.

The following data will be recorded:

(a) Demographic data (all patients) including patient's initials, age, sex, date of birth, ethnic origin, and the referring hospital and clinician;
(b) Tumour Details including anatomical location of the primary, tumour stage, histological type and grade of differentiation, and the sites of any metastases should also be recorded;
(c) Treatment Details including the chemotherapy regimen administered, number of courses and the dates administered, the date of any blood samples taken for this study, and tumour response as defined by the RECIST criteria (38).
(d) Follow up Details including the date of disease progression and date of death.
(e) Results of the pre-treatment full blood count and biochemistry, especially for determining emerging prognostic factors such as NLR (neutrophil – lymphocyte ratio).

Case Report Forms (CRFs) will be supplied by the Clinical Trials Unit, Beatson West of Scotland Cancer Centre, Glasgow.

These forms should be completed in accordance with the CRF completion guidelines issued for the study. Queries should be handled as described in the study data-flow section of the CRF completion guidelines. Specific questions about data management should be addressed to the Clinical Trial Co-ordinator for the study.

All CRFs must be returned to the Cancer Research UK Clinical Trials Unit, Glasgow for data entry and ultimately, statistical analysis.

CRFs from the study will be stored in line with current regulatory requirements, that is, until 5 years after completion of the study or as long after this as is agreed between the sponsor and investigators. Other essential documents, including source data, consent forms, and regulatory documents, will be archived by or for the Investigator in an appropriate archive in line with current regulatory requirements and made available for monitoring, audit and regulatory inspection.

9.2. Trial Management Group

A Trial Management Group (TMG) will oversee the running of the trial and meet if and when necessary. Members of the TMG will include the Chief Investigator, Principal Investigator, Project Manager, Clinical Trial Co-ordinator, Trial Statistician, IT Staff, Quality Assurance Manager and TPL staff.

10.WITHDRAWAL CRITERIA

Patients will be withdrawn from the study for the following reasons:

1. Patient decision to withdraw consent
2. Patient decision to refuse venesection
3. Development of a medical or psychiatric condition that would make venesection contra-indicated in the opinion of the investigator
4. Discontinuation of chemotherapy for any reason (including disease progression).

11. STATISTICS

The primary study endpoint is the association of baseline telomere length with outcome. All other analyses are exploratory. The sample size calculation is based on splitting the patients into two equally-sized groups (high and low) based on the median telomere length at baseline. The primary analysis would be based on fractional polynomials (treating the data as continuous) so the actual power obtained

is actually likely to be higher than 80%. The prognostic significance of telomere length would be assessed in the context of other existing recognised prognostic factors.

The assumptions regarding outcome with chemotherapy in this patient population is based on the results of the Real-2 study[37]. The overall objective response rate with ECX (or EOX) is 45%. To be of prognostic interest the difference between the high and low RTL groups would have to be at least 25% (32.5% v 57.5%). To detect this magnitude of difference with 80% power at the 1.7% 2-sided level of statistical significance (a more stringent level of statistical significance is used to adjust for the fact that 3 primary comparisons are being made) requires 155 patients. Allowing for a modest level of correlation between RTL and other prognostic factors (0.25) means that to detect this as an independent prognostic factor requires that the sample size should be increased by 6.25%, which give a sample size of approximately 165 patients. The median progression-free survival (PFS) with ECX (or EOX) is 6.5 months. To be of prognostic interest the hazard ratio between the high and low RTL groups should be at least 2 (median PFS 4.35 v 8.65 months). To detect this magnitude of difference with 80% power at 1.7% 2-sided level of statistical significance requires 120 patients. Allowing for a modest level of correlation between RTL and other prognostic factors (0.25) means that to detect this as an independent prognostic factor requires that the sample size should be increased by 6.25% to approximately 128 patients.

The median overall survival (OS) of patients in the Real-2 study was 10 months. To be of prognostic interest the hazard ratio between the high and low RTL groups should be at least 2 (median OS 6.65 v 13.35 months). To detect this magnitude of difference with 80% power at 5% 2-sided level of statistical significance requires 130 patients. Allowing for a modest level of correlation between RTL and other prognostic factors (0.25) means that to detect this as an independent prognostic factor requires that the sample size should be increased by 6.25% to approximately 138 patients.

The sample size for the study will target the largest number required for the above comparisons, that is 165 patients.

In order to allow independent validation of prognostic models developed using secondary or exploratory biomarkers in the initial cohort of 165 patients a subsequent validation set of 165 patients will also be recruited. The validation power of this depends on the size of prognostic effects observed in the prognostic models developed, but it is thought this number should be sufficient, especially as 1-sided testing could be employed. Any validation work will be preceded by formal framing of hypotheses and associated statistical calculations of power.

Thus the total sample size for this study will be 330 patients assuming all patients are evaluable for response and have a baseline telomere length measurement. On the assumption that 17.5% of patients will not fulfil these criteria we will recruit 400 patients to the study

12. REGULATORY ISSUES

12.1. Ethics Approval

All patients will give written informed consent on entering the study. The protocol will require approval by a coordinating Research Ethics Committee, and by a Research Governance and Management Committee (e.g. Research and Development Office) of all the participating centres.

The study will be carried out in accordance with ICH GCP and the World Medical Association Declaration of Helsinki (1964) and its' revisions (Tokyo 1978, Venice 1983, Hong Kong 1989, South Africa 1996 and Edinburgh 2000).

12.2. Informed Consent

Consent to the study must be sought from each participant only after a full explanation has been give, the participant has been given an information sheet and time allowed for consideration. Signed participant consent should be obtained, the consent forms should also be signed by the person undertaking the consent procedure at site, who must be detailed on the site contact and responsibility log as having this authorisation. The Principal Investigator is responsible for ensuring if taking consent is delegated to a designee, the designee is suitable qualified by training or experience to take informed consent.

The right of the participant to refuse to participate without giving reasons must be respected. All participants are free to withdraw at any time from the study without giving reasons and without prejudicing further treatment.

Completed consent forms must be retained at each site, with one copy placed in the patient's casenote and another in the appropriate section of the Investigator Site File. All patients must be given a copy of the signed patient information sheet and consent form for their records. Consent forms must be retained on site and not submitted to the Trials Office.

In the event that new patient information sheets/consent forms are produced throughout the duration of the study, it may be that patients already participating in the study should be re-consented to the updated version. However, if the Principal Investigator decides that this is not in the best interest of the patients, re-consent is not required.

12.3. Patient Confidentiality

National Health Service Guidelines for storage, transmittal and disclosure of patient information will be followed at all times. Data on patients treated in this course of the study will be documented anonymously. Patients will only be identified by a patient number and initials.

Following formal admission to the study, patient data will be recorded in the hospital case records in the usual way including the circumstances of their entry into the study. Additionally data will be held in hard copy study case record files (CRF). These files will be identified as belonging to a particular patient identified only by their unique study ID. Results of the study may be communicated at scientific meetings and will contribute to the scientific literature. At no time will this be done in such a way that an individual patient may be identified.

12.4. Audit/Inspection

It is the sponsor's responsibility to inform the investigator(s) of all intended study centre audits and regulatory inspections involving the study centre. It is the investigator's responsibility to ensure appropriate resources at site and that the inspector(s) have access to all study personnel, documentation and patient medical notes as appropriate.

12.5. Liability, Indemnity and Insurance

The Hospital Trust at each participating site is responsible for the following:

1. Acts and omissions of its own staff and others engaged by it, including the Clinical Trials Unit and PI;
2. Ensuring the appropriate insurance administered by the National Health Service Litigation Authority is in place;
3. Ensuring any non-NHS employees involved in the clinical trial have Honorary Contracts with the Trust to cover access to patients and liability arrangements.

These responsibilities are outlined and agreed within the Clinical Study Agreement. No special insurance is in place for patients in this study other than standard NHS liability insurance providing indemnity against clinical negligence. This does not provide cover for non-negligence e.g. harm caused by an unexpected side effect of participating in a study.

13. QUALITY ASSURANCE

Quality Assurance will be maintained by the following requirements and activities:

- Trial Investigators and Site Staff must ensure that the trial is conducted in compliance with the protocol, Good Clinical Practice (GCP) and the applicable regulatory requirements.
- No on site monitoring will be performed however central monitoring of trial data will be performed by the Trial Statistician and the Clinical Trial Co-ordinator.
- The CTU will control data consistency and data quality by entering trial data onto the CTU trial database. Computerised and manual consistency checks will be performed and queries issued in cases of inconsistency or missing information. A full audit trail of any changes to the database will be maintained.
- The Trial Management Group will ensure the trial is being managed according to protocol, GCP and regulatory requirements.

14. ALLOCATION OF STUDY RESPONSIBILITIES

The Research Governance Sponsor of this clinical trial is NHS Greater Glasgow and Clyde.

A Clinical Study Agreement will be put in place between NHS Greater Glasgow and Clyde and each of the participating sites. This agreement outlines the responsibilities of each party in the running of the trial as well as the Chief Investigator (CI), the Cancer Research UK Clinical Trials Unit, Glasgow (CTU), and the Principal Investigator at the Participating Site.

14.1. Sponsor Responsibilities (NHS Greater Glasgow and Clyde)

The responsibilities will be for ethics opinion, GCP and conduct. The majority of the Sponsor's responsibilities have been delegated to the Chief Investigator and these are performed by the CTU as the co-ordinating centre for the study. As such, the main role of the Sponsor is to ensure that the CTU fulfil their responsibilities as outlined in the Clinical Study Agreement.

14.2. Clinical Trials Unit (CTU)

The CTU is responsible for the overall management of the clinical trial. This includes all regulatory submissions (Ethics and R&D), all administration relating to the submissions, circulation and correspondence to participating sites, data management, monitoring of data quality and ongoing communication with participating sites.

14.3. Chief Investigator (CI)

The Chief Investigator has delegated the majority of his/her responsibilities to the CTU. The CI is directly responsible for ensuring that the protocol and any amendments are in place and to provide any advice and recommendations on management of the patients on study.

14.4. Principal Investigator (PI)

The PI is responsible for the delegation of study activities within their unit and ensuring all personnel are adequately trained and qualified to carry out their responsibilities. Regarding the management of patients within their site, the PI is responsible for the safety and well being of trial patients, reporting any deviations from the protocol to the CTU.

Full details of the responsibilities are outlined in the Clinical Study Agreement. Two original copies of this will be held – one with the Sponsor and the other at Participating Site. A photocopy of the signed agreement will also be held within the CTU.

15. DEFINITION OF END OF STUDY

For the purposes of the Main REC approval, the study end date is deemed to be the date of the last data capture.

16. PUBLICATION OF THE TRIAL RESULTS

It is anticipated that manuscripts will be prepared from the results of this study and that these will be submitted for publication, with authorship according to the requirements for manuscripts in the Vancouver Statements.

No Centre will publish data from an individual centre without prior approval of the Trials Management Group.

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APPENDIX I: DECLARATION OF HELSINKI

DECLARATION OF HELSINKI
WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the
52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.
2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."
4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.
5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.
6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the etiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.
7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.
8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.
9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.
12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.
13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.
14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.
15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.
16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.
17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.
18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.
19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.
20. The subjects must be volunteers and informed participants in the research project.
21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed

consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.
23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.
24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.
25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.
26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.
27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negate as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.
30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.
31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.
32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.