PROTOCOL

Hypersensitivity Study

A Mechanistic Investigation into Drug and Chemical Induced Hypersensitivity Reactions

Personnel Involved

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1. Protocol Statements

1.1. General Information

This document describes the Hypersensitivity study and provides information regarding procedures for identification and recruitment of participants. The protocol should not be used as an aide-memoir or guide for the assessment and treatment of other patients. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to the registered investigators in the study. Clinical problems relating to this study should be referred to the Chief Investigator via the Wolfson Centre for Personalised Medicine.

1.2. Statement of Compliance

This study is designed to comply with the guideline developed by the International Conference on Harmonisation (ICH) for Good Clinical Practice (GCP), Research Governance Framework and local NHS Trust and University policies and procedures.

1.3. UK registration

This study will have National Research Ethics Service (NRES) approval, undergo relevant Site Specific Assessment by the relevant Trust Research and Development department and be granted Research and Development Approval from each participating NHS Trust.

Contents

1. Background

Adverse drug reactions (ADRs) are a major cause of patient morbidity and mortality and are also a key reason for drug attrition in drug development[1-3] ADRs can be classified as 'on target' or 'off target' reactions. On target reactions can be predicted from the known primary or secondary pharmacology of the drug and often represent an exaggeration of the pharmacological effect of the drug. They show simple dose-response relationships and, therefore, can usually be avoided by dose reduction; rarely, they are life-threatening. In contrast, off target reactions cannot be predicted from the known basic pharmacology of the drug, are often dose-independent and can exhibit marked inter-individual variability. They are often referred to as idiosyncratic reactions. Although off- target reactions are less common than on-target reactions, they can be serious and life-threatening[4,5].

An epidemiological study conducted in an adult population showed that 6.5% of all hospital admissions were caused by ADRs[2] while 95% of these were 'on target' reactions, 'off target' reactions still remain a clinical problem accounting for 1 in 3000 hospital admissions.

Idiosyncratic "off target" ADRs can be divided into two categories[6]:

- Immune-mediated, where the reaction has either the clinical or laboratory features which suggest that the immune system is involved in the pathogenesis; and
- Non-immune mediated reactions, or "metabolic idiosyncrasy", where non-immune, often poorly characterized pathways are affected by the drug leading to the reaction.

One of the presently accepted mechanisms of drug hypersensitivity is based on the hapten hypothesis[7,8]. The hapten hypothesis states that low molecular weight compounds must first bind to a macromolecular carrier, such as a protein, in order to become immunogenic. Central to this hypothesis is drug metabolism. Although the physiological role of drug metabolism is detoxification, oxidation of certain drugs can lead to the generation of chemically reactive metabolites and protein haptenation. The body responds to the drug-protein conjugate by initiation of an immune response, characterised by either specifically committed T-cells and/or antibodies (produced by B-cells)[9]. T-cells play a central role in the regulatory and effector arms of the immune response. T-cell receptors recognise small peptide fragments of the drug protein conjugate that are presented by MHC molecules, after cellular processing[10]. Other studies have shown an alternative pathway of immune recognition, which is independent of drug metabolism and cellular processing[11,12]. Laboratory studies have detected drug-specific antibodies, T-cells and protein conjugates from hypersensitive and non-hypersensitive individuals.

Protein haptenation has been the focus of many immunopharmacological studies. In a recent study by Meng *et al.*[13] it was shown that benzylpenicillin and its re-arrangement product, benzyl penicillenic acid, can form haptens with human serum albumin (HSA); these haptens have been detected in plasma isolated from patients receiving benzylpenicillin therapy. Physiologically relevant synthetic conjugates were prepared and were shown to stimulate T-cells derived from patients that are hypersensitive to benzylpencillin. Other studies have investigated the haptens formed with flucloxacillin[14] and piperacillin in patients[8].

Hypersensitivity reactions can also occur with chemicals found in commonly used products; due to this, the hypersensitivity project also encompasses any chemical that may cause a person to have an allergic reaction; examples include chemicals contained in hair dyes and fragrances. Therefore, this hypothesis is not exclusively applicable to drugs, but also to chemicals that may cause allergic reactions, for example para-phenylenediamine (ppd) in hair dyes.

In the context of this study, the term chemical is defined as a compound/substance that is either found in a drug or a product.

An improved mechanistic understanding of allergy is crucial as currently available methods to diagnose reactions lack sensitivity and there are no established methods to predict the immunogenicity of a drug. Characterization of drug antigens and definition of the role of protein modification with respect to sensitization is important for the safe development of new drugs and reducing the risk of drug toxicity. An appreciation of quality and intensity of the drug-specific immune response will pave the way for the development of improved diagnostic assays that will increase drug safety by stratification of drug use.

Recently a new group of oncology therapeutics known as checkpoint inhibitors (CPI) and targeted treatments have started to be used in mainstream clinical practice[15]. The toxicities of these drugs appear to characterise a specific subset of 'off-target' hypersensitivity effects leading to the development of a well characterised group of immune-related adverse events (irAEs)[16]. These treatments have revolutionised the management of historically poor-prognostic malignancies. However immune related adverse effects (irAE) associated with their use are increasingly concerning, particularly when these drugs are used in combination. The irAEs caused by CPIs mimic endogenous autoimmune disease (AID) in their clinical manifestations and apparent response to treatment[17]. The impact of irAEs will become increasingly significant as their use increases. Thus, understanding of the underlying mechanisms of immune mediated, 'off-target' hypersensitivity is vital. Insight into the immune processes involved in toxicity will inform further research, further drug design, reduce toxicity induced morbidity and optimise patient management and outcomes.

It is clear that the changes that occur at the end-organs targeted by hypersensitivity reactions and the comparison of those changes to the ones seen in blood are essential to complete the picture of hypersensitivity and therefore HYSTs aims and objectives. Thus, the collection and analysis of end-organ tissue, paired with blood samples, will give insight and understanding of what is occurring at the end-organ but also the relationship between systemic and local changes in the presence of hypersensitivity.

2. Study Rationale, Objectives and Design

2.1 Rationale

Drug and chemical hypersensitivity remains a major clinical problem in the UK and USA. Methods for diagnosis of drug hypersensitivity are currently only available for penicillins but not for other drugs. Moreover, current methods for diagnosing penicillin hypersensitivity appear to have low sensitivity and specificity. It is, therefore, important to develop an assay that exhibits high sensitivity and

specificity to confidently diagnose drug hypersensitivity. The mechanistic studies described herein may help to identify novel diagnostic reagents for drug hypersensitivity, and provide valuable information that will further our understanding of hypersensitivity reactions with the potential for the development of novel diagnostic assays.

Immune related adverse events (irAE) are having a significant impact on cancer patients who are receiving checkpoint inhibitor (CPI) immunotherapy as part of their oncological treatment[15]. These drugs cause novel toxicities. The constellation of irAEs includes colitis, hepatitis, endocrinopathies, pneumonitis, nephritis and dermatitis[17]. irAEs clinically mimic autoimmune diseases (AID), in which CTLA-4 and PD-1/PDL1 has an established role, and can be severe, permanent and multi-organ effecting. National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Grade 3/4 irAEs are apparently indication-independent and seen in 5-30% (monotherapy) to ~55% (combination therapy) of patients[16,18]. Insight into the immune processes involved in toxicity will inform further research, further drug design, reduce toxicity induced morbidity and optimise patient management and outcomes.

By nature of their mechanism of action, oncological treatments other than CPIs can also result in profound toxicities. In particular targeted treatments and cytotoxic agents disturb pathways fundamental to epithelial homeostasis, resulting in cutaneous toxicities including but not limited to folliculitis, vitiligo, bullous pemphigoid, non-specific maculopapular rashes and alopecia. Such toxicities can impair patients' quality of life and negatively affect clinical decision-making, which can result in early treatment cessation. Indeed, many of these treatments are increasingly being utilised in conjunction with CPIs supporting the need for better insight into the on and off target effect of these treatments.

2.2 Aims and objectives

The aim of this project is to investigate the mechanism(s) of drug-induced and/or chemical-induced hypersensitivity reactions. In order to study the mechanistic aspects of drug/chemical hypersensitivity novel proteomic mass spectrometric-based methods together will cell-based assays will help us to determine the chemical and cellular mechanisms as follows:

(1) Retrospectively using a number of drug (including but not limited to sulfamethoxazole, carbamazepine, lamotrigine, phenytoin, abacavir) and beta-lactams (including but not limited to penicillins, cephalosporins, carbapenems, monobactams and beta lactamase inhibitors)), chemical (including, but not limited to, hair dye [para-phenylenediamine]) and fragrance [including, but not limited to, cinnamic alcohol, eugenol]) allergens as paradigms.
(2) Prospectively using patients admitted to hospital with any drug induced hypersensitivity.
(3) Prospective pharmacokinetic study to investigate protein haptenation over a stipulated time-frame.
(4) Use prospectively and retrospectively identified patients to understand the mechanism ofoncological treatments including CPIs 'off-target', immune mediated adverse events (irAEs).

The study will define the basic mechanisms of hypersensitivity in terms of drug metabolism, hapten formation, and immunology of both the innate and adaptive immune system (examples include T-cells, B cells, dendritic cells etc) to investigate overall aspects of immune responses to drugs/chemicals.

The critical questions to be addressed are:

- What is the interaction between the hapten and the immune system?
- Is there a threshold of hapten formation to trigger immune responses?
- What is the mechanism of cellular presentation of the drug?
- What is the role of immune cells, for example T cells, and their specific phenotype in the immune response?
- Is there potential to use haptenated peptides as diagnostic reagents?

The integration of chemical, biochemical and immunological expertise will provide insight into the critical question of how low molecular weight chemicals cause serious tissue injury in man.

With regards to oncological therapies, the hypothesis is that there are identifiable immune parameters that are altered in response to CPIs and targeted treatments resulting in IRAEs that can be modelled to determine underlying immunopathological mechanisms. Thus this extends focus within 'What is the role of immune cells, for example T cells, and their specific phenotype in the immune response?' Another critical question is what happens to non-cellular immune mediators in this situation. The main aim of this project is to determine the molecular and cellular parameters affected by CPIs and other oncological treatments in the patient population and those that experience irAE toxicity. We will also investigate the immunophenotypical changes seen in involved end-organs. The project will also establish if there is a dose-toxicity relationship, identify person-specific factors that may predispose/predict susceptibility to irAEs and identify autoantibodies involved in irAEs.

For each drug / chemical, or any drug class, we will aim to recruit a maximum of 100 complete cases.

2.3 Study Design

The following studies will define the basic mechanisms of drug hypersensitivity in terms of drug metabolism and hapten formation, in combination with immunological assays.

- Proteomics investigation into hapten formation in patient plasma.
- Evaluation of the cellular mechanism of hypersensitive reactions induced by drugs and/or chemicals.
- Identification of MRNA profiles in patient cells
- Evaluation of the cellular mechanism of 'off-target' hypersensitivity irAE induced by drugs including oncological immunotherapies using cytometry techniques and cytokine arrays
- Evaluation of the humoral response to drugs including oncological immunotherapy and their role in 'off-target' hypersensitivity irAEs using protein arrays
- Evaluation of end organ tissue markers of hypersensitivity using histopathological, cytological, immunohistochemical and cytometry techniques
- Identification of patient HLA and genomic profiles (including single cell characterisation)

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The overall study design is outlined in Figure 1.

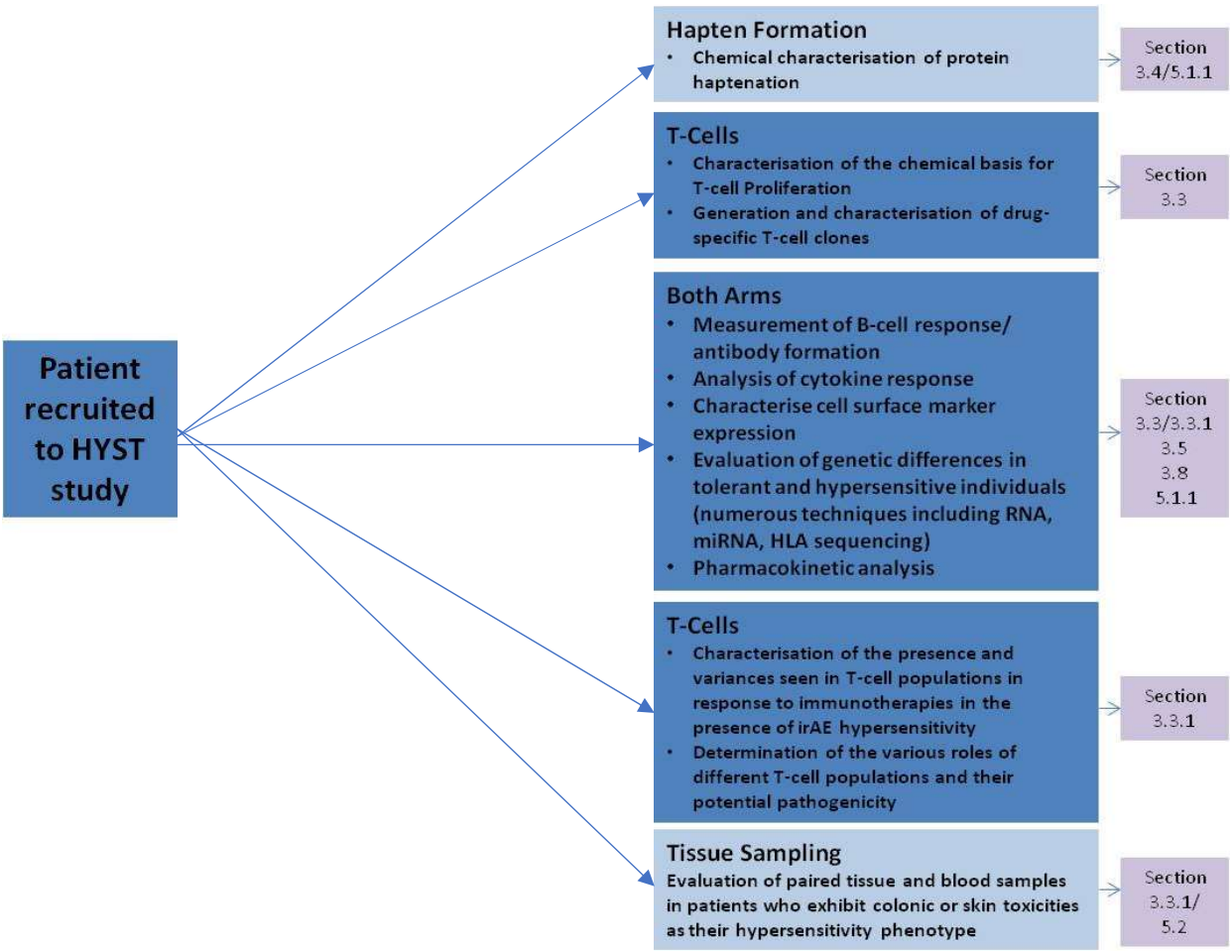


Figure 1: Summary of study design incorporating hypersensitivity to oncological immunotherapy

2.4 Patient Identification

There are two separate arms to the clinical protocol, a case-control study and a more limited cohort study

2.4.1 Case-Control Study

T-lymphocytes are thought to be involved in the pathogenesis of certain immunological drug reactions, causing tissue damage indirectly through the action of cytokines or directly by the secretion of cytolytic molecules. To stimulate a T-cell response a drug must act as an antigen and in some way ligate specific T-cell receptors[8,9,11]. Despite this, the paucity of studies that define drug antigens has severely restricted mechanistic studies that relate the chemistry of antigen formation to immune function. In order to evaluate the relationship between hapten formation and the immune response, lymphocytes (T- and B-cells and other immune cell types) will be isolated from subjects with a history of hypersensitivity along with cells from appropriate non-hypersensitive control patients. Whole blood samples will be collected to:

- Characterise drug antigen-specific T-cell and B-cell responses
- Characterise the relative involvement of different T-cell populations
- Measure effector molecules secreted from stimulated T-cells (e.g., cytokines) and B-cells (antibodies)
- Quantify regulatory T-cell expression and activity
- Generate and characterize the phenotype and function of drug antigen specific T-cell clones.
- Generate B-cell lines and dendritic cells for use as antigen presenting cells in functional assays.
- Characterise an overall immunophenotype that occurs in response to therapy with oncological therapies (CPIs, targeted treatments and chemotherapy)
- Characterise the impact of changes to the microbiota on the immune system in the setting of adverse drug reactions
- Transcriptomics, HLA typing and single cell work will also be undertaken

All methods needed to complete the proposed experiments are established and performed routinely[11,19-22].

In addition, whole blood samples from cases and controls that are undergoing drug treatment will be collected and the hapten formed in plasma will be identified and quantified by mass spectrometry methods established in CDSS[8,13,19]. This will allow us to evaluate the relationship between hapten formation and drug hypersensitivity.

Finally, in order to investigate and identify genomic factors associated with hypersensitivity reactions to certain drugs, genomic-based experiments are planned that will require the extraction of RNA from cells. Following RNA extraction, microarray and other assays will be performed to evaluate whether there are differences in the transcriptome between subjects with and without ADRs.

2.4.1.1 Case control Study – Drug Class NOS

Case group

- Panels of patients that have had hypersensitivity to the paradigm drugs/chemicals will be identified retrospectively through clinic attendances, referrals or existing clinical databases/systems.
- Prospectively patients that are attending hospital with any drug or chemicals induced skin reactions will be recruited locally and/or nationally.

Control group

2 groups of controls will be recruited

- Panels of patients that are undergoing drug therapy without adverse reactions (Control group 1)
- Healthy volunteers who may or may not have been exposed to paradigm drugs/ chemicals without ADRs (Control group 2)

Because adverse drug reactions affect children and adults, we will also allow children and families to be recruited to the Case/Control section of this study to ensure that they are not excluded from the process of improving medication safety.

2.4.1.2 Case Control Study – Oncological treatments

Case Group

- Identification of patients on the drug at the point of commencement of therapy
- Identification of patients admitted with a toxicity via tumour-group specific teams, acute triage logs, and real-time acute oncology on-line registry.
- Prospective patients that are attending irAEs≥grade 2 or endocrinopathies of any grade will be approached and assessed for eligibility both locally and nationally

Control Group

- Cases and controls will be recruited in a 1:1 manner
- Only one group of controls (patients receiving the drug but not exhibiting toxicity) will be recruited for this section
- Controls will be required to be receiving treatments for a minimum period outlined in the eligibility section

Due to the very limited use of oncological immunotherapies in children, only adults will be recruited to this arm of the case control study.

2.4.2 Cohort Studies

2.4.2.1 Cohort Study – Drug Class NOS

For each drug, and where appropriate, a small cohort of non-allergic control patients that are being administered the drug as part of their normal clinical care will be recruited to investigate the pharmacokinetics of hapten formation and clearance. Blood sampling will be detailed in section 5.

For each drug / chemical, or any drug class, we will aim to recruit no more than ten participants.

Currently, we have no plans to recruit participants under the age of 18 years to the cohort aspect of this study.

2.4.2.2 Cohort Study – Oncological Immunotherapy

Oncological immunotherapies mechanism of action lies in the upregulation of the immune system to recognise and act on tumour antigens presented to T-lymphocytes[23]. This is done by inhibition of so called checkpoints. The role of these checkpoints is to act as co-inhibitory monitors to prevent T-cell activation when a t-cell receptor is bound[23]. By inhibiting these, an immune reaction is propagated for malignant control. However, in a relatively high proportion of patients these drugs lead to 'off-target' irAEs, sometimes leading to death. Whilst it is thought that there is a sophisticated set of immunological effects that lead to both efficacy and toxicity, they have yet to be characterised into an immunophenotype that can lead to the identification of differences between those who are experiencing toxicity and those who do not.

The cohort study aims to characterise the immunological changes that occur following introduction of these drugs both alone in monotherapy and when two agents are used together in combination

Patients will have blood sampled prior to treatment and then at set time points throughout treatment with the analysis parameters reflecting those of the case-control studies.

Currently, we have no plans to recruit participants under the age of 18 years to the cohort aspect of this study

2.4.3 End Organ Specific Toxicity

End organ tissue biopsies from colon, skin, thyroid and liver will be taken to determine the hypersensitivity immunophenotype in the affected organs. Patients will have biopsies taken with a paired blood sample to determine the correlation of immunoparameter changes between blood and tissue in the presence of toxicity.

Analysis will involve:

- Standard histopathological and immunohistochemical testing
- Cytometry techniques
- Serum, plasma and cellular material analysis
- Transcriptomics, HLA typing, genomics and single cell work

2.4.3.1 Skin Toxicity secondary to drug hypersensitivity

The mechanism by which rash and skin blistering (including widespread skin cell) death occurs in skin adverse drug reactions occurs is not fully understood. In order to investigate the mechanism behind skin cell death histological characterisation, cell subtype/cytokine characterisation and genetic factors (including HLA) will be analysed. Additionally, 3D skin models from patients either having previously experienced an adverse drug reaction from any drug or from matched controls will be constructed.

3. Scientific Methodologies

In order to gain a comprehensive understanding of the complex mechanisms of drug hypersensitivity we need to perform scientific experiments at the RNA, DNA, protein and cellular levels.

Methodologies to be used in the investigation include:

3.1 RNA Based Experiments

- Extraction of RNA from subject cells will allow us to evaluate differences in gene expression levels (either for specific genes or globally using gene expression microarrays) between hypersensitive and tolerant patients. Numerous papers have identified a role for microRNAs (miRNA) as regulatory molecules in many cellular processes and so we also aim to evaluate miRNA levels in these samples. Additional RNA analysis techniques eg Nanostring will be undertaken in a subset of patient samples. Single cell RNA sequencing will also be undertaken in a subset of patient samples.
- Characterisation of the microbiome in the setting of adverse drug reactions will also be undertaken

3.2 Protein Based Experiments

- Identification and quantification of metabolites and haptens linked to the mechanism of drug hypersensitivity by mass spectrometric and immunoassays according to methodologies established within CDSS.

3.3 Cell Based Experiments

- Definition of the chemical basis of lymphocyte proliferation; T-cell responses to the paradigm drugs will be determined from freshly isolated peripheral blood mononuclear cells and cells previously frozen in liquid nitrogen. In order to investigate the role of parent drug or reactive metabolite(s) in T-cell activation, we will develop a reproducible assay that incorporates a metabolising system.
- Generation and characterisation of drug-specific T-cell clones from hypersensitive individuals; the cross reactivity of drug-specific T-cell clones with drug metabolites can be investigated since all cells in a cloned population have the same receptors and have been selected for proliferation in response to a known drug. Furthermore, the full range of activation events, including signal transduction, cytokine expression, cytolytic activity and T-cell receptor downregulation can be measured in T-cell clones.[1] T-cell clones will be maintained in culture and restimulated using established methodology[22,24]. Measurement of proliferation of the clones will determine (a) the nature of the drug signal to the immune system, and (b) the mechanism of cellular presentation of the drug.
- Measurement of cytokine levels in the T-cell clones and T-cell lines from drug hypersensitive individuals; the phenotype of responding T-cell clones and lines in the proliferation assays will be characterised by immunofluorescence flow cytometry using specific CD4+ and CD8+ antibodies. Helper T-cells will be characterised further by investigating the cytokine pattern in order to determine whether helper 1 or helper 2 response is predominant.
- Measurement of antibody and B-cell responses; to measure plasma IgG isotypes and total IgG levels, ELISA plates will be coated with drug-modified albumin (unmodified albumin as a control). Hapten inhibition experiments to definitively define the presence of anti-drug antibodies will be conducted. To track the development and maintenance of antigen-specific memory B-cells, an ELIspot assay[25], recently established in our laboratory, will be used. Briefly, blood lymphocytes will be stimulated with polyclonal mitogens or drug for 6 days, prior to transfer to IgG (or IgG isotype) coated ELIspot plates. Plates will then be developed for the ELIspot reader.
- Dendritic cell culture[26]; dendritic cells will be isolated directly from peripheral blood using magnetic bead separation methods or generated from monocytes by culturing the cells with cytokine. Dendritic cell activation (expression of MHC class II and co-stimulatory receptors, cytokine secretion and endocytosis) will be measured by flow cytometry.
- T cell responses will be determined from freshly isolated blood mononuclear cells and cells previously frozen following collection of whole blood from participants. This will utilise CyTOF and flow cytometry depending on the number of parameters under investigation at different points. Established T cell proliferation assays will also be undertaken. Specific antibodies will be used to determine the presence of different T-cell populations and subpopulations, to include but not limited to, CD8+, CD4+,NK cells and Tregs

- The full range of activation events including signal transduction, cytokine expression and cytolytic activity will be examined. This will utilise cytometry techniques and cytokine assays.
- The B-cell responses to these drugs will be analysed using antibody detecting protein arrays in serum. All samples (cohort and case-control) will be analysed for autoantibodies using immunome protein array to determine differences between patients with and without irAEs. The array identifies early-stage antibody biomarkers associated with cancer and autoimmune disease. ELISA techniques will also be employed.
- Tissue biopsies will be analysed for the presence of immune cell populations and subpopulations in addition to the presence of relevance cell surface receptors.

Biopsies may be utilised for cell and organ culture and treated with pharmacological agents to perturb toxicity.

Induced pluripotent stem cells (iPSCs) will be derived from isolated peripheral blood mononuclear cells (PBMCs). iPSCs are a type of cell which has the ability to be differentiated into a number of different cell types. In this instance, they will be differentiated to create 2 types of cells found in skin (keratinocytes and fibroblasts). Keratinocytes (epidermis) and fibroblasts (dermis) will be subsequently used to create 3D models of the skin using validated cell culture methods.
Patient iPSCs, keratinocytes and fibroblasts will be placed in liquid nitrogen for long-term storage in order to preserve them.

Skin punch biopsies will be taken from patients in order to isolate keratinocytes and fibroblasts. Skin biopsy samples will be utilised to compare their molecular (protein) profile to the iPSC-derived keratinocytes and fibroblasts described above.

3.4 Protein and Cell - Combined Experiments

- Evaluation of the relationship between haptenation and cellular proliferation; we will monitor the level of hapten formation and the clearance at different time points in venous blood samples and in cell culture to define the relationship between haptenation and cellular proliferation. This study will determine whether there is a threshold of haptenation for triggering immune responses.
- Evaluation of the sensitivity and specificity of the novel peptide reagents; experiments including analysis of the T cell specificity and phenotype and cytokine profiles upon stimulation with peptide reagents will be performed to determine whether there is significant difference in the immune responses between allergic versus control subjects.

3.5 HLA Typing

HLA subtypes will be determined using sequenced based typing methodology. Sequence based HLA-typing will be performed using the Histogenetics genotyping service which implements rigorous genotyping quality control assured by built-in redundancies. State-of the art software and bioinformatic tools will be utilized in sequence alignment and allele identification.

3.6 Statistical analysis

At present, we are unable to provide a total sample size of patients to be recruited. Given the different adverse drug reactions and the different frequencies at which they occur, it is not possible to give an exact number. However, given the rarity of some of the reactions, in most cases it will be unlikely that we will recruit more than 100 cases per culprit drug. Our general target will be 100 cases for each reaction.

For patients receiving oncological immunotherapy we will initially aim to recruit approximately 10 patients per drug to the cohort study and 25 cases and 25 controls per drug to the case-control study; however the study will institute a Bayesian inference with regard to sample size, using it to adjust the sample size within a dynamic analytical approach to the data analysis. Thus, we will recruit up to but no more than 100 patients (who complete the study) per drug as per the original protocol.

Given our case target it is vital we recruit patients both locally and nationally. As we are unable to state the exact number of patients to be recruited, we will provide figures for the number of patients recruited following an ADR for all the drugs that are studied to the ethics committee in the annual report.

3.7 Study endpoints:

- Chemical characterisation of protein haptenation.
- In response to exposure to suspect drug, the degree of:
    - lymphocyte proliferation
    - cytokine secretion
    - change in cell surface marker expression
    - antibody release
    - changes in microbiota
- Identification of genetic / genomic/ transcriptomic factors as biomarkers of hypersensitivity

3.8 Pharmacokinetic sub-study

Plasma drug levels will be determined using enzyme-linked immunosorbent assay **(**ELISA**)** or electrochemiluminescent (ECL) assays. For the case-control study, plasma will be taken from patients, but because they will present at different times, it may not always be possible to detect drug levels. The data from the prospective and case-control studies will be combined to develop a model to determine the relationship between dose and irAE. Analysis of specific cytokines, including but not limited to TNF, IL6, IL17, and IL23 in subsequent samples taken for PK analysis will be undertaken to inform the systems pharmacology knowledge base of immunotherapies.

4.Selection, Recruitment and Withdrawal of Participants

4.1 Participant Selection

4.1.1 Case Control study – Drug Class NOS

Inclusion Criteria for Case-Control Studies (Adult)

Case (Age ≥18 years):

a. Subject is willing to take part
b. 18 years or over
c. Diagnosis of drug hypersensitivity as per criteria laid out by the Phenotype Standardization Project.[2] or clinician decision
d. Written informed consent obtained

Control groups 1 and 2:

a. Participant willing to take part
b. Currently taking the drug in question without adverse reactions (Control group 1)
c. Healthy volunteers who may or may not have been exposed to paradigm drugs/ chemicals without ADRs (Control group 2)

d. Ethnically matched to the index patient
e. Written informed consent obtained

Exclusion Criteria for Adult Case-Control Studies

a. Unwilling to take part
b. Subject is unable to consent
c. Subject is, in the opinion of the Investigator, not suitable to participate in the study.

Inclusion Criteria for Case-Control Studies (Paediatric)

Case (Age <18 years):

a. Family are willing to take part
b. Age less than 18 years at time of the recruitment
c. Age 5 years or over at time of recruitment

d. Diagnosis of drug hypersensitivity as per criteria laid out by the Phenotype Standardization Project. [25] or clinician decision
e. Written informed consent obtained
f. Assent from older participants (judged on a case by case basis)

Control groups 1 and 2:

A control for the paediatric study may either be a paediatric or adult patient

a. Participant/family willing to take part
b. Currently taking the drug in question without adverse reactions (Control group 1)
c. Healthy volunteers who may or may not have been exposed to paradigm drugs/ chemicals without ADRs (Control group 2)

d. Ethnically matched to the index patient
e. Written informed consent (and assent if older paediatric participant) obtained

Exclusion Criteria for Paediatric Case-Control Studies

a. Unwilling to take part (or older participant unwilling to assent)
b. Subject is, in the opinion of the Investigator, not suitable to participate in the study.

4.1.2 Case-Control Study- Oncological treatments

Inclusion Criteria for Case-Control Studies

Case (Age ≥18 years):

a. Subject is willing to take part
b. 18 years of age or over
c. Diagnosis of drug hypersensitivity, defined as any grade 2 irAE with a requirement for steroid treatment/ recurrence of symptoms/ >1 break in treatment OR grade 3/4 irAE, as per criteria laid out in the NCI CTCAE (v4)[18] OR clinician decision OR an endocrinopathy of any NCI CTCAE grade
d. Administration of at least one dose of either monotherapy or combination oncological treatment (including CPI, targeted treatments and chemotherapy)
e. Any AE experienced up to 24 months after commencement of treatment
f. Written informed consent obtained

Control (Age ≥18 years):

Controls will be matched for age and duration of treatment to cases

a. Participant willing to take part
b. Currently taking oncological treatments without AE (grade 1 toxicity is acceptable) or

c. Previously received oncological treatment, did not have >grade one toxicity and have stopped for reasons other than toxicity
d. Ethnically matched to the index patient
e. Written informed consent obtained

Exclusion Criteria for Case-Control studies

a. Unwilling to take part
b. Subject is unable to consent

Subject is, in the opinion of the investigator, not suitable to participate in the study

4.1.3 Cohort Study – Drug Class NOS

Inclusion Criteria for Cohort Studies

a. Subject is willing to take part
b. 18 years or over
c. Subject is willing to donate "pre-dose" blood sample(s) and follow specified sampling regime
d. Subject is being administered the drug

Exclusion Criteria for Cohort Studies

a. Unwilling to take part
b. Subject is unable to consent
c. Subject is, in the opinion of the Investigator, not suitable to participate in the study

4.1.4 Cohort Study – Oncological Immunotherapy

Inclusion Criteria for Adult Cohort Study

a. Subject is willing to take part
b. 18 years or over
c. Subject is willing to donate "pre-dose" blood sample (s) and follow a specified sampling regime
d. Subject is being administered the drug

Exclusion Criteria for the Adult Cohort Study

a. Unwilling to take part
b. Subject is unable to consent
c. Subject is, in the opinion of the investigator, not suitable to participate in the study

4.2 Patient Recruitment

4.2.1 *Recruitment target*

At present, we are unable to provide a total sample size of patients to be recruited. Given the different adverse drug reactions and the different frequencies at which they occur, it is not possible to give an exact number. However, given the rarity of some of the reactions, in most cases it will be unlikely that we will recruit more than 100 cases per culprit drug. Our general target will be 100 cases for each reaction. It is, therefore, vital we recruit patients both locally and nationally.

As we are unable to state the exact number of patients to be recruited, we will provide figures for the number of patients recruited following an ADR for all the drugs that are studied to the ethics committee in the annual report.

4.2.2 Study Population

The study population will be recruited from a number of sources:

(a) Direct referrals from health professionals within any NHS Hospital Trust or primary care practice. Regular news letters will be sent to professional societies / Clinicians / NHS Trusts / GP practices, to disseminate project specific information. The flowchart in ***Appendix 2*** of the protocol demonstrates the recruitment via direct health professional referral.

(b) Searching hospital database records and case notes to identify potential participants. Research nurses working on the project will actively search for eligible patients.

(c) Direct approach to previous study populations known to have experienced ADRs, via the healthcare professionals normally involved in the care of the patient.

(d) The Yellow Card Reporting System. Following approval of this method of recruitment by the MHRA, we will utilise the yellow card database to identify potential patients. The flowchart in ***Appendix 5*** of the protocol demonstrates the recruitment process via the Yellow Card Reporting System.

(e) Referrals from industry partners via health professionals from clinical research facilities.

(f) Direct referrals from private healthcare providers.

(g) Imported samples from national and international collaborators collected under their own protocols and ethical approvals which have permission to share and ship samples. Cryopreserved peripheral blood mononuclear cells and other relevant biological samples collected will be transferred to Liverpool using an MTA stating that the external samples will be used to contribute to analysis for HYST.

4.2.3 *Withdrawal of Subjects*

Subjects will be informed that they are free to withdraw from the study at any time. Subjects may ask for their samples to be destroyed up to the time the samples and data are coded but not anonymised. However, once the sample is fully anonymised, this will not be possible. The Investigator may remove a subject if, in his / her opinion, it is in the best interests of the subject. If a patient permanently withdraws from the study, or is lost to follow-up, the reason will be recorded.

4.2.4 Loss to follow up

If any of the study patients are lost to follow up, contact will initially be attempted through the pharmacology research nurses at each participating NHS site. If the Principal Investigator at the study NHS site is not the patient's usual clinician responsible for their speciality care then follow-up will also be attempted through this clinician. Where all of these attempts are unsuccessful, the patient's GP may be asked to provide follow-up information to the recruiting centre.

4.2.5 *Co-enrolment Guidelines*

Patients registered for the Hypersensitivity study are not restricted to entry onto any other clinical trial, study or research project as this study consists of blood sampling. However information regarding any trial participation will be collected on the follow-up CRF forms.

4.2.6 *Concomitant medications*

There are no restrictions on medication for this study. All current medications and medications taken at time of reaction will be recorded in the CRF.

5. Sampling and Data Collection

5.1 Blood Sampling

Hypersensitive patients and patients that can tolerate the drug of interest without hypersensitivity reactions will also be recruited; the samples provided by this control patient cohort will act as experimental controls.

The adult patients will be asked initially to provide 50mL venous blood sample from which we will isolate the plasma and mononuclear cells using density gradient centrifugation. The volume of blood from paediatric patients will be less than this, and will conform to EMA guidelines and will not exceed 1% of total body circulating volume at a single sample, or 3% in a 4 week period. The current laboratory techniques require a minimum volume of blood for a successful sample analysis, although this will decrease as the technologies evolve during the study period. Children and young people whose weight requires a sample less than the minimum volume required for successful analysis will not be recruited. The minimum volume required will be determined by regular consultation with the laboratory team during the study. When biological samples including peripheral blood mononuclear cells (from hypersensitive and tolerant patients) are imported from collaborators under their own protocols and ethical approval, cells will be cryopreserved at the collaborating site and transported in a cryogenic environment (eg. dry ice, liquid nitrogen). The cells will be defrosted in Liverpool and used in the same way as samples collected from other sources in the experiments described below.

Plasma will be used for bioanalysis; DNA will be used for HLA typing and profiling drug specific changes in gene expression. Cells will be used for:

(a) Standard lymphocyte proliferation assays and an assay that incorporates a metabolising system.
(b) Generation and characterisation of drug specific T cell clones.
(c) Generation of immortalised Epstein-barr virus transformed B cell lines to be used an autologous population of antigen presenting cells.

Storage in liquid nitrogen to restimulate responding T cell lines and clones as and when required. Adult patients that produce the most vigorous response in the lymphocyte transformation test (LTT) may be asked to donate further blood samples; the patients that are re-called will be asked to donate up to 60mL of venous blood. We believe it is important to make these patients aware of the LTT result to their medication(s), so that severe reactions can potentially be avoided in the future. Paediatric patients will be assessed on a case by case basis. Any further requests for blood samples will be guided by the EMA guidelines (and the wishes of the patient/family) but will not exceed 3% total circulating volume in a 4-week period (approx. 2.4ml/kg).

For patients taking oncological immunotherapies, those displaying hypersensitivity and those tolerant to the drug will both be recruited. The samples provided by this control patient cohort who do not display hypersensitivity will act as experimental controls. Patients receiving PD-1 inhibitor monotherapy or in combination with a CTLA-4 will be recruited into the study.

The adult patients will be asked to donate up to 50mL venous blood samples (schedule seen in 5.1.2.2) from which we will isolate the plasma and mononuclear cells using density gradient centrifugation. This arm of the study will not be recruiting paediatric patients

Samples will be analysed as follows:

a. Whole blood (including PBMCs post isolation) samples will be analysed for the presence of T-cell populations and subpopulations, cell surface and intracellular immune markers using Mass Cytometry using Time of Flight technology (CyTOF) and/or Flow cytometry
b. Whole blood samples and PBMCs (isolated from whole blood) will be analysed for the presence of cytokines using CyTOF
c. Plasma will be used for cytokine analysis using cytokine arrays and ELISA techniques
d. Serum will be used to determine the presence of autoantibodies utilising protein array technology and ELISA techniques
e. Transcriptomic analysis
f. HLA typing

5.1.1 Pharmacokinetic sub-study to investigate hapten formation and clearance

The following outlines a detailed sampling procedure for the purpose of beta-lactam antibiotic hapten formation and clearance assessment. For other types of drugs, we would follow a similar procedure and ensure that no more than 20 samples (4mL per sample) are collected over 3 weeks.

(a) Blood sampled in this sub-study will be collected into lithium/heparin coated vacuettes.
(b) Blood will be sampled a maximum of 4 times a day; each blood sample will not exceed 4mL.

(c) Sampling will be performed / defined on the basis of Cmax for the drug under study to enable blood to be sampled on the basis of peak and trough measurements of the free drug. Peak measurements will be taken 5 minutes after the drug had been administered intravenously; this will be in keeping with Tmax for drugs administered in this way.

(d) Peak and trough blood sampling will be conducted twice a day for 2 days (i.e, 4 samples per day). For the following three days, blood sampling will be conducted to obtain two subsequent trough samples (i.e 2 samples per day) that will not exceed 4mL. Trough blood samples will be taken just before the administration of the next dose of drug.

(e) After this initial 5-day period of blood sampling, blood will be sampled once a week for 3 weeks after the treatment has finished; the samples will not exceed 4mL. This is to measure the clearance of the drug-haptenated protein. During this time patients may have been discharged from hospital and, therefore, patients will be reimbursed reasonable travel expenses.

(f) Plasma, required for bioanalysis, will be isolated within 60 minutes of each blood sample being taken, or as close as possible to this time point

Please note that on the basis of the initial assessments, participant availability and flexibility, we may change the sampling schedule but we will keep the total number of samples per patient constant. We will not exceed the total number of samples stated (seventeen samples (52 mls in total) over a four week period); however on occasion we may not obtain the full set of samples due to participant availability, withdrawal or Loss to Follow Up.

5.1.2 Patient Visit Schedule

5.1.2.1 Patient Visit Schedule for Drug Class NOS studies – see Appendix 2

5.1.2.2 Patient Visit Schedule for Oncological Immunotherapy studies – see Appendix 3

5.1.2.3 Patient Visit Schedule for Pharmacokinetic Sub-study- see Appendix 4

5.2.1 Microbiome Sampling

5.2.1.1 Gut Microbiome

The gut microbiome will be characterized via the collection of faecal samples according to standard operations procedures. The will then undergo sequencing analysis characterize bacterial populations during treatment and toxicity. Samples may also be taken during a clinically indicated flexible sigmoidoscopy.

5.2.1.2 Oral Microbiome

The oral microbiome will be characterized via the collection of oral cavity swab samples/ 5ml saliva according to standard operations procedures. The will then undergo sequencing analysis characterize bacterial populations during treatment and toxicity.

5.2.1.3 Skin Microbiome

The skin microbiome will be characterized via the collection of skin swabs (upto 3 areas) according to standard operations procedures. The will then undergo sequencing analysis characterize bacterial populations during treatment and toxicity.

5.3 Tissue Sampling

Given the off-target end-organ immunopathophysiology of adverse events, further mechanistic insight can be gained through the collection of tissue samples from the end-organ site of toxicity involved.

5.3.1Colonic Biopsy

Collection of colonic tissue samples is considered standard of care for diagnostic purposes in the case of colitis and forms part of the clinical care algorithm for CPI induced colitis. Sampling for this study would occur during a clinically indicated endoscopy but we would request a further biopsy samples (up to 12 in number) to be taken for research purposes. This will allow comprehensive sampling, even if the inflammation is patchy. These would be placed in either in cell medium and processed fresh or appropriate freezing media with a view to future work using cytometry techniques and other forms of cellular/ cytokine analysis or analysis of bacteria or proteins or genes. Additionally a request for permission to retrieve the clinical blocks after clinically indicated analyses have been performed will be made and histopathological/ immunohistochemical analysis performed. The patients will also have a paired bloods sample taken (50mls) at the time of endoscopy. **NO** endoscopic procedures would be undertaken for research purposes alone.

There may be some minor but short-lasting discomfort from having a blood test. Patients will not feel the biopsies being taken. There is a very small risk of complications (such as bleeding or perforation) from having any biopsy of the bowel, but it is important to note that the biopsy will be taken by an experienced endoscopist and will be done at sites with extensive endoscopic experience with established clinical practice. If patients have experienced colitis but are unable to give endoscopy samples a single (upto 50mls) blood sample can be given as part of the colitis substudy.

5.3.2 Skin Biopsy

Skin biopsies will be taken from patients who have skin hypersensitivity following any drug treatment including treatments used in oncology e.g CPIs, targeted treatments and chemotherapy (with a paired blood sample (up to 50mls)). If patients receiving CPIs wish to only give a blood sample and not a biopsy they will be approached for enrolment in the case control oncological immunotherapy study arm. If patients have experienced skin toxicity but are unable to give/do not wish to give skin biopsy samples a single (up to 50mls) blood sample can be given as part of the skin substudy.

Patients will only be given Patient Information Sheet A if they agree to have a skin sample taken. Otherwise patients will be given a PIS for the case control study. Blood and skin biopsy samples will be collected for research purposes. In a Stevens Johnson Syndrome (SJS) Patient and Public Involvement (PPI) group meeting held at the Wolfson Centre for Personalised Medicine (University

of Liverpool), the concept of skin biopsies for purposes of research was discussed. All SJS/PPI members were consulted and were unanimously in favour of biopsies being conducted in order to increase knowledge about cutaneous hypersensitivity reactions.

The Biopsy procedure shall take place either in out-patient Dermatology or Oncology Clinics at experienced centres, on the ward, or established Clinical Research Units (CRU). This will involve a simple procedure performed by a trained health professional whereby a full skin examination will be performed and classified. Subsequently up to two punch biopsies (up to 6mm) shall be taken from the reaction area and non-involved skin. If patients wish only to give a single punch biopsy then this will be taken from the involved skin. If patients develop reactions involving the scalp i.e. alopecia or folliculitis then we will ask the patient if they would be willing to have two smaller punch biopsies (up to 5mm) taken from the scalp in addition to the punch biopsy of normal skin. The rationale for this is that histological evaluation of immune reactions surrounding the hair follicle requires both vertical and horizontal sectioning which cannot be performed from a single biopsy. 50mls of blood and plucked hair follicles (up to 20) may be taken from the scalp for biomarker studies. If a patient has a blistering rash then the blister fluid (up to 5mls) can be taken either without or alongside a punch biopsy. Clinical photographs may be taken.

Skin biopsies/blood samples may also be taken following resolution of the hypersensitivity reaction to increase our mechanistic understanding and to compare with earlier samples. All skin biopsies shall be taken in line with appropriate Trust policy. Additionally, a request for permission to retrieve the clinical blocks after clinically indicated analyses have been performed will be made and histopathological/ immunohistochemical/transcriptomic analysis performed.

Patients may be asked to complete a 5 minute questionnaire to assess the impact of their skin on their quality of life. This assessment is known as the Dermatology Life Quality Index Questionnaire (DLQI) and is a validated questionnaire used regularly in dermatology outpatient clinics.

In order to understand the processes occurring in skin from patients experiencing adverse drug reactions (ADRs), it is important to analyse healthy skin from donors. This allow us to compare healthy skin biology with that of ADR skin to identify markers or signatures of ADRs. It also allows us to undertake studies using explants (excised skin tissue cultured in the laboratory), in which we can replicate the blistering skin ADR conditions in healthy skin. This will give us insight into the mechanisms at play but also allow us investigate novel therapies for blistering skin reactions.

Skin biopsies will be obtained from excess skin tissue collected from patients already undergoing excisions as part of their routine dermatology care. Patients will be identified and approached by their attending dermatologist who will also obtain informed consent. The collected skin will be utilised in *ex-vivo* experimental investigations examining factors that predispose individuals to adverse drug reactions. In order to further investigate biological risk factors and mechanisms of ADRs in the skin, biopsies will additionally be obtained, subject to informed consent, from individuals who have previously experienced a skin ADR.

5.3.2.2 Prospective skin arm (See 12.11)

In addition to those patients experiencing skin toxicity and recruited at the time of toxicity, patients who are due to be commenced on a oncological treatment will be recruited to a prospective observational cohort study. They will be provided Prospective Punch Biopsy Patient Information Sheet. All visits and procedures shall take place either in out-patient Dermatology or Oncology

Clinics at experienced centres or established Clinical Research Units (CRU). Patients will be seen on two occasions by a trained health professional.

Visit 1: A representative 6mm punch biopsy will be taken from the patient's skin prior to treatment commencing. Furthermore, patients may also have a paired blood sample (up to 50mls) and plucked hair follicles (up to 20) taken.

Visit 2: A second review will be made within the first 4 weeks after treatment has been commenced at which point patients will be reviewed and a full skin examination performed. Patients will be grouped into those who develop skin reactions and those who do not. In those patients who develop a skin reaction, a 6mm punch biopsy will be taken from the affected skin in addition to a 6mm punch biopsy of the adjacent non-affected skin. If patients wish only to give a single punch biopsy then this will be taken from the involved skin. In patients who do not develop a skin reaction, a 6mm punch biopsy will be taken from a representative area of skin. In those patients who develop reactions involving the scalp we will ask the patient if they would be willing to have two smaller punch biopsies (up to 5mm) taken from the scalp in addition to normal skin (as above). In addition, 40mls of blood and up to 20 plucked hair follicles may be collected.

Should a patient develop a skin reaction subsequent to their follow up visit they will be offered the opportunity for a further skin biopsy (with paired blood sampling). As with the recruitment of patients at the point of toxicity a subsequent sample (with a paired blood sample) may be taken at the point of resolution.

At all points in skin sampling patients may be asked to complete a 5 minute questionnaire to assess the impact of their skin on their quality of life. This assessment is known as the Dermatology Life Quality Index Questionnaire (DLQI) and is a validated questionnaire used regularly in dermatology outpatient clinics. Clinical photographs may be taken at each time point.

5.3.2.3 Biopsy procedure

After the procedure patients will require 1 or 2 sutures, removable after 7 days for the arms, 10 days for the legs and 14 days for the back. We will arrange a follow up visit to remove the sutures; removal of sutures shall take place at the CRU RLBUHT or at dermatology out-patients departments. The risks associated with punch biopsies are considered very small and may include bleeding, infection and small scarring. Although the risks are considered small and treated simply, participants will be advised to visit their General Practitioner (GP) if concerned. If a participant consents to the biopsy procedure as part of the study, a letter will be sent to the GP with notification of their participation. The biopsies and plucked hair follicles may be processed in a number of ways depending on the intended investigations planned. Therefore, samples will be either snap frozen in liquid nitrogen and immediately stored at -80ºC, fixed in formaldehyde for paraffin embedding (FFPE), stored in RNAlater® (Quiagen, Manchester, UK) to preserve RNA integrity, or kept as fresh samples in transport media to either be processed fresh or slow frozen.

5.3.3 Thyroid Biopsy

Whilst irAEs can manifest in almost any organ CPI-associated endocrinopathies are irreversible and thus of particular interest[27]. Endocrine irAEs include hypophysitis, hypopituitarism, hypoadrenalism, thyroiditis and new onset type 1 diabetes mellitus[27]. Whilst thyroid irAE (TirAEs) were only seen in a small minority of patients treated with ipilimumab (4%) the incidence is 6-11% in patients treated with PD-1 inhibitors, nivolumab and pembrolizumab[15,16,17]. When CPIs are used in combination the TirAE incidence is increased further with the Checkmate 067 trial reporting an incidence of 15%[17].

This figure has consistent across subsequent combination trials irrespective of CTLA-4 dosing[28]. Whilst it is broadly accepted that the mechanism of CPI-induced TirAEs is immune in origin further characterisation of the underlying mechanisms within the immune setting is required.

Drug induced thyroiditis is caused by relatively few drugs and even fewer cause toxicity by an immune mediated mechanism. Characterization of the histopathological, cellular and transcriptomic features of TirAEs is an entirely novel area of research. Undertaking FNAs to acquire thyroid material and associated immune cells is likely to greatly inform mechanistic understanding of the events and potential mechanisms of TirAEs. In addition to this the knowledge and understanding of TirAEs will convey insight into mechanisms in more complex organ systems and thus CPI irAEs as a group.

Fine needle aspiration (FNA) of the thyroid gland in patients experiencing TirAEs will be undertaken by experience interventional radiologists under image guidance. Samples will be taken following the commencement of thyroid dysfunction for research purposes. A single lobe, double pass technique will be undertaken to ensure that sufficient cellular material gained whilst maintaining patient comfort. The use of local anaesthetic will be utilised where indicated. There are minimal risks of FNA investigation. Side effects may include a small area of bruising. Risk of infection is minimised by the use of aseptic conditions. The use of image guidance minimises the risk of any additional effects.

A paired blood sample (50mls) will be taken alongside the FNA sample. Analysis utilising cytometry techniques, other forms of cellular/ cytokine analysis, transcriptomics and additional protein or gene analysis will be undertaken to characterise CPI TirAEs including potential mechanism. If patients do not wish to give a thyroid biopsy then a single blood test can also be taken as part of this study arm.

5.3.4 Liver Biopsy

Standard toxicological studies during the preclinical phase of drug development do not reliably detect potential hepatotoxicity of novel agents[29]. Hepatotoxicity has been the 2nd most common reason for withdrawals worldwide, accounting for 32% of 47 such drug withdrawals in 3 decades[30]. Lack of specific tests means that the diagnosis of drug induced liver injury (DILI) is often delayed or left unrecognised[31]. In a recent prospective population-based study, the annual incidence of DILI was 19 per 100,000, of which 23% are hospitalised and 1% died[32]. Mortality from liver failure among DILI patients who present with jaundice is approximately 10%[33]. Hepatotoxicity from chemotherapy and other systemic anticancer therapies occurs commonly and in an unpredictable or idiosyncratic fashion[34]. CPIs are also associated with unpredictable hepatotoxicity affecting 7-33% of patients across all grades[16.] The mechanisms of toxicity are varied and currently largely unclear. It is well know that tissue biopsy and histological analysis are essential in fully understanding the pathological processes occurring and thus necessary to gain a complete understanding of toxicity. This is particularly the case in drug classes where animal models are unable to provide insight due to complex immunological differences between species such as the case with CPIs.

Radiologically guided biopsy of the liver will be undertaken in patients who have clinically significant drug induced hepatotoxicity (CTCAE Grade 3 or 4 and/or high risk clinical phenotype or toxicity behavior eg those with pre-existing liver disease or patients with atypical blood test derangement which are not captured by the currently used CTCAE criteria) whom are considered low risk (INR <1.5 and Platlets >150). Upto 2 cores of liver tissue (upto 25mm in length) will be taken at biopsy. In low risk patients having a non-targeted liver biopsy carries low risk of complications associated with the

procedure (<0.1%; <1 in 1000[36]). Samples will be either fixed in formaldehyde for paraffin embedding (FFPE), stored in RNAlater® (Quiagen, Manchester, UK) to preserve RNA integrity, or kept as fresh samples in transport media to either be processed fresh or slow frozen.

A paired blood sample (50mls) and urine sample (25mls) will be taken alongside the tissue sample. The urine will be frozen and/or freeze dried. Analysis utilising cytometry techniques, other forms of cellular/ cytokine analysis, transcriptomics and additional protein or gene analysis will be undertaken to characterise hepatotoxicity including potential pathological mechanisms. Alongside liver biopsy and blood/urine sampling a liver stiffness assessment will be undertaken. This is a non-invasive ultrasound based modality which carries no procedural risk.

If patients do not wish to give a liver biopsy then a single blood +/- urine +/- stiffness test can also be taken as part of this study arm.

5.4 Sample Storage and Data Collection

Samples will be processed at the relevant institution with the necessary facilities and regulatory approvals in place and then transferred/delivered to the University of Liverpool, Department of Pharmacology Laboratories. We have implemented a new bar coding system in the Department which adds further security in terms of sample tracking and also for confidentiality purposes. All biological samples stored within the Department are in keeping with the Human Tissue Act (license held by the University of Liverpool).

Representative data to be collected will include diagnosis of ADR, symptomatic presentation and type of drugs involved as defined above. Age, weight, height, ethnicity (in the form of a questionnaire), medical history, concomitant medication at time of reaction, current medication, and if available, clinical blood sample and investigational results conducted at time of reaction will also be collected (***Appendix 5***). The patient's clinical care will not be affected in any way by taking part in the study.

6. Safety Assessment

The study will be conducted with close attention to patient safety. Participants recruited will have their blood sampled by venepuncture. Donating blood by venepuncture produces minimal risk of bruising or bleeding. This procedure will be carried out by trained, competent health professionals.

7. Direct Access to Source Data and Documents

The Case Record Forms (CRFs) will be supplied by the Investigator for recording all data collected during the study. All CRFs are to be completely filled out by personnel administering the study procedures and once final review has been conducted and signed by the Chief Investigator or nominated designee. All CRFs are to be completed in a clear, legible manner. Black ink must be used to ensure accurate interpretation of data. Any changes or corrections made by drawing a line through the data to be changed, entering corrected information, and signing (or initialling) and dating the change. Erasing, overwriting, or use of "liquid paper" is not permitted on CRFs.

As this study will be recruiting patients from a wide variety of sources, once the CRF is returned to the University and entered onto a specially designed database, the CRF may then also be classed as the source documentation. Data collected into the CRF's and subsequently entered onto the database will be reviewed for discrepancies, missing data and queries. Research nurses and data managers will liaise with the relevant clinicians regarding data corrections. The Chief Investigator will be updated on a regular basis and will review the reports produced by the statistician in order to ensure consistency and accuracy of the data. Once CRF's and corresponding queries and reports are reviewed, the CRF will be signed off by the Chief Investigator or designated person.

The database especially designed for this project will be held at the University of Liverpool in the Department of Pharmacology and Therapeutics Research Office. It will be password protected and is backed up on a daily basis.

The following abbreviations are for use when values or answers cannot be provided: NA = Not Applicable, NK = Not Known, ND = Not Done, NR= Not Retrievable or Not Available.

Every effort should be made to have the CRFs completed and as soon as possible following a subject's study visit.

All study documentation, as defined above will also be made available to the Ethics Committee and to regulatory authorities for inspection on request.

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties other than those noted below is prohibited. Records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations will not be made publicly available. All study centre personnel will comply with the privacy rules of their institutions and /or professional groups and with the ICH Guideline for Good Clinical Practice.

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited, except for inspection on request by one or more of the following:

- The Research Ethics Committee
- Auditors (including those instructed by regulatory bodies)

Documents relating to the trial that contain personal data that may disclose the identity of the subject should remain with the Investigator in a locked filing cabinet. The Investigator should not provide any personal data that may identify the subject to any third party at any time during or after the study. Subject confidentiality will be further assured by utilising unique subject identification code number. The link between the patient's name and code will be broken and the sample completely anonymised when the study is completed and all clinical data have been obtained.

The Investigator must maintain adequate records for the study including completed CRFs, volunteer medical records, laboratory reports, worksheets, nursing notes, signed Consent Form/Subject Information Sheet, drug disposition records, adverse experience reports, information regarding

subjects who discontinued, all correspondence with the Research Ethics Committee and other pertinent data.

All records are to be retained by the Investigator for the period of 15 years.

8. Ethics

Potential subjects for the study will have been identified by one of the sources of recruitment highlighted in section 4. The majority of potential subjects will be given a detailed oral presentation of the study nature and receive detailed written information. All subjects will be informed of the nature and purpose of the study, its requirements and possible hazards, and their rights to withdraw at any time from the study without prejudice and without jeopardy to any future medical care at the study site. They will have adequate opportunity to ask the Investigator or nominated designee about any aspect of the study. Each subject must agree to co-operate in all aspects of the study and must be given informed written consent to the Investigator for participation.

Three Consent Forms will be signed, one for the researcher, one for the casenotes and the other for the participant. Signed Consent Forms must remain in the study file and be available for monitoring purposes at any time.

The study will be conducted in compliance with the guidelines of the Declaration of Helsinki on biomedical research involving human volunteers (Hong Kong revision, 1989 and the 48th General Assembly, Somerset West, Republic of South Africa, October 1996, updated in October 2000), ICH-GCP guidelines, relevant regulatory guidelines, and the study protocol. The protocol and relevant substantive data will be submitted for consideration by the Local Research Ethics Committee and written approval from the Chair of the Ethics Committee is required before the study is initiated and clinical activities of the study can commence.

The Ethics Committee will be notified promptly by the Investigator of the following:

a) Deviations from, or changes of, the protocol to eliminate immediate hazards to the trial volunteers.
b) Changes increasing the risk to volunteers and/or affecting significantly the conduct of the trial.
c) New information that may affect adversely the safety of the volunteers or the conduct of the trial.
d) As the research consists of blood sample and data collection only, there will be no adverse event reporting. However for safety reasons, if a problem occurs during or following venepuncture when collecting a blood sample, this will be recorded in the CRF. Although the risks associated with this intervention are minimal and occur very rarely.

Any major changes to the protocol will be made by means of a formal written protocol amendment and submitted for approval by the Research Ethics Committee. The Committee will also be kept informed of study progress and will receive a copy of the final study report.

9. Quality Assurance, Data Handling, Publication Policy and Finance

The Principal Investigator will take overall responsibility for the internal monitoring of all CRFs, taking care to ensure that entries are complete and legible and to otherwise ensure compliance to the protocol, and to ICH GCP. The Investigator will permit representatives of the regulatory authorities to inspect facilities and records relevant to this study.

The Investigator will be responsible for preparing the final study report. All results generated in this study may be submitted for publication or presentation.

The study has been funded by the Medical Research Council.

10. Data Management

Case report forms (CRFs) will be developed by the Data Management team at the Wolfson Centre for Personalised Medicine along with the lead Investigator, the Trial statistician and the lead research nurse. A bespoke database will be produced using specific clinical trials database design software. CRF data entered into the database designed for this study will be centrally monitored by the data management team, to ensure that data collected is consistent in accordance to the study protocol. The database used for this study will include validation features that notify the user to when certain validations are violated. If any irregularities are identified via automated validation or central monitoring, a discrepancy will be raised within the database and a notification will appear in the users login screen to action. A complete log of discrepancies and data amendments is automatically generated, including the date of each change, the reason for the change and the user who action the change, thus providing a complete audit trail. Additional site training will be carried out if recurring problems are noted with data from a certain site, such as consistently incorrect or incomplete data, a backlog of unresolved discrepancies, or unacceptable time delays in submitting CRFs.

11. Data Monitoring

A generic study monitor is employed by the department. Through a variety of processes the monitor will ensure; that the study meets IHC-GCP compliance, that all staff working on the study are trained competent and authorised to do so, that study data integrity is maintained, that patient safety and data protection are not compromised, that all documents and approvals are in place prior to and during the period of the study etc

12. Appendices

12.1 **Appendix 1:** Abbreviations

ADR:	Adverse Drug Reaction
CRF:	Case Report Form
DNA:	Deoxyribonucleic acid
GPRD:	General Practitioner Research database
MHRA:	Medicines for Health Regulatory Authority
PIL:	Patient Information Leaflet
PMH:	Patient Medical History
RNA:	Ribonucleic acid
YCC:	Yellow Card Centre

12.2 Appendix 2: Patient Visit Schedule for Drug Class NOS studies

Visit 1: Patient Recruitment (Identification of patients on active drug treatment either with or without evidence of ADR to drug).

1. Consent	Written and verbal consent for inclusion into study
2. Blood sampling	Blood sample (up to 500mL) collected into a lithium/heparin / EDTA vacuette
3. Initial assessment	PMH/admission details Current medications (including herbal and over the counter medications) Alcohol/smoking/recreational drug use Height/weight/ethnicity

Visit 2: Patient Recall

1. Consent	Verbal consent for continuation in the study
2. Blood sampling	1 blood sample (Up to 60mL) collected into a lithium/heparin vacuette

For each adult case or control, up to a total of 180mL of blood will be collected per year for a maximum of 3 years. For paediatric patients, the minimal blood volume possible will be used, and will not exceed amount stated in EMA guidelines.

If, after 3 years of blood donation, the data generated from a particular adult patient warrants further investigation, we will approach the subject to reconsent for further blood donations to continue as specified above (a maximum of 180mL per year).

12.3 Appendix 3: Patient Visit Schedule for Oncological Immunotherapy studies

As per the pharmacokinetic subsection please note that on the basis of the initial assessments, participant availability and flexibility, we may change the sampling schedule but we will keep the total number of samples per patient constant.

Patient Visit Schedule for the Case-Control Study

A flexible sampling window of +/- 7 days will be allowed in both case and control groups.

Cases

Day 0: Patient Recruitment (Identification of patients who have had an irAE to an immunotherapy CPI or non-hypersensitive control patient)

Patients will ideally be recruited and a blood sample taken prior to the introduction of initial steroid therapy; however patients can still be recruited if it is within 7 days of commencement of steroid therapy.

Visit 1: Day of recruitment (Day 0)

1. Consent	Written and verbal consent for inclusion into the study
2. Blood sampling	50mls of blood collected into lithium/heparin (or CPT), EDTA and serum tubes (+/- PAXgene). Optional Stool Sample
3. Initial Assessment	Malignant site and current disease status Therapeutic history PMH (particularly of autoimmune disease) FH (particularly of autoimmune disease) Current medications (including herbal and over the counter medications) Alcohol/smoking/ recreational drug use Height/weight/ethnicity

Subsequent Sampling (All Organ Sites) – if antibody analysis is not needed at each point then can reduce the amount of blood needed at each point.

Week 0 (Day 1)	Blood Sampling	40mls Blood
Week 1 (~Day 7)	Blood Sampling	40mls Blood

Week 2 (~Day 14)	Blood Sampling	40mls Blood
Week 3 (~Day 21)	Blood Sampling	40mls Blood
Week 4 (~Day 28)	Blood Sampling	40mls Blood
Point of resolution of symptoms or biochemical disturbance (as applicable)[1]	Blood Sampling	40mls Blood
Point of recurrence (if applicable)	Blood Sampling	40mls Blood
Week 8 (~Day 56)	Blood Sampling	40mls Blood

[1]Resolution of symptoms to be used in the case of colitis and dermatitis and biochemical restoration to be used in the case of hepatitis and thyroid disturbance.

Patients recruited with biochemical thyroid dysfunction or hepatitis will have an additional sample taken if/when their biochemical profiles begin to normalise

The sampling schedule may be modified in terms of the number of visits on clinical, patient choice or geographical grounds as not all patients will be able to attend for all visits. On occasion a single sample at the point of toxicity will be taken if no further samples are possible on clinical grounds. Additionally if a patient experiences an additional toxicity during enrolment an additional blood sample may be taken.

Microbiome samples may be taken throughout enrolment

Controls

A single sample will be taken at a matched time point to cases and subsequent samples will be taken at the start of month 4 (~day 84) following the first sample (even if CPI has been discontinued on non-toxicity ground eg disease progression). Patients will have 40mls of blood taken at each visit and may have microbiome samples taken alongside. If control patients wish to contribute further to the study and give further blood samples at different durations of therapy for further matching this will be permitted.

We will not request that subjects donate more than 475mL of blood in a period of 16 weeks. This is the blood donation volume and rate adopted by the National Blood Service for England and Wales (www.blood.co.uk) and is thus a well recognised national standard.

Patient Visit Schedule for Cohort study

Baseline Visit: Patient Recruitment (Identification of patients due to commence PD-1 inhibitor monotherapy or PD-1/CTLA-4 combination therapy). A flexible sampling window of +/- 14 days will be allowed as long as it remains prior to the commencement of therapy.

1. Consent	Written and verbal consent for inclusion into the study
2. Blood Sampling	50 mls of blood collected into lithium/heparin (or CPT) / EDTA /serum tubes (+/- PAXgene). Optional Stool Sample
3. Initial assessment	Malignant site and current disease status Therapeutic history PMH (particularly of autoimmune disease) FH (particularly of autoimmune disease) Current medications (including herbal and over the counter medications) Alcohol/smoking/ recreational drug use Height/weight/ethnicity

Visit 1: Pretreatment sample within 0-2 days of commencing treatment

1. Consent	Verbal consent for continuation of the study
2. Blood Sampling	50mls of blood collected into lithium/heparin (or CPT), EDTA and serum tubes (+/- PAXgene).
3. Current circumstances	Determine changes to current state of health and medication

Visit 2: Week 0 (Day 1) – the day of initial CPI treatment

4. Consent	Verbal consent for continuation of the study
5. Blood Sampling	40mls of blood collected into lithium/heparin (or CPT), EDTA and serum tubes (+/- PAXgene).
6. Current circumstances	Determine changes to current state of health and medication

Subsequent Visits

Week 1 (~Day 7) Visit 3	Blood sampling	40mls of blood
Week 2 (~Day 14) Visit 4	Blood sampling	40mls of blood

Week 3 (~Day 21) Visit 5	Blood Sampling	40mls of blood
Week 4 (~Day 28) Visit 6	Blood Sampling	40mls of blood
Week 8 (~Day 56) (Start of treatment month 3) Visit 7	Blood Sampling	40mls of blood
Week 12 (~Day 84) (Start of month 4) Visit 8	Blood sampling	40mls of blood

Patients will have their consent to continue in the study and their current status regarding medical circumstances and medications verbally confirmed at the beginning of each visit.

The sampling schedule may be modified in terms of the number of visits on clinical, patient choice or geographical grounds as not all patients will be able to attend for all visits. If patients experience toxicity during time on the cohort study a further blood sample will be taken at that point. If patients experience toxicity after the conclusion of the sampling schedule a patient will be offered the opportunity of donating an additional sample of blood or enrolment on to the case-control study.

Microbiome samples may be taken throughout enrolment on the study

We will not request that subjects donate more than 475mL of blood in a period of 16 weeks. This is the blood donation volume and rate adopted by the National Blood Service for England and Wales (www.blood.co.uk) and is thus a well recognised national standard.

12.4 Appendix 4: Patient Visit Schedule for Pharmacokinetic Sub-Study

Day 0 : Patient Recruitment (Identification of patients who have previously had an ADR to a drug of interest or non-hypersensitive control patient).

1. Consent	Written and verbal consent for inclusion into study

2. Initial assessment	PMH/admission details Current medications (including herbal and over the counter medications) Alcohol/smoking/recreational drug use Height/weight/ethnicity

Days 0/1-2: Preliminary Blood Samples

1. Consent	Verbal consent for continuation in the study
2. Blood sampling	1 blood sample (4mL) immediately prior to drug dose = trough measurement
3. Blood sampling	1 blood sample (4mL) 5 minutes after drug dosing = peak measurement
4. Blood sampling	1 blood sample (4mL) immediately prior to subsequent drug dose = trough measurement
5. Blood sampling	1 blood sample (4mL) 5 minutes after drug dosing = peak measurement

Days 3-5: Secondary Blood Samples

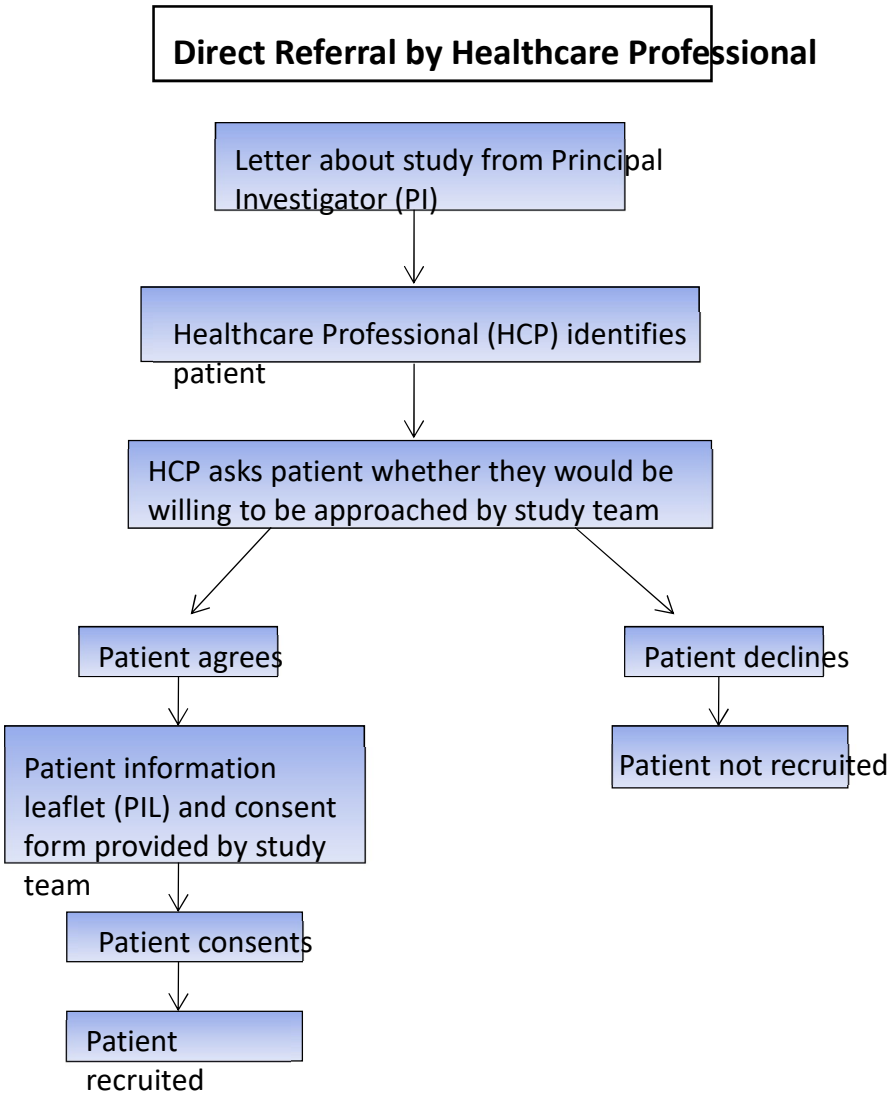
1. Consent	Verbal consent for continuation in the study
2. Blood sampling	1 blood sample (4mL) immediately prior to drug dose = trough measurement
3. Blood sampling	1 blood sample (4mL) immediately prior to subsequent drug dose = trough measurement

Weeks 2-4: Single weekly blood samples

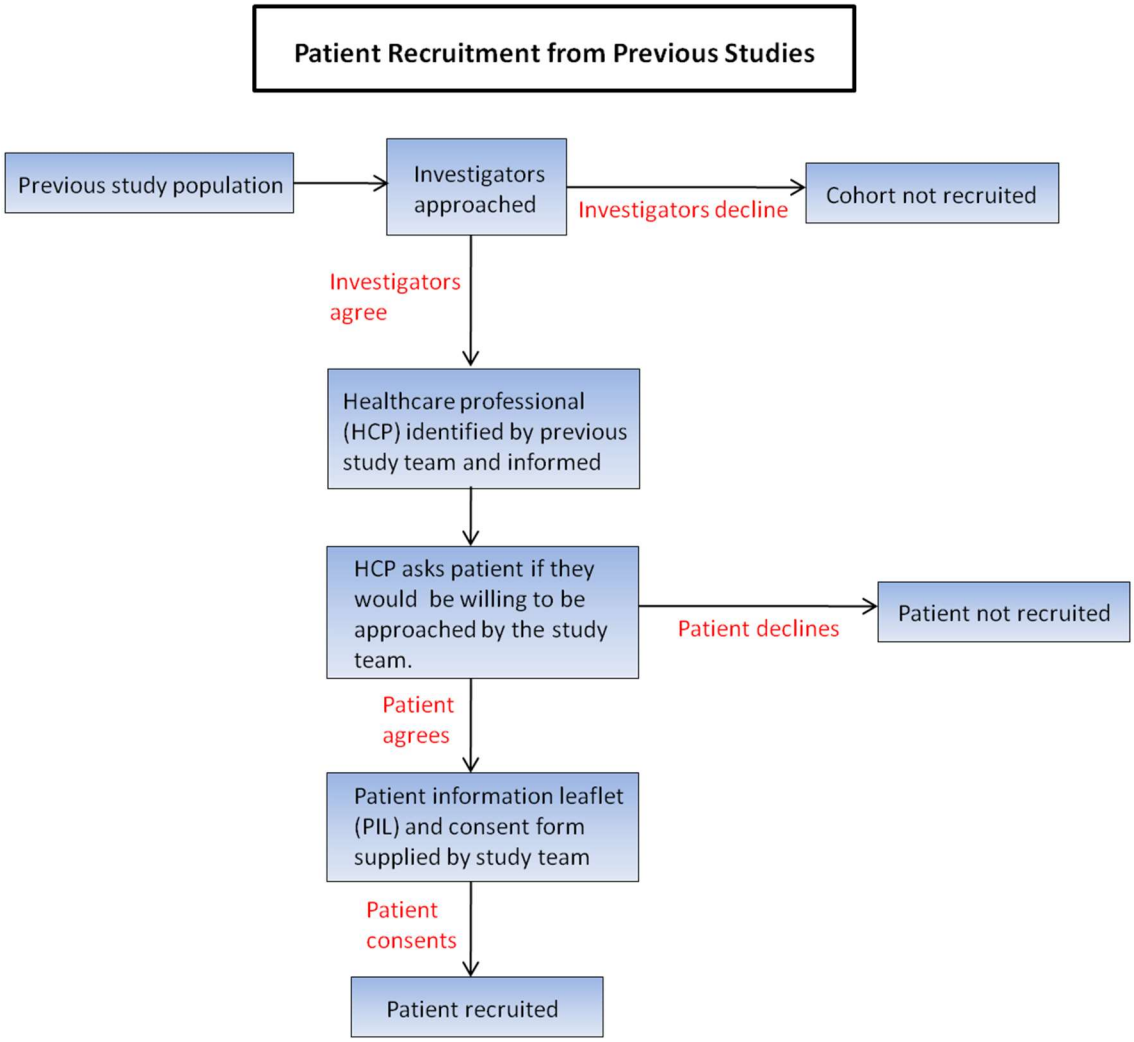
1. Consent	Verbal consent for continuation in the study
2. Blood sampling	1 blood sample (4mL)

For patients taking oncological immunotherapies Plasma drug concentration samples will be taken prior to commencement of treatment, on the day of the initial treatment at Cmax, day 7, day 14 and day 21 of the initial treatment cycle. This is in keeping with the rest of the cohort schedule (Section 5.1.2.2.2) and will be taken at the same time.

12.5 Appendix 5: Flow chart for recruitment via direct healthcare professional referral



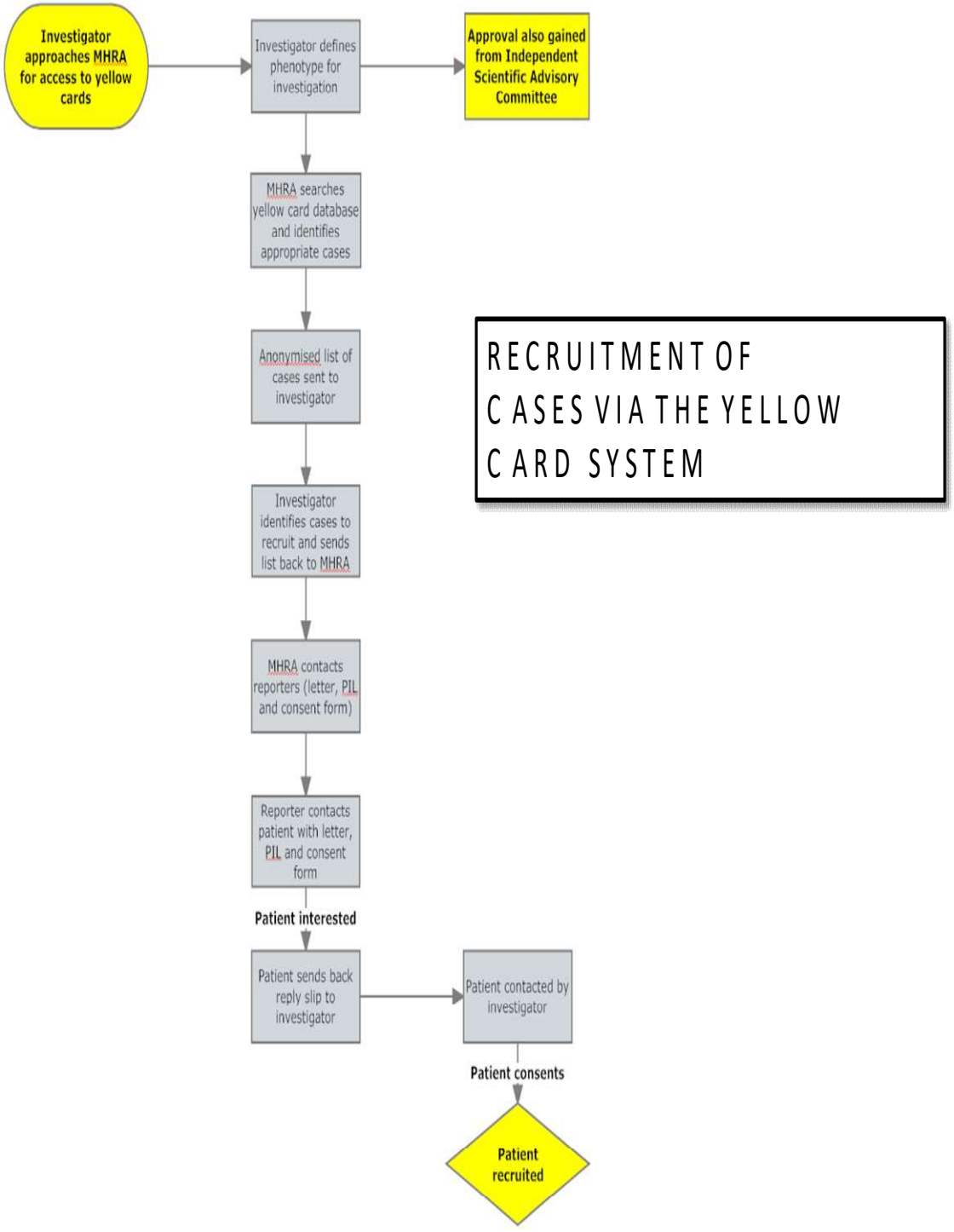
12.6 Appendix 6: Flow chart for recruitment from previous studies



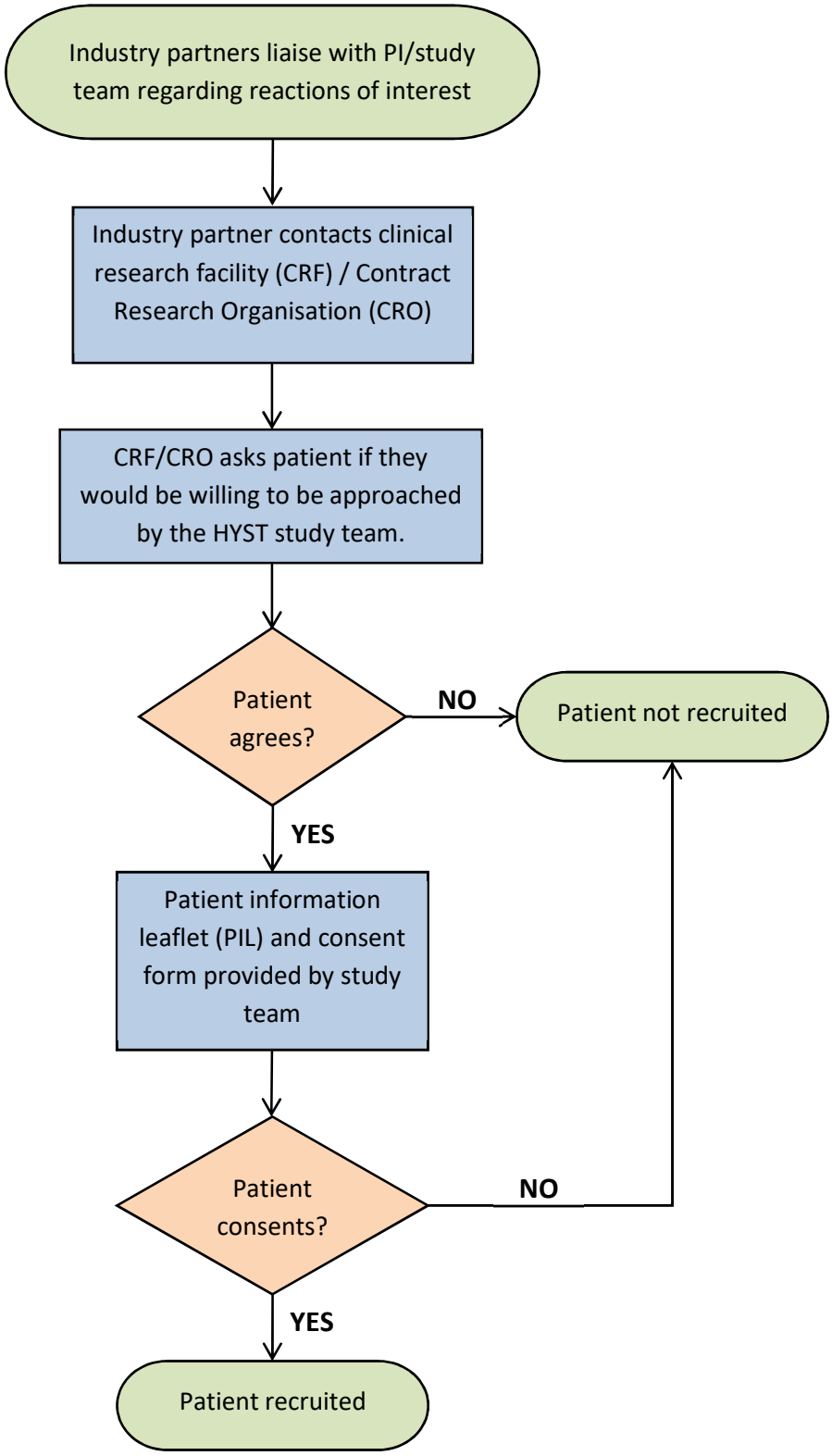
12.7 Appendix 7: Data to be collected in the CRF

- Date of Birth
- Weight (current and, if available, historical values including birth weight)
- Height (current and, if available, historical values)
- Past medical history including autoimmune disease
- Allergies
- Family history including allergies and autoimmune disease
- Smoking history (of older participants, and of home environment)
- Alcohol intake (of older participants)
- Ethnicity questionnaire
- Current medications
  - Dose
  - Duration of treatment
- Medications at time of reaction
  - Dose
  - Duration of treatment
- Other medications (herbal, over the counter, recreational
  - Dose
  - Duration of treatment
- Information regarding drug reaction
  - Dose at time of reaction
  - Duration of treatment
  - If the drug/s was re-challenged
  - Recurrent reactions
  - Ongoing supportive therapy
- Clinical samples and investigational results
  - Haematological
  - Clinical Chemistry
  - Coagulation
  - Skin patch test
  - Biopsy

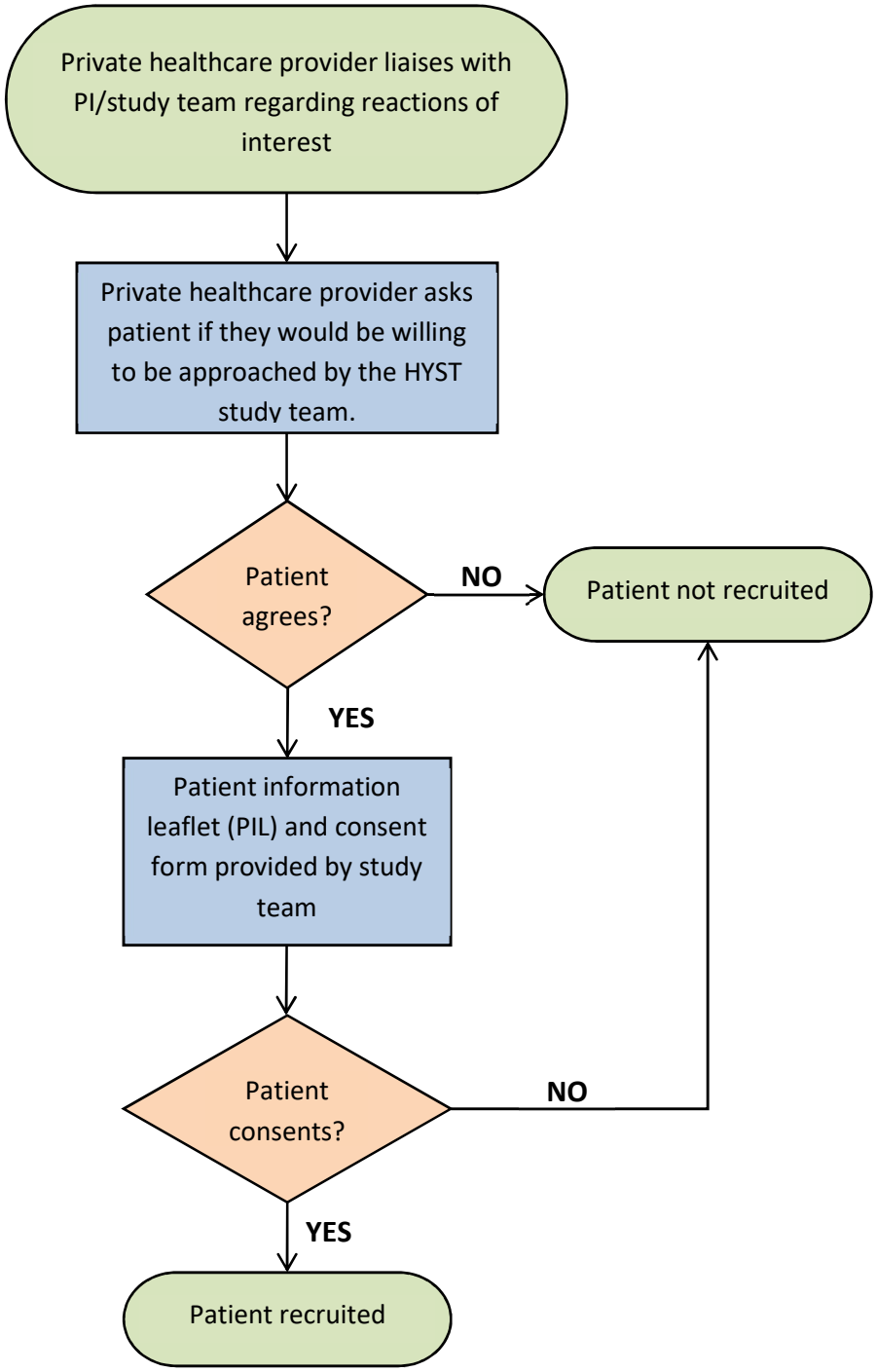
12.8 Appendix 8: Flow chart for recruitment via the Yellow Card Reporting System



12.9 Appendix 9: Flow chart for recruitment via industry partners



12.10 Appendix 10: Flow chart for recruitment via private healthcare providers



12.11 Appendix 11: **Flow chart for prospective cohort study for patients on treatments in oncology**

Procedures	**Visit 1**	**Visit 2 (Up to 4 weeks)**
Informed consent	X	
Demographics	X	
Medical history	X	
Punch biopsy (pre or post treatment)	X	x
Observation of treatment		x
40ml blood sample	X	x
DLQI	X	x

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