Portsmouth Hospitals University NHS Trust

Qualitative Protocol Development Tool

Full/Long Title of Trial

Characterising biomarkers and clinical algorithms to identify oncology patients on immune checkpoint inhibitors (ICPI) that are at greater risk of developing immune-related adverse events (irAE).

Short Trial title/ Acronym

BIO-CHECKPOINT

Protocol version number and date

Version 1.3, 2nd January 2024

RESEARCH REFERENCE NUMBERS

IRAS Number:	329576
SPONSORS Number:	Not applicable
FUNDERS Number:	Not 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 Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

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 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 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):

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LIST of CONTENTS

KEY STUDY CONTACTS

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Funder(s)	National School of Healthcare Science

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STUDY SUMMARY

Study Title	Characterising biomarkers and clinical algorithms to identify oncology patients on immune checkpoint inhibitors (ICPI) that are at greater risk of developing immune-related adverse events (irAE).
Internal ref. no. (or short title)	BIO-CHECKPOINT
Study Design	Retrospective cohort study
Study Participants	Adult oncology patients at Queen Alexandra hospital, who are prescribed FDA approved ICPI for the first time
Planned Size of Sample (if applicable)	120
Follow up duration (if applicable)	6 months post initiation of ICPI
Planned Study Period	Sample collection = 18 months Data collation and analysis = 6 months Total time = 24 months
Research Question/Aim(s)	Can a clinical algorithm be created to flag high risk Oncology patients that have a greater risk of developing irAE? In addition, can novel biomarkers such as malondialdehyde be incorporated into this algorithm to aid stratification.

FUNDING AND SUPPORT IN KIND

FUNDER(S)	**FINANCIAL AND NON-FINANCIAL SUPPORT GIVEN**
National School of Healthcare Science	£13,000 per year for 5 years £65,000 total
Royal College of Pathologists	Awarded £1015.60 2nd of August 2023

ROLE OF STUDY SPONSOR AND FUNDER

Queen Alexandra Hospital is the sponsor of this research study. However, the responsibility for the design, conduct, data analysis, interpretation, manuscript writing, and dissemination of results falls to the lead researcher Mrs Louise E Duvall. The sponsor will have oversight of the study and ensure that it continues to operate within legal boundaries. The chief investigator and lead researcher are responsible for ensuring that the sponsor is kept informed of changes and updated to the BIO-CHECKPOINT study.

Funder(s) do not control the final decision regarding any of these aspects of the study.

ROLES AND RESPONSIBILITIES OF STUDY MANAGEMENT COMMITEES/GROUPS & INDIVIDUALS

Study Steering Groups

No study steering groups have been involved in the study coordination and conduct.

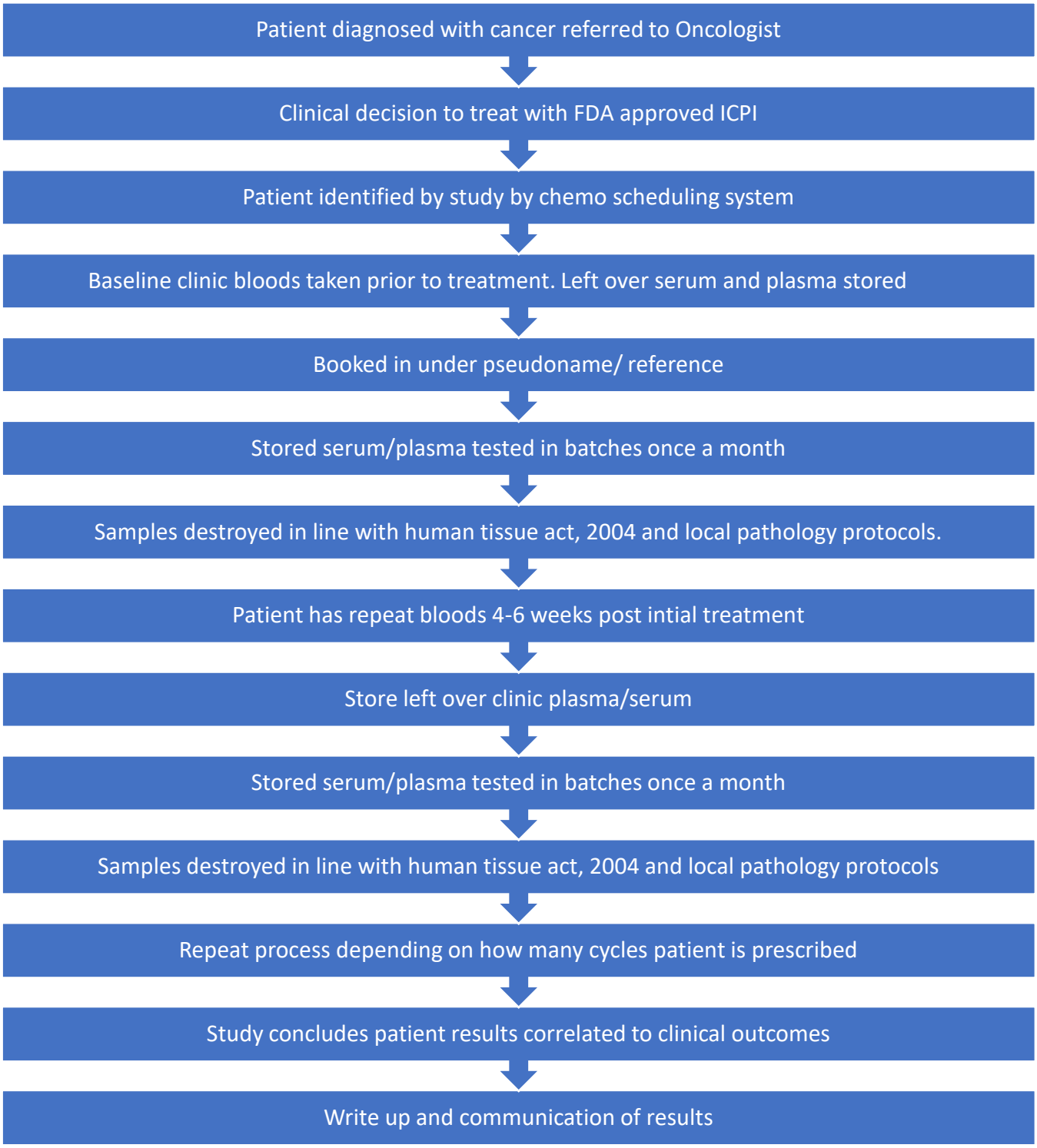
Patient and Public Involvement group has been engaged at Portsmouth and research study will be presented to this group. Public presentation will also be given at Manchester May 2024 as part of Louise E Duvall's Doctorate in Clinical Biochemistry.

PROTOCOL CONTRIBUTORS

The study design is principally the responsibility of the lead researcher Louise E Duvall and Chief investigator Laura Wainwright. The funder the National School of Healthcare Science does not control any aspect of the study. The sponsor the Research and Development office at Portsmouth will review and feedback on the protocol when indicated. The study design will be discussed and review with the Portsmouth Patient involvement group, facilitated by Sharon Court.

KEY WORDS: Check point inhibitors, oncology, cancer, immune related adverse events, blood biomarkers

STUDY FLOW CHART



STUDY PROTOCOL

1.0 LAY SUMMARY –

Immune checkpoint inhibitors (ICPI) are a type of cancer treatment. Unlike traditional chemotherapy and radiation, which damages both the cancer and the healthy tissue, ICPI targets cancer directly by altering the immune system. Cancer cells produce high levels of protein called checkpoint proteins, which bind to white blood cells (which are part of the immune system) and stops them from working. Effectively the cancer is pushing a stop button on the immune system and the body can no longer fight it off. ICPIs block and remove these cancer checkpoint proteins, which allows the immune system to target the cancer again removing this stop button.

ICPI has great success in treating cancer and 10% of oncology NHS patients receive this treatment, although this number is increasing. However, ICPI carries a risk of a type of side effect called immune related adverse events (irAE). irAEs can be life threatening and present with similar symptoms to the patient's cancer. For example, a patient may have kidney cancer and after treatment with ICPI develop kidney failure. It is difficult for the doctor to tell if this is the cancer progressing or a side effect of the treatment. Delays in diagnosing irAE can lead to unnecessary hospitalisation, unnecessary breaks in treatment, lifelong side-effects, and death.

Currently there is not a unified blood test panel for ICPI patients, and the cancer societies have produced little guidance for the doctors to use. At Portsmouth what blood tests you get as an ICPI patient depend on which clinician you see. There is also little research as researchers are focussing on using blood tests to predict ICPI treatment success rather than the chance of a patient developing an irAE.

We intend to conduct a study where we collect leftover blood from routine clinical blood draws on oncology patients being treated with ICPIs for the first time. We will freeze the leftover samples and test them a month later for different routine blood tests as well as malondialdehyde. Malondialdehyde is a blood test that can assess the body's oxidative stress level, a condition where your body lacks antioxidants. Testing for malondialdehyde is not available in the NHS and we would produce a new NHS blood test as part of this study. Once testing is completed the samples will be destroyed as per our normal protocol. At the end of the study, we will correlate the blood test results to the patient's outcome i.e., did they have an irAE or not and assess if there are any differences in their blood test results. From this information we hope to understand which blood tests help to highlight if a patient is at a risk of developing irAE before it occurs. It also aims to develop a new method for measuring malondialdehyde.

2.0 BACKGROUND

This study aims to identify biomarkers that stratify Oncology patients on immune check point inhibitor (ICPI) that have an increased risk of developing autoimmune toxicity known as immune-related adverse events (irAE).

ICPIs are being increasingly prescribed and 10% of NHS Oncology patients receive treatment from this drug group (1,2). The reported rate of total irAE triggered by ICPI varies but is thought to be between 40-66% (3–5), a third of which are endocrine related (6,7).

Several bodies including the European Society for Medical Oncology, American Society for Clinical Oncology and the National Comprehensive Cancer Network have produced guidance for irAE management, but no unified screening protocol exists to stratify patients at high-risk (8). Currently the literature primarily centres around looking for prognostic biomarkers for treatment success rather than characterising toxicity.

Individual studies review the utilisation of some routine clinical blood tests, i.e., albumin, rheumatoid factor, lactate dehydrogenase, IL-6, etc, to screen and stratify high-risk patients (9,10). However, these tests are used in isolation and no singular clinical algorithm using a variety of routine tests has been developed for initial screening during ICPI therapy. A variety of protocols have been developed for screening endocrine irAE, although these are not consistently adopted, and practice varies.

ICPIs induce an enhanced immunological response to promote anti-tumour activity (11). There are a few forms of ICPI including CTLA-4 antibodies that upregulate T-cell activation pathways to enhance the tumour immune response. There are also antibodies that act as programmed death receptor agonists, which promote cytokine release and T-cell activation. (12)

An array of studies has shown that reactive oxygen species (ROS) are involved in the regulation of T-cell anti-tumour immunity (13–20). ICPI are active therapies that alter T-cell intracellular signalling, which can increase ROS production and proinflammatory cytokine secretion, thus exerting an immunotherapeutic effect (13).

irAE refers to a spectrum of autoimmune toxicities triggered by ICPI. ROS are implicated in the initiation, amplification, pathogenesis of autoimmune diseases (21,22). Are ROS heavily upregulated in irAE due to a dysregulated immune response and could the measurement of an oxidative stress marker act as a marker of toxicity?

Malondialdehyde is generated from ROS driven lipid peroxidation of polyunsaturated fatty acids and is an indirect marker of oxidative stress. Malondialdehyde can be detected within plasma, serum and urine via liquid-chromatography mass-spectrometry (LC-MS/MS). (23–25) Malondialdehyde has been suggested as a future marker of tissue level thyroid dysfunction, the most common irAE is hypothyroidism (26).

At present, Portsmouth does not have a unified screening process for patients about to commence ICPI and tests requested are clinician dependent. This project seeks to produce a unified clinical algorithm.

3.0 Objectives and Outcomes Measures/Endpoints

3.1 Primary objectives

Can a clinical algorithm be created to flag high risk Oncology patients that have a greater risk of developing irAE? In addition, can novel biomarkers such as malondialdehyde be incorporated into this algorithm to aid stratification.

P	Oncology adult patients on ICPI
I	No intervention – retrospective analysis
C	Comparing blood test results of those who develop irAE to those who don't
O	Are any of the blood tests significantly different in those who develop irAE
T	6 months follow up post starting treatment, 18 testing period.

3.2 Secondary objectives

This principal research question will be broken down to the following sub aims:

1. Are there any established biochemistry, haematology and immunology blood tests that produce significantly different results in those who develop irAE when compared to those that don't. Including IL-6 (requires in-house verification). Observational study

2. Can a malondialdehyde LC-MS/MS assay suitable for clinical laboratory use be developed based on a method development by Sobsey *et al*.

3. Is malondialdehyde significantly raised in those who develop irAEs compared to those that do not. An ELISA kit has been located in the event an LC-MS/MS method is not successfully developed.

4. Can a blood test clinical algorithm be developed to stratify patients at high-risk of developing an irAE.

3.3 Outcome

This project could produce a clinical algorithm and/or blood test panel that successfully highlights patients at a high risk of developing irAE, prior to commencing therapy. This would allow closer monitoring of high-risk patients, leading to earlier diagnosis of irAE. Currently irAE can be tricky to diagnose and have overlapping symptoms with the malignancy and related concomitant treatments (27). Earlier diagnosis would lead to reduced hospitalisation, better outcomes, and reduced

interruptions to the anti-cancer therapy, which may lead to better malignancy curative rates. There is also a potential to reduce morbidity and mortality risk for this subset of patients, as well as an overall reduction in resource burden.

4.0 STUDY DESIGN

4.1 SUMMARY OF DESIGN

This is a retrospective cohort study that looks at blood test results correlated to clinical outcomes.

Oncology patients, whom have been prescribed ICPIs for the first time, will be identified via the chemotherapy scheduling system. This study does not require extra blood draws or tubes to be taken. Left over serum and plasma from routine clinical blood draws will be retrieved. This serum and plasma will be aliquoted into tube labelled with a pseudonym reference. The samples will be booked into our laboratory information management system under a pseudonym reference. This system is password protected and only those involved in this research should be accessing these records. Left-over samples will be frozen at -80C and stored for a period of 1 month before being tested in batches. Before testing a list of NHS numbers of the patients that have been recruited will be sent to the Trust's data office. All samples for patients that have opted out of having their records used for research purposes will be destroyed in line with local pathology policy. Once testing is completed the left-over samples will be disposed of as in line with the Human Tissue Act, 2004 and local Portsmouth Pathology protocols.

Louise E Duvall lead researcher will correlate the results to the patients' identifiers in a password protected sheet. Results will then be clinically correlated to patient's records to deem patient outcomes and if they had an irAE. Data will be correlated into a password protected excel sheet kept on Trust computer system. Data will then be de-identified and patient identification will be removed. Identifiable patient data will be removed for data analysis the spread sheet would contain age in years, sex, cancer type, ICPI type, if they had an irAE, irAE type and when it occurred. This data will be in a password protected excel sheet. Statistics will be carried out via R.

Minimal patient data will be used in the write up of this study i.e., age in years, sex, cancer type, ICPI treatment type, and if they had an irAE. Information about irAE will include type of event, and when it occurred post treatment.

4.2 ELIGIBILITY CRITERIA

Inclusion criteria

- ≥18 years of age
- All sex
- Oncology patients
- Who is prescribed FDA approved check point inhibitors: (ipilimumab, nivolumab, pembrolizumab, cemiplimab, atezolizumab, avelumab, and durvalumab)

- All cancer subtypes are included

Exclusion criteria

- <18 years of age
- Those previously been treated with checkpoint inhibitors.
- Those with a previous medical history of autoimmune disease
- Those with previous medical history of endocrine diseases
- Those with a pre-existing malignancy
- Non-Queen Alexandra hospital oncology patients
- Lacking capacity to consent

4.3 ASSESSMENT AND MANAGEMENT OF RISK

This is a low risk study, and is categorised as a study limited to working with human tissue samples and/or analysis of data. There will be no research specific participant contact or observation and therefore no AEs/SAEs would occur because of study participation.

4.4 STUDY PROCEDURES

Please refer to the flow chart on page viii for a summary of the study procedures. The study procedures described below will be conducted in accordance with SOPs and/or work instructions provided by the CI.

4.5 STUDY INTRODUCTION

This study seeks to gain consent from patients remotely via the telephone. Samples will be scavenged from routine care samples (no additional procedures will occur i.e. blood draws etc.). The study is being designed to limit the burden on clinical staff. The NHS is currently facing massive pressures and unprecedented demand. However, we should continue to seek improvement this study has been designed so we can continue to improve our services while putting minimum burden on the Oncology staff. This project is part of a service improvement project to highlight which blood tests are best at predicting irAEs.

4.6 STUDY OPT OUT

Patients that have been recruited on the study will have their NHS numbers extracted into a list. Once a month before running of the sample batches, this list of NHS numbers will be raised on the Trust's internal system called Mycall http://MyCall as a National Data Opt out. Any patients who have opted out of having their data and records used for research will be removed from the study

and the samples destroyed as in line with local pathology procedures. This is in line with local trust policy that has been created to ensure compliance with the NHS National Data Opt-out policy.

4.7 STAFF TRAINING

Information about the study training will be provided by the research team. Local in house training procedures will be followed for the operation of the analysers. Louise E Duvall will receive additional research based training as part of her Doctorate with the University of Manchester. Louise E Duvall has also completed Good Clinical Practice basic research training.

5.0 SAMPLE PROCESSING

5.1 SAMPLE COLLECTION AND STORAGE

Patients will be identified via the chemotherapy scheduling system, which will be reviewed and checked by lead researcher Louise Duvall daily Monday-Friday. Patients who are yet to start ICPI will be listed in a password protected sheet with the expected day of their chemo. The lead researcher will then check to assess that if the patients listed meet the inclusion criteria and if they have any of the features of the exclusion criteria. Those that meet the exclusion criteria will be deleted off the list. The laboratory information management system will be reviewed daily to assess if we have received their clinical bloods. Left over serum and plasma will be stored frozen and tested in batches once a month.

5.2 PATIENT CONSENT

Enrolment onto this study required patient consent; this will be achieved by telephone. Samples will not be tested unless patient consent has been obtained. Once eligible patients have been selected and their samples have been stored a member of the research team will contact the patient as per BIO-CHECKPOINT Telephone Consent Script, version 1.0. The research team will offer to send the patient the PLS BIO-CHECKPOINT adults version 1.0 information leaflet and arrange to call back at a suitable time to take consent recorded via the BIO-CHECKPOINT version 1.0 Consent Form. Participants expressing an interest in the study will have their eligibility assessed, receive a PIS and will have adequate time to read this information. The information will also be presented to them verbally to ensure they understand. If they would like to, they will be able to talk to relevant health care professionals or family members about involvement in the study and will have the opportunity to ask any questions they may have before consent is taken. If the patient does not consent to the study the samples will be destroyed and not tested.

5.3 SAMPLE ANALYSIS

Residual serum and plasma that will be anonymised will be defrosted and run at the Blood Sciences laboratory in Portsmouth.

Blood tests will be run on UKAS ISO15189 accredited blood analysed by stated registered Clinical Scientists and Biomedical Scientists. Malondialdehyde levels will be assessed via isotope dilution mass spectrometry method or an ELISA kit in the event of method validation failure. The method will be fully validated and installed at Portsmouth Pathology laboratories. It is not anticipated that there will be any sample remaining at the end of the analysis procedure. Any sample that remains at the end of the analysis procedure will be destroyed as per standard NHS Pathology Department guidelines.

5.4 REPORTING OF ACTIONABLE RESULTS

Critically abnormal results may be produced during this study. To avoid moral dilemma of if to report results that the clinician did not request the following mitigating factors have been put into place. Samples will be labelled and booked in under a pseudonym and only the lead researcher will have access to the key and relevant patient identifiers. Samples will be batched and run once a post collection, making these results historic values. Results will not be clinically correlated to patient outcomes until May 2025 meaning outcomes of any abnormal results would have likely presented by this point. Clinicians will not see or be able to look up the study's results.

6. DATA PROCESSING

6.1 DATA TRANSFER AND STORAGE

Results will be anonymised under a pseudonym/reference on the laboratory information management system (APEX). This is a password protected system that only authorised personnel can access on password protected Trust accounts, which are also password protected. The patients' clinicians will not be able to see these results or link them to their patients. Only those on the research team will be able to de-anonymise the data. Results will be stored on APEX during the study. Post study completion the results will be extracted from APEX into a password protected excel sheet. Results and patient identifying data will not be sent outside of the Trust.

6.2 SOURCE DATA

Laboratory results, CVs, delegation logs will be deemed source data.

6.3 DATA CONFIDENTALITY

The central research team Portsmouth Pathology i.e., Laura Wainwright and Louise Duvall will have access to patient data and anonymised result data. This data will be stored securely on password protected computers at the NHS Blood Sciences laboratory in Portsmouth. Patient identifiable data will not be stored outside of the Trust computers. Linkage of test results to personal identifiable information will only be possible through the pseudoname/reference. Linkage will only be carried out by Louise E Duvall. Statistics will be carried out by Louise E Duvall as this study is part of their Doctorate in Clinical Science awarded by the University of Manchester. Statistics will be carried out using R on a password protected personal computer. Data for statistics will not contain patient identifiable data i.e., first name, surname, date of birth, hospital number, NHS number, post code, etc. Data will be stored for 1 year post completion of the study before being archived or deleted in line with the sponsors' policy.

6.4 DATA ARCHIVING

At the end of the study, archiving will be carried out in accordance with sponsor protocols and, where relevant, local site governance requirements.

7.0 SAMPLE SIZE

All adult oncology patients at Queen Alexandra Hospital who are prescribed FDA approved ICPI for the first time, providing they don't meet the exclusion criteria will be recruited. The sample size is expected to be around 120 patients, based on ICPI patient Portsmouth Hospital University Trust data from 2022. It is estimated that around 40-66% of patients on ICPI will have an irAE based on the literature.

Assuming an incidence rate of 40% for irAE, this study would require at least a sample size of 47 individuals assuming a significance criterion of α=0.05 and power =80.

The study will run for 18 months and hopes to obtain at least a sample size of 47 individuals, depending on how many patients are referred for ICPI therapy. The study will end before 18 months if we recruit 150 patients before the end date.

The period of testing was decided upon discussion with the Oncology department, based on ICPI prescriptions provided by the pharmacy department.

8.0 STUDY MANAGEMENT

The chief investigator and lead researcher are responsible for the day-to-day operation of the study. Management support for this study will be provided by the Research and development department at Portsmouth Hospital University Trust.

9.0 HEALTH RELATED FINDINGS

Samples will be stored and tested under a pseudonym/reference one month post initial collection. Clinicians will not have access to these results and those testing the samples will not know who the sample belongs too. Results will not be linked to the patients' records until completion of the data collection period i.e., May 2025.

10.0 END OF STUDY

The study will finish 6 months post the final analysis of samples has been undertaken.

11.0 FUNDING

This study will be funded by the Higher Specialist Scientist Training (HSST) program via the National

School of Healthcare Science and by the Royal College of Pathologists Fellowship Research Start-up Grant.

12.0 MONITORING AND INDEMNITY

Monitoring of this study will ensure compliance with Good Clinical Practice. The Investigator (s) will permit monitoring, audits, REC review and regulatory inspections by providing the Sponsor, Regulates and REC direct access to source data and other appropriate documents (e.g., study data collection forms).

NHS Indemnity will apply.

13.0 IMPORTANCE AND POTENTIAL BENEFIT

This study is aiming to act as service improvement collaboration between Oncology and Pathology services at Portsmouth Hospital University trust. To help highlight which blood tests help to highlight ICPI at risk of developing irAE. On the basis of the results produced we aim to produce a blood test panel that can be ordered on our blood requesting software (ICE) for all ICPI patients. This will help to increase consistency in blood test requesting and diagnosis of ICPI irAE faster.

This study will also further the progression of Louise E Duvall's career and professional development. This will allow development of mass-spectrometry skills, data processing and analysis as well as journal publications. Completion of this project will also feed in to Louise E Duvall's Royal College of Pathologists exams for her fellowship.

14.0 PUBLIC AND PATIENT INVOLVEMENT

Research was presented to research team at Portsmouth Hospital University trust design was enthusiastically received.

Sharon Court patient public involvement coordinator has been approached; research will be discussed at an upcoming meeting including the acceptability, design, management, undertaking, and analysis of results.

Results will also be presented to the Portsmouth Patient Involvement Group. Research will also be presented to patient representatives as part of Louise E Duvall's doctorate in Clinical Science with the University of Manchester.

15.0 DISSEMINATION/ IMPLEMENTATION OF RESEARCH

Results will be written up and submitted for publication in a peer-reviewed journal. Abstracts will be submitted to national conferences. Results will be presented to clinical colleagues at regular in-house meetings. Results will also be presented to the Portsmouth Patient Involvement Group. This study will be submitted as part of a Doctorate in Clinical Science (DClinSci) with the University of Manchester and as part of Fellowship exams with the Royal College of Pathologists for the student Louise E Duvall.

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17.0 APPENDICIES

17.1 Appendix 2 – Amendment History

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
1	1.1	10/08/2023	L Duvall	Amended frequency of when to send list of NHS numbers to MyCall
2	1.2	12/09/2023	L Duvall	Included patient consent process

List details of all protocol amendments here whenever a new version of the protocol is produced.

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC.