Study Title: **The"MEDALLION" - The *M*onitoring *immun*E *D*ysregul*A*tion *fo*LL*owing* I*mmune checkp*O*int-inhibitio*N** **Study** Short Title: MEDALLION-PILOT

Acronym: MEDALLION-PILOT

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SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted.

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STUDY SUMMARY

Clinical Phase	Pilot observational study
Summary of Study Design	Open-label non-randomised longitudinal cohort study
Summary of Participant Population	Advanced cancer patients receiving immune checkpoint inhibitors as part of their standard treatment
Planned Sample Size	80 patients
Number of Sites	1 (The Newcastle upon Tyne Hospitals NHS Foundation Trust)
Follow Up Duration	Up to 10 months of treatment
Planned Study Period	102 months
Primary Objective	To establish a clinically well-characterised and immune-phenotyped inception cohort of patients with advanced cancer who are commenced on immune checkpoint inhibitor (CPI) therapy, thereby enabling the study of immune dysregulation that precedes immune-related adverse event (irAE) development.
Secondary objective	To determine whether STAT3 phosphorylation in circulating CD4+ T cells of CPI recipients predicts irAE development at baseline and/or following initiation of treatment.
Primary Outcome Measure (with respect to secondary objective)	CD4+ T cell phospho-STAT3 measurement by flow cytometric analysis.
Study Intervention:	None (observational).

1 Contents

GLOSSARY OF ABREVIATIONS

ABBREVIATION	**DEFINITION**
AE	Adverse event
ANCA	Antineutrophil cytoplasmic antibodies
anti-CCP	Anti-cyclic citrullinated peptide
anti-dsDNA	Anti-double stranded deoxyribonucleic acid
CI	Chief Investigator
CPI	Checkpoint Inhibitor
CRP	C-reactive protein
CRF	Case Report Form
eCRF	Electronic Case Report Form
CTCAE	Common terminology criteria for adverse events
CTIMP	Clinical Trial of an Investigational Medicinal Product
EDTA	Ethylenediaminetetraacetic acid
ESR	Erythrocyte sedimentation rate
FBC	Full blood count
GCP	Good Clinical Practice
hcG	Human chorionic gonadotropin
HTA	Human Tissue Act
IMID	Immune-mediated inflammatory diseases
ICF	Informed Consent Form
irAE	Immune-related adverse event
JRO	Joint Research Office
LFT	Liver function tests
MDT	Multi-Disciplinary Team
MRC	Medical Research Council
NCCC	Northern Centre for Cancer Care, Freeman Hospital, Newcastle

NHS	National Health Service
NSCLC	Non-small cell lung carcinoma
NuTH	The Newcastle upon Tyne Hospitals NHS Foundation Trust
NSirAE	Non-Significant Immune-Related Adverse Event
PI	Principal Investigator
PIS	Participant Information Sheet
RF	Rheumatoid Factor
SirAE	Significant Immune-related adverse event
SoC	Standard of Care
SOP	Standard Operating Procedure
SSMDT	Specialist skin cancer multidisciplinary team
SST	Serum Separator tube
TFT	Thyroid Function Test
TPO	Thyroid Peroxidase
TTG	Tissue transglutaminase
U&E	Urea and Electrolytes

1. BACKGROUND

Immune mediated inflammatory diseases (IMIDs) include common illnesses like rheumatoid arthritis (RA), inflammatory bowel disease and multiple sclerosis. They are conditions in which the immune system attacks an otherwise healthy body as if it were harmful. Treatments for them work better for some people than others and are not curative. Indeed, despite knowing a lot about *how* the immune system can damage the body, scientists understand remarkably little about *why* these diseases begin in the first place. Studying this "initiation phase" is difficult because "autoimmunity" typically begins before patients develop symptoms. Now, the use of a new class of drugs in the field of cancer medicine, called immune checkpoint inhibitors (CPIs), offers a way of overcoming this barrier for the first time.

CPIs are being used in an ever widening number of cancer types, being standard first line treatment in metastatic melanoma, non-small cell lung cancer, and with licensed indications in bladder, renal, head and neck and a sub-set of colo-rectal cancers, with multiple other tumour areas being explored in clinical trials.

CPIs work by interfering with signals transmitted by the body's cells that tell its own immune system they should not be targeted for cell death. By blocking the ability of cancer cells to do this, CPIs allow the immune system to attack and kill these cells – an approach that has transformed the outlook of many cancer patients[1]. In some patients, however, this strategy also makes the body's *normal* cells vulnerable to attack, and depending on the treatment used, between about 15 and 50% of CPI-treated

patients develop significant side effects that look like IMIDs – called immune-related adverse events (irAEs)[2]. Where these occur they usually do so within the first 10 weeks of treatment[3].

Studying these side effects in cancer patients may lead to a better understanding of why these adverse events are triggered, and which patients might also be vulnerable to the severe toxicities. It may also enable better understanding of these events in the general population who develop spontaneous auto-immune diseases as described above.

An MRC-funded study "BIOFLARE" is recruiting patients with stable IMID, who are monitored following the stopping of their immunosuppressive treatment for the development of a disease flare. It is likely that similar immune mechanisms or genetic vulnerabilities are also involved in patients who develop irAEs on CPIs. We plan to explore this concept investigating the immune system and genetics of patients receiving CPIs who do or do not develop immune-related side effects, using many of the same research techniques established for BIOFLARE. Allied to this, there is increasing evidence that changes in the balance of bacteria that live the skin and gut (the "microbiome") may affect people's risk of developing IMIDs in the general population. The microbiome may be just as important in explaining why some people receiving CPIs develop irAEs and others do not. We will therefore investigate this, because it may in future be possible to alter people's microbiome (for example by modifying the diet) to help prevent the development of irAEs and IMIDs more generally in at-risk individuals.

2. RATIONALE

Having established the required sampling techniques within an oncology clinic setting, the purpose of this study is to generate data from a substantive pilot cohort of 80 patients to support hypotheses that may be tested and/or validated in future investigations.

Our over-arching hypothesis is that a continuum of "latent auto-reactivity" exists within the general population, and immune and/or microbiome perturbation as a result of CPI therapy lowers the threshold above which transition to IMID occurs. We therefore propose that patients with advanced cancer receiving CPI treatment present a vital opportunity to close the aforementioned knowledge gap through study of a "synchronised human model of autoimmune induction." Specifically, our hypotheses are:

1. Prior to CPI commencement, measurable cellular immune parameters in the peripheral blood, and/or the microbiome of the skin or gut, predict irAE development.

2. Dynamic alterations in peripheral blood immune parameters and/or the microbiome presage irAE development.

3. Changes in peripheral blood immune parameters mirror tissue infiltrates observed in subsequent organ-specific immune pathology.

Although the main purpose of the current study is to develop a body of pilot data for future investigation, the investigators' published work has shown that pSTAT3 expression in circulating CD4+ T cells of early, untreated RA patients is increased compared to that seen in disease controls (Anderson and Pratt, Annals of the Rheumatic Diseases 2016). Our sample size is sufficient to address hypothesis 1, above, in relation to the measurement CD4+ T cell pSTAT3 specifically (see Section 10 of protocol).

3. OBJECTIVES AND OUTCOME MEASURES

3.1. Primary Objective

To establish a clinically well-characterised and immune-phenotyped inception cohort of patients with advanced cancer who are commenced on immune checkpoint inhibitor (CPI) therapy, thereby enabling the study of immune dysregulation that precedes immune-related adverse event (irAE) development.

3.2. Secondary Objective

To determine whether STAT3 phosphorylation in circulating CD4+ T cells of CPI recipients predicts irAE development at baseline and/or following initiation of treatment

3.3. Exploratory Objectives

i. To determine whether baseline and/or dynamic changes in microbiome composition in the skin or gut precede irAE development in CPI recipients.

ii. To identify immune cellular phenotypes whose dynamic frequency amongst CPI recipients predicts irAE development.

iii. Amongst CPI recipients who develop clinically significant colitis and/or skin dermatoses as adverse reaction(s) to treatment, to characterise immune infiltrates of lesional *versus* non-lesional tissue**.**

iv. To determine whether circulating cytokine profiles of CPI recipients predicts irAE development at baseline and/or following initiation of treatment

4. STUDY DESIGN

4.1. Study type

A single centre, prospective, longitudinal observational cohort study. All enrolled patients will receive treatment with combination or single agent CPI therapy (see below) routinely as Standard of Care (SoC; non-CTIMP).

4.2. Follow-up

Patients on combination (one of two ipilimumab and nivolumab regimens) CPI therapy, or single agent nivolumab, single agent atezolizumab, or one of two single agent pembrolizumab (3-weekly or 6-weekly) regimens will be followed up for up to 6 visits (9-10 months) of treatment (depending on treatment regimen), or until they develop a significant irAE (SirAE) or CPI treatment is stopped for any reason – whichever is sooner. Patients will undergo formal study clinical review at baseline and for the remainder of their participation in the study at each study visit, to coincide with routine care visits to hospital or at an End of Study visit if reached.

Study visits will be scheduled as shown in the tables below:

MELANOMA COHORT

STUDY VISIT	**2**	**3**	**4**	**5**	**6**
	Weeks				
Ipilimumab/Nivolumab	3	6	9	15	39
Nivolumab	4	8	12	16	40
Pembrolizumab (start 3-weekly)	3	6	9	15 or 18	36 or 39
Pembrolizumab 6-weekly	6	12	18	24	36

NON-SMALL CELL LUNG CANCER COHORT

STUDY VISIT	**2**	**3**	**4**	**5**	**6**
	Weeks				
Pembrolizumab 3-weekly x4 then 6 weekly	3	6	9	18	36
Atezolimumab 4-weekly	4	8	12	16	40

MESOLTHELIOMA COHORT

STUDY VISIT	**2**	**3**	**4**	**5**	**6**
	Weeks				
Nivolumab (3-weekly)/Ipilimumab (6-weekly)	3	6	9	15	36

In the event of any treatment delays the relevant weeks of the delay in treatment will be added to for study visits (e.g. if a melanoma patient was receiving Ipilimumab/Nivolumab and their 9 week treatment was deferred 3 weeks, then their study visit 4 would take place at 12 weeks (9 weeks + 3 weeks) with subsequent visits delayed to correlate with the new treatment schedule as closely as possible (e.g. study visit 5 would happen at 18 weeks and study visit 6 at 42 weeks). For visits 1 to 5, where omission of a treatment cycle for any reason means a scheduled study visit does not take place, that study visit may be rescheduled to occur at the subsequent treatment cycle if not already linked to any other study visit (+/- 7 days from when that cycle is scheduled).

If patients change CPI therapy prior to completing the scheduled 6 study visits, including a change between the therapies above, or to another anti-PD-1, anti-PD-L1 or anti-CTLA-4 CPI treatment/regimen, then they can continue on the study from cycle 1 of their new treatment until they have completed 6 scheduled study visits in total. Clinical reviews will include collation of detailed symptom directed questionnaires from patients, physical examinations and routine blood tests. Significant irAEs (SirAEs) will be defined according to strict criteria (see *Section 7.6.2*). Incident SirAEs may be recorded at an unscheduled visit. Research specific biological sampling will take place at baseline, the time of incident SirAE and, providing follow-up is on-going, at a maximum of 6 time-points per patient.

5. STUDY SETTING

Patients with malignant melanoma, non-small cell lung carcinoma (NSCLC) or mesothelioma, referred through the Specialist Skin Cancer or Lung Cancer Multidisciplinary Teams for systemic treatment at the Northern Centre for Cancer Care, will form the study population. Patients will be offered enrolment into the study if they are considered suitable for treatment *either* with combination ipilimumab and nivolumab *or* with single-agent (nivolumab, atezolizumab or pembrolizumab) therapy for their melanoma, NSCLC or mesothelioma.

For melanoma, combination therapy is currently the international standard-of-care (SoC) as first line treatment in metastatic disease for melanoma and is offered to all patients considered fit enough to receive it, with less fit patients, or stage 3 patients in the adjuvant setting, being offered single-agent therapy. Patients receiving the combination regime will have a maximum of 4 3-weekly doses of combination therapy, then, in responding patients, move to receiving maintenance nivolumab alone every 4 weeks, until disease progression, 6 weeks after completion of combination treatment. Those receiving a single-agent regime will have either**:** 3-weekly doses of pembrolizumab moving to 6-weekly after 9 or 12 weeks; 6 weekly pembrolizumab; or 4–weekly doses of nivolumab for the duration of follow-up.

For NSCLC, single-agent therapy is currently the international standard-of-care (SoC) as first line treatment in metastatic disease for NSCLC and is offered to all patients considered fit enough to receive it, in patients with >/=50% PDL1 expression. Patients receiving a single-agent regime will have either**:** 3-weekly doses of pembrolizumab moving to 6-weekly after 12 weeks; or 4–weekly doses of atezolizumab for the duration of follow-up.

For mesothelioma, combination therapy is currently an international standard-of-care (SoC) first line treatment in metastatic disease and is offered to all patients considered fit enough to receive it. Patients receiving the combination regime will receive 3-weekly Nivolumab and 6-weekly Ipilimumab.

The study will be managed from the Northern centre for Cancer Care, Freeman Hospital, Newcastle upon Tyne. Patient identification, recruitment and sample collection will take place in the Newcastle upon Tyne Hospitals NHS Trust, specifically the outpatient departments of the Northern Centre for Cancer Care, Freeman hospital, Newcastle upon Tyne.

Processing of biological samples will take place in the Translational and Clinical Research Institute, Newcastle University, UK and Clinical Laboratory Medicine, The Newcastle upon Tyne Hospitals NHS Foundation Trust. The majority of analyses will take place in these settings, although some specialist analyses (for example of stool samples) may take place at collaborating institutions.

6. RECRUITMENT OF PARTICIPANTS

6.1. Eligibility Criteria - Inclusion Criteria

1. Male or female patient ≥18 years of age.
2. Confirmed diagnosis of malignant melanoma, NSCLC or mesothelioma.

3. Shared decision by oncologist and patient to proceed with CPI treatment, *either* with the combination of ipilimumab and nivolumab, *or* with single-agent nivolumab, pembrolizumab *or* atezolizumab as standard of care.
4. Patient is judged as being capable of understanding the information sheet and of giving informed consent according to the Mental Capacity Act 2005.
5. Written informed consent to participate in the study.

6.2. Eligibility criteria - Exclusion Criteria

1. Known pre-existing autoimmune or immune-mediated inflammatory disease requiring immunomodulatory treatment, including (but not limited to) inflammatory bowel disease (Crohn's disease, ulcerative colitis) autoimmune endocrinopathy or hepatitis, vitiligo and inflammatory arthritis.
2. Received enteral or parenteral steroids within past month (topical, inhaled or intranasal permitted).
3. Previous treatment with CPI therapy.
4. Vaccination within the past 4 weeks, except COVID-19 vaccination permitted.
5. Known chronic infection.
6. Current pregnancy, or pregnancy planned within next 6 months
7. Inability to provide informed consent and/or undergo any of the procedures mandated by the study.

6.3. Note on vaccinations

Patients should continue to receive standard vaccinations per the UK vaccination schedule, and as clinical need dictates. Examples include seasonal influenza vaccination, travel immunisations, tetanus toxoid, shingles (herpes zoster vaccination). For all patients on CPI therapy it is advised that live vaccines are avoided, and this standard of care advice will be given to study participants also.

If a patient receives an immunisation during the course of the study, the date and formulation must be recorded on the case report form. Date and formulation of COVID-19 vaccine administered at any point prior to be baseline must be recorded on the case report form

6.4. Target Recruitment Size and Enrolment Overview

Local real-world data together with that presented as part of the European Society for Medical Oncology (ESMO) Guidelines for immunotherapy toxicity management[4] indicate that approximately 50% of combination CPI recipients and 10-15% of anti-PD-1 monotherapy recipients (nivolumab or pembrolizumab) will develop SirAEs, broadly classified as grade 3 or above by National Cancer Institute - Common Terminology Criteria for Adverse Events (CTAEv4). The current study is intended to generate a body of pilot data as a precursor to definitive, possibly multicentre studies, to corroborate predictive biomarkers of toxicity and/or targetable mechanisms of autoimmune induction amongst CPI recipients. From current projections we expect to enrol an approximately 40:60 ratio of combination:single-agent CPI recipients into our study. Based on the above information, and allowing for drop-outs and the exclusion of a small proportion of individuals who develop irAEs that are clinically significant but do not fulfil SirAE classification criteria (intermediate, or "non-significant irAEs," *NSirAEs*; see also Section 7.7), we

anticipate that a total of 80 enrolled participants will represent a suitable sample size for this pilot study, yielding a target of 19 patients experiencing SirAEs and 37 with unequivocally absent SirAEs. Further justification of this sample size is provided in *Section 10*. An overview of the study is depicted in *Figure 1*.

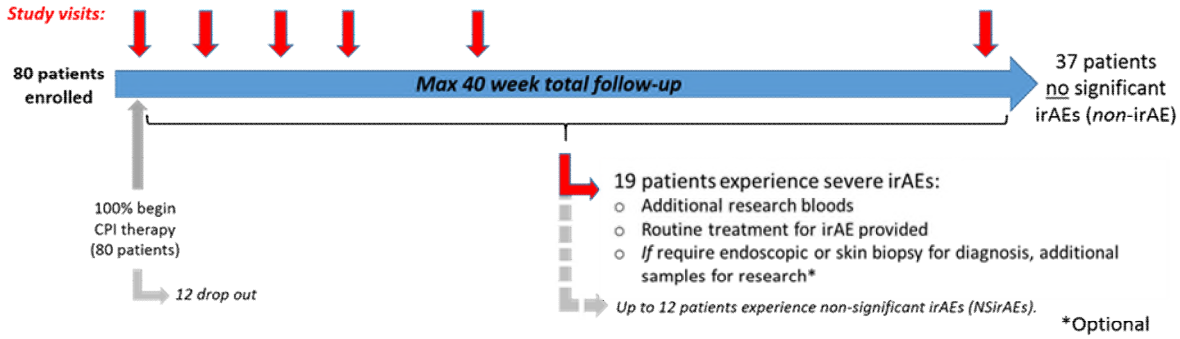

***Figure 1**. Enrolment plan for clinical study. Clinical assessments will take place at all patient visits, planned to coincide with routine hospital visits, and red arrows indicate time points at which research bloods will be drawn (providing irAE has not occurred); additional research bloods +/- biopsy will be obtained at the time of incident irAE.*

6.5. Closure of Study.

Recruitment to the study will close upon recruitment of the 66th evaluable patient. Evaluable patients are those who complete a baseline visit. The study will close on completion of all study visits and 18 month clinical follow-up data collection from last study visit for each participant, laboratory processing of participant samples and data analysis that address the study's primary, secondary and exploratory objectives.

7. STUDY PROCEDURES

7.1. Patient identification.

Potentially eligible patients attending the Northern centre for Cancer Care, Freeman Hospital, Newcastle upon Tyne will be identified by the doctor or nurse specialist that they see in an outpatient setting (for example this may be an oncology outpatient clinic or by the Specialist Skin Cancer Multidisciplinary Team (SSMDT)). The patient's direct care team who will identify patients for the study will all be part of the study team and on the study delegation log. Such patients may be provided with a participant information leaflet, and/or put in contact with a member of the clinical research team who may provide the same. Anyone identifying patients for the study will receive verbal consent from the patient to being contacted about being given further information about the study from a member of the study team, if not given directly at a standard clinic visit, and this will be documented in the patient's medical records and /or clinic letter. If the patient is interested in joining the study, then a member of the research team will arrange for an initial study baseline appointment, usually timed to coincide with a routine hospital appointment.

Alternatively, the referring healthcare professional may provide contact details (name, address and telephone number) of the patient to a study researcher, who will contact the patient by telephone to discuss the study further. If the patient would like to join the study, a participant information sheet will be posted to the patient and the researcher will arrange the initial study

appointment. Potentially eligible patients who are retrospectively identified by their clinical team can also be recruited to the study. In this instance, a member of their clinical team may arrange for a participant information sheet to be posted to the patient together with contact information for the research team to discuss further. If the patient wishes to join the study, then they can inform the research team who will arrange for the baseline appointment to proceed.

7.2. Consent

All potential participants will have the opportunity to discuss the study further with a study investigator before signing the consent form. At each study visit, the patient will be asked to confirm their willingness to continue participation in the study. Patients may withdraw their consent to participate in the study at any time, and this is made clear in both the informed consent form (ICF) and participation information sheet (PIS) (see corresponding versions of the ICF and PIS accompanying this document).

7.3. Baseline visit.

Potential participants attending their baseline visit will have the opportunity to discuss the study further with an investigator before signing the consent form. At this point patients are also given the opportunity to consent to the donation of stool samples, as an additional, optional procedure at each study visit (see additional consent form for optional stool sample donation). Baseline procedures below will take place within 14 days of receiving administration of the first dose of ipilimumab/nivolumab combination therapy or single agent nivolumab, atezolizumab or pembrolizumab. In view of the skin swab procedure at baseline and subsequent visits, patients are asked to avoid washing the forehead and any areas where they have a rash or skin changes 12 hours before study visit appointments, and to avoid the use of cosmetics on the day of visits requiring skin swab**s**.

The following procedures will be undertaken during the baseline period:

7.3.1 Collection of Demographic & Clinical Information

i. Date of birth
ii. Sex
iii. Ethnicity
iv Parity
v. General medical assessment

Includes:

a) Menopausal status/date of last menstruation
b) Past medical history
c) Current medications including dose and duration.
d) Immunisation status within the last year (approximate dates of relevant immunisations if known) including, but not limited to:
   (i) seasonal influenza and pneumococcal vaccination
   (ii) shingles (herpes zoster) vaccination
   (iii) travel immunisations
   (iv) COVID-19
e) Relevant allergies.

f) Tobacco smoking status
   (i) Current / previous / never smoker.
   (ii) If ever smoker: average daily cigarettes or quantity tobacco
   (iii) If ever smoker: year started and year stopped smoking (if applicable).
g) Current alcohol intake
h) General physical examination.
   (i) Height
   (ii) Weight
   (iii) Cardiovascular
   (iv) Respiratory
   (v) Abdominal
   (vi) Neurological
   (vii) Endocrine
   (viii) Skin
   (ix) Musculoskeletal
   (x) Other

7.3.2 Adverse event recording (this is performed on a continuous basis throughout the trial).

Skin adverse events may be photographed by a medical photographer or member of the study team.

7.3.3 Symptom-directed questionnaire (patient-reported data; Appendix 1)

7.3.4 Baseline blood samples (to be processed in NHS laboratory).

Standard of care bloods

i. FBC
ii. U&E
iii. LFT
iv. TFT
v. Magnesium
vi. hCG

Research bloods

i. ESR
ii. CRP

7.3.5 Research blood tests to be processed in Newcastle University research laboratory

i. Serum separator tube (SST) sample – 1 x 8.5 ml
ii. EDTA sample – 4 x 10ml
iii. Heparin sample – 1 x 10ml

7.3.6* Stool collection *(stool collection applicable only for the first 53 evaluable participants)

Supply of stool collection kit, including stool sample participant questionnaire and sampling instructions (for patients consenting to optional stool collection only; see *7.3*

and *Appendix 2* & *Appendix 3*). The stool sample should be collected within 7 days of receiving the stool kit and returned by post within 24 hours of collecting.

7.3.7 **Skin swabs *(skin swabs applicable only for the first 53 evaluable participants)***
Collected from the forehead, upper chest, upper back, dorsum of the hand and forearm with potential additional swab from a site of vitiligo-like depigmenting rash if occurs.

7.3.8 **Administration of SoC CPI drug to be recorded**

7.4. Follow-up visits

Follow-up visits will occur at:

Visits 2, 3, 4, 5 and 6 (9-10 months) of CPI treatment, scheduled as shown below at:

i. Weeks 3, 6, 9, 15 and 39 for patients receiving 3-weekly nivolumab in combination with ipilimumab (3- or 6-weekly) (melanoma)
ii. Weeks 4, 8, 12, 16 and 40 for patients receiving single agent nivolumab (melanoma)
iii. Weeks 3, 6, 9 then weeks 15 or 18 and 36 or 39 for single-agent 3-weekly pembrolizumab (melanoma)
iv. Weeks 6, 12, 18, 24 and 36 of treatment with single agent 6-weekly pembrolizumab (melanoma)
v. Weeks 3, 6, 9, 18 and 36 of treatment with pembrolizumab, 4x 3-weekly followed by 6 weekly (NSCLC)
vi. Weeks 4, 8, 12, 16 and 40 of treatment with single agent atezolizumab (NSCLC)
vii. Weeks 3, 6, 9, 15 and 36 for patients receiving) nivolumab in combination with ipilimumab (mesothelioma)

and

End of Study Visit, if applicable, i.e. if a patient's treatment ends before completing the follow up visits and for a reason other than a siRAE

Follow-up visits will always be within +/-7 days of the planned SoC CPI dose, and within 14 days of the decision to stop treatment if the patient has a SoC visit within this time, so will not require an additional hospital visit; timings have been selected to reflect this. At each follow-up visit, visits 2, 3, 4, 5 and 6, the participant will undergo the following procedures:

i. Consultation with a research clinician to confirm that the patient is happy to continue in the study.

ii. Documentation of current medications.

iii. Adverse event recording (this is performed on a continuous basis throughout the trial). AE assessment includes a requirement for the investigator to record whether, in their opinion, the AE under consideration represents a definite or possible irAE and, if definite, whether it fulfils criteria to be classified as a SirAE (see Section **7.6**, below).

iv. Female patients will be asked whether they might be pregnant at each study visit. Further urine samples for hCG (pregnancy) testing may be necessary after this discussion; if standard practice at local site is to use serum measurement then this is also taken.

v. Blood tests to be processed in NHS laboratory:

Bloods at each scheduled visit:

Standard of care bloods

a) FBC
b) U&E
c) LFT
d) Magnesium
e) TFT

Research Bloods

a) ESR
b) CRP

Research bloods within 14 days of decision to End treatment:

a) FBC (as research blood, if not being done as SoC)
b) ESR
c) CRP

vi *Research blood tests to be processed in Newcastle University research laboratory:*
a) Serum (SST) sample – 1 x 8.5ml
b) EDTA sample – 4 x 10ml
c) Heparin sample – 1 x 10ml

vii. Symptom-directed questionnaire (patient-reported data; *Appendix 1*)

viii. Physical exam, as applicable as per standard of care

ix. In the event of skin irAEs, these may be photographed by a medical photographer or member of the study team.

x. Administration of SoC CPI drug to be recorded.

At the visits shown below patients will also undergo the following procedures:

At visits 2, 3, 4 and 5 and End of Study:

xi. Supply of stool collection kit (for patients consenting to optional stool collection; see *Appendix 2* and *Appendix 3*). ***Stool collection applicable only for the first 53 evaluable participants***

xii. Skin swab collected from forehead, upper chest, upper back, dorsum of the hand and forearm with potential additional swab from a site of vitiligo-like depigmenting rash if occurs. ***Skin swabs applicable only for the first 53 evaluable participants***

7.5. Follow up post-end of study visits

Clinical data will be collected from participant's medical records, for up to 18 months following their last study visit, including:

i subsequent therapies

ii cancer response to treatment(s)

iii irAEs.

7.6. Unscheduled visits.

Patients will be continued to be managed by their usual care team, who will also be part of the study team. Patients may contact a member of their usual care team if they develop symptoms of concern between routine assessments, whereupon unscheduled visits may be undertaken at an oncologist's discretion, to determine whether a SirAE has occurred (see Section 7.**6** for definition of SirAE). Procedures to be undertaken at unscheduled visits include:

i. Consultation with a clinician from their routine care team, who will also be a member of the study team, to confirm that the patient is happy to continue in the study.

ii. Documentation of current medications

iii. Adverse event recording. AE assessment includes a requirement for the investigator to record whether, in their opinion, the AE under consideration represents a definite or possible irAE and, if definite, whether it fulfils criteria to be classified as a SirAE (see Section 7.6 below).

iv. Blood tests to be processed in NHS laboratory:

Standard of care bloods

a) FBC
b) U&E
c) LFT
d) Magnesium
e) TFT

Research Bloods

a) ESR
b) CRP

v. Symptom-directed questionnaire (patient-reported data; *Appendix 1*)

vi. Physical exam, as applicable as per standard of care

7.7. Incident irAEs & SirAE Classification.

At each visit (scheduled or unscheduled) investigators will be asked to record whether, based on the information available, a study participant has been the subject of an irAE and, if so, the extent to which the irAE is causally related to the CPI therapy they are receiving and whether, for the purposes of the study, it represents an irAE of special interest. Study investigator's reporting irAEs will all be members of the participants usual care team. It is important to note that recording of irAEs (including causality) in relation to CPI therapy is a requirement of the study but, since CPI therapy is SoC (and the study is non-CTIMP) there is *no* requirement to report these events to the sponsor / Research Ethics Committee (see also *Section 9*). Hence, irAEs are classified and recorded for research purposes according to the following criteria, being updated as necessary at subsequent study visits:

7.7.1 Severity and Relatedness classification

IrAEs are classified in terms of their severity and relatedness to CPI therapy as follows:

Severity:i. Mild: Symptoms noted but no disruption to normal daily activities
ii. Moderate: Symptoms sufficient to disrupt normal daily activities iii. Severe: Symptoms sufficient to prevent normal daily activities

Relatedness:
Causality of an irAE is defined according to relatedness to CPI therapy as follows:

Relationship	***Description***
Unrelated	There is no evidence of any causal relationship
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

7.7.2 Classification of irAEs: Significant and Non-significant irAEs.

IrAEs are further classified according to their "significance." Significant irAEs (SirAEs) are irAEs of special interest and represent an important outcome determinant of the study. Individuals who experience SirAEs will form one of 2 comparator groups in the analysis phase. The primary analysis will compare groups who developed SirAEs with those who did not at the end of the study. A secondary analysis will exclude individuals who developed possible/equivocal irAEs from the analysis; it is therefore necessary to develop criteria to identify such patients, and these are termed "Non-Significant irAEs" (nSirAEs).

SirAE Criteria:

SirAEs for the purposes of the current study are those affecting one of the organ systems indicated, considered "definitely" or "probably" related to administration of the CPI therapy, and meeting the criteria specific for that organ system as outlined below.

- Skin:
  - *Physician-diagnosed vitiligo*
  - *CTCAE≥grade 2 irAE, warranting topical/systemic steroids* and/or *warranting diagnostic skin biopsy in judgment of consulting dermatologist.*
- Gastrointestinal:
  - *Diarrhoea, CTCAE≥grade 2 persisting ≥3 days, warranting steroid treatment and/or endoscopically/histologically confirmed colitis. (Severity grade 2 is determined as per European Society for Medical Oncology [ESMO] guidelines[3]: 4-6 liquid stools per day over baseline or abdominal pain or nausea or nocturnal episodes).*
  - *CTCAE≥grade 2 hepatitis warranting steroids.*
- Endocrine:
  - *Symptomatic autoimmune thyroid disease confirmed biochemically.*
  - *Hypophysitis confirmed biochemically and/or radiologically.*
- Musculoskeletal
  - Objective inflammatory arthritis
- Other
  - *CTCAE Grade 3 or above.*

Where a definite irAE is determined to have occurred by the investigator but the above SirAE criteria are not fulfilled, the patient will remain in follow-up.

Non-significant irAEs (nSirAE) criteria:
In order to ensure clearly dichotomous groups for comparison at the end of the study, it may be necessary to exclude from downstream analysis data derived from individuals who experience irAEs of intermediate clinical significance, including aEs where relatedness to CPI therapy use is in doubt (possible). These include:

- AEs *not* considered immune-related in opinion of investigator, or those for which relatedness to CPI therapy is considered no more than "possible."

- irAEs of intermediate severity, defined:
  - Skin:
    - *irAE not requiring topical/systemic steroid.*

- Gastrointestinal:
  - *Possible irAE* not *requiring steroid treatment unless biopsy-proven.*
  - *Persistent CTCAE grade 1 hepatitis unless histologically confirmed.*
  - *Asymptomatic biochemical dysthyroidism not warranting treatment*
- *CTCAE grade 2 otherwise not covered by the SirAE criteria listed in* Section 7.6.

7.8. SirAE Procedures.

7.8.1 Primary and subsequent siRAEs

Where SirAE criteria are fulfilled the procedures outlined below should be undertaken, after which no further scheduled study visit procedures will normally be undertaken. However, should a study participant continue to take CPI therapy after this point and be subsequently noted, during routine assessments, to fulfil criteria for a gastrointestinal and/or skin SirAE (i.e. where the event[s] is/are different to the primary SirAE), or are having standard of care gastrointestinal or skin biopsies for either the same or different event to the primary siRAE, additional SirAE procedures specific to this/these events may be performed within the follow-up period of up to 6 visits (9-10 months) of CPI treatment (see Section 7.7 and 7.8 for details).

If an investigator determines that a patient fulfils criteria for a significant immune-related adverse event (SirAE), as defined in *Section 7.6*, additional *SirAE-specific procedures* will be undertaken as outlined below. SirAE-specific procedures will usually be undertaken at the time of the scheduled/unscheduled visit at which the incident SirAE is determined. However, there may be circumstances in which the SirAE can only be confirmed after the visit (e.g. where it is based on symptomatic thyroid dysfunction requiring biochemical confirmation); in such cases patients will be asked to attend for an additional "SirAE visit," specifically to undertake SirAE-related procedures. Optional research gut or skin biopsies (taken at the same time as standard of care biopsies) may also be obtained at a SirAE visit, for participant's developing a SirAE that falls into the gastrointestinal or dermatological category (see Section 7.6.2). SiRAE biopsy procedures are outlined in Section 7.8.

All SirAE visits should take place as close as possible to identification of the SirAE, and within 7 days in all cases, except for the collection of standard of care gut or skin biopsies due to SirAE(s) when a research biopsy is also being collected, when a delay of up to 14 days is permitted to complete all of the SirAE visit procedures listed below:

i. Symptom-directed questionnaire (patient-reported data; Appendix 1)

ii. Physical exam, as applicable as per standard of care

iii Research blood tests to be processed in Newcastle University research laboratory:

a. Serum (SST) sample – 1 x 8.5ml
b. EDTA sample – 4 x 10ml
c. Heparin sample – 1 x 10ml

iv. Supply of stool collection kit (for patients consenting to optional stool collection; see *7.3*, *Appendix 2* and *Appendix 3*). ***Stool collection applicable only for the first 53 evaluable participants***

v Skin swabs collected. ***Skin swabs applicable only for the first 53 evaluable participants***

The procedures outlined above are to be undertaken in addition to all other procedures mandated at the index scheduled/unscheduled visit at which the SirAE is determined to have occurred, unless a scheduled or unscheduled visit has already been performed within post 7 days (or 14 days if a research gut or skin biopsy is being performed) of identification of the siRAE, in which instance the procedures above should be carried out alone.

7.8.2 Optional 'special case' siRAE biopsies

If a standard of care gut or skin biopsy occurs later than 14 days post initial identification of an siRAE (for example, due to worsening or recurrence of the primary siRAE), or subsequent standard of care gut or skin biopsies are taken for any siRAEs whilst on CPI treatment, optional research biopsies will be allowed at any time point that standard of care biopsies occur, until what would have been the patient's final scheduled study visit if no siRAEs had occurred.

If a research biopsy occurs outside the 14 day initial identification of siRAE window for other siRAE study procedures, then research procedures below will be performed on the same day as the research biopsy (if not already performed within 7 days of the biopsy):i. Documentation of current medications.

ii Adverse event recording

iii. Female patients will be asked whether they might be pregnant at each study visit. Further urine samples for hCG (pregnancy) testing may be necessary after this discussion; if standard practice at local site is to use serum measurement then this is also taken

iv Blood tests to be processed in NHS laboratory:

Standard of care bloods, if applicable (FBC, U&E, LFT, Magnesium, TFT)

Research Bloods (ESR, CRP)

v Research blood to be processed in Newcastle University research
- a. Serum (SST) sample – 1 x 8.5ml
- b. EDTA sample – 4 x 10ml
- c. Heparin sample – 1 x 10ml

vi. Symptom-directed questionnaire (patient-reported data; Appendix 1)

vii. Physical exam, as applicable as per standard of care

Wherever possible/practical, research blood samples should take place prior to/simultaneous with administration of any systemic steroid therapy for treatment of a SirAE.

7.9. Optional SirAE Procedures: donation of gut biopsy at routine endoscopy and/or skin tissue at routine diagnostic skin biopsy.

7.9.1 Gut biopsies

If a participant develops a SirAE that falls into the gastrointestinal category (see *Section 7.6.2*) specialist input from a gastroenterologist will normally be requested as part of routine care according to internationally agreed guidelines[3]. If a lower gastrointestinal tract endoscopy (colonoscopy or flexible sigmoidoscopy) is recommended as part of this routine specialist gastroenterology input, participants will be asked by the study team if they would be willing for gut biopsies to be obtained for research during the procedure, over and above any samples obtained for routine diagnostic purposes (see additional consent form for optional gut biopsy).

Endoscopic procedures will be scheduled to take place on routine or acute lists at an endoscopy suite within NuTH by the consulting gastroenterologist and their team, and will be undertaken in accordance with local routine practice. Sedative medication may be administered to patients as part of these procedures according to local practice

7.9.2 Skin biopsies

If a participant develops a SirAE that falls into the skin category (see *Section 7.6.2*) specialist input from a dermatologist may be requested as part of routine care, resulting in a decision to undertake a skin biopsy for diagnostic purposes. Under such circumstances, participants will be asked by the study team if they would be willing for a single punch skin biopsy to be obtained for research during the procedure, over and above any samples obtained for routine diagnostic purposes (see additional consent form for optional skin biopsy).

7.10. Patient autonomy.

The patient will have full autonomy throughout this study.

The participant information sheet makes it clear that the patient may withdraw their consent at any time; for example they may opt to discontinue CPI therapy. The wishes of the patient will be respected, though the patient should then be discharged from the study and referred back to the clinical (referring) team.

Investigators should try to ascertain the reason for withdrawal and document this reason within the Case Report Form and participant's medical notes.

7.11. Withdrawal criteria

The clinical investigator may withdraw a participant from the trial at any time if this is considered necessary, and for any reason including:i. Symptomatic deterioration

ii. Participant withdrawal of consent or inability (through incapacity or otherwise) to provide consent for study-specific procedures to proceed.

iii. Significant protocol deviation or non-compliance, including failure to attend for >2 consecutive visits.

iv. An adverse event (AE) such that continuation of CPI therapy is no longer appropriate, even if SirAE criteria are not fulfilled

v. Termination of the clinical trial by the sponsor

vi. Investigator's discretion that it is in the best interest of the participant to withdraw

7.12. Schedule of events:

VISITS	VISIT 1[a]	VISIT 2[b]	VISIT 3[b]	VISIT 4[b]	VISIT 5[b]	VISIT 6[b]	END OF STUDY VISIT[c]	Unsched-uled Visit[d]	sirAE Visit[e]	Optional 'special case' siRAE biopsies[f]
TREATMENT CYCLE (Combination ipilimumab/nivolumab, single agent pembrolizumab, Nivulumab or atezolizumab, or switch between these therapies or to another CPI treatment / regimen	**CYCLE 1 -14 days (Baseline)**	**CYCLE 2 +/-7 days**	**CYCLE 3 +/-7 days**	**CYCLE 4 +/-7 days**	**CYCLE 5/ alternatively CYCLE 6 for NSCLC Pembrolizumab +/-7 days**	**CYCLE 6/7/9/11 or 12 (**dependent upon drug)[b] **+/-7 days**				
Procedures										
Discuss Study	X									
Sign consent form	X[g]								X[h]	X[h]
Pregnancy test	X	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]
Concomitant meds recording	X	X	X	X	X	X	X	X		X
General physical examination	X	X[j]	X[j]	X[j]	X[j]	X[j]	X[j]	X[j]		X[j]
Adverse event assessment	X	X	X	X	X	X	X	X		X
Questionnaire[k]	X	X	X	X	X	X	X	X		X
Blood test (NHS Lab)[l]	X	X	X	X	X	X	X	X		X
Research blood test[m]	X	X	X	X	X	X	X		X	X
Skin swabs[n]	X	X	X	X	X		X		X	
Stool Sample (optional)[o]	X	X	X	X	X		X		X	
Photography of skin irAEs (optional)[p]		X	X	X	X	X	X	X		
Skin Biopsy for research (optional)[q]									X	X
Endoscopic biopsies for research (consenting patients with GI SirAEs only)[r].									X	X

a Baseline procedures should be performed within 14 days of receiving of administration of the first dose of combination ipilimumab/nivolumab, or single agent pembrolizumab, nivolumab or atezolimumab

b Study visits to occur within +/-7 days of receiving CPI for visits 2, 3, 4, 5 and 6. Cycles of CPI treatment are scheduled to occur at:

i Nivolumab/Ipilimumab (melanoma) cycles 2, 3, 4, 5 and 6 (i.e. weeks 3, 6, 9, 15 and 39).

ii Nivolumab (melanoma) cycles 2, 3, 4, 5 and 6 (i.e. weeks 4, 8, 12, 16 and 40)

iii Pembrolizumab (start 3-weekly) (melanoma) cycles 2, 3, 4, 5 and 7 (i.e. weeks 3, 6 and 9, then week 15 or 18 and week 36 or 39)

iv. Pembrolizumab (6-weekly) (melanoma) cycles 2, 3, 4, 5 and 7 (i.e. weeks 6, 12, 18, 24 and 36)

v Pembrolizumab (NSCLC) cycles 2, 3, 4, 6 and 9 (i.e. weeks 3, 6, 9, 18 and 36)

vi Atezolizumab (NSCLC) cycles 2, 3, 4, 5 and 11 (i.e. 4, 8, 12, 16 and 40)

vii Nivolumab/Ipilimumab (Mesothelioma) cycles 2, 3, 4, 6 and 12 (i.e. weeks 3, 6, 9, 15 and 36)

c End of Study Visit, if applicable, i.e. if a patient's treatment ends before completing the follow up visits and for a reason other than a siRAE Visit to be completed within 14 days of the decision to end treatment

d An unscheduled visit is only required at the discretion of the investigator if the participant contacts the research team with a new possible irAE (see *Section 7.6*)

e Wherever possible the SirAE visit (and all of the listed procedures except optional gut biopsy) should be combined with the scheduled or unscheduled visit at which the SirAE is determined to have occurred, unless a scheduled or unscheduled visit has already been performed within post 7 days (or 14 days if a standard of care gut or skin biopsy with a research biopsy is being performed) of identification of the siRAE (see section 7.7), in which instance the siRAE procedures should be carried out alone. Some of the SirAE procedures may require a separate visit when the SirAE event can only be confirmed when blood results are available or for gastrointestinal or skin SirAEs where the patient consents to gut or skin biopsy.

f Optional 'special case' siRAE biopsies when standard of care gut or skin biopsy occur later than 14 days post initial identification of an siRAE whilst on CPI treatment. Optional research biopsies will be allowed at any time point that standard of care biopsies occur, until what would have been the patient's final scheduled study visit if no siRAEs had occurred. Research procedures listed in the above table will be performed on the same day as the research biopsy (if not already performed within 7 days of the biopsy)

g Main study and (optional) for stool sample donation and also (optional) for a skin biopsy where indicated

h For obtaining gut biopsies for research from patients experiencing gastrointestinal SirAEs undergoing lower gastrointestinal tract endoscopies as part of routine care under direction of consulting gastroenterologist (optional), or for a skin biopsy where indicated (optional)

i Female patients will be asked whether they might be pregnant at each study visit and a pregnancy test conducted if relevant

j Physical exam as applicable, as per standard of care

k Symptom-directed questionnaire; Appendix 1

l FBC, U&E, LFT, TFT, Magnesium (standard of care tests) ESR, CRP (non-standard of care tests). At an End of Study visit FBC to be done as a research blood, if not being done as standard of care.

m 4x10ml EDTA, 1x8.5ml serum, 1x10ml25eparinn

n. Skin swabs from forehead, upper chest, upper back, dorsum of the hand and forearm at each visit with potential additional swab from a site of vitiligo-like depigmenting rash if occurs. ***Skin swabs applicable only for the first 53 evaluable participants***

o Only for patients who consented at baseline to stool sample collection. ***Stool collection applicable only for the first 53 evaluable participants***

p Only for patients who consented at baseline to photography of skin irAEs

q Only for patients who consent to optional skin biopsy

r Only for patients who consent at time incident irAE determined

8. LABORATORY PROCEDURES

8.1. Blood sample processing and storage

Blood samples for FBC, U&E, LFT, TFT, Magnesium (standard of care tests) and ESR, CRP to be processed in by the clinical laboratory medicine services at The Newcastle upon Tyne Hospitals NHS Foundation Trust).

Research blood samples will be transported, processed and stored according to the procedures detailed in the research laboratory SOP booklet. Briefly, all samples will be pseudo-anonymised using a study-specific labelling system (e.g. MP-E001v1, denoting MEDALLION_Pilot E (EDTA) 001 (unique identifier of patient) v1 (visit 1). Samples will be maintained at room temperature for a maximum of 2 hours before processing and storage, during which time they will be transferred to the Translational and Clinical Research Institute laboratories, Newcastle University, for this purpose. Following analysis any residual sample will be stored at Newcastle University for future research, by transfer to the Newcastle Biomedicine Biobank. Peripheral blood will be extensively phenotyped, focussing on immune cell subsets and signalling pathways, using either conventional fluorescence-based flow cytometry and/or mass cytometry. Molecular biology techniques will be used to genotype patients and seek relevant transcriptomic signatures, in whole blood, that may be relevant

in the development of Immune-mediated inflammatory diseases. Storage of research blood samples will be in accordance with the Human Tissue Act 2004. Storage of these samples would be for a maximum of 10 years from the date of closure of the study. Research samples may also be analysed at specialist UK research laboratories (including commercial laboratories) elsewhere in the country, in Europe, or the United States.

8.2. Biopsy processing and storage

8.2.1 Skin biopsies

Skin biopsies will be fixed in formalin for tissue microscopy e.g. H&E, immunohistochemistry or immunofluorescence to understand immune architecture*8.2.2* Gut biopsies

Gut mucosal biopsy samples have a variable yield. Tissue collected will therefore be handled and stored according to the following hierarchy:

1. Approx. 2 biopsies fixed in formalin for tissue microscopy e.g. H&E, immunohistochemistry or immunofluorescence
2. Approx. 2-6 biopsies collected in storage media for either fresh disaggregation, or cryopreservation and later disaggregation, of cellular component for immune phenotyping by mass cytometry or high dimensional fluorescence cytometry
3. Approx. 2 biopsies in storage medium e.g. RNAlater for subsequent RNA extraction
4. Any additional samples left over from above may be used for additional assays e.g. 16S sequencing of tissue, or for other ethically approved projects

Skin and gut biopsy tissue will be transported, processed and stored according to the procedures detailed in the research laboratory SOP booklet, employing a labelling and pseudo-anonymisation procedure analogous to that described above (*Section 8.1*). Storage of samples will be in accordance with the Human Tissue Act 2004. Storage of these samples would be for a maximum of 10 years from the date of closure of the study.

8.3. Stool sample processing and storage (*applicable only for first 53 evaluable participants)*

Participants who consent to donate stool samples will be provided with written instructions on how to do this together with a stool collection kit which includes a unique, pseudo-anonymised study identification and visit number (to be assigned at the time of the visit). The kit includes a questionnaire capturing dietary intake information (see Stool collection kit instructions in Appendix 2 and questionnaire document in Appendix 3). Stool samples are collected into labelled sample tubes containing 70% ethanol, and patient volunteers will be asked to send these together with completed questionnaires in a pre-paid envelope, addressed for the attention of the research team at Newcastle University where samples will be stored in the freezer until the time of analysis. These samples may be transferred to a collaborating research team at another institute for specialist analyses, subject to a collaborative agreement and materials transfer agreement.

8.4. Skin swabs (*applicable only for first 53 evaluable participants*)

Prior to the study visit patients are asked not to wash their forehead, and any areas where they have a rash or skin changes, 12 hours before study visit appointments, and to avoid the use of cosmetics on the day of visits requiring skin swabs. Single-use sterile swabs are moistened in sterile medium before being rubbed gently over a 5x2cm area over the middle of the forehead, upper chest, upper back, dorsum of the hand and forearm at each visit with potential additional swab from a site of vitiligo-like depigmenting rash if occurs; cotton tips of the swabs are then broken off into MoBio Power Bead tubes filled with Powerbead solution. Tubes are pre-labelled with the participants' unique identifier and visit number, transported to the Newcastle University Translational and Clinical Research Institute laboratory and frozen at -80◦C until processing for bacterial DNA extraction.

9. SAFETY & GOVERNANCE

As this is a non-CTIMP study AEs and SAEs that are unrelated to study procedures (including those potentially related to CPI therapy as part of SoC) are not subject to reporting requirements.

The procedures that form part of the study are considered safe, and information about them is provided to patients in the PIS. Research blood sampling as part of the study will happen, wherever possible when blood is collected as part of participants' routine care, otherwise will be collected separately. Some people experience some mild discomfort and bruising as a result of the blood sample collection. Skin swabs are moistened with a solution containing salt and a mild detergent, and there is a small risk of localised skin irritation after the procedure.

Some participants who experience gastrointestinal irAEs will undergo lower gastrointestinal tract endoscopy with diagnostic biopsies as part of their routine care, and may consent to donate additional biopsies for research. Accidental perforation of the bowel is a rare but potentially serious complication of such procedures, including where biopsies are undertaken, occurring in approximately 1/1,500 colonoscopy procedures and 1/40,000 flexible sigmoidoscopy procedures. Accidental perforation of the bowel would normally lead to a requirement for emergency surgery and stoma formation. The other risks pertinent to this procedure are bleeding, discomfort, and risks associated with sedation for the procedure including drug reactions. Patients will be fully informed of all of these risks prior to signing the ICF for this voluntary aspect of the study.

All adverse events in relation to any study procedure will be recorded. In addition, adverse events that occur in association with the procurement, transport, testing and disposal of study specimens will be recorded; these may include:

- Samples used without appropriate consent
- Breach of data protection/confidentiality (e.g. sample bearing patient identifiers)
- Sample collected in incorrect sample tube
- Sample taken from wrong patient
- Sample labelling error
- An event which compromises sample integrity/sample damage
- Failure to trace sample/loss of sample

- Failure to dispose of samples appropriately
- Harm to staff or visitors

For the purposes of safety reporting any serious adverse event (SAE) that occurs in relation to any study *procedure* will be reported by the Chief Investigator or sponsor to the REC within 15 days of the CI becoming aware of the event.

The reporting of adverse events will be in adherence to:

1) The study Sponsor Newcastle Joint Research Office (NJRO) Standard Operating Procedure (SOP), NJRO-GOV-SOP-005 'Adverse Event Recording and Reporting for non-CTIMP studies v5', web-link:

https://g14784.ideagenqpulse.com/QPulseDocumentService/Documents.svc/documents/active/attachment?number=NJRO-GOV-SOP-005

2) Human Tissue Act-Research Sector SOP 'Adverse Event Reporting under the Newcastle University research sector Human issue Act licence (Ref. 12534), NJRO-TISS-SOP-003web-link: https://g14784.ideagenqpulse.com/QPulseDocumentService/Documents.svc/documents/active/attachment?number=NJRO-TISS-SOP-003

10. STATISTICAL CONSIDERATIONS

The primary objective of the study is to generate pilot data from a substantive cohort to support hypotheses that may be tested and/or validated in future investigations. A broad view of those measurable elements of immune activation (including the microbiome) that may presage autoimmune inset will be taken, but our secondary objective addresses a specific hypothesis in relation to CD4+ T cell pSTAT3. Exploratory objectives are hypothesis-generating. The statistical considerations have been revised in light of information that has been published during the course of the study and in the context of the impact from the Covid-19 pandemic.

10.1. Sample size justification.

Samples from patients with malignant melanoma, non-small cell lung carcinoma or mesothelioma, eligible for treatment with immunotherapy including combination ipilimumab and nivolumab, single-agent nivolumab, atezolizumab or pembrolizumab will be recruited and prospectively followed for up to a 10 month follow-up period. The pilot data arising from this study will provide proof-of-principle that biological differences measurable amongst the immune parameters of CPI recipients distinguish individuals with incipient irAEs from those who do not develop irAEs. The longitudinal nature of our study is a unique aspect, it will allow taking account of the exact time the irAEs occur. There are very few relevant similar datasets but one recent report found in a Swiss population 3 immune-related predictors of irAEs in melanoma patients treated with ICI: CXCL10, IL10 and Treg+ with hazards ratios of respectively 12.55, 4.0 and 3.41 (Nunez et al, 2023, PMID 36693381). Detecting effect sizes broadly similar to these with power 80% at significance level 0.05 requires a minimum of 21 events. At current rate (16 irAEs in 50), this means recruiting and following up a minimum of 66 patients.

With regard to Objective 2 (cross-sectional analysis) observations made on pSTAT3 expression measured in a subset of circulating CD4+ T cells of RA patients in remission who develop flare following treatment cessation versus those who do not, from the BIOFLARE study[5], were used to

model adequacy of the sample size. Assuming melanoma patients with and without irAEs will display a difference of similar magnitude in pSTAT3 at baseline, MEDALLION will have 80% power at alpha 0.05 (pilot data used currently in preparation for publication).

10.2. Analysis plan.

The primary analysis will evaluate immune markers' association with time-to-irAE using Cox proportional hazards regression. Significantly predictive markers will be graphically displayed by Kaplan-Meier curves after dichotomisation. Mixed effect logistic regression, a form of general linear mixed model (GLMM), will be applied to repeated measurements to test association between irAE occurrence and marker longitudinal trends. Statistically significant trends will be depicted graphically. GLMM framework is flexible and will allow to exploratively adjust key clinical covariates although their number will be limited by available sample size. Careful selection of these such as in stepwise or penalised modelling will be prioritised. We will also explore the ability to build predictive models for future irAEs using re-sampling techniques such as bootstrap, and cross-validation. Variable combination giving the highest prediction will be combined into a weighted score. Multivariable methods that do not assume linear relationships and exploit variable correlations such as supervised clustering and principal components analysis (PCA) will also be used.

Descriptive statistics will be used to describe recruitment rates, reasons for refusal to participate, refusal rates for gut biopsies and missing data. Recognising that the unavoidable need to permit Covid-19 vaccination during the course of recruitment and follow-up, preliminary cross-sectional and longitudinal analyses will focus on identifying whether parameters under investigation are unduly perturbed by vaccination, enabling modification of the foregoing analysis plan as appropriate. In respect of Objective 2, descriptive analyses (e.g. Mann Whitney-U test) will be applied to compare baseline CD4+ T cell pSTAT3 between patients developing a SirAE (as defined in *Section 7.7.2*) *versus* those that do not. We will also compare this variable at the time of incident SirAE (in those individuals that experience them) with those in non-irAE individuals at matched time point and the change between those time points. Exploratory analyses in relation to a wide range of immune parameters and the microbiome will be undertaken in a similar manner. The study design thereby ensures that, in addition to testing a specific hypothesis, our unique cohort will form a substrate for a wide range of exploratory analyses in relation to cellular immunity and the microbiome during the development of irAEs, which will afford new knowledge in their own right, whilst forming a basis for future validation studies. and seek to replicate key predictors reported in the Swiss cohort (Nunez et al, 2023, PMID 36693381).

11. DATA HANDLING

11.1. Data Collection Tools and Source Document Identification

Data including the number of patients screened, approached and interested in taking part will be collected via a log completed by staff conducting screening. Data for an individual patient will be collected by the study CI/PI or their delegated person and recorded in the secure, password-protected electronic case report form (eCRF) for the trial. Patient identification on the eCRF will be through a unique trial identifier number. A record linking the patient's name to the unique identifier number will be held securely at the trial site, and is the responsibility of the CI. As such, patients cannot be identified from eCRFs. The CI or delegated person will monitor completeness and quality of data recording in eCRFs and will correspond regularly with site CI (or their delegated team member) with the aim of capturing any missing data where possible, and ensuring continuous high quality of data. Patients will complete the paper assessment tools as required. The tools will also only be identified using the unique patient identifier number.

Data collected will reflect procedures outlined in Section 7, and will include:

- Date of birth
- Patient initials
- Gender
- Stage of disease
- Type of cancer
- Previous treatment
- Parity
- Details of CPI treatment given including dose and number of cycles
- irAEs according to classification criteria (*irAEs*, *SirAEs*, *NSirAEs*) outlined in *Section 7.**6*** with reference to CTCAE Criteria version 4.03, including their severity and relatedness to CPI therapy. This information will be serially updated by visit for all patients who remain in follow-up.
- Concomitant medication, including treatment for irAEs.
- Past medical history of auto-immune disease
- Outcome of irAEs
- Questionnaire responses
- Sample analysis results

Overall responsibility for data collection lies with the CI. Data collected on paper assessment tools will be entered onto a secure validated clinical data management system. A study identifier number will be used to identify participants on all paper data collection forms throughout the duration of the trial. Data will be handled, computerised and stored in accordance with the Data Protection Act 2018. No participant identifiable data will leave the study site. The quality and retention of study data will be the responsibility of the CI. All study data will be retained in accordance with the latest Directive on Good Clinical Practice (2005/28/EC) and local policy.

11.2. Access to data

Staff involved in the conduct of the trial, including the CI/PIand study staff involved in screening and intervention will have access to the site files for patients at the hospital study site. Clinical information shall not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor or regulatory authorities.

Secure pseudonymised electronic data may however be released to named members of the study team for analysis purposes. The CI/PI and trial site staff involved with this trial may not disclose or use for any purpose other than performance of the trial, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the trial. Prior written agreement from the Sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

11.3. Archiving

All trial data will be stored securely in accordance with Good Clinical Practive (GCP) and the Sponsor guidelines (Newcastle JRO SOPs). Any personal identifiable information will be stored at the study site or NuTH archiving facilities, for up to 5 years before secure disposal.

12. MONITORING, AUDIT & INSPECTION

The conduct of this trial will follow the most current approved version of this protocol, and must satisfy the requirements of GCP and relevant policies and guidelines issued by The Newcastle upon Tyne Hospitals NHS Foundation Trust and Newcastle University. Auditors from either partner institution will be able to request access to all study data as necessary for quality assurance purposes.

A serious breach of the project protocol or GCP is deemed to have occurred when a breach is likely to affect to a significant degree:

1. The safety or physical or mental integrity of the subjects of the study; or

2. "The scientific value of the study"

Suspected serious breaches should be notified to the Sponsor without delay. Following consultation with the Chief Investigator the Sponsor should, if it is deemed that a serious breach is suspected, notify the Research Ethics Committee and appropriate regulatory authorities within 7 calendar days of original notification to the Sponsor.

The Study may be subject to audit by representatives of the Sponsor or inspection by the Human Tissue Authority (HTA). The CI will permit trial-related monitoring, audits and regulatory inspection including access to all essential and source data relating to the trial.

13. ETHICAL AND REGULATORY CONSIDERATIONS.

This is an observational study that seeks to determine the feasibility of the proposed procedures ahead of a substantive multicentre investigation, as well as undertake a preliminary analysis of immune parameters that presage irAEs in malignant melanoma patients in receipt of CPI therapy. The majority of study procedures are extremely low risk to participants, their main consequence being the minor inconvenience of a prolonged hospital visit and, rarely, the requirement for an additional hospital visit.

A small proportion of patients are expected to undergo lower gastrointestinal tract endoscopy procedures as part of their routine care, and biopsies may be taken for research over and above those required for routine diagnostic purposes. This additional procedure may carry a very small additional risk of serious complication (see *Section 9*). All of the potential risks will be explained fully to patients at the time of informed consent.

a. Research Ethics Committee Review

The study has received favourable opinion from East of England - Essex Research Ethics Committee.

b. Regulatory Compliance

The study will be conducted in compliance with the approved study protocol, Good Clinical Practice (GCP) guidelines, the relevant Standard Operating Procedures and policies and procedures of the Sponsor (The Newcastle upon Tyne Hospitals NHS Foundation Trust).

c. Notification of Serious Breaches or serious adverse events

A serious breach is a breach of the protocol which is likely to effect to a significant degree the safety or physical or mental integrity of the subjects of the trial or the scientific value of the trial. The sponsor must be notified immediately of any incident that may be classified as a serious breach. The Sponsor (The Newcastle upon Tyne Hospitals NHS Foundation Trust) should be notified as soon as possible of any serious adverse event, any serious breach of security, confidentiality or approval conditions.

d. Data Protection and Patient Confidentiality

Patient-identifiable information will be kept strictly confidential with access limited to responsible personnel named on the study delegation log and who hold a suitable substantive/honorary contract or letter of access with the NHS Trust hosting that research site. Any data which is transferred out of the research site will be link-anonymised by use of a unique identifier specific to the MEDALLION-Pilot

study. Study CRFs and site files must be kept in a secure locked location within the research site. Storage of study data must comply with the 2018 Data Protection Act, and the storage and transfer of study data must also be approved by the Caldicott Guardian of the NHS Trust that hosts the research site.

Biological samples which are sent for processing at a clinical NHS laboratory will be labelled with patient identifiable information in line with local NHS Trust policy. Samples which are processed within a research laboratory must be labelled with the unique study identifier and not with any patient-identifiable information. Research samples must be stored in accordance with the 2004 Human Tissue Act.

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APPENDIX 1 *Symptom-directed questionnaire. Version 1.3 2nd June 2020*

Patient symptom checklist

Study:_____

Participant Study ID: MP__ __ __ Date:_____ Visit No. _____

We hope this check list will help you let us know about any potential side effects you may have from your immunotherapy treatment, and therefore allow the medical and nursing team looking after you advise on how to treat these. Your doctor will have explained to you that the side effects of immunotherapy can be very different to "traditional" cancer treatments because of the way it works. Immunotherapy allows your own immune cells to attack the cancer cells, unfortunately these cells can also attack other part of the body causing side effects. Not all patients get side effects, and when these happen which part of the body is affected can vary.

Symptom**s**	Enter Y =Yes N =No	If yes, score out of 10 (1=mild, 10=worst)	How often: R=rarely, O=occasionally, F=frequently, C=constantly
Skin			
Is your skin dry or itchy?			
Do you have a new or worsening rash?			
Is any of your skin peeling?			
Do you have any blisters or mouth ulcers?			
Digestive system			
Have you had any nausea or vomiting?			
Have you had any diarrhoea?			
Has there been any blood or mucus in your stools?			
Do you have any abdominal pain or cramps?			
Joints			

Have you had any new pains in your joints?			
Do you have any swollen joints?			
Liver			
Have you noticed any yellowing of your eyes			
Is your urine darker in colour			
Have you had any right sided abdominal pain			
Do you bruise more easily?			
Lungs			
Have you become more short of breath?			
Do you have a new dry cough?			
Endocrine system			
Have you had increased thirst?			
Have you felt hungrier?			
Are you feeling more tired than usual?			
Are you gaining weight easily?			
Have you had any headaches?			
Nervous system			
Have you noticed any weakness or tingling in your arms and legs?			
Have you developed any numb patches?			

Questionnaire reviewed by: PRINT NAME:_____

Signature: _____ Date: _____

APPENDIX 2 *Stool Collection Proforma. Version 1.2 10th Feb 2022*

Participant Study ID: MP___ __ ___ Date questionnaire completed:_____

Visit No. _____ Sample ID: MPS__ __ __ __

STOOL SAMPLE PARTICIPANT QUESTIONNAIRE

(For postage with stool sample)

Please complete the following short questionnaire and return it with your specimen in the postal box provided. Please DO NOT send us any personal information (e.g. name, address etc.).

Sample collection

1. Date you collected your stool sample:

2. Time you collected your stool sample:

3. Date you sent the stool sample:

4. How often do you pass a bowel movement (stool)? *Please tick as appropriate*

a. Over 3 times a day ☐ b. 1-3 times a day ☐

c. 1-3 times per week ☐ d. Less than once a week ☐

Current medication

1. Have you changed any medication use (including over-the-counter medications) since your last visit to the Oncology clinic? If yes, please provide details below:

2. In the last 6 months have you used antibiotic tablets by mouth?

a. Yes ☐

b. No ☐

c. Not sure ☐

If yes, what were the antibiotics called?

If yes, what were the antibiotics used for?

If yes, what date did you start the course and what was the duration of the course?

Diet *Please tick as appropriate*

1. Do you have any dietary restrictions (e.g. allergy, intolerances)?

a. Yes ☐

b. No ☐

If yes, please provide details?

2. What are your dietary preferences with respect to meat?

a. Standard diet (includes red meat, poultry and seafood) ☐

b. Standard diet with poultry and seafood (no red meat) ☐

c. Pescetarian (no red meat or poultry) ☐

d. Vegetarian (no meat) ☐

e. Vegan (no meat, dairy or animal products) ☐

3. Do you regularly take probiotics (yakult etc.)?

a. No ☐ b. rarely (1-3 times per week) ☐

c. 4-6 times per week ☐ d. Daily ☐

If you answered b, c or d please provide the probiotic supplement name: _____

4. In the last week have you consumed products that contain live cultures (e.g. yoghurt, kefir, sauerkraut)

a. No ☐ b. 1-3 times ☐

c. 4-6 times ☐ d. Daily ☐

5. In the last week how often did you eat the following food groups? *(Please tick the boxes that apply)*	0 times	1-3 times	4-6 times	Daily	If daily, please give the approximate number of times (e.g. 1)
Dairy products (e.g. milk, cheese etc.)					
Acidic fruits (e.g. oranges, apples, berries)					
Non-acidic fruits (e.g. bananas)					
Brassica vegetables (includes cauliflower, broccoli, cabbage and brussel sprouts)					
Non-brassica vegetables (those not listed above)					
Breads, cereals and starches (includes bread, rice, pasta)					
Red meat (includes beef, pork and lamb)					
White meat (e.g. chicken, turkey etc.)					
Fish					
Eggs					
Simple sugars in processed foods (includes chocolate, sweets, fruit juice, fizzy drinks, sugar in hot drinks etc.)					
Products containing caffeine (E.g. tea, energy drinks etc.)					

6. In the last week how often did you take vitamin / other supplements?	0 times	1-3 times	4-6 times	Daily	If daily, please give the approximate number of times (e.g. 1)
Vitamin B6					
Vitamin B12					
Vitamin C					
Vitamin D					
Zinc					
Magnesium					
Selenium					
Folate					

7. In the last week have you taken the following?	0 times	1-3 times	4-6 times	Daily	If daily, please give the approximate number of times (e.g. 1)
Antacids (heart burn tablets) or other stomach acid reducing drugs					
Laxatives					
Protein supplements					
Dietary meal replacements (e.g. dietary shakes)					

Further Information

If you would like more information or have any queries please contact the local study team on the information below:

Professor Ruth Plummer or the study team, Sir Bobby Robson Cancer Trials Research Centre, Freeman Hospital, Newcastle upon Tyne 0191 213 8476

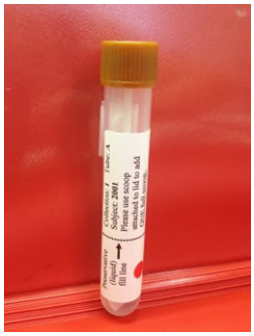
APPENDIX 3

Stool Sampling Instructions for Participants. Version 1.0 19th November 2018

Instructions for collecting stool samples

Please follow the instructions below on collecting your stool sample. If you have any questions please do not hesitate to contact Study team, Sir Bobby Robson Cancer Trials Research Centre, Northern Centre for Cancer Care, Freeman Hospital, Newcastle upon Tyne. 0191-**213 8476**. **Please collect your sample ideally within 7 days of receiving the kit and return your sample by post within 24 hours of collection.**

 + 	**Package contents.** You will have received a postal kit (box) that contains a labelled collection tube wrapped inside absorbent cushioning material, a pair of gloves and a paper stool catcher in a plastic envelope. There will also be a "DO NOT ACCEPT IF SEAL IS BROKEN" sticky label that will be used to secure the postal box after stool collection. The tube in the pack contains a small amount of liquid (ethanol) that is used to preserve the sample. The following is a step by step guide on how to collect and return your stool sample.
	**STEP 1**. Remove the stool catcher from its envelope and open it out according to its instructions. Paste it to the back of the toilet seat by using the adhesive strips without letting it touch the inside of the toilet. **STEP 2**. Deposit your stool directly onto the collection paper. Do **not** urinate onto the collection paper.

	**STEP 3.** Put on gloves. From the bio bag unscrew the tube that comes wrapped in absorbent cushioning. **Important:** *If there is no liquid in the tube do not continue to take a sample. Place the whole kit in general waste and contact Clinical Research Nurse team at the Bobby Robson Unit, Freeman Hospital, Newcastle upon Tyne.* 0191-**2138353** *to obtain a replacement kit.* *Please take care not to remove the backing from the yellow seal of the bio bag.* *Please take care not to spill any of the liquid preservative contained in the tube*. If you get any preservative (ethanol) on your skin, please wash with soap and water. Use the provided scoop (found within the tube) to transfer a SINGLE scoop of your stool from the collection container into the collection tube and screw the cap back on. There is no need to shake the tube. **STEP 4**. After collecting your sample, loosen the stool catcher at both ends, bring the ends together and deposit remaining stool and the paper collector into the toilet allowing a few minutes for the paper to soak.
	**STEP 5.** Replace the collection tube into the absorbant cushioning. Replace tube into the bio bag that comes as part of the postal pack and seal. Place the bio bag containing the tube into the postal box.
	STEP 6. Fill out the participant questionnaire and place in the postal box. Seal the box using the "DO NOT ACCEPT IF SEAL IS BROKEN" sticky label contained in the kit.
	**STEP 7.** Post the postal kit using regular royal mail services. There is no need to add any postage to the box.