FULL/LONG TITLE OF THE STUDY

*Prote*omic Profiling of *O*esophageal and Gastroesophageal Junction *A*denocarcinoma *N*eoantigens

ACRONYM

PROTEAN

PROTOCOL VERSION NUMBER AND DATE

PROTEAN Protocol V1 17-05-22

University of Dundee

This protocol has regard for the HRA guidance and order of content V1.2 March 2016

RESEARCH REFERENCE NUMBERS

IRAS Number:	**309452**
ISRCTN Number:	TBC
SPONSORS Number:	2-014-22
FUNDERS Number:	Generated by the funder. Enter if applicable

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Good Clinical Practice Guidelines (GCP) guidelines, the Sponsor's (and any other relevant) Standard Operating Procedures (SOP), and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest, accurate and transparent account of the study will be given; and that any discrepancies and serious breaches of GCP from the study as planned in this protocol, will be explained.

For and on behalf of the Study Sponsor:

Signature: Date:

..../...../.....

Name (please print):

Position:

.....

Chief Investigator:

Signature: Date:

..../...../.....

Name: (please print): Prof Russell Petty

Signature:

.....

Study Statistician:

Name: (please print):

Signature: Date:

..../...../.....

Position:

.....

KEY STUDY CONTACTS

Chief Investigator	Prof Russell Petty Chair of Medical Oncology Deputy Head, Division of Molecular and Clinical Medicine Ninewells Hospital and Medical school University of Dundee James Arnott Drive, Dundee DD1 9SY 01382 383542/383125 r.petty@dundee.ac.uk
Study Co-ordinator	Margaret Band Senior Trial Manager, Tayside Clinical Trials Unit (TCTU) 01382 383297 m.band@dundee.ac.uk
Sponsor	Patricia Burns Research & Development Office, Tayside Medical Science Centre (TASC), Residency Block Level 3, George Pirie Way, Ninewells Hospital, Dundee DD1 9SY, 01382 383297, TASCgovernance@dundee.ac.uk
Funder(s)	Platinum Informatics, The Vision Building 20, Greenmarket, Dundee DD1 4QB
Clinical Trials Unit	Tayside Clinical Trials Unit, TASC, Residency Block, Level 3, Ninewells Hospital, Dundee, DD1 9SY 01382 383581, TCTU@dundee.ac.uk
Key Protocol Contributors	Prof Russell Petty – CI Prof Angus Lomond- Trial Scientific Adviser Margaret Band – Senior Trial Manager Hasi Bandara – Database Manager
Statistician	To be confirmed, will be a member of the CI's research team

I. LIST OF ABBREVIATIONS

AE	Adverse Event
CART	Chimeric Antigen receptor T-Cell therapy
CI	Chief Investigator
CNORIS	Clinical Negligence and Other Risks Insurance Scheme
DMT	Data Management Team
eCRF	Case Report Form
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
ICF	Informed Consent Form
ISF	Investigator site file
ISRCTN	International Standard Randomised Controlled Trials Number
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
MS	Mass Spectrometry
MHC	Major Histocompatibility Complex
NHS R&D	National Health Service Research & Development
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
PIS	Participant Information Sheet
REC	Research Ethics Committee
SAE	Serious Adverse Event
SLS	School of Life Sciences
SOP	Standard Operating Procedures
TASC	Tayside Medical Science Centre
TBR	Tayside Biorepository
TCTU	Tayside Clinical Trials Unit
TMB	Tumour Mutational Burden
TMF	Trial Master File
TMG	Trial Management Group
TSA	Trial Scientific Adviser

II. STUDY SUMMARY

Study Title	***Prote*omic Profiling of *O*esophageal and Gastroesophageal Junction *A*denocarcinoma *N*eoantigens**	
Short title	PROTEAN	
Study Design	Basic science	
Study Participants	Patients with oesophageal or gastroesophageal junction adenocarcinomas	
Planned Sample Size	75 (50 oesophageal samples/25 lower oesophageal-gastric samples)	
Follow up duration	18 weeks	
	Objectives	Outcome Measures
Primary	To characterise neoantigen profiles of oesophageal or gastroesophageal junction adenocarcinomas	Neoantigen profiles for individual patients with oesophageal or gastroesophageal junction adenocarcinomas
Secondary	1- To determine the feasibility of neoantigen profiling using tumour biopsies of oesophageal or gastroesophageal junction adenocarcinomas 2- To identify oesophageal or gastroesophageal junction adenocarcinoma neoantigens as candidate targets for immunotherapy 3- To investigate the relationship between neoantigen profiles and clinical outcomes 4- Recovery of RNA and DNA from samples	1- Proportion of patients in which neoantigen profiles are successfully generated from biopsies 2- Characterise pre-existing T cell responses to identified neoantigens 3- Correlate objective response rate, disease control rate, progression free survival and overall survival with neoantigen profiles 4- Genomic and transcriptomic studies; neoantigen validation experiments.

III. FUNDING AND SUPPORT IN KIND

FUNDER(S)	FINANCIAL AND NON-FINANCIAL SUPPORT GIVEN
Platinum Informatics, The Vision Building 20, Greenmarket, Dundee DD1 4QB	Funding for the trial. Scientific expertise in immunoprecipitation, liquid chromatography-tandem mass spectrometry, and analysis of data to identify tumour neoantigens.

IV. ROLE OF STUDY SPONSOR AND FUNDER

The roles and responsibilities of the Sponsor and Funder will be detailed in the Clinical Research Agreement.

V. ROLES AND RESPONSIBILITIES OF STUDY MANAGEMENT COMMITEES/GROUPS & INDIVIDUALS

The Chief Investigator (CI) will be responsible for the conduct of the study. Site delegate(s) will oversee the study and will be accountable to the CI. A study-specific Delegation Log will be prepared for each Site, detailing the duties of each member of staff working on the study.

The study will be conducted in accordance with the principles of Good Clinical Practice.

In addition to Sponsorship approval, a favourable ethical opinion will be obtained from an appropriate NHS Research Ethics Committee (REC). Appropriate NHS R&D permission(s) will be obtained prior to commencement of the study.

The study will be co-ordinated by a Trial Management Group (TMG), consisting of the CI and Trial manager, other appropriate members will be invited. TMG membership details and minutes of the TMG meetings will be maintained in the Trial Master File (TMF).

VI. PROTOCOL CONTRIBUTORS

CI: Prof Russel Petty, Initial draft, review and final approval

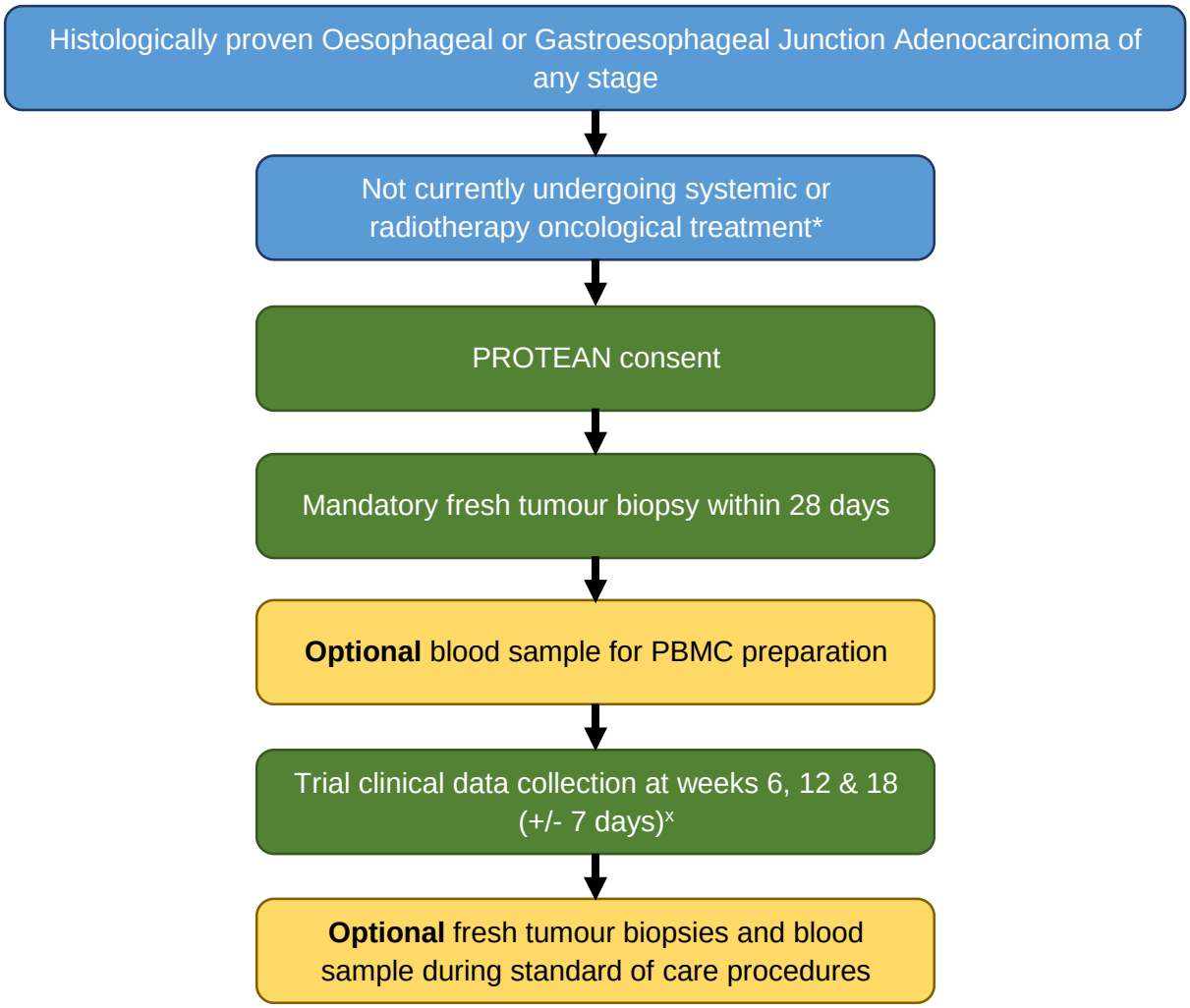
TCTU Senior Trial Manager: Margaret Band, Review

TCTU Database Manger: Hasithi Bandara, Review

VII. KEY WORDS:

Oesophageal Adenocarcinoma, Gastroesophageal junction carcinoma, neoantigens

VIII. STUDY FLOW CHART



*Treatment can be planned to occur subsequently or recently completed. Eligible participant may be pre-, post- or between treatments.

[x]May be by telephone if participant is not attending a standard of care clinic visit.

[¥]A maximum of 2 additional optional research blood samples and 3 research biopsy samples may be collected. This includes during standard of care procedures at any time during the study period and also research biopsy / PBMC up to 28 days after radiological and/or clinical progression.

1. BACKGROUND & RATIONALE

1.1. Immunotherapy in Oesophageal Adenocarcinoma

Clinical outcomes for gastroesophageal adenocarcinomas with standard approaches involving cytotoxic chemotherapy, radiotherapy and surgery have limited effectiveness, with the majority of patients dying within a year of diagnosis, and only 15% surviving 5 years [1]. Immune checkpoint inhibitors have provided durable clinical responses in patients with advanced solid tumours [2]. Recently clinical trials have extended this observation to gastroesophageal adenocarcinomas and established checkpoint inhibitors as a standard of care (see Table 1.1) [3-15]. The durable responses seen to checkpoint inhibitors in advanced stage gastroesophageal adenocarcinomas are hitherto unprecedented and have had a transformative impact on patients in this clinical setting.

However, the majority of gastroesophageal cancer patients are resistant to checkpoint inhibitors and do not benefit [16]. Predictive biomarkers Programmed Death Ligand-1 (PDL1),Tumour mutational burden (TMB) and Microsatellite instability-high (MSI-H), can identify sensitive patients and enrich treated cohorts for those most likely to benefit clinically, but new and alternative immunotherapy strategies are needed to extend the benefits of durable 'immune type' responses to a larger proportion of patients with gastroesophageal adenocarcinoma [16].

The tumour immune microenvironment has been identified as a key factor in determining the outcome of immunotherapies. Immune checkpoint inhibitors show better clinical response in tumours (including oesophageal adenocarcinomas), with high levels of infiltrating T cells together with more antigens, including neoantigens compared to those lacking tumour-reactive infiltrating T cells [17]. A number of studies have focussed on converting immune tumour microenvironments from 'cold' with low levels of T cell infiltration into 'hot' with high levels of infiltrating T cells to enhance sensitivity to immune checkpoint inhibitors [16].

T cell–based immunotherapy has been successfully used to treat human solid cancers including metastatic melanoma where complete, durable tumour regressions have been observed [18-24]. However, in solid tumours broadly, success has been more limited, with durable responses an uncommon occurrence, and this clinical experience has highlighted a number of barriers to the development of effective T cell–based immunotherapy including a limited array of targetable antigens and heterogeneous antigen expression between patients [25]. More recently an early phase trial demonstrated safety and promising efficacy for Chimeric Antigen receptor T-Cell therapy (CART-T) in gastric cancer. In advanced stage gastric cancer patients treated with at least 2 previous lines of treatment including checkpoint inhibitors in 44%, CT041, an anti-CLDN18.2 CAR-T cell therapy, reported objective response rate of 61.1% median progression free survival of 5.4 months and median overall survival 9.5 months [26]. This study provides a proof of concept for the effectiveness of an adoptive T cell-based approach in gastroesophageal adenocarcinoma, importantly including in those patients resistant to checkpoint inhibitors.

Similar challenges have been encountered in clinical studies with cancer vaccines, although recent technological advances with mRNA vaccines provide solutions if suitable targets can be defined [27-30]. Vaccines present tumour-specific antigens and activate cytotoxic T cells to recognise tumour cells and infiltrate into tumours, thereby training the immune system to target

and kill tumour cells, and also convert tumour immune microenvironments from ‘cold’ into ‘hot’ which could enhance effectiveness of immune checkpoint inhibitors as well [27-30].

1.2. Targeting tumour neoantigens as a therapeutic strategy

Increasing evidence suggests that T cells specific for tumour neoepitopes (neoantigens) derived from the products of tumour specific gene aberration stimulate a strong cytotoxic T cell–mediated immune response and are important for tumour regression in patients receiving tumour infiltrating lymphocytes (TILs) therapy, and immune checkpoint inhibitors [31-33]. Neoantigen profiles may therefore have value as predictive biomarkers for immune checkpoint inhibitors to guide optimised personalised treatment strategies, for example as either monotherapies or in combination regimens.

Neoantigen-specific T cells generated de novo from tumour-specific somatic mutations, are not subject to central and peripheral tolerance and cannot target normal tissues. Thus, neoantigens represent ideal targets for T cell–based cancer immunotherapy and other therapeutic strategies including cancer vaccines, that harness a T cell response against neoantigens could also significantly benefit cancer patients. Therefore, targeting neo-antigens promises high therapeutic specificity but since in solid tumours, due to between patient genomic heterogeneity they are largely patient-specific, a precision medicine approach may be required built upon neoantigen identification in individual patients. Oesophageal adenocarcinoma has high TMB and high predicted neoantigen loads associated with increased CD8+T cell density, and genomically exhibits high levels of inter-patient heterogeneity [34, 35].

1.3. Neoantigen identification

Current methods to identify tumour neo-antigens have a number of limitations and identifying patient specific, individualised, targetable neoantigens remains a major obstacle to neoantigen based therapies for cancer. Neoantigen identification at present mainly involves whole-genome, whole exome, and transcriptome sequencing [36-42]. However systematic immunogenicity assessment of neoepitopes for common driver mutations in solid tumours is currently lacking. Current methods for immunogenic neoantigen identification often require synthesis of dozens to hundreds of peptides and are time-consuming and costly and have low positive rates. Narrowing down the list of potential neoepitopes and reducing the time of the identification process are currently major unresolved clinical challenges.

Direct identification of neoantigens by the analysis of the tumour ligandome using mass spectrometry (MS) offers a number of advantages, for example translational errors and post-translational modifications (found to be more efficient in triggering an immune response), that cannot be predicted by genomic or transcriptomic approaches, but can be characterized by mass spectrometry analysis [43-45]. Recently dissimilarity to the non-mutated proteome has been shown to identify a subset of neoantigens with distinct hydrophobic properties, high likelihood of immunogenicity, and correlation with benefit from immune checkpoint inhibitors [46].

Table 1.1. Practice changing randomised Phase 3 trials with immune checkpoint inhibitors in gastroesophageal adenocarcinoma (adapted from [16])

Trial	Phase	Population	Setting	Agent	Response rate	PFS	OS
>2nd line							
ATTRACTION-2 Kang et al. [5]	3	Advanced GOJ or GC (*n* = 493) Asian population	≥2nd line	Nivolumab (*n* = 330) vs placebo (*n* = 163)	11% (8–16%) DCR 40% (34–46%)	1.61 m (1.5–2.3)	5.3 m vs 4.1 m (HR 0.63, *P* < 0.0001)
KEYNOTE-059 (cohort 1) Fuchs et al. [7]	2	GC/GOJ (*n* = 259)	≥2nd line	Pembrolizumab (all patients)	12%[8–17] PD-L1 + ve 16% PD-L1 –ve 6% DCR 27	2.0 m (2.0–2.1)	5.5 m (4.2–6.5)
CHECKMATE-032 Janjigian et al. [12]	1/2	Advanced OC, GOJ or GC (*n* = 160)	≥2nd line	Nivolumab 3 mg/kg (*n* = 59) nivolumab 1 mg/kg + Ipilimumab 3 mg/kg (*n* = 49) nivolumab 3 mg/kg + Ipilimumab 1 mg/kg (*n* = 52)	12% vs 24% vs 8%	12 month: 8% vs 17% vs 10%	12 month: 39% vs 35% vs 24%
JAVELIN-300 Bang et al. [13]	3	GOJ (*n* = 111) or GC (*n* = 260)	3rd line	Avelumab vs TPC	2.2% vs 4.3% DCR 22.2 vs 44.1%	1.4 m vs 2.7 m	4.6 m vs 5.0 m (HR 1.1, NS)
2nd line							
KEYNOTE-061 Shitara et al. [6]	3	GOJ (*n* = 185) or GC (*n* = 407)	2nd line	Pembrolizumab (*n* = 296) vs paclitaxel (*n* = 296)	CPS ≥ 1: 16% vs 14% CPS ≥ 10: 24.5% v 9.1% CPS < 1: 2% vs 10.4%	1.5 m vs 4.1 m (HR 1.27) CPS < 1: HR 2.05	9.1 m vs 8.3 m (HR 0.82, *P* = 0.0421) CPS ≥ 10: 10.4 m v 8.0 m (HR 0.64) CPS < 1: 4.8 m vs 8.2 m (HR 1.20)
KEYNOTE-181 Kojima et al. [14]	3	Advanced OAC (*n* = 227) or OSCC (*n* = 401)	2nd line	Pembrolizumab vs TPC	–	–	ITT: 7.1 m vs 7.1 m SCC: 8.2 m vs 7.1 m CPS > 10: 9.3 m vs 6.7 m

Trial	Phase	Population	Setting	Agent	Response rate	PFS	OS
1st line							
KEYNOTE-062 Shitara et al. [15]	3	GC/GOJ (PD-L1 CPS ≥ 1%)	1st line	Pembrolizumab (P) (*n* = 256) pembrolizumab + chemotherapy (P + CTx) (*n* = 257) chemotherapy (CTx) (*n* = 250)	P v CTx: 14.8% vs 37.2% CPS ≥ 10: 25.0% vs 37.8%	P v CTx CPS ≥ 1: 2.0 m v 6.4 m (HR 1.66), CPS ≥ 10: 2.9 m v 6.1 m (HR 1.10)	P v CTx ITT: 12.5 m v 11.1 m (HR 0.85) CPS ≥ 1: 10.6 m v 11.1 m (HR 0.91) CPS ≥ 10: 17.4 m vs 10.8 m (HR 0.69)
ATTRACTION-4 Boku et al. [8]	2	GC/GOJ (*n* = 724)	1st line	Nivolumab (*n* = 362)+CTx (SOX/CapOX) vs CTx alone (*n* = 362)	58% vs 48%	10.5 vs 8.4 (HR 0.68)	17.5 m vs 17.2 (HR 0.90)
KEYNOTE-590 Kato et al. [9]	3	OAC or OSCC (*n* = 749)	1st line	Chemotherapy + /pembrolizumab	OSCC: 45% vs 29.3%	–	OSCC (all): 12.6 m vs 9.8 m (HR 0.72) OSCC (CPS ≥ 10): 13.9 m vs 8.8 m (HR 0.57) ITT 12.4 m vs 9.8 m
CHECKMATE 649 Janjigian et al. [3]	3	GC/GOJ (*n* = 1266, including Ipilimumab/nivolumab arm)	1st line	Nivolumab+chemotherapy (*n* = 473) chemotherapy alone (*n* = 482)	CPS > 5: 60% vs 45%	CPS ≥ 5: 7.7 m vs 6.0 m (HR 0.68)	CPS ≥ 5: 14.4 m vs 11.1 m (HR 0.71, *P* < 0.0001)

DCR disease control rate; PFS progression free survival; OS overall survival; HR hazard ratio; NS non-significant; m month; SOX S-1/oxaliplatin; CapOx capecitabine/oxaliplatin; GOJ gastroesophageal junctional; GC gastric cancer; OAC oesophageal adenocarcinoma; OSCC oesophageal squamous cell carcinoma; PD-L1 programmed death ligand 1; TPC treatment of physician's choice; CPS combined positivity score.

2. OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

The aims of this study are to characterise neoantigen profiles in oesophageal and gastroesophageal adenocarcinomas and to generate neoantigen profiles for individual patient tumours. This will be achieved by analysing the Major Histocompatibility Complex (MHC) - associated 'immunopeptidome', using a state-of-the-art liquid chromatography-tandem mass spectrometry (LC-MS/MS) based method for direct neoantigen identification. Proprietary software tool DESIMAL, created by Platinum Informatics Ltd. will underpin this work and establish an automated pipeline for raw MS data processing and downstream data interpretation. This study will determine the feasibility of neoantigen identification using this approach in gastroesophageal adenocarcinoma patients, using routinely available clinical biopsy materials. The study will evaluate neoantigens as therapeutic targets for individualised adoptive T cell and/or cancer vaccine approaches for the treatment of oesophageal adenocarcinoma. Additional objectives include the characterisation of pre-existing T cell responses to identified neoantigens and the recovery of RNA and DNA from samples that will be used for genomic and transcriptomic studies and for neoantigen validation experiments.

2.1. Table of endpoints/outcomes

Primary Objective:	**Outcome Measure:**	**Timepoint**
To characterise neoantigen profiles of oesophageal or gastroesophageal junction adenocarcinomas	Neoantigen profiles for individual patients with oesophageal or gastroesophageal junction adenocarcinomas	End of study

Secondary Objectives	Outcome Measures	Timepoint
1- To determine the feasibility of neoantigen profiling using tumour biopsies of oesophageal or gastroesophageal junction adenocarcinomas	1- Proportion of patients in which neoantigen profiles are successfully generated	1- End of study
2- To identify oesophageal or gastroesophageal junction adenocarcinoma neoantigens as candidate targets for immunotherapy	2- Characterise pre-existing T cell responses to identified neoantigens	2- End of study
3- To investigate the relationship between neoantigen profiles and clinical outcomes	3- Correlate objective response rate, disease control rate, progression free survival and overall survival with neoantigen profiles.	3- End of study
4- Recovery of RNA and DNA from samples	4- Genomic and transcriptomic studies; neoantigen validation experiments.	4- End of study

3. STUDY DESIGN

Observational study with specimen collection.

4. STUDY SETTING

The study, which will characterise tumour proteomes and tumour-specific neoantigens, will benefit from work contributed by NHS hospitals and NHS cancer centres across the UK. Up to 15 sites will be involved in recruiting participants and collecting samples. Tumour specimens will be transported to the School of Life Sciences, University of Dundee, for neoantigen analysis by Platinum informatics Ltd. utilising laboratory facilities at the University of Dundee.

5. PARTICIPANT ELIGIBILITY CRITERIA

5.1. Inclusion criteria

- 18 years of age or older
- Willing and able to provide written informed consent
- Histologically confirmed oesophageal or gastroesophageal junctional adenocarcinoma.
- Able to provide a tumour biopsy sample as per protocol requirements (see section 6.4)

5.2. Exclusion criteria

- Concurrent systemic anti-cancer treatment and/or radiotherapy (participants undergoing cancer surgery are eligible.)
- Any significant medical condition that in the opinion of the Principal Investigator (PI) would impair the ability of the participant to complete the requirements of the protocol

6. STUDY PROCEDURES

6.1. Recruitment

75 participants across all sites will be recruited. Where it was not possible to obtain a tumour biopsy further participants will be recruited to ensure tumour biopsies are obtained from up to 75 participants (approximately 50 oesophageal samples/25 lower oesophageal-gastric samples).

6.1.1. Participant identification

Patients will be identified during routine clinical care pathways by the existing clinical care team. Patients may be identified as part of case reviews at upper gastrointestinal cancer multidisciplinary team meetings and during other routine clinical care contacts. Subsequently, patients will be reviewed as part of their routine care by the PI or a sub-investigator, also part of the patient's clinical care team, who will confirm if the patient meets the study inclusion criteria and will then make the initial approach to discuss the study with them.

Suitable patients will be first approached at clinic by the PI or sub-investigator who is part of their clinical care team. They will be provided with information about the study (Participant Information Sheet) and the consent form. They will have the opportunity to ask questions, to think about their involvement, to talk to others about the study and if willing to take part, will return to give consent at a later date. Participants will be given a minimum of 24 hours from initial approach and discussion to consider whether they wish to take part in the study. The time given to consider taking part is unlimited as long as standard of care treatment is not started. The start of their standard care treatment will not be delayed.

6.1.2. Screening

Screening will be performed by local sites under the overall responsibility of the local PI who will be required to confirm that the participant satisfies all the approved inclusion and exclusion criteria of the protocol. No post-consent screening will be conducted.

6.1.3. Ineligible participants

After a patient has been approached about the study if they are found to be ineligible for study participation, they will be thanked and the reasons for the ineligibility fully explained. Any queries or questions will be answered by an appropriate member of the research team.

6.1.4. Payment

No payment for participation in the study will be made. Where any research visits are not conducted at the time of a clinical visit, travel expenses will be given.

6.2. Consent

The PI retains overall responsibility for the conduct of research at their site and this includes taking of informed consent at each site. They must ensure that any person delegated responsibility for seeking informed consent is duly authorised, trained, and competent according to the approved protocol and principles of GCP.

Participants will receive a copy of the Participant Information Sheet (PIS) and will be given at least 24 hours to consider participation in the study prior to consent. Prior to consent participants will be given the opportunity to ask any questions about the study. Where a participant requests to speak with a physician from the study team the consent process will not be completed until the participant has spoken to the physician and had all questions answered to their satisfaction. Once a patient agrees to participate, written informed consent will be obtained and eligibility confirmed.

At time of consent participants will be asked if they only consent to give tissue samples when this is feasible during standard clinical procedures or, where this is not possible, if they give consent to have a biopsy solely for the purpose of obtaining research samples.

Participants may provide additional consent for further biopsies to be taken during further routine clinical care procedures as an optional part of the study.

Written informed consent will be obtained from participants for blood and tissue collection as well as for genomic analysis of these specimens.

The original Informed Consent Form (ICF) will be filed in the TMF or Investigator Site File (ISF), a copy will be given to the participant and a further copy will be filed in the participant's medical notes.

For adults who lose capacity their previous wishes will remain valid unless the protocol changes significantly. If this occurs and further consent is required from a participant who has lost capacity, an appropriate person will be asked for their consent. This will be fully documented in the patient's notes. Data or tissue already collected with consent will be retained and used in the study.

6.2.1. Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable

Consent for retention of surplus biospecimen material remaining after the completion of PROTEAN study analysis for future ethically approved research into the causes of or treatments for oesophageal/gastroesophageal adenocarcinoma will be requested. Surplus material will be stored in the Tayside Biorepository (TBR) and registered with Tayside Biorepository (REC approval17/ES/0130) to ensure traceability and to provide support required for any services involving the tissue. TBR is part of the Scottish Biorepository network which operates as a unified network and is accredited by NHS Research Scotland Central Management Team to

ensure that best practice for human tissue research within Scotland is carried out to the equivalent standards of the Human Tissue Authority. Request to access samples for further research will be made by the CI and Trial Scientific Adviser (TSA) or will be made in writing by other investigators using a standardised format, to the CI and TSA who will review the request. An application will be submitted to the TBR detailing the requirement for further use. This will undergo scientific and ethical review by the biorepository tissue access committee who will confirm suitable tissue and samples are available. The access committee will ensure that use of the tissue is compliant with the consent provided by patients and the REC delegated ethical authorisation to TBR (17/ES/0130).

6.3. Baseline data

Baseline data will be collected as per Schedule of Procedures, Appendix 4. Only information directly related to the objectives and outcome measures detailed in the protocol shall be collected.

6.4. Study assessments

Study assessments will be carried out as per Schedule of Procedures, see Appendix 4

Medical history

- To confirm eligibility
- Location of primary tumour
- Histological and molecular diagnosis details – Human epidermal growth factor receptor 2 (HER2), PD-L1 testing
- Tumour stage according to AJCC/UICC version 8 for cancers of the oesophagus and stomach
- Stage and location of metastases
- Progression assessment – RECIST Criteria 1.1
- Medical co-morbidities
- Smoking history

Demographics

- Age, gender, body mass index

Concomitant medication

- Details of any scheduled or prior cancer treatment

Biopsy

- A fresh tumour biopsy will be collected:
    - Prior to the commencement of any subsequent scheduled anticancer treatments*; OR
    - After the completion of the latest line of anticancer treatment*; OR
    - During follow up when a participant is not receiving or is between scheduled anti-cancer treatments*.

*Anti-cancer treatments excluding surgery

- The biopsy may be obtained from any site of tumour, primary or metastatic as considered the most appropriate and feasible at the time of the procedure.
- Biopsies from more than one tumour site may be obtained at the same time point and procedure, but this is not mandatory, only biopsy of a single tumour site is required for study participation.
- Up to 10 biopsies, approximately 2mm each, will be taken at each timepoint and if tumour biopsy is being undertaken by endoscopy 2 biopsies approximately 2mm each of adjacent normal oesophageal or gastric mucosa.
- As far as possible, tumour biopsies for the study will be obtained during routine clinical care procedures e.g. endoscopy, surgery, stenting. However, where this is not possible, consent will be requested for study specific procedures for biopsy to obtain tumour material.
- Participants may provide additional consent for further biopsies during further routine clinical care procedures to be taken as an optional part of the study.
- Details of sample collection, processing, storage and transfer will be provided in PROTEAN Clinical Sample Handling Manual.

Research bloods

- A maximum of 20ml of blood will be collected at each visit.
- Sites which have the facilities to carry out peripheral blood mononuclear cell preparations (PBMC) analysis will process and complete analysis on site. These samples will be processed at sites for PBMCs.
- Details of sample collection, processing, storage and transfer will be provided in PROTEAN Clinical Sample Handling Manual.

Outcome assessment

- Tumour response status according to RECIST v1.1
- Overall Survival

Adverse events (AE)

- Will be recorded as per Section 8, Pharmacovigilance.

6.5. Long term follow-up assessments

Nil

6.6. Qualitative assessments

Nil

6.7. Withdrawal criteria

Participants are free to withdraw at any time and are not obliged to give reason(s). It will be clearly stated in the PIS that the participant is free to withdraw from the study at any time for any reason without prejudice to future care. The CI, PI or delegate will make a reasonable effort to ascertain the reason(s), both for those who express their right to withdraw and for those lost to

follow up, while fully respecting the individual's rights. Following a request to withdraw from the study this will be acted on immediately by the local study team and communicated to the CI.

The investigator may withdraw a participant at any time if it is in the best interest of the participant and continuation would be detrimental to the participant's well-being. A full explanation will be provided.

If a participant withdraws or is withdrawn from the study, the research team will retain any data and samples obtained up until the time of the point of withdrawal for use in the study analysis. Participants do not have the right to withdraw their data or any research tissue collected. No further data or samples will be collected after withdrawal.

6.8. Storage and analysis of clinical samples

The collection, local processing, storage and transportation of participant samples, including tumour and PBMCs; histopathological quality assurance and processing in the TBR and transportation to Platinum Informatics Ltd. at the School of Life Sciences, University of Dundee, along with return of surplus material to the TBR for storage and additional future analyses, will be detailed in the PROTEAN Clinical Sample Handling Manual, which will be available to all participating sites.

The patient tumour samples and PBMCs (if site is undertaking) will be temporarily stored at the respective collection sites, before transportation to the TBR, at Ninewells Hospital in Dundee.

TBR will catalogue samples and histopathological characterisation and quality assurance checks will be undertaken. Subsequently, tumour specimens will be transported to the School of Life Sciences (SLS), University of Dundee, for neoantigen analysis by Platinum Informatics Ltd. Utilising laboratory facilities at the University of Dundee, SLS, including at the FingerPrints Proteomics and Mass Spectrometry Facility, sample processing, (comprising extract preparation, immunoprecipitation and LC-MS/MS analysis) will be performed according to optimised SOPs established by Platinum Informatics Ltd. Raw MS data will be processed and used by Platinum Informatics for neoantigen identification.

Surplus tumour material will be returned to the TBR for storage and will be available for additional genomic and transcriptomic work, allowing both validation of predicted neoantigens and for additional future proteo-genomic studies undertaken in laboratory facilities at the University of Dundee, SLS with analysis of data undertaken by Platinum Informatics.

PBMC preparations will be used for characterisation of pre-existing T cell responses to identified neoantigens in the Immunoassay Biomarker Core laboratory at Ninewells Hospital and Medical School.

All material will be handled in accordance with the Human Tissue Act 2004 and the 2006 Human Tissue (Scotland). Access to them will be tightly controlled through the CI.

6.9. End of study

The end of study at all sites is defined as last participant last visit. The Sponsor, CI and/or the TMG have the right to terminate the study at any time for clinical or administrative reasons.

The end of the study will be reported to the Sponsor, REC and NHS R&D Office(s) within 90 days, or 15 days if the study is terminated prematurely.

A final clinical study report will be published within 1 year of the end of study and will also be provided to the Sponsor and REC.

7. STUDY INTERVENTION

There will be no treatment intervention during this study. The study will not interfere with the participant's routine treatment pathway and the participant's standard of care treatment will continue. The participant's care will not be affected by any of the data generated.

8. PHARMACOVIGILANCE

8.1. Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence
Serious Adverse Event (SAE)	A SAE is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above.

8.2. Operational definitions for (S)AEs

As this study involves the collection of blood and tissue samples with no intervention, AEs will only be recorded where, in the opinion of the CI/PI, they are directly associated with the collection of these samples. AEs occurring during the collection of samples, where this is being done at the same time as a standard care procedure, will only be recorded if, in the opinion of the CI/PI, this is directly associated with the additional sampling required for the study.

8.3. Recording and reporting of AEs/SAEs

All AEs will be recorded on the AE Log in the electronic case report form (eCRF) and will be assessed for severity by the CI or PI. AEs will be recorded from the time a participant consents to join the study until the participant's last study visit. An AE may be classified as a SAE.

AEs/SAEs will be followed up until recovered/recovered with sequelae/death/30 days after participant's last visit - whichever is the soonest.

SAEs which are both unexpected and related to study participation will be submitted on an HRA non-CTIMP Safety Report form to the REC by the CI, within 15 days of becoming aware of the SAE, and copied to the Sponsor Research Governance Office.

8.4. Responsibilities

CI / delegate:

- Clinical oversight of the safety of patients participating in the study, including an ongoing review of the risk/benefit.

9. STATISTICS AND DATA ANALYSIS

9.1. Sample size calculation

This study will generate neoantigen profiles of oesophageal adenocarcinomas and determine the feasibility of identification of neoantigens by the direct analysis of the tumour ligandome using MS and evaluate neoantigens as targets for future cancer immunotherapies including vaccine-based strategies. Characterisation of the neoantigen profiles of oesophageal or gastroesophageal junction adenocarcinomas is the primary objective of this study which to our knowledge this has not been investigated to any significant extent previously. Gastroesophageal adenocarcinomas are recognised as being genomically heterogeneous and this is likely to be reflected in the heterogeneity of their neoantigen profiles between patients. In this context and at this stage in the investigational process, it is not possible to perform a formal sample size calculation. Nevertheless it is important that the cohort of patients in whom neoantigen profiles will be determined is sufficiently representative to account for expected between patient heterogeneity; provide a clinically useful estimate of the possible frequencies of different neoantigen targets in patients (to guide the future development of personalised cancer vaccine strategies); to provide a useful estimate of the feasibility of our approach to determine neoantigen profiles in individual patients tumours; and be feasible to recruit within a timeframe that is useful to the development of the programme of research with regard to subsequent use to the information generated in this study to develop new therapies and meet the unmet clinical need to develop improved treatments for gastroesophageal cancer. Based upon these considerations, we estimate that analysis of 75 samples (50 oesophageal samples/25 lower oesophageal-gastric junction samples) will be sufficient to determine the feasibility of this method and considering will allow a clinically useful representative profile of neoantigen expression in this tumour type to be undertaken to identify candidate targets for further investigation for cancer immunotherapy. This study will also determine the feasibility of our

assay and method for neoantigen determination in clinical practice and inform the design of , including providing the data needed for sample size calculations for future studies.

9.2. Planned recruitment rate

75 participants over 12 months.

9.3. Statistical analysis plan

A detailed statistical analysis plan will be developed prior to commencement of the analysis of study data. Outcome analysis will be undertaken once all patient samples have been collected and LC-MS/MS immunopeptidome analysis completed and raw MS data generated for subsequent processing. The outcome analysis will be performed by the CI, their research team, trial scientific adviser and Platinum Informatics Ltd.

9.3.1. Primary outcome analysis

The primary outcome is to generate neoantigen profiles of oesophageal adenocarcinomas in individual patients. This will be achieved by analysing the MHC-associated 'immunopeptidome', using a liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based method for direct neoantigen identification with proprietary software tool DESIMAL, created by Platinum Informatics Ltd. This will establish an automated pipeline for raw MS data processing and downstream data interpretation. This outcome of this analysis is qualitative and will describe the detail of the neoantigen expression profile in oesophageal adenocarcinoma.

9.3.2. Secondary outcome analysis

The feasibility of neoantigen identification with the described approach in gastroesophageal adenocarcinoma patients, using routinely available clinical biopsy materials will be evaluated by assessing the proportion of patients in whom neoantigens are identified. The minimal amount of oesophageal adenocarcinoma tissue, and the qualitative and quantitative histological features associated with successful neoantigen identification will be evaluated to provide data on the features of optimal tumour specimens for future trials. Characterisation of pre-existing T cell responses to identified neoantigens will enhance evaluation of their potential utility as therapeutic targets for adoptive T cell and/or cancer vaccine approaches for the treatment of oesophageal adenocarcinoma. An exploratory analysis of the relationship between tumour neoantigen profiles and objective response rate, progression free and overall survival will be undertaken.

9.4. Participant population

All samples received of a sufficient quality to perform the tissue analyses will be included.

9.5. Procedure(s) to account for missing or spurious data

Samples with failed sample processing at any point (no tumour cells in provided biopsy, failed protein extract preparation, immunoprecipitation or LC-MS/MS analysis) will be withdrawn from the analysis of the primary outcome but incorporated in the secondary outcome regarding the

feasibility of neoantigen identification with the described approach in gastroesophageal adenocarcinoma patients, using routinely available clinical biopsy material.

10. DATA MANAGEMENT

10.1. Data collection tools and source document identification

Medical records will be used as source data. The medical record will be flagged to state that the patient is participating in the PROTEAN study.

The PI or delegate will maintain source documents for each participant in the study, consisting of hospital medical records containing demographic and medical information.

An eCRF, using CASTOR Electronic Data Capture system, will be provided by TCTU. The study system will be based on the protocol for the study. Development and validation of the study database and quality control will be done according to TCTU procedures.

The eCRF will not collect more information than is required to meet the aims of the study and to ensure the eligibility and safety of the participant.

The PI may delegate eCRF completion but is responsible for completeness, plausibility and consistency of the eCRF. Delegated study staff will enter the data required by the protocol into the eCRFs following training in the definitions and methods used in completing the eCRF. Any queries will be resolved by the CI or delegated member of the study team. On completion of data collection, the PI must certify that the data entered into the eCRFs is complete and accurate. PI sign-off of the eCRF will be embedded in the eCRF with the PI using their own login.

Data verification, cleaning and extraction of data will be performed as per TCTU local procedures and detailed in the Data Management Plan.

Data preservation and sharing will be in accordance with established procedures at the University of Dundee. All electronic data will be stored on secure University of Dundee or cloud-based servers which have restricted access and have disaster recovery systems in place.

General laboratory data methods and results will be documented in laboratory notebooks and then analysed and written up for publication for dissemination to the scientific community. All electronic data will be stored on password-protected computers in secure staff access - controlled offices at investigator sites. All data and laboratory notebooks will be retained for at least ten years, in accordance with general RCUK guidelines.

10.2. Access to Data

The CI, PIs and all institutions involved in the study will permit study-related monitoring, audits, and REC review. In the event of an audit, the CI, PIs and all institutions involved in the study will allow the Sponsor, representatives of the Sponsor or REC direct access to all trial records and source documentation. The company analysing the samples (Platinum Informatics) will have no access to electronic health care records. Data shared with Platinum informatics will be fully anonymised prior to transfer.

10.3. Archiving

Essential documents will be archived for 5 years post end of study. The research dataset will be archived by the University of Dundee according to local policy. Medical case notes will be maintained in compliance with local NHS Policy on Retention of Medical Case notes.

11. MONITORING, AUDIT & INSPECTION

11.1. Monitoring

The study may be selected for audit and/or monitoring by the Sponsor.

12. ETHICAL AND REGULATORY CONSIDERATIONS

12.1. Research Ethics Committee (REC) review & reports

Before the start of the study, approval will be sought from REC for the study protocol, ICF and other relevant documents.

Substantial amendments that require review by REC will not be implemented until the REC grants a favourable opinion for the study (note that amendments will need to be reviewed and accepted by NHS R&D departments before they can be implemented in practice at sites).

All correspondence with the REC will be retained in the TMF/ISF.

An annual progress report will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the study is declared ended.

It is the CI's responsibility to produce the annual reports as required.

The CI will notify the REC of the end of the study.

If the study is ended prematurely, the CI will notify the REC, including the reasons for the premature termination.

Within one year after the end of the study, the CI will submit a final report with the results, including any publications/abstracts, to the REC.

This project has been peer reviewed by The Ninewells Cancer campaign scientific committee comprising senior clinical academics from the School of Medicine at the University of Dundee.

12.2. Public and Patient Involvement

The PIS and ICF have been reviewed by Patient and Public Involvement representatives and their views extensively considered in drafting these documents.

The CI is a Board member of OCHRE (https://www.ochrecharity.org.uk/), the Scottish Oesophageal Cancer charity that provides support for patients and their families and promotes awareness of oesophageal cancer to the public. The CI also has established relations with

gastroesophageal cancer UK national patient support groups, the Oesophageal Patients Association (www.opa.org.uk), Action Against Heartburn (https://www.actionagainstheartburn.org.uk/), and Heartburn Cancer UK (http://www.heartburncanceruk.org/) and a Tayside oesophageal cancer patient support group established in the Dundee Maggie's Centre. These interactions have provided the views of oesophageal cancer patients and their carers regarding clinical research into the disease and have been considered in the development of the clinical trial proposal and trial documentation. These interactions indicate that our research aims in this project are highly relevant to patient needs. The CI will continue to engage with these groups as results become available to ensure a patient view can contribute to interpretation of results and outcomes and planning future research.

12.3. Regulatory Compliance

The study will not commence until a favourable REC opinion is in place. Before enrolling participants into the study, the CI/PI or delegate will ensure that appropriate approvals from participating organisations are in place.

For any amendment to the study, the CI, PI or delegate, in agreement with the sponsor, will submit information to the appropriate body for them to issue approval for the amendment. The CI, PI or delegate will work with sites (NHS R&D departments at sites as well as the study delivery team) so they can put the necessary arrangements in place to implement the amendment to confirm their support for the study as amended.

12.4. Protocol compliance

The CI will not implement any breach of the protocol except where necessary to eliminate an immediate hazard to study participants. In the event that there is a breach of the protocol, the nature of and reasons for the breach will be recorded in the study Breach Log.

It is Sponsor policy that waivers to the Protocol will not be approved.

12.5. Notification of Serious Breaches to GCP and/or the protocol

If a breach of the protocol or GCP is suspected, this will be reported to the Sponsor immediately using the TASC Breach Reporting Form and documented in the study Breach Log. The Sponsor SOP for reporting breaches will be followed.

If a breach necessitates a subsequent protocol amendment, this will be submitted to the Sponsor for approval and then to the appropriate REC, and NHS R&D for review and approvals as appropriate.

12.6. Data protection and patient confidentiality

The CI and study staff will comply with the requirements of the Data Protection Act 2018 and UK General Data Protection Regulation (UK GDPR) or any subsequent amendment or replacement thereof with regard to the collection, storage, processing and disclosure of personal information and will uphold the Directive's core principles.

The CI and study staff will also adhere to the NHS Scotland Code of Practice on Protecting Participant Confidentiality or equivalent.

All study records and data will be managed in a manner designed to maintain participant confidentiality. All records, electronic or paper, will be kept in a secure storage area with access limited to appropriate study staff only. Computers used to collate data will have limited access measures via usernames and passwords.

Personal clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor, its designee or regulatory authorities.

The CI and study staff will not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the Sponsor will be required for the disclosure of any said confidential information to other parties.

Access to collated participant data will be restricted to the CI and appropriate delegated study staff.

Where data requires to be transferred, an appropriate Data Transfer Agreement will be put in place.

Published results will not contain any personal data that could allow identification of individual participants.

12.7. Financial and other competing interests for the CI, PIs at each site and committee members for the overall study management

The CI, PIs at each site will be required to disclose any potential conflicts of interest in relation to the PROTEAN study. This will require disclosure of:

- Ownership interests that may be related to products, services, or interventions considered for use in the study or that may be significantly affected by the study
- Commercial ties requiring disclosure include, but are not restricted to, any pharmaceutical, behaviour modification, and/or technology company
- any non-commercial potential conflicts.

This information will be collected prior to site activation and the commencement of any trial related activities at the site and collated in TCTU.

12.8. Indemnity

The University of Dundee are sponsoring the study.

Insurance. – The University of Dundee will obtain and hold Professional Negligence Clinical Trials Insurance cover for legal liabilities arising from the study.

Tayside Health Board will maintain its membership of the Clinical Negligence and Other Risks Insurance Scheme (CNORIS) which covers the legal liability of Tayside in relation to the study.

Where the study involves University of Dundee staff undertaking clinical research on NHS participants, such staff will hold honorary contracts with Tayside Health Board which means they will have cover under Tayside's membership of the CNORIS scheme.

Indemnity. The Sponsor does not provide study participants with indemnity in relation to participation in the Study but have insurance for legal liability as described above.

Where other Scottish Health Boards are participating as study sites, those other Scottish Health Boards will maintain membership of CNORIS to cover their liability in relation to their conduct of the study.

Where other UK NHS organisations are participating as study sites, those other UK NHS organisations will maintain membership of a scheme similar to CNORIS.

12.9. Amendments

The CI will seek Sponsor approval for any amendments to the Protocol or other approved study documents. Amendments to the protocol or other study documents will not be implemented without approval from the Sponsor and subsequent approval from the appropriate REC and NHS R&D Office(s) when required, dependent on classification of amendment.

12.10. Post study care

Participants will be treated and followed up as per the local cancer service.

12.11. Access to the final study dataset

The CI and Platinum Informatics will have access to the final study dataset. Access to the final study dataset to others will be approved by the CI.

13. DISSEMINIATION POLICY

13.1. Dissemination policy

The study will be registered on International Standard Randomised Controlled Trials (ISRCTN) register. Details of the study will be published on ISRCTN no later than 12 months after the end of the study. The report will be made available to the funder. The report can be used for publication and presentation at scientific meetings. Study investigators have the right to publish study results orally or in writing. The criteria for authorship will follow the criteria of the International Committee of Medical Journals.

Publications will be reviewed according to the agreed contractual terms but will not restrict the general rights outlined above for the Investigators to publish the results of the study.

13.2. Authorship eligibility guidelines and any intended use of professional writers

The data arising from this study resides with the study team and ownership with University of Dundee. On completion of the study, the study data will be analysed and tabulated, and a final report will be prepared.

14. REFERENCES

Oesophageal Cancer Survival statistics 2015 [cited 2016 May]; Available from: http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/oesophageal-cancer/survival#heading-Zero.

2. Larkin, J., et al., *Five-Year Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma.* New England Journal of Medicine, 2019. **381**(16): p. 1535-1546.
3. Janjigian, Y.Y., et al., *First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a randomised, open-label, phase 3 trial.* The Lancet, 2021. **398**(10294): p. 27-40.
4. Shitara, K., et al., *Efficacy and Safety of Pembrolizumab or Pembrolizumab Plus Chemotherapy vs Chemotherapy Alone for Patients With First-line, Advanced Gastric Cancer: The KEYNOTE-062 Phase 3 Randomized Clinical Trial.* JAMA Oncology, 2020. **6**(10): p. 1571-1580.
5. Kang, Y.-K., et al., *Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial.* The Lancet, 2017. **390**(10111): p. 2461-2471.
6. Shitara, K., et al., *Pembrolizumab versus paclitaxel for previously treated, advanced gastric or gastro-oesophageal junction cancer (KEYNOTE-061): a randomised, open-label, controlled, phase 3 trial.* The Lancet, 2018. **392**(10142): p. 123-133.
7. Fuchs, C.S., et al., *Safety and Efficacy of Pembrolizumab Monotherapy in Patients With Previously Treated Advanced Gastric and Gastroesophageal Junction Cancer: Phase 2 Clinical KEYNOTE-059 Trial.* JAMA Oncology, 2018. **4**(5): p. e180013-e180013.
8. Boku, N., et al., *LBA7_PR Nivolumab plus chemotherapy versus chemotherapy alone in patients with previously untreated advanced or recurrent gastric/gastroesophageal junction (G/GEJ) cancer: ATTRACTION-4 (ONO-4538-37) study.* Annals of Oncology, 2020. **31**: p. S1192.
9. Kato, K., et al., *LBA8_PR Pembrolizumab plus chemotherapy versus chemotherapy as first-line therapy in patients with advanced esophageal cancer: The phase 3 KEYNOTE-590 study.* Annals of Oncology, 2020. **31**: p. S1192-S1193.
10. Takashi, K., et al., *Pembrolizumab versus chemotherapy as second-line therapy for advanced esophageal cancer: Phase III KEYNOTE-181 study.* Journal of Clinical Oncology, 2019. **37**(4_suppl): p. 2-2.
11. Kelly, R.J., et al., *Adjuvant Nivolumab in Resected Esophageal or Gastroesophageal Junction Cancer.* New England Journal of Medicine, 2021. **384**(13): p. 1191-1203.
12. Janjigian, Y.Y., et al., *CheckMate-032 Study: Efficacy and Safety of Nivolumab and Nivolumab Plus Ipilimumab in Patients With Metastatic Esophagogastric Cancer.* Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 2018. **36**(28): p. 2836-2844.
13. Bang, Y.J., et al., *Phase III, randomised trial of avelumab versus physician's choice of chemotherapy as third-line treatment of patients with advanced gastric or gastro-oesophageal junction cancer: primary analysis of JAVELIN Gastric 300.* Annals of oncology : official journal of the European Society for Medical Oncology, 2018. **29**(10): p. 2052-2060.
14. Kojima, T., et al., *Randomized Phase III KEYNOTE-181 Study of Pembrolizumab Versus Chemotherapy in Advanced Esophageal Cancer.* J Clin Oncol, 2020. **38**(35): p. 4138-4148.
15. Shitara, K., et al., *Efficacy and Safety of Pembrolizumab or Pembrolizumab Plus Chemotherapy vs Chemotherapy Alone for Patients With First-line, Advanced Gastric*

Cancer: The KEYNOTE-062 Phase 3 Randomized Clinical Trial. JAMA Oncol, 2020. **6**(10): p. 1571-1580.
16. Baxter, M.A., et al., *Resistance to immune checkpoint inhibitors in advanced gastro-oesophageal cancers.* British journal of cancer, 2021. **125**(8): p. 1068-1079.
17. Paijens, S.T., et al., *Tumor-infiltrating lymphocytes in the immunotherapy era.* Cell Mol Immunol, 2021. **18**(4): p. 842-859.
18. Rosenberg, S.A., et al., *Durable Complete Responses in Heavily Pretreated Patients with Metastatic Melanoma Using T-Cell Transfer Immunotherapy.* Clinical Cancer Research, 2011. **17**(13): p. 4550-4557.
19. Junghans, R.P., et al., *Phase I Trial of Anti-PSMA Designer CAR-T Cells in Prostate Cancer: Possible Role for Interacting Interleukin 2-T Cell Pharmacodynamics as a Determinant of Clinical Response.* Prostate, 2016. **76**(14): p. 1257-70.
20. Wang, Y., et al., *CD133-directed CAR T cells for advanced metastasis malignancies: A phase I trial.* Oncoimmunology, 2018. **7**(7): p. e1440169.
21. Guo, Y., et al., *Phase I Study of Chimeric Antigen Receptor-Modified T Cells in Patients with EGFR-Positive Advanced Biliary Tract Cancers.* Clin Cancer Res, 2018. **24**(6): p. 1277-1286.
22. Pule, M.A., et al., *Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma.* Nat Med, 2008. **14**(11): p. 1264-70.
23. Liu, Y., et al., *Anti-EGFR chimeric antigen receptor-modified T cells in metastatic pancreatic carcinoma: A phase I clinical trial.* Cytotherapy, 2020. **22**(10): p. 573-580.
24. Hegde, M., et al., *Tumor response and endogenous immune reactivity after administration of HER2 CAR T cells in a child with metastatic rhabdomyosarcoma.* Nat Commun, 2020. **11**(1): p. 3549.
25. Wagner, J., et al., *CAR T Cell Therapy for Solid Tumors: Bright Future or Dark Reality?* Molecular Therapy, 2020. **28**(11): p. 2320-2339.
26. C. Qi, Y.Q., D. Liu, J. Gong, S. Ge, M. Zhang, Z. Peng, J. Zhou, X. Zhang, X. Peng, H. Wang, C. He, J. Xiao, Z. Li, L. Shen, *CLDN 18.2-targeted CAR-T cell therapy in patients with cancers of the digestive system.* Annals of Oncology 2021. **32**: p. S1040-S1075.
27. Sahin, U. and Ö. Türeci, *Personalized vaccines for cancer immunotherapy.* Science, 2018. **359**(6382): p. 1355-1360.
28. Zhang, X., et al., *Personalized cancer vaccines: Targeting the cancer mutanome.* Vaccine, 2017. **35**(7): p. 1094-1100.
29. Ott, P.A., et al., *An immunogenic personal neoantigen vaccine for patients with melanoma.* Nature, 2017. **547**(7662): p. 217-221.
30. Sahin, U., et al., *Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer.* Nature, 2017. **547**(7662): p. 222-226.
31. Gubin, M.M., et al., *Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens.* Nature, 2014. **515**(7528): p. 577-581.
32. Rizvi, N.A., et al., *Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer.* Science, 2015. **348**(6230): p. 124-128.
33. Allen, E.M.V., et al., *Genomic correlates of response to CTLA-4 blockade in metastatic melanoma.* Science, 2015. **350**(6257): p. 207-211.
34. Secrier, M., et al., *Mutational signatures in esophageal adenocarcinoma define etiologically distinct subgroups with therapeutic relevance.* Nat Genet, 2016. **48**(10): p. 1131-1141.
35. Pectasides, E., et al., *Genomic Heterogeneity as a Barrier to Precision Medicine in Gastroesophageal Adenocarcinoma.* Cancer Discovery, 2018. **8**(1): p. 37-48.
36. Linnemann, C., et al., *High-throughput epitope discovery reveals frequent recognition of neo-antigens by CD4+ T cells in human melanoma.* Nat Med, 2015. **21**(1): p. 81-5.

37. van Rooij, N., et al., *Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma.* J Clin Oncol, 2013. **31**(32): p. e439-42.
38. Tran, E., et al., *Immunogenicity of somatic mutations in human gastrointestinal cancers.* Science, 2015. **350**(6266): p. 1387-90.
39. Lennerz, V., et al., *The response of autologous T cells to a human melanoma is dominated by mutated neoantigens.* Proceedings of the National Academy of Sciences of the United States of America, 2005. **102**(44): p. 16013-16018.
40. Robbins, P.F., et al., *Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells.* Nat Med, 2013. **19**(6): p. 747-52.
41. Pritchard, A.L., et al., *Exome Sequencing to Predict Neoantigens in Melanoma.* Cancer Immunol Res, 2015. **3**(9): p. 992-8.
42. Cohen, C.J., et al., *Isolation of neoantigen-specific T cells from tumor and peripheral lymphocytes.* J Clin Invest, 2015. **125**(10): p. 3981-91.
43. Yadav, M., et al., *Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing.* Nature, 2014. **515**(7528): p. 572-576.
44. Kalaora, S., et al., *Use of HLA peptidomics and whole exome sequencing to identify human immunogenic neo-antigens.* Oncotarget, 2016. **7**(5): p. 5110-5117.
45. Bassani-Sternberg, M., et al., *Direct identification of clinically relevant neoepitopes presented on native human melanoma tissue by mass spectrometry.* Nature Communications, 2016. **7**(1): p. 13404.
46. Richman, L.P., R.H. Vonderheide, and A.J. Rech, *Neoantigen Dissimilarity to the Self-Proteome Predicts Immunogenicity and Response to Immune Checkpoint Blockade.* Cell Systems, 2019. **9**(4): p. 375-382.e4.

15. APPENDICIES

15.1. Appendix 1-Risk

Risks associated with study interventions

A ≡ Comparable to the risk of standard medical care

B ≡ Somewhat higher than the risk of standard medical care

C ≡ Markedly higher than the risk of standard medical care

Justification:

The main risk for participants is from the biopsy of tumour tissue. Where possible this will be done at the same time as standard care biopsy or tumour resection.

What are the key risks related to assessments you plan to undertake in this study?

Intervention	Hazard	How will these risks be minimised?
Blood samples	Discomforts of drawing blood include temporary discomfort from the needle stick, the possibility of pain or bruising at the site of the blood draw, occasional feelings of light headedness and, rarely, infection at the site of the blood draw	Blood samples will be taken at the same time as blood draw for routine care purposes whenever possible. Staff taking blood will be experienced in venepuncture.
Biopsy	Risk of serious complications, such as bleeding, pneumothorax, infection, or even death, depending on the site of disease. Risks vary according to the technical aspects of the biopsy (e.g. size of needle and location or size of the required specimen), the experience or training of the medical professional performing the biopsy, and participant characteristics. The risks for both major complications and AEs increase with multiple biopsies.	Previously collected samples will be used when available, or samples will be taken at the same time as clinically indicated procedures whenever possible. In other instances, the biopsies will be only performed if the risk to the participant is low or moderate. Only samples at baseline are required as per protocol, other samples will only be collected as far as possible during standard care procedures. In considering the study design we have reflected upon the key ethical guidance on research biopsies in cancer research provided in the American Society of Clinical Oncology Research Statement: Ethical Framework for Including Research Biopsies in Oncology Clinical Trials. Journal of Clinical Oncology 37, no. 26 (September 10,

		2019)2368-2377. Our approach in this study is consistent with this guidance and accordingly is consistent with the practice of oncology studies of this type in a global context.
Outline any other processes that have been put in place to mitigate risks to participant safety (e.g. DMC, independent data review, etc.) Nil		

15.2. Appendix 2 - Study management / responsibilities

Responsibilities will be detailed in the co-sponsorship, site agreements and TCTU Statement of Services.

15.2.1. Participant registration/randomisation procedure

Participants will be allocated a unique study number by sites; the format will be as directed by TCTU Trial Management Team.

No randomisation included.

15.2.2. Data management

Data management will be overseen by TCTU Data Management Team (DMT).

Local sites will be expected to enter data directly on to the eCRF. After data entry, site staff will be expected to visually verify data entered. Worksheets will be provided to facilitate this process, but their use is not mandatory. Worksheets, where used, will not record source data and will not be archived.

All data from participants should be entered on the eCRF within 14 days of the last data collection point for that participant, data entry out with this timeline will not constitute a breach.

Data queries will be generated by the DMT and emailed to sites, return of queries should be within 2 weeks, return of queries out with this timeline will not constitute a breach.

15.2.3. Preparation and submission of amendments

TCTU Trial Management Team will be responsible for working with the CI to submit any amendments.

15.2.4. Preparation and submission of Annual Safety Report/Annual

TCTU Trial Management Team will be responsible for liaising with the CI to submit REC annual reports.

15.2.5. Data protection/confidentiality

The CI and study staff will comply with the requirements of the EU General Data Protection Regulation and the Data Protection Act 2018 or any subsequent amendment or replacement thereof with regard to the collection, storage, processing and disclosure of personal data and will uphold the Principles of GDPR in Article 5.

The CI and study staff will also adhere to the NHS Scotland Code of Practice on Protecting Participant Confidentiality or local equivalent.

15.2.6. Study documentation and archiving

Archiving trial site data will be the responsibility of individual sites. Payment for archiving will be provided as per site agreement.

15.3. Appendix 3 – Authorisation of participating sites

15.3.1. Required documentation

The following data should be made available to TCTU Trial Management Team prior to site initiation:

- PI CV, signed and dated
- PI GCP certificate
- Protocol signature page, signed and dated by PI
- Copy of signed Participating Site Agreement
- Copy of R&D confirmation of capacity and capability

15.3.2. Procedure for initiating/opening a new site

Site Initiation will be carried out remotely.

Site Initiation will be performed by TCTU Trial Management Team after R&D confirmation of capacity and capability.

15.3.3. Principal Investigator responsibilities

The PI's legal responsibilities will be listed in the Participating Site Agreement. A summary is given below:

- Attendance at the Site Initiation teleconference
- Training of new members of study staff in the protocol and its procedures
- Ensuring that the ISF is accurately maintained
- Dissemination of important safety or study related information to all stakeholders within their site
- Safety reporting within the required timelines
- Ensuring data entry to eCRF and responses to data clarification queries are completed within the required timelines
- Certify data entered on eCRF is correct and complete
- Archiving of site study data

15.4. Appendix 4 – Schedule of Procedures

Procedures	Visits				
	V1[a]	V2[a]	V3[bc]	V4[bc]	V5[b]
	Screening	Day 1 Up to 28 days post screening	Week 6 +/- 7 days	Week 12 +/- 7 days	Week 18 +/- 7 days
Eligibility check	X				
Informed consent	X				
Demographics	X				
Medical history	X				
Height & weight	X				
Concomitant medications	X	X	X	X	X
Research biopsy		X			
Research biopsy(optional)			X A maximum of 3 further research biopsy samples may be collected between day 1 and week 18		
Research bloods		X			X
Research bloods (optional)			X A maximum of 2 further research blood samples may be collected between day 1 and week 18		
Progression assessment		X	X	X	X
AE assessments		X	X	X	X

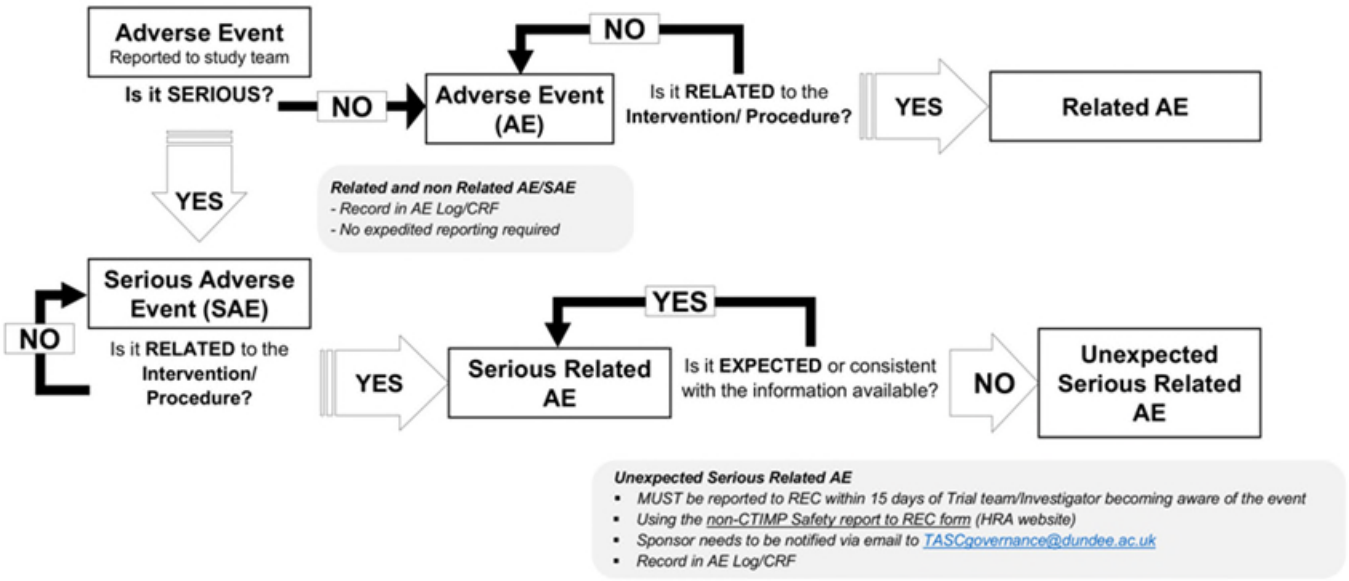
[a] Visits 1 & 2 may be combined

[b] Visits will be conducted at the same time as standard care procedures and may not fall in line with visit windows, this will not be considered a breach.

[c] May be by telephone if participant is not attending a standard care clinic visit.

15.5. Appendix 5 – Safety Reporting Flow Chart

Activity	Responsibility	Timing	Comments
Review for AEs	Trial staff	Each visit	Recorded on eCRF system.
Review of recorded AEs for relatedness and seriousness	PI (or delegate)	Within 10 days of recording	Recorded on eCRF and/or medical records.
Reporting SAEs: All SAEs need to be assessed by the PI or delegated doctor.	PI (or delegate)	Within 10 days of becoming aware of SAE	SAE form: Non-CTIMP safety report Report to: CI TCTU Trial Manager
Reporting SAEs: All SAEs need to be signed off by the CI.	CI TCTU Trial Manager	Within 15 days of PI becoming aware of SAE	Report to: REC Sponsor



15.6. Appendix 6 – Amendment History

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made