CLINICAL STUDY PROTOCOL

Study Title:	A Phase II, Two-Stage, Trial of Pembrolizumab in Cancer of unknown primary - CUPem
Protocol Number:	C/37/2017
Product:	Pembrolizumab
Sponsor:	Imperial College London
EudraCT Number:	2018-001327-39
Development Phase:	II
Version Number:	V7.0
Protocol Date:	8th December 2021

This protocol has regard for the HRA guidance

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ABBREVIATIONS

Abbreviation	Explanation
AE	Adverse Event
ALC	Absolute Lymphocyte Count
ALT	Alanine aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate aminotransferase
CI	Chief Investigator
CNS	Central Nervous System
CR	Complete response
CRUK	Cancer Research UK
CSR	Clinical study report
CT	Computerised tomography
CTAAC	Clinical Trials Awards and Advisory Committee
CTCAE	Common Terminology Criteria for Averse Events
CTS	Clinical Trials Section
CTU	Clinical Trials Unit
CUP	Carcinoma of unknown primary
DCR	Disease Control Rate
DNA	Deoxyribonucleic acid
DSUR	Development Safety Update Report
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EDC	Electronic data capture
EORTC	European Organisation for Research and Treatment of Cancer
EUDRACT	European Clinical Trials Database
ECI	Event of Clinical interest
FFPE	Formalin Fixed Paraffin Embedded
GCP	Good Clinical Practice
GMC	Genomic Medicine Centres
HIV	Human Immunodeficiency Virus
HRA	Health Research Authority
IB	Investigator Brochure
ICD	International Classification of Disease
ICHTB	Imperial College Healthcare NHS Tissue Bank
ICMJE	International Committee of Medical Journal Editors
IEC	Independent Ethics Committee
IUD	Intrauterine Device
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
INR	International normalized ratio
IRB	Institutional Review Board
ir-RECIST	Immune-related
ITIM	Immunoreceptor tyrosine-based inhibition motif

ITSM	Immunoreceptor tyrosine-based switch motif
IV	Intravenous
JRO	Joint Research Office
LDH	Lactate dehydrogenase
mAb	Monoclonal Antibody
MDT	Multidisciplinary Team
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare Products Regulatory Agency
MRI	Magnetic resonance imaging
MSI	Microsatellite instability
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NHSE	National Health Service England
NSAIDs	Non-steroidal anti-inflammatory drugs
OS	Overall Survival
OTC	Over-the-counter
PD	Progressive Disease
PFS	Progression Free Survival
PR	Partial Response
PS	Performance Status
PT	Prothrombin Time
APTT	Activated Partial Thromboplastin Time
QLQ	Quality of Life Quotient
QoL	Quality of Life
Q2W	Every 2 weeks
Q3W	Every 3 weeks
RBC	Red Blood Cell
RECIST	Response Evaluation Criteria in Solid Tumours
SAE	Serious adverse event
SD	Stable disease (RECIST)
SSAR	Suspected Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Bacillus Tuberculosis
TILs	Tumour-infiltrating lymphocytes
TMG	Trial Management Group
TSC	Trial Steering Committee
ULN	Upper Limit of Normal
WBC	White Blood Cell
WGS	Whole Genome Sequencing

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TRIAL SUMMARY

Abbreviated Title	CUPem
Clinical Indication	A Phase II, Two-Stage, Trial of Pembrolizumab in Cancer of unknown primary
Trial Type	Single Arm, non-randomised; Two-stage; Hypothesis generating
Type of control	None
Route of administration	IV
Trial Blinding	N/A
Treatment Groups	The study initially opened with a two-cohort design: (i) First Cohort: One or more lines of prior therapy (ii) Second Cohort: First Line untreated CUP patients A substantial amendment in February 2022 has formally closed the second cohort, due to the significant challenges in recruitment caused by the ongoing COVID-19 pandemic as well as associated clinical factors.
Number of trial subjects	Following the closure of the second cohort, the first cohort sample size has been expanded to 31-67 patients. Recruitment will end once 67 patients have been recruited into cohort 1 or at the end of December 2022, whichever is sooner. The precise statistical implications of the eventual recruitment total are detailed (section 8).
Eligibility Criteria	The Eligibility Criteria are the same as used in the CUP-ONE trial in the UK, please see below. • Histologically confirmation of a diagnosis of CUP, with imaging and all diagnostic investigations confirmed as CUP within a CUP MDT. • ECOG Performance Status 0-2 • Patients must have disease that is not amenable to potentially curative options such as resection or radical radiotherapy • If patient's disease presentation precludes tumour biopsy (inaccessible or biopsy thought not to be in the patient's best interest), the patient is not study eligible.
Estimated recruitment period	2 years
Estimated duration of trial	3.9 years including set up, recruitment, follow up and close down.
Duration of Participation	6-8 months

1 BACKGROUND AND RATIONALE

1.1 Introduction

Carcinoma of unknown primary (CUP) is one of the ten most frequent cancer diagnoses and the 5th commonest cause of cancer death in the UK (CRUK mortality data for 2012 (ICD-10 codes C77-80)). Every day in the UK alone, 30 people die from CUP. The ICD-10 (C77-80) coding data show that since 2010, the incidence and mortality have remained static at 9,500 to 10,500 per year. Despite improving diagnostic techniques, only a 13% decline in mortality was achieved in the 6 years between 2006 and 2012. CUP remains a poor prognosis cancer, with median survivals of 8 months from diagnosis and a one year survival probability of 15-35%. Despite this, there are very few randomised clinical treatment studies and therefore little consensus on the optimal treatment regimens. There is no established second-line treatment regimen. The highly metastatic clinical presentation and heterogeneity of CUP at the clinical and pathological level, as well as the possible overlaps with some known primary solid cancers, has also been a practical and intellectual hindrance to the successful classification and treatment strategies.

In 2009/2010 CRUK funded the CUP-ONE Trial (EUDRACT ref 2008-000657-35) which was the first prospective clinico-translational study of cancers of unknown primary with the aim of collecting tissue biopsies in consenting patients and developing (with industry partners) the best rapid molecular classification for carcinomas of unknown primary, initially as a diagnostic validation, and correlation with clinical outcomes. (Wasan HS: CUP-ONE phase II clinico-translational study of Cancers of unknown primary – CRUK project grant C18607/ TRICC grant A7967 & CTAAC grant C18607 Glasgow CTU C18607).

The recruitment in the UK between 2010 and 2014 has led to a successful collaboration between many Cancer Networks and a comprehensive clinical and tissue database of 640 tissue samples. These data are currently being analysed. Preliminary data from the clinical treatment outcomes of the CUP-ONE Trial has been presented (*Wasan HS, J. Paul, M.C. Nicolson et al Ann Oncol (2014) 25 (suppl 4): iv397. doi: 10.1093/ annonc /mdu345.8)* and demonstrates that two-thirds of patients do not have any treatment offered because of a combination of performance status, lack of efficacy and toxicity of current cytotoxic regimens. Of the treated patients only about a third responds, with a median survival of 8 months for all the treated patients. There is thus a significant unmet need to change the treatment paradigm in CUP.

There has been recent agreement (March 2017) from UK NHSE to prospectively (deep) whole genome sequence 750 genomes from 250 patients within the 100k-genome project. The combination of this and integration with global projects (see below) will allow a more detailed understanding of the biology of CUP, which should apply in a tumour agnostic manner, potentially to all solid cancers that are highly metastatic at presentation. Within the NHS 100k genomes project, there is the ability to link the data to therapeutic interventions including clinical trials.

In addition, (Wasan HS) is a co-applicant in the current Australian Study (**SUPER: Solving Unknown Primary cancER;** *Professor David Bowtell, and Penny Schofield, are PI's for the Australian CUP genome sequencing project APP1048193– SUPER 2013 to 2016*

*Funding from Cancer Australia)***),** which have and are developing tools that might better assess this sensitively in a way that is meaningful to patients with one of its co-primary aims being a comprehensive psychosocial analysis of CUP Patients. We are thus working on better QOL tools for CUP patients.

1.1.1 Pharmaceutical and Therapeutic Background

Please also refer to the Investigator's Brochure (IB)/approved labelling for detailed background information on MK-3475. (*Edition 13, February 2017 and any subsequent revisions*).

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumour-infiltrating lymphocytes (TILs) in cancer tissue and favourable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / Fox P3+ regulatory T-cells seem to correlate with improved prognosis and long-term survival in many solid tumours.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumours to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signalling upon engagement of its ligands (PD-L1 and/or PD-L2).

The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signalling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signalling motifs, an immune-receptor tyrosine-based inhibition motif (ITIM) and an immune-receptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD 1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signalling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signalling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumours.

Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signalling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic

inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumour-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumour immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab has recently been approved in the United Stated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

1.1.2 Preclinical and Clinical Trial Data

Refer also to the pembrolizumab Investigator's Brochure *(Edition 16, 16 June 2018 and any subsequent revisions)* for Preclinical and Clinical data.

1.2 Rationale

1.2.1 Rationale for the Study and Selected Subject Population

The pathogenesis of CUP is poorly understood. The clinical presentation of a high and poorly differentiated, rapidly progressive tumour burden, without an obvious primary site, leads to an intriguing hypothesis that early transforming malignant and invasive CUP phenotypes, have preferential ability to metastasize rapidly, rather than grow locally initially as in most cancers. One possible explanation of these CUP phenotypes are 'immuno-evasive" leading to unchecked and rampant early spread. This would make the clinical testing of PD-1 inhibitors compelling given their remarkable success in other aggressive phenotypes to date.

The current standard of care for patients, as recently verified in the CUP-ONE trial is ECX (Epirubicin, Cisplatin and Capecitabine (Xeloda®)) chemotherapy administered intravenously and orally in the UK. Each cycle of ECX takes 21 days (3 weeks). However, this standard varies worldwide with alternatives being Gemcitabine and Cisplatin (or Carboplatin) and in the USA – the common first choice option is Paclitaxel with Carboplatin. However, the majority of patients do not get any treatment due to their diminished performance status at presentation (66% of CUP patients are PS2 or more). Overall, the prognosis for CUP patients remains very poor at a median of 6-9 months with conventional chemotherapy, which is poorly tolerated in the PS2 patients, which form the majority of CUP patients. There is thus an urgent need for newer therapeutic approaches in this high area of unmet need. Additionally, CUP is a paradigm for the heterogeneity seen in many solid tumours, when they become highly metastatic, as therapy resistance occurs and become rapidly lethal. Equivalent to ECX is the newer variation EOX (Epirubicin, Oxaliplatin and Capecitabine (Xeloda®)), where the cisplatin is substituted to Oxaliplatin, which is more convenient, shorter, less emetogenic and requires less intravenous hydration.

1.2.2 Rationale for Dose selection/Regimen/Modification

An open-label Phase I trial (Protocol 001) is being conducted to evaluate the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumours. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumour size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD has been identified to date. 10 mg/kg Q2W, the highest dose tested in PN001, will be the dose and schedule utilized in Cohorts A, B, C and D of this protocol to test for initial tumour activity. Recent data from other clinical studies within the MK-3475 program has shown that a lower dose of MK-3475 and a less frequent schedule may be sufficient for target engagement and clinical activity.

PK data analysis of MK-3475 administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life (refer to IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the proposed dose regimen of 2 mg/kg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumour burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

The rationale for further exploration of 2 mg/kg and comparable doses of pembrolizumab in solid tumours is based on: 1) similar efficacy and safety of pembrolizumab when dosed at either 2 mg/kg or 10 mg/kg Q3W in melanoma patients, 2) the flat exposure-response relationships of pembrolizumab for both efficacy and safety in the dose ranges of 2 mg/kg Q3W to 10 mg/kg Q3W, 3) the lack of effect of tumour burden or indication on distribution behaviour of pembrolizumab (as assessed by the population PK model) and 4) the assumption that the dynamics of pembrolizumab target engagement will not vary meaningfully with tumour type.

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab

showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

1.3 Rationale for Endpoints

1.3.1 Efficacy Endpoint

There are no established standards of care, consensus, or known benefits to second or more lines of therapy in CUP. Hence a new standard of care needs to be established which is well tolerated and beneficial. Any objective responses or disease control of any significant duration, seen in second line or further are rare so the simple observation of Pembrolizumab (as the test drug here) demonstrating any clinical benefit, where there are no other known treatment options would be an important step in further investigating this strategy in the first line setting.

Initially conceived as a two-cohort study, recruitment had proved challenging for cohort 2 (due to COVID issues). A decision was made in January 2021 for a substantial amendment to lift the recruitment cap in cohort 1 as the minimum effect in $\geq$ 1 participant had been observed and that, as there was no standard of care treatment worldwide for second-line CUP, there would be major interest in understanding meaningful standard of efficacy in cohort 1, as well as in cohort 2 for immunotherapy strategies.

Since this amendment recruitment continued to be difficult, and a new substantial amendment in February 2022 has formally closed the second cohort, due the effect of ongoing COVID-19 pandemic as well as associated clinical factors.

Following the closure of the second cohort, the first cohort sample size is now expanded to a range of 31-67 patients. Recruitment will end once 67 patients have been recruited into cohort 1 or at the end of December 2022, whichever is sooner.. The precise statistical implications of the eventual recruitment total are detailed in full within section 8.

2 OBJECTIVES AND HYPOTHESIS(ES)

2.1 Primary Objectives and Hypothesis

Objectives:

- Overall response rates by immune-related (irRECIST) and RECIST criteria:
  - Disease Control Rate (DCR), Overall Survival (OS) & Progression Free Survival (PFS).

Hypothesis:

Pembrolizumab has Efficacy in CUP:

- as a second-line treatment in terms Response which translates into improved PFS and OS.
- and treatment leads to rapid improvement in QOL in high metastatic disease burden.

2.2 Secondary Objectives and Hypothesises

Objective:

- Incidence of adverse events up to 8 weeks after the last dose of Pembrolizumab.
- Incidence of adverse events up to 8 weeks after the last dose of Pembrolizumab in Performance Status 2 (PS2) patients.

Hypothesis:

- Pembrolizumab is much better tolerated than conventional chemotherapy in CUP patients and PS2 patients may also benefit.

2.3 Exploratory Objective

Objective:

- Identification of genomic biomarkers predictive of immune response to Pembrolizumab.

Hypothesises:

- CUP patients have identifiable biomarkers predictive for Pembrolizumab efficacy.
- CUP patients have high mutational loads and this correlates with increased efficacy with Pembrolizumab.

3 STUDY DESIGN

3.1 Overall Study Design

An open label, non-randomised, single arm, sequential phase (two sequential cohorts) study, evaluating the preliminary efficacy of Pembrolizumab in Cancer of unknown Primary (CUP).

A substantial amendment in February 2022 has formally closed the second cohort, due to the significant challenges in recruitment caused by the ongoing COVID-19 pandemic as well as associated clinical factors.

Cohort 1:
Cohort 1 will enrol a minimum of 31 patients and a maximum of 67 patients, who have had at least one prior line / regimen of chemotherapy (at least 2 cycles) appropriate for CUP and who have not had a RECIST response to first-line chemotherapy, or are progressing after an initial response, or are treatment intolerant to first-line chemotherapy, due to unacceptable toxicity.

Cohort 2:
Cohort 2 is now formally closed. This cohort had sought to enrol patients who are chemo-naïve (first-line setting) for CUP, with a PS 0-2.
****

Patients will undergo screening procedures during a standard 28-days time window from initiation of the study, under standard GCP and informed consent. Restaging will be performed by computerized tomography using ir-RECIST criteria at 3 months from initiation of systemic treatment and on an 8 weekly basis thereafter until radiological proven disease progression or intolerance or patient choice.

The EORTC QLQ-C30 questionnaire (to assess the quality of life) will be done at baseline after 3 months and then at discontinuation of study treatment.

Correlative translational study samples (blood) and tissue (used for histological confirmation and to document PD-L1 expression) will be collected at baseline, and blood and serum samples monthly (after an informed optional consent and banked for retrospective immune-modulating and other biomarkers for future research and analysis). Patients will also be separately consented to the NHSE CUP 100K genome project that will be running in the UK by 2018, for deep sequencing of tumor tissue and germ-line, in centers participating in, or with access to the UK framework for this programme.

3.2 Study Treatment (Dosage, Administration and Duration)

The treatment to be used in this trial is outlined below in Table 1, further details can be found in Section 6.

Table 1: Trial Treatment

Drug	Dose/ Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period	Use
Pembrolizumab	200 mg	Q3W	IV infusion	Day 1 of each 3 week cycle	Experimental

Trial treatment should begin on the day of study entry (screening and consent completed) or as close as possible to the date on which treatment is allocated.

3.3 Dose Selection

The rationale for selection of doses to be used in this trial is provided in Section 1.2.2. Details on preparation and administration of pembrolizumab (MK-3475) are provided in the Pharmacy Manual.

3.3.1 Dose Modification (Escalation/Titration/Other)

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic aetiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 2 below. Also see Section 6.9 for supportive care guidelines.

Table 2: Dose Modification Guidelines for Drug-Related Adverse Events

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Diarrhoea/Colitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	Recurrent 3 or 4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose
	3-4	Permanently discontinue (see exception below)[a]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycaemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycaemia associated with evidence of beta cell failure	Resume pembrolizumab when patients are clinically and metabolically stable
Hypophysitis	2-4	Toxicity resolves to Grade 0-1. Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism	2-4	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted
Infusion Reaction	2[b]	Toxicity resolves to Grade 0-1	Permanently discontinue if toxicity develops despite adequate premedication
	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks. Treatment will be permanently discontinued if recurrent grade 2 pneumonitis
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	3-4	Permanently discontinue	Permanently discontinue
Neurological Toxicities	3-4	Permanently discontinue	Permanently discontinue
Myocarditis	1	Toxicity resolves to Grade 0	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	2-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[c]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.

[a] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.

[b] If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose.

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
[c] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.			

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

3.4 Timing of Dose Administration

Trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed in the Table of Assessments (Section 5.0). Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons.

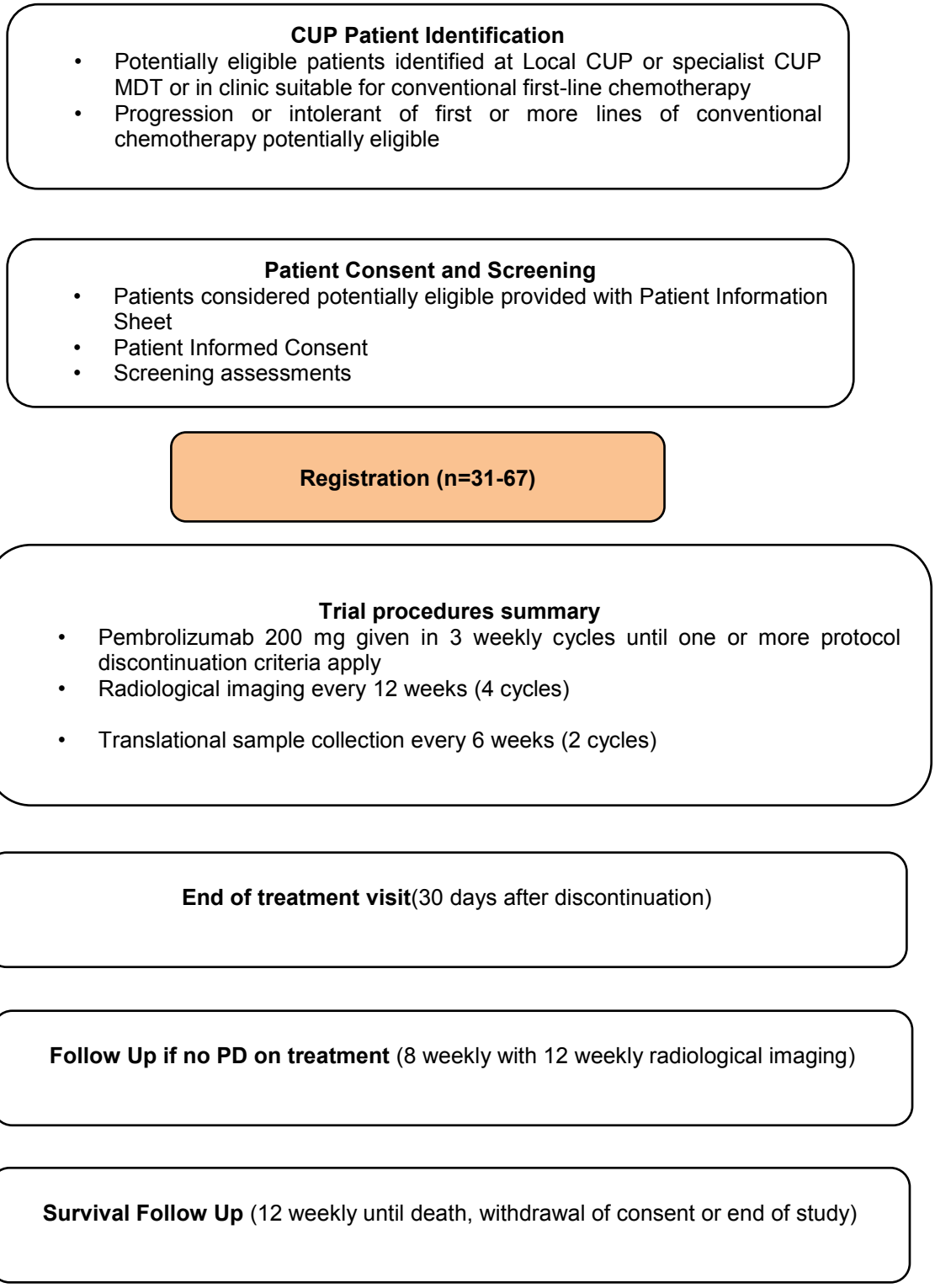
All trial treatments will be administered on an outpatient basis. Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution. This is an open-label trial; therefore, the Sponsor, investigator and subject will know the treatment administered from the outset.

Treatment will continue until unacceptable treatment-related toxicity, disease progression or patient and/or Investigator decision.

3.5 Study Flow Chart

Figure 2: Study Flow Chart



4 PARTICIPANT ENTRY

4.1 Study setting and population

This trial will be performed at three investigational sites in England, and recruit patients with cancer of an unknown primary. See full Eligibility Criteria below.

4.2 Inclusion Criteria

Patients who meet all of the following inclusion criteria will be considered eligible for this study:

1. Be willing and able to provide written informed consent/assent for the trial.
2. Be ≥ 18 years of age on day of signing informed consent.
3. Have measurable disease based on RECIST 1.1.
4. Be willing to provide consent for archival tumour (in the form of formalin fixed paraffin embedded (FFPE) block) or fresh tumour material (if judged technically feasible by radiologist) is mandatory for diagnosis and biomarker analysis.
5. Have a performance status of 0-2 on the ECOG Performance Scale.
6. Have had at least one prior line / regimen of chemotherapy appropriate for CUP (at least 2 cycles). Either did not have a RECIST response to first-line chemotherapy or progressing after an initial response, or are treatment intolerant to first-line chemotherapy due to unacceptable toxicity.
7. Adequate organ and bone marrow function (all screening tests should be performed within 10 days of treatment initiation):
   a. Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L
   b. Platelets ≥ 100 x 10^9/L
   c. Haemoglobin ≥ 9 g/dL (≥90 g/L) without transfusion or EPO dependency (within 7 days of assessment)
   d. Serum creatinine ≤ 1.5 x Upper Limit of Normal (ULN) or Creatinine clearance* ≥ 60 mL/min for patients with creatinine levels > 1.5 x ULN
   * Creatinine clearance should be calculated per institutional standard
   e. Serum total bilirubin ≤ 1.5 x ULN or direct bilirubin ≤ ULN for patients with total bilirubin levels >1.5 ULN
   f. Aspartate aminotransferase [AST] ≤ 2.5 x ULN (< 5 x ULN if liver metastases are present)
   g. Alanine aminotransferase [ALT]) ≤ 2.5 x ULN (< 5 x ULN if liver metastases are present)
   h. Albumin ≥ 2.5g/dL
   i. International Normalized Ratio (INR) or Prothrombin Time (PT) ≤1.5 X ULN (unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants)
   j. Activated Partial Thromboplastin Time (APTT) ≤1.5 X ULN (unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants).
8. Female subject of childbearing potential should have a mandatory negative serum pregnancy within 72 hours prior to receiving the first dose of study medication.
9. Female subjects of childbearing potential must be willing to use an adequate method of contraception as outlined in Section 6.9.2 – Contraception, for the course of the study through 120 days after the last dose of study medication.

Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

10. Male subjects of childbearing potential must agree to use an adequate method of contraception as outlined in Section 6.9.2 - Contraception, starting with the first dose of study therapy through 120 days after the last dose of study therapy.
    Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.

4.3 Exclusion Criteria

Patients who meet any of the following exclusion criteria will **not** be eligible for this study:

1. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.
2. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.
3. Has a known history of active TB (Bacillus Tuberculosis).
4. Hypersensitivity to pembrolizumab or any of its excipients.
5. Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
6. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or have not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.
    - Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.
    - Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.
7. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.
8. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.
9. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

10. Has a history of (non-infectious) pneumonitis/interstitial lung disease that required steroids or has current pneumonitis/interstitial lung disease.
11. Has an active infection requiring systemic therapy.
12. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.
13. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
14. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.
15. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.
16. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).
17. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).
18. Has received a live vaccine or live-attenuated vaccine within 30 days of planned start of study therapy.
    Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.

5 PROCEDURES AND MEASUREMENTS

5.1 Identification and recruitment of patients

Potential patients will be identified either by their direct care team at a participating investigational site (i.e. principal and/or co-investigator), or as the result of referral to the principal and/or co-investigator by another doctor based within or outside of that investigational site. Recruitment will take place as part of routine hospital outpatient clinic visits at participating investigational sites.

5.2 Study Screening Assessments

Written informed consent will be obtained before the patient undergoes any study specific procedures. Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

Each patient will undergo screening during the 28 days prior to admission to confirm eligibility, as per Table 3. Tumour assessments and other clinical data obtained as standard of care prior to consent may be used for the study provided they comply with the protocol specified timelines.

Once consent has been obtained the patient should be added to the InForm Electronic Case Report Form (eCRF), where a unique Screening ID will be allocated, which should be used in all correspondence during the screening period.

A complete record of all patients who enter screening for the study, and also those who go on to be enrolled, must be maintained at each site. The local investigator is responsible for ensuring that this record includes the allocated trial ID as well as the patient identifiable data including name, hospital number and date of birth.

Eligible patients who take part in the study must meet all of the listed inclusion criteria and none of the exclusion criteria.

5.3 Treatment Period

Patients will receive their study treatment until disease progression, unacceptable toxicity or withdrawal. Patients will be required to attend clinic for assessments to be performed during treatment as per Table of Assessments (Table 3).

5.4 End of Study Treatment Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. Subjects who are eligible for retreatment with pembrolizumab (Section 5.6) may have up to two safety follow-up visits, one after the Treatment Period and one after the Second Course Phase.

5.5 Follow Up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 8 weeks (± 7 days) clinically and before / by radiological imaging 12 weekly by radiologic imaging to monitor disease status. Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study or if the subject begins retreatment with pembrolizumab (see Section 5.6). Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

Subjects who are eligible to receive retreatment with pembrolizumab according to the criteria in Section 5.6 will move from the follow-up phase to the Second Course Phase when they experience disease progression.

5.5.1 Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be contacted by telephone every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.6 Second Course Phase (Retreatment Period)

Subjects who stop pembrolizumab with Stable Disease (SD) or better may be eligible for up to one year of additional pembrolizumab therapy if they progress after stopping study treatment. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the subject meets the following conditions:

- **Either**
  - Stopped initial treatment with pembrolizumab after attaining an investigator-determined confirmed Complete Response (CR) according to RECIST 1.1, and
    - Was treated for at least 24 weeks with pembrolizumab before discontinuing therapy
    - Received at least two treatments with pembrolizumab beyond the date when the initial CR was declared
      - **OR**
  - Had SD, Partial Response (PR) or CR and stopped pembrolizumab treatment after 24 months of study therapy for reasons other than disease progression or intolerability

AND

- Experienced an investigator-determined confirmed radiographic disease progression after stopping their initial treatment with pembrolizumab
- Did not receive any anti-cancer treatment since the last dose of pembrolizumab
- Has a performance status of 0 or 2 on the ECOG Performance Scale
- Demonstrates adequate organ function as detailed in Section 4.2
- Female subject of childbearing potential should have a negative serum or urine pregnancy test within 72 hours prior to receiving retreatment with study medication.
- Female subject of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study medication (Section

6.10.2). Subjects of child bearing potential are those who have not been surgically sterilized or have been free from menses for > 1 year.

- Male subject should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy.
- Does not have a history or current evidence of any condition, therapy, or laboratory abnormality that might interfere with the subject's participation for the full duration of the trial or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

Subjects who restart treatment will be retreated at the same dose and dose interval as when they last received pembrolizumab. Treatment will be administered for up to one additional year.

5.7 Study Termination

The study will be terminated 6 months after the last patient stops study treatment or up to 12 months after the last patient is enrolled, whichever is sooner.

5.7.1 Treatment after Study Termination

Following participation in the study, patient management will be according to local standard of care.

5.7.2 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of the criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete
2. Poor adherence to protocol and regulatory requirements
3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
4. Plans to modify or discontinue the development of the study drug.

In the event of MSD decision to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

5.8 Study Schedules during Treatment

Table 3 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. Treatment is divided into cycles of 21 days, with visits and assessments specified according to the relevant cycle.

Unless otherwise indicated, scheduled assessments may take place within ±3 days of the specified visit. Assessment days are relative to the start of study treatment i.e. Cycle 1 Day 1.

If a patient has a treatment break, please contact the Clinical Trials Section (CTS) for advice regarding the timing of research blood assessments. All other assessments, including laboratory safety assessments, vital signs and radiological imaging should continue to be performed.

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor and/or MSD for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

During the COVID-19 pandemic, patients whom are under ongoing treatment can be offered longer intervals of 1 to 2 weeks between treatment to avoid peak COVID-19 capacity issues.

Table 3: Table of Assessments

Trial Period:	Screening Phase	Treatment Cycles								End of Treatment	Post-Treatment		
						To be repeated beyond 8 cycles							
Treatment Cycle/Title:	Study Screening	1	2	3	4	5	6	7	8	Discon	Safety Follow-up	Follow Up Visits	Survival Follow-Up
Scheduling Window (Days):	-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 8 weeks post discon	Every 12 weeks
Administrative Procedures													
Pre-screening Visit													
Informed Consent	X												
Inclusion/Exclusion Criteria	X												
Demographics and Medical History	X												
Prior and Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X		
Trial Treatment Administration		X	X	X	X	X	X	X	X				
Trial Treatment Compliance		X	X	X	X	X	X	X	X				
Quality of Life Questionnaire (EORTC QLQ-C30)	X					X				X			
Post-study anticancer therapy status											X	X	X
Survival Status		X	X	X	X	X	X	X	X	X	X	X	X
Clinical Procedures/Assessments													
Review Adverse Events	X	X	X	X	X	X	X	X	X	X	X		
Full Physical Examination	X[a]	X			X				X	X	X		
Directed Physical Examination	X[a]	X	X	X	X	X	X	X	X	X	X	X	
Vital Signs and Weight	X[a]	X	X	X	X	X	X	X	X	X	X	X	
ECOG Performance Status	X[a]	X	X	X	X	X	X	X	X	X	X	X	
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory													
Pregnancy Test –Serum β-HCG	X[b]		X[c]		X[c]		X[c]		X[c]				
PT/INR and aPTT	X[d]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X	X	X	
CBC with Differential	X[d]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X	X	X	

Trial Period:	Screening Phase	Treatment Cycles								End of Treatment	Post-Treatment		
						To be repeated beyond 8 cycles							
Treatment Cycle/Title:	Study Screening	1	2	3	4	5	6	7	8	Discon	Safety Follow-up	Follow Up Visits	Survival Follow-Up
Scheduling Window (Days):	-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 8 weeks post discon	Every 12 weeks
Comprehensive Serum Chemistry Panel including full or directed tumor marker panel (+/- NET serum markers)	X[d]	x[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X	X		
Urinalysis	X[d]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X	X		
T3, FT4 and TSH	X[d]	x[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X	X	X	
Efficacy Measurements													
Tumor Imaging	X[f]					X[g]			X	X (if not done within 12 weeks prior)		X	
Tumor Biopsies/Archival Tissue Collection/Correlative Studies Blood													
Archival or Newly Obtained Tissue Collection	X[h]												
Correlative Studies Blood & Urine Collection	X		X		X		X		X	X	X		

Trial Period:	Screening Phase	Treatment Cycles								End of Treatment	Post-Treatment		
						To be repeated beyond 8 cycles							
Treatment Cycle/Title:	Study Screening	1	2	3	4	5	6	7	8	Discon	Safety Follow-up	Follow Up Visits	Survival Follow-Up
Scheduling Window (Days):	-28 to -1		± 3	± 3	± 3	± 3	± 3	± 3	± 3	At time of Discon	30 days post discon	Every 8 weeks post discon	Every 12 weeks

[a]In circumstances where screening assessments are conducted within 3 days prior to treatment initiation these can be used to cover both screening and C1D1.

[b]Mandatory negative blood serum pregnancy test at screening for patients of childbearing potential.

[c]In patients of childbearing potential:

- Mandatory negative serum pregnancy test within 72 hours prior to receiving study treatment
- Mandatory negative urine pregnancy test result available prior to receiving study treatment

[d] Lab tests (blood samples for haematology, clinical chemistry and urinalysis) for screening should be performed within 10 days prior to the first dose of treatment.

In circumstances where blood samples for haematology and clinical chemistry and urinalysis fulfils both the timeline of day -10 to 0 and 72 hours prior to C1D1 dosing these can be used for both screening and C1D1.

[e]On dosing days for all cycles, samples must be taken pre-dose (up to 72 hours prior to dosing).

[f] Baseline assessment should be performed no more than 28 days before the start of study treatment and ideally as close as possible to the start of the study treatment.

[g]Subsequent tumour assessments will be conducted every 12 weeks, with window for each assessment is ± 7 days.

[h]Tumour blocks / slides should be made available within 60 days of patient enrolment.

5.9 Procedures and Measurements

5.9.1 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

5.9.2 Demographic Data

Patient date of birth, race / ethnicity will be collected at screening.

5.9.3 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

5.9.4 Prior and Concomitant Medications Review

5.9.4.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication

5.9.4.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial and 30 days after the last dose of trial treatment. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.

The following details will be collected: drug name, reason for therapy, therapy dosage / units, frequency of therapy, route of administration, start and end date of therapy.

5.9.5 Disease Details and Treatments

5.9.5.1 Disease Details

The investigator or qualified designee will obtain prior and current details regarding disease status.

5.9.5.2 Prior Treatment Details

The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation and surgeries.

5.9.5.3 Post Study Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30 day Safety Follow-up visit must occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated the subject will move into survival follow-up.

5.9.6 Survival Status

A record of overall survival will be made.

5.9.7 Adverse Events (AEs) Review

Potential new or worsening AEs will be monitored throughout the study. Refer to section 7 for detailed information regarding the assessment and recording of AEs.

5.9.8 Physical Examination

5.9.8.1 Full Examination

A complete physical examination will be performed at screening and at other time-points, as indicated in Table 3. The following examinations should be undertaken in the complete physical examinations: general appearance, skin, head and neck, lymph nodes, thyroid, musculoskeletal/extremities, cardiovascular, respiratory, abdomen and neurological. The outcome of the examinations will be assessed as normal, abnormal (clinically significant or not clinically significant) or not done. Clinically significant abnormal findings should be recorded as medical history.

5.9.8.2 Direct Examination

For cycles that do not require a full physical exam as indicated in Table 3, a directed physical exam will be preformed as clinically indicated prior to trial treatment administration.

During the COVID-19 pandemic, this can be omitted if the risk of COVID-19 is higher than omitting the examination however, this should be conducted should other study assessments reveal the need for one.

5.9.9 Vital Signs and Weight

Vital signs including height, weight, blood pressure, body temperature, pulse and respiratory rate will be measured as indicated in Table 3. Vital signs may be assessed at any time during the visit; however, supine blood pressure and pulse should be measured after 10 minutes rest. Height will be measured at initial screening visit only.

5.9.10 ECOG Performance Status

Performance status will be assessed as indicated in Table 3 according to ECOG criteria as follows:

Table 4: ECOG Performance Status

0	Fully active, able to carry on all pre-disease activities without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature. For example, light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair 50% or more of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair

5	Death

5.9.11 Pregnancy Tests

A blood serum pregnancy test will be performed for women of childbearing potential at screening. In patients of childbearing potential, a urine pregnancy test must be performed prior to study treatment on Day 1 of every cycle with negative results available before study treatment is administered. A blood serum pregnancy test may be performed within 3 days prior to study treatment in place of the Day 1 urine test.

5.9.12 Laboratory Evaluations

Blood samples for haematology and clinical chemistry and urinalysis will be taken at scheduled visits and analysed at the local laboratory using standard methods for routine tests. **NB. Laboratory tests for screening or entry into the trial should be performed within 10 days prior to the first dose of treatment, o*n dosing days for all cycles, samples must be taken pre-dose (up to 72 hours prior to dosing)***. Samples must be processed, and results available, prior to study treatment administration to ensure and confirm an assessment of patient suitability. In circumstances where blood samples for haematology and clinical chemistry and urinalysis fulfils both the timeline of day -10 to 0 and 72 hours prior to C1D1 dosing these can be used to cover both screening and C1D1.

The following variables will be measured at the time-points indicated in Table 3.

- Biochemistry: Sodium [Na], potassium [K], chloride [Cl], bicarbonate [CO_2]‡, Uric Acid, calcium, phosphate, glucose), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase, albumin, Lactate dehydrogenase (LDH), total bilirubin, direct bilirubin (*If total bilirubin is elevated above the upper limit of normal)*), total protein, magnesium
- Haematology: White blood cell count (WBC) with differential (to include neutrophils, lymphocytes, monocytes, eosinophils, basophils), red blood cell (RBC) count haemoglobin (Hgb), haematocrit (Hct), Absolute neutrophil count (ANC), Absolute lymphocyte count (ALC) and platelet count.
- Coagulation: PT/INR and aPTT
- Urinalysis: Macroscopic assessment of the amount of protein, glucose, white blood cells and blood if they are present (levels should be recorded if available) and microscopic analysis should be performed if an abnormality is noted.
- Pregnancy test for patients of child-bearing potential (***If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required).*** Serum β-human chorionic gonadotropin (β-hCG) for patients of child-bearing potential
- Thyroid: Total triiodothyronine (T3), Free thyroxine (FT4), Thyroid stimulating hormone (TSH)
- Tumour Markers for CUP +/- any serum markers deemed to be relevant to disease monitoring, e.g. PSA, b-HCG, CEA, Ca19.9, Ca125, Ca153, AFP, NSE, SCC, S100, and Chromogranin A and B.
- ‡ ***If considered standard of care in your region.***

Laboratory values that have changed significantly from baseline and are considered to be of clinical concern must be recorded as an adverse event and followed up as appropriate.

5.9.13 Treatment Compliance (Patient Diary)

Patients will be required to keep a detailed record of all study treatment that they take in a patient diary. Date and time of administration and drug dose will be collected.

5.9.14 Quality of Life

The QOL instrument, EORTC QLQ-C30 version 3 will be administered as specified in Table 3 and must be completed before other assessments are performed or study drug is administered.

5.9.15 Tumour Assessments

Tumour assessments will be performed using CT contrast or MRI scans of the chest, abdomen and pelvis at the time-points indicated in Table 3. The same method used for assessment at baseline must be used at all subsequent time points.

Tumour assessment will include: if disease is measurable or non-measurable (at least one lesion must be measurable), date of assessment, imaging method used, site (breast, lung, liver, pleural effusion, local lymph nodes, distant lymph nodes, bone, brain or other site), longest diameter of each target lesion and sum of longest diameters for all target lesions.

Patient response to treatment will be assessed using RECIST v1.1. Tumour size, progression free survival and objective response rate will all be determined. The RECIST v1.1 (January 2009) guidelines for measurable, non-measurable, target and non-target lesions and the objective tumour response criteria (complete response, partial response, stable disease or progression of disease) are detailed in Appendix A.

Tumour size will be assessed at baseline and on scans at subsequent time points and recorded as the sum of the longest diameters of the target lesions. Baseline assessment should be performed no more than 28 days before the start of study treatment and ideally as close as possible to the start of study treatment; it should include all areas known for possible breast cancer metastases. Subsequent tumour assessments will be conducted every 12 weeks from C1D1, the window for each assessment is ± 7 days. During the period of the COVID-19 pandemic, this window period can be increased from -1 to a 2 week interval.

5.9.16 Tumour Biopsies and Archival Tumour Samples

Archival tumour (in the form of formalin fixed paraffin embedded (FFPE) block) or fresh tumour material (if judged technically feasible by radiologist) is mandatory for diagnosis and biomarker exploratory analysis. The tumour biopsy procedure will be performed by core needle, under radiological guidance if indicated, or surgically, if appropriate.

Tissue should preferably be from diagnosis; however, FFPE blocks from secondary debulking surgery and/or recurrence are also acceptable. If it is not possible to obtain the entire tumour block, 20 ordinary unstained slides (5µm sections) may be provided instead.

Detailed instructions for the collection, processing, and shipment of the fresh and archival tumour biopsy samples are provided in the Laboratory Manual.

Tumour blocks / slides should be made available within 60 days of patient enrolment for histopathology review and further biomarker analysis as detailed in the Laboratory Manual. The analysis may vary as new evidence and technologies come to light during the trial process and will be updated in the Laboratory Manual for this study.

5.9.16.1 Tissue Biomarkers

Pre-treatment diagnostic tumour biopsy (2 cores minimum) at screening must be available. This is also mandated within the NHSE 100K genome project which all patients will be offered a separate consent.

Tissue specimens will be utilized for as a minimum:

1) Immunohistochemical studies for intra-tumoural expression PD-L1.
2) MSI by immunohistochemistry and DNA POL mutations
3) Part of the tissue will be flash frozen in liquid nitrogen and utilized for genomic sequencing within the 100K-genome project (patients are offered a separate optional consent for the 100k- genome project), the data from which can be linked to this and other CUP trials. (This is not costed in this application).

5.9.17 Research Blood and Urine Samples

Research bloods and urine sample for this study are optional, and are as follows:

- Correlative translational study samples (blood and serum) will be collected at screening, cycle 2 and then every other cycle, at end of treatment and then at safety follow up visit. The samples will be banked for retrospective immune-modulating and other biomarkers.

- Serum and urinary metabolomic profiling – at screening, cycle 2 and then every other cycle, at end of treatment and then at safety follow up visit. 1H NMR Global Metabolic Phenotyping followed by validation using mass spectrometry (LC-MS) will be carried out in house taking advantage of the Imperial College expertise within the National Phenome Centre. This sub-project is already running within the cancer department at Imperial for many tumour types. In this context, metabolomic profiling will be looked at in the context of responders to Pembrolizumab.

The date of collection and sample IDs will be recorded on the eCRF.

5.9.18 Chain of Custody of Biological Samples

In all cases, patients will be consented for the collection and use of their biological samples and a full chain of custody will be maintained for all samples throughout their lifecycle.

The investigator at each site is responsible for maintaining a record of full traceability of biological samples collected from patients while these are in storage at the site, either until

shipment or disposal. Any person(s) responsible for temporarily holding samples, e.g. sub-contracted service provider keeps full traceability of samples from initial receipt of sample to further shipment or disposal (as appropriate).

Imperial College keeps overall oversight of the entire lifecycle through internal procedures and monitoring of study sites.

Archival tumour blocks/slides will be returned to source at the end of the study or earlier, upon request, if required.

Samples retained for further use will be registered with the Imperial College Healthcare NHS Tissue Bank (ICHTB).

6 TREATMENTS

6.1 Investigational Medicinal Product Details

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations. Clinical Supplies will be provided by MSD as summarised in Table 5.

Table 5: Product Descriptions

Product Name & Potency	Dosage Form
Pembrolizumab 100 mg/ 4mL	Solution for Injection

6.2 Labelling and Packaging

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

6.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

6.4 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Receipt and dispensing of trial medication must be recorded by an authorised person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

6.5 Compliance

The importance of treatment compliance should be emphasised to the patient. Each patient will be given a patient dairy to note the date of their treatment and the dose of study treatment administered.

6.6 Accountability

The investigator is responsible for keeping accurate records of the clinical supplies received from MSD or designee and the amount administered to each patient.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

6.7 Concomitant Medications/Vaccinations (allowed and prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or

vaccination may be required. The investigator should discuss any questions regarding this with the Chief Investigator of this study, who will seek advice from the MSD Clinical Team if required. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

6.7.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the electronic case report form (eCRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

6.7.2 Prohibited Concomitant Medication

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response, relapse) of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy
- Live or attenuated vaccines within 30 days before the first dose of study intervention and while participating in the study. Note: Any licensed COVID-19 vaccine (including for Emergency Use) in a particular country is allowed in the study as long as they are mRNA vaccines, adenoviral vaccines, or inactivated vaccines. These vaccines will be treated just as any other concomitant therapy. Investigational vaccines (i.e., those not licensed or approved for Emergency Use) are not allowed.
- Systemic glucocorticoids except when used for the following purposes:
    - To modulate symptoms of an AE that is suspected to have an immunologic etiology
    - For the prevention of emesis
    - To premedicate for IV contrast allergies
    - To treat COPD exacerbations (only short-term oral or IV use in doses >10 mg/day prednisone equivalent)
    - For chronic systemic replacement not to exceed 10 mg/day prednisone equivalent
- Other glucocorticoid use except when used for the following purposes:
    - For topical use or ocular use
    - Intraarticular joint use
    - For inhalation in the management of asthma or chronic obstructive pulmonary disease

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial.

Subjects may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describes other medications which are prohibited in this trial.
There are no prohibited therapies during the Post-Treatment Follow-up Phase.

6.8 Rescue Medication and Supportive Care

6.8.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation the event is determined not to be related, the investigator does not need to follow the treatment guidance (as outlined below). Refer to Section 3.3.1 for dose modification.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

- **Pneumonitis**:
  - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
  - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
  - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

- **Diarrhea/Colitis**:
  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).
  - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
  - For **Grade 2 diarrhea/colitis,** administer oral corticosteroids.

- For **Grade 3 or 4 diarrhea / colitis,** treat with intravenous steroids followed by high dose oral steroids.
- When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**
  - For **T1DM** or **Grade 3-4** Hyperglycemia
    - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
    - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis**:
  - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
  - For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hyperthyroidism or Hypothyroidism**:
  Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.
  - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyronine, is indicated per standard of care.
  - **Grade 3-4** hyperthyroidism
    - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hepatic**:
  - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).

- Treat with IV or oral corticosteroids
- For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
- When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- **Renal Failure or Nephritis**:
  - For **Grade 2** events, treat with corticosteroids.
  - For **Grade 3-4** events, treat with systemic corticosteroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.
  Table 4 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 6: Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion);	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen	No subsequent dosing

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

6.9 Diet, Activity and Other Consideration

6.9.1 Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhoea, nausea or vomiting.

6.9.2 Contraception

Pembrolizumab may have adverse effects on a foetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.
For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they are either:
(1) Postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);
OR
(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;
OR
(3) has a congenital or acquired condition that prevents childbearing.
Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:
(1) practice abstinence† from heterosexual activity;
OR
(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are‡:

- Single method (one of the following is acceptable):

- intrauterine device (IUD)
- vasectomy of a female subject's male partner[¥]
- contraceptive rod implanted into the skin
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion

[†]Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and IECs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

[‡]If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

[¥]Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the female subject of child-bearing potential land that the vasectomised partner has received medical assessment of the surgical success.

Subjects should be informed that taking the study medication may involve unknown risks to the foetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

6.9.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy should be reported to the Sponsor and to MSD without delay; within 24 hours of site becoming aware to the Sponsor and within 2 working days to MSD if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the foetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor, who will then report to MSD (see Section 7.8).

6.9.4 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrolment.

6.9.5 PERMANENT DISCONTINUATION OF STUDY TREATMENT AND WITHDRAWAL FROM STUDY

Subjects may withdraw consent at any time for any reason or be permanently discontinued from study treatment at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrolment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons.

6.9.6 PERMANENT DISCONTINUATION OF STUDY TREATMENT

Patients may discontinue study medication for the following reasons:

- Confirmed radiographic disease progression[1]
- Unacceptable adverse experiences as described in Table 2
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Non-compliance with trial treatment or procedure requirements
- Completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later[2]
- Confirmed complete response (CR) and treated for at least 24 weeks with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared.

[1]A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved.

[2]24 months of study medication is calculated from the date of first dose. Subjects who stop pembrolizumab after 24 months may be eligible for up to one year of additional study treatment if they progress after stopping study treatment provided they meet the requirements detailed in Section 5.6.

Date of permanent discontinuation and the reason will be recorded.

6.9.7 Withdrawal from Study

Withdrawal from the study refers to discontinuation of study treatment and study procedures and can occur for the following reasons:

- Patient decision
- Loss to follow-up
- Death
- Administrative reasons.

After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7. Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. Subjects who are discontinued from treatment, but have not progressed at that time, will still be under follow-up for study events of interest (unless lost-to-follow-up or withdrawal of consent). After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

If a patient dies whilst participating in the study a "Statement of Death" eCRF must be completed. The following details will be collected: date of death, whether autopsy performed, whether death was related to the disease under investigation, primary cause of death, secondary cause of death, and any other details.

6.9.8 Procedures for Withdrawal from Study

If the patient is withdrawn from the study the date of withdrawal and the reason must be recorded. When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation, unless the patient is too unwell to attend the clinic. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7. Subjects who a) attain a CR or b) complete 24 months of treatment with pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 5.6. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (Section 5.4) and then proceed to the Follow-Up Period of the study (section 5.5).

6.9.9 Discontinuation of Study Therapy after Complete Response

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR that have been treated for at least 24 weeks with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared. Subjects who then experience radiographic disease progression may be eligible for up to one year of additional treatment with pembrolizumab via the Second Course Phase at the discretion of the investigator if no cancer treatment was administered since the last dose of pembrolizumab, the subject meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is open. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation. Additional details are provided in Section 5.6.

7 PHARMACOVIGILANCE

7.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the trial medication, whether or not considered related to the IMP.

Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a pre-existing condition that is temporally associated with the study treatment, is also an adverse event.

7.1.1 Disease Progression

Disease progression is a worsening of a patient's condition attributable to the disease for which the study medication is being given. This may be an increase in severity of the disease or increase in the symptoms of the disease. The development of new, or progression of existing metastasis to the primary cancer under study should be considered as disease progression and not an AE. Events that are unequivocally due to disease progression should not be reported as AEs during the study.

7.2 Adverse Event recording

AEs will be collected throughout the study, from the point of consent until the Safety Follow Up visit*; they will be followed up according to local practice until the event has stabilised or resolved, or the follow-up visit, whichever is sooner. Serious Adverse Events (SAEs) will also be recorded throughout the study. See Appendix D for further details.

At the Safety Follow Up visit, any AEs of Grade > 1 which remain unresolved will be followed up by the Investigator until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first, but without further recording in the eCRF.

*SAEs, including death that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

Details to be collected in the eCRF for each AE will include:

- AE description
- Date of onset and date of resolution
- Severity
- Seriousness
- Relationship to study treatment

7.2.1 Severity of Adverse Events

Severity is a measure of intensity; whereas seriousness is defined by the criteria in section 7.4.

Severity will be assessed using the grading scales found in the National Cancer Institute CTCAE version 4.03 (June 2010) for all adverse events with an assigned CTCAE term.

For those events without assigned CTCAE grades, the recommendation on page 1 of the CTCAE that converts mild, moderate and severe into CTCAE grades should be used. A copy of the CTCAE version 4.03 can be downloaded from the Cancer Therapy Evaluation Program website (http://ctep.cancer.gov).

Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event eCRF.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

7.2.2 Causality of Adverse Events

The Investigator will assess causal relationship between the study treatment and each AE.

Unrelated:	No evidence of any causal relationship
Unlikely:	There is little evidence to suggest there is a causal relationship (e.g. 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 patient'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 patient's clinical condition, other concomitant treatments).
Probable:	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definite:	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

7.3 Abnormal Laboratory Test Results

All clinically important abnormal laboratory test results occurring during the study will be recorded as adverse events. The clinically important abnormal laboratory tests will be repeated at appropriate intervals until they return either to baseline or to a level deemed acceptable by the investigator and the clinical monitor, or until a diagnosis that explains them is made.

7.4 Serious Adverse Events (SAE)

7.4.1 Definition of SAE

An SAE is defined as any event that

- Results in death;
- Is life-threatening*****;
- Requires hospitalisation or prolongation of existing inpatient's hospitalisation******;
- Results in persistent or significant disability or incapacity;
- Is a congenital abnormality or birth defect;
- Is another important medical event (that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

N.B. In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to MSD in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by MSD for collection purposes:

- A new cancer (that is not a condition of the study)
- Is associated with an overdose (whether accidental or intentional).

***** "Life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

****** "Hospitalisation" means any unexpected admission to a hospital department. It does not usually apply to scheduled admissions that were planned before study inclusion or visits to casualty (without admission).

7.4.2 Reporting of SAEs

Rapid reporting of all SAEs, i.e. within 24 hours of the Principal Investigator or designee becoming aware of the event, occurring during the study must be performed as detailed in the Pharmacovigilance Manual. If the investigator becomes aware of safety information that appears to be drug related, involving a patient who participated in the study, even after an individual patient has completed the study, this should be reported to the Sponsor.

The SAE should be reported electronically to the study team at the Imperial Clinical Trials Section via the CUPem inform database as detailed in the Pharmacovigilance study manual.

All SAEs will be reviewed by the Chief Investigator (CI) or a designated medically qualified representative to confirm expectedness and causality. Reporting of SAEs and review by

the CI will be via the trial data collection system (eCRF) as detailed in the Pharmacovigilance Study Manual.

Following documented assessment by the CI, the completed SAE form will be sent by email to the Sponsor by the study team at the CTS within the pre-specified timelines. Regardless of expectedness or causality, all SAEs must also be reported to MSD by the Sponsor, within 2 working days (to meet certain local requirements).

7.5 Definition of a Serious Adverse Reaction (SAR)

A SAR is defined as a SAE that is judged to be related to any dose of study drug administered to the subject.

7.6 Definition of Suspected Unexpected Serious Adverse Reaction (SUSAR)

Any SAR that is NOT consistent with the applicable product information as set out in the Investigator Brochure (IB) or Summary of Product Characteristics (SmPC).

7.6.1 Reporting of SUSARs

SUSARs should be notified to the appropriate regulatory authority, the relevant REC and the Sponsor in accordance with regulatory requirements. SUSARs which are fatal or life-threatening will be reported not later than seven days after alerting the sponsor to the reaction. Any additional relevant information will be sent within eight days of the report.

A SUSAR which is not fatal or life-threatening will be reported within 15 days.

Follow up of patients who have experienced a SUSAR should continue until recovery is complete or the condition has stabilised.

7.7 Development Safety Update Reports (DSURs)

Development Safety Update Reports (DSURs) will be submitted to the Sponsor, the relevant Ethics Committees and Regulatory Authorities in accordance with regulatory requirements.

7.8 Reporting of Pregnancy and Lactation

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and lactations that occur from the time of treatment allocation through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination

of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor, who will then report to MSD Global Safety within 2 days.

7.9 Definition of Overdose and Reporting

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity.

If an adverse event(s) is associated with ("results from") the overdose of a MSD product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of MSD's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor, who will then report it to MSD Global Safety. Within 2 days.

7.10 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the Sponsor and within 2 working days to MSD Global Safety.

For the time period beginning when the consent form is signed until treatment allocation, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to MSD Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to MSD product, must be reported within 24 hours to the Sponsor and within 24 hours to MSD Global Safety.

Events of clinical interest for this trial include:
An overdose of MSD product, as defined in Section 7.2.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

An elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*****

****Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying aetiology.***

7.11 Reporting urgent safety measures

If any urgent safety measures are taken the CI/Sponsor shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the MHRA and the relevant REC of the measures taken and the circumstances giving rise to those measures.

8 STATISTICAL ANALYSES

8.1.1 Statistical Analysis Plan

Response rates can be assessed in patients who have completed at least 2 cycles of trial therapy

- Overall response rates by immune-related (irRECIST) & RECIST criteria:
  - Disease Control Rate (DCR), Overall Survival (OS) & Progression Free Survival (PFS).

- Overall response rates by immune-related (irRECIST) & RECIST criteria in a First-line setting
  - DCR, OS & PFS.

- QOL analysis by EORTC-QLQC30.

- Incidence of adverse events up to 8 weeks days after the last dose of Pembrolizumab.
  - incidence of adverse events up to 8 weeks days after the last dose of Pembrolizumab in PS2 patients.

- Exploratory Identification of genomic biomarkers predictive of immune response to Pembrolizumab in CUP patients and correlates of efficacy, toxicity and QOL.
  - Where patients are co-registered and consented to the NHSE 100k genome project – the data from this project will be requested to cross-correlate with this study.

8.2 Sample size, power considerations and planned recruitment rate

An interim futility analysis was originally pre-planned after recruiting 24 (out of 57) patients in Cohort 2 and a final analysis after 77 participants completed the study (20 for Cohort 1 and 57 for Cohort 2). $\geq$1 documented response in Cohort 1 was achieved in May 2020, enabling Cohort 2 to open and Cohort 1 to close once sufficient evaluable participants had been enrolled. Due to the unexpected challenges recruiting study participants outlined in the Trial Summary and sections 1.3.1 and 3.1, particularly for Cohort 2, Cohort 1 was reopened and expanded. Version 7.0 of the protocol has formally closed Cohort 2 with approval from the TMG, MSD and combined TSC/IDMC. Cohort 1 (pre-treated patients) remains a crucial area of unmet need with no standard of care treatment existing.

The study statistics were originally modelled on the UK CUP-ONE trial based on the primary study end-point of response in cohort 2.

The sample size has thus been recalculated to accommodate the shift of focus to assessing PFS and OS in Cohort 1 and complete removal of Cohort 2. The sample sizes were recalculated based on the Brookmeyer-Crowley test for median survival time (Brookmeyer R and Crowley JJ. A confidence interval for the median survival time. Biometrics. 1982: 38, 29-41).

Assuming a historic null PFS of 2 months and an alternative PFS of 4 months, the minimum required sample size to determine the median PFS for the study cohort is 46 (at 0.05 level of significance, one-sided, 90% power). For the OS, assuming a null OS of 4 months and an alternative OS of 7 months, the required minimum sample size is 67 with the same level of significance and power. Therefore, the final sample size needed to determine both the median PFS and OS is 67 (the higher sample size). In the event that this sample size will be difficult to recruit within the additional accrual period of 12 months, a sample size of 31 will still be able determine the median PFS and 45 the median OS with a power of 80%.

Power	Null Median Survival		Alternative Median Survival	Min Sample Size
0.80	2 months	PFS	4 months	31
0.90	2 months		4 months	**46**
0.80	4 months	OS	6 months	83
0.80	4 months		7 months	45
0.90	4 months		6 months	122
0.90	4 months		7 months	**67**

The study will therefore aim to recruit between 31 and 67 patients to Cohort 1 within the allotted study period. This sample size range is estimated to have a power of 80% to 90% in determining the PFS and/or the OS of the study cohort.

8.3 Patient Replacement Strategy

Patients assessable for the primary end-point for both cohorts of the study are those that have received at least 2 cycles of Pembrolizumab and are assessable for toxicity. If there is documented clinical or radiological progression, prior to the first planned radiological CT assessment at 12 weeks then the subject(s) are classified as treatment failures. Enrollment of additional subjects to fulfill the study targets can only be considered in those that have ***not*** progressed but have not completed the minimum of 2 cycles for whatever reason. This will be discussed on a case by case basis, by the lead investigators in participating sites via the chief investigator and the TMG.

9 REGULATORY, ETHICAL AND LEGAL ISSUES

9.1 Declaration of Helsinki

The investigator will ensure that this study is conducted in full conformity with the 1964 Declaration of Helsinki, and any relevant revisions.

9.2 Good Clinical Practice

The study will be conducted in accordance with the guidelines laid down by the International Conference on Harmonisation for Good Clinical Practice (ICH GCP E6 guidelines).

9.3 Independent Ethics Committee Approval

9.3.1 Initial Approval

Prior to the shipment of IMP and the enrolment of subjects in each country, an IEC must provide written approval of the conduct of the study in that country at named sites, the protocol and any amendments, the Patient Information Sheet and Consent Form, any other written information that will be provided to the subjects, any advertisements that will be used and details of any subject compensation.

9.3.2 Approval of Amendments

Proposed amendments to the protocol and aforementioned documents must be submitted to the IEC for approval as instructed by the Sponsor. Amendments requiring REC approval may be implemented only after a copy of the IEC's approval letter has been obtained.

Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving Sponsor or IEC approval. However, in this case, approval must be obtained as soon as possible after implementation.

Amendments, including whether the changes are substantial or non-substantial, will be made in accordance with HRA and/or IEC guidance as appropriate, with the decision being made by the Trial Management Group (TMG). Changes will be appropriately version controlled. In the case of protocol amendments, the amended protocol must be reviewed by all members of the Protocol Development Group prior to finalising, while amendments affecting stakeholders e.g. patient groups will require review prior to finalising where appropriate.

9.3.3 Annual Progress Reports

The IEC will be sent annual progress reports in accordance with national requirements.

9.3.4 Annual Safety Reports and End of Trial Notification

The IEC will be sent annual safety updates in order to facilitate their continuing review of the study (reference. ICH GCP E6 Section 3.1.4) and will also be informed about the end of the trial, within the required timelines.

9.4 Regulatory Authority Approval

The study will be performed in compliance with the UK regulatory requirements. Clinical Trial Authorisation from the appropriate Regulatory Authorities must be obtained prior to the start of the study. In addition, the Regulatory Authorities must approve amendments prior to their implementation (as instructed by the Sponsor), receive SUSAR reports, DSURs, and be notified of the end of the trial.

9.5 HRA Approval

Health Research Authority (HRA) approval will be obtained prior to starting the study. Each participating site will confirm capacity and capability prior to commencing.

The HRA and all participating sites also need to be notified of all protocol amendments to assess whether the amendment affects the institutional approval for each site.

9.6 Other Required Approvals

This trial includes the use of ionising radiation. The procedures are compliant with the Ionising Radiation (Medical Exposure) Regulations, and appropriate review by a Medical Physics Expert and Clinical Radiation Expert has been undertaken.

9.7 Non-Compliance and Serious Breaches

All protocol deviations and protocol violations will be reported via the eCRF and reviewed by the Chief Investigator and reported to the JRO on a monthly basis.
An assessment of whether the protocol deviation/violation constitutes a serious breach will be made.

A serious breach is defined as a breach of the conditions and principles of GCP in connection with a trial or the trial protocol, which is likely to affect to a significant degree:

- The safety or physical or mental integrity of the UK trial subjects; or
- The overall scientific value of the trial.

The Sponsor will be notified within 24 hours of identifying a likely Serious Breach. If a decision is made that the incident constitutes a Serious Breach, this will be reported to the MHRA, UK REC and other participating countries within 7 days of becoming aware of the serious breach.

9.8 Insurance and Indemnity

The Sponsor has civil liability insurance, which covers this study in the UK.

9.9 Trial Registration

The study will be registered on a trial database in accordance with requirements of the International Committee of Medical Journal Editors (ICMJE) regulations.

9.10 Informed Consent

The Principal Investigator at each site will:

- Ensure that each patient is given full and adequate oral and written information about the study including the background, purpose and risks/benefits of participation
- Ensure that each patient is notified that they are free to withdraw from the study at any time
- Ensure that each patient is given the opportunity to ask questions, allowed sufficient time to read and understand the information sheet, and given at least 24 hours to decide whether or not to take part
- Ensure each patient provides signed, dated informed consent before undergoing any study specific procedure
- Ensure the original copy of the signed, dated Informed Consent Form is stored in the patient's medical records and a copy is also filed in the Investigator site file
- Ensure that each patient receives a copy of the signed, dated Informed Consent Form.

9.11 Contact with General Practitioner

It is the investigator's responsibility to inform the patient's General Practitioner (where applicable) by letter that the patient is taking part in the study provided the patient agrees to this, and information to this effect is included in the Patient Information Sheet and Informed Consent. A copy of the letter should be filed in the Investigator Site File.

9.12 Patient Confidentiality

The investigator must ensure that the patient's confidentiality is maintained. On the eCRF or other documents submitted to the Sponsors, patients will be identified by a subject ID number only. Documents that are not submitted to the Sponsor (e.g., signed informed consent form) should be kept in a strictly confidential file by the investigator.

The investigator shall permit direct access to patients' records and source documents for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the Sponsor, Regulatory Authorities and RECs.

9.13 Data Protection and Patient Confidentiality

The investigator will preserve the confidentiality of all participants taking part in the study, which will be conducted in accordance with the Data Protection Act. The Patient Consent form will identify those individuals who will require access to patient data and identifiable details and obtain appropriate permission from the consenting patient.

9.14 End of Trial

The end of the trial is defined as the last data capture for the last patient on study; this will be 6 months after the last patient stops study treatment or up to 12 months after the last patient is entered whichever is sooner.

9.15 Study Documentation and Data Storage

The investigator must retain essential documents until notified by the Sponsor, and for at least ten years after study completion. Patient files and other source data (including copies of protocols, CRFs/eCRFs, original reports of test results, IMP dispensing logs,

correspondence, records of informed consent, and other documents pertaining to the conduct of the study) must be retained. Documents should be stored in such a way that they can be accessed/data retrieved at a later date. Consideration should be given to security and environmental risks.

No study document will be destroyed without prior written agreement between the Sponsor and the investigator. Should the investigator wish to assign the study records to another party or move them to another location, written agreement must be obtained from the Sponsor.

10 DATA MANAGEMENT

10.1 Source Data

All original records and certified copies of original records of clinical findings, observations, or other activities necessary for the reconstruction and evaluation of the trial are classified as source data. Source data are contained in source documents; these are defined as: original documents, data, and records e.g. hospital records, clinical and office charts, laboratory notes, memoranda, patient diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, patient files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial.

10.2 Language

eCRFs will be in English and must be completed in English. Generic names for concomitant medications should be recorded in the eCRF wherever possible. All written material to be used by patients must use vocabulary that is clearly understood, and be in the language appropriate for the study site.

10.3 Database

The study eCRF will be built in InForm. Data management will be performed using the InForm electronic data capture (EDC) and management system. The system allows for real time oversight of trial activity including adverse event reporting, rapid data validation and data aggregation.

AE data will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term, and CTCAE grade.

Data queries will be raised for inconsistent, impossible or missing data. All entries to the study database will be available in an audit trial.

10.4 Data Collection

In compliance with Good Clinical Practice (GCP), the medical records/medical notes should be clearly marked and allows easy identification of a patient's participation in the clinical trial.

The Investigator (or delegated member of the site study team) must record all data relating to protocol procedures, IMP administration, laboratory data, safety data and efficacy data into the trial InForm electronic data collection (EDC) system.

Details of procedures for eCRF completion will be provided in the eCRF Completion Manual.

10.5 Archiving

All trial documentation, including that held at participating sites and the trial coordinating centre, will be archived for a minimum of 10 years following the end of the study.

11 STUDY MANAGEMENT STRUCTURE

11.1 Trial Oversight Committees

11.1.1 Trial Steering Committee

A Trial Steering Committee (TSC) will be convened as a joint Committee with the Independent Data Monitoring Committee (IDMC).

11.1.2 Trial Management Group

A Trial Management Group (TMG) will be convened including the Chief Investigator, co-investigators and key collaborators. The TMG will be responsible for day-to-day conduct of the trial and operational issues. Details of membership, responsibilities and frequency of meetings will be defined in a separate Charter.

11.1.3 Independent Data Monitoring Committee

An Independent Data Monitoring Committee (IDMC) will be convened as a joint committee with the Trial Steering Committee (TSC) to monitor data collected during the study, and make recommendations to the TMG on whether there are any ethical or safety reasons as to why the trial should not continue. It will consist of an independent Chair, an independent statistician and an independent clinician. Details of membership, responsibilities and frequency of meetings will be defined in a separate Charter.

11.2 Early Discontinuation of the Study

In case of early discontinuation of the study, the end of treatment assessments should be performed for each patient remaining on study treatment.

11.3 Risk Assessment

A study-specific risk assessment will be performed prior to the start of the study to assign a risk category of ‘low’, ‘medium’ or ‘high’ to the trial. Risk assessment will be carried out by the Joint Research Office in collaboration with the Trial Coordinator and the result will be used to guide the monitoring plan. The risk assessment will consider all aspects of the study and will be updated as required during the course of the study.

11.4 Monitoring

The study will be monitored periodically by trial monitors to assess the progress of the study, verify adherence to the protocol, ICH GCP E6 guidelines and other national/international requirements and to review the completeness, accuracy and consistency of the data. Monitoring procedures and requirements will be documented in a Monitoring Plan, in accordance with the risk assessment.

11.5 Quality Control and Quality Assurance

Quality Control will be performed according to internal procedures. The study may be audited by a Quality Assurance representative of the Sponsor. All necessary data and documents will be made available for inspection.

The study may be subject to inspection and audit by regulatory bodies to ensure adherence to GCP and the UK Policy Framework for Health and Social Care Research

11.6 Trial Registration

This trial will be registered on http://www.clinicaltrials.gov, as well as other relevant websites. Information posted on this Clinical Trials Data Bank will allow patients to identify potentially appropriate trials for their disease conditions and allow them to gather further information on appropriate trial locations and trial site contact information.

11.7 Peer Review

The study outline was presented to the NIHR CGS, HB sub group and also discussed at the London Cancer Alliance CUP guidelines group of London Alliance. The study has also undergone peer review by: Merck Sharp & Dohme Limited; Imperial College London Cancer Clinical Trials Committee and the research team at Imperial College London (Chief Investigator's host institution).

11.8 Patient and Public Involvement

CUP Foundation – Jo's friends have reviewed the study and endorsed the QoL questionnaire to be used as part of the study. Lay members will be invited as members of the Trial Oversight Committees as required.

11.9 Publication and Dissemination Policy

Information concerning the study, patent applications, processes, scientific data or other pertinent information is confidential and remains the property of the Sponsor. The investigator may use this information for the purposes of the study only.

It is understood by the investigator that the Sponsor will use information developed in this clinical study in connection with the development of the IMP and, therefore, may disclose it as required to other clinical investigators and to Regulatory Authorities. In order to allow the use of the information derived from this clinical study, the investigator understands that he/she has an obligation to provide complete test results and all data developed during this study to the Sponsor.

Verbal or written discussion of results prior to study completion and full reporting should only be undertaken with written consent from the Sponsor.

Therefore all information obtained as a result of the study will be regarded as CONFIDENTIAL, at least until appropriate analysis and review by the investigator(s) is completed.

Permission from the TMG is necessary prior to disclosing any information relative to this study outside of the TMG.

The results may be published or presented by the investigator(s), but only with the permission of the Sponsor.

12 SIGNATURE PAGES

SIGNATURE PAGE 1 (CHIEF INVESTIGATOR)

The signature below constitutes approval of this protocol by the signatory and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol including all statements regarding confidentiality.

Study Title: A Phase II, Two-Stage,Trial of Pembrolizumab in Cancer of unknown primary (CUP) - CUPem

Protocol Number: C/37/2017

Signed:

_____

Dr. Harpreet Wasan
Title - Reader in Medical Oncology & Lead GI trials Unit

Date: _____

SIGNATURE PAGE 2 (SPONSOR)

The signatures below constitute approval of this protocol by the signatory.

Study Title: A Phase II, Two-Stage, Trial of Pembrolizumab in Cancer of unknown primary (CUP) - CUPem

Protocol Number: C/37/2017

Signed: _____

Miss Ruth Nicholson
Head of Research Governance & Integrity
Imperial College London

Date: _____

SIGNATURE PAGE 3 (STATISTICIAN)

The signatures below constitute approval of this protocol by the signatory.

Study Title: A Phase II, Two-Stage, Trial of Pembrolizumab in Cancer of unknown primary (CUP) - CUPem

Protocol Number: C/37/2017

Signed: _____
Dr Jingky Pamesa Lozano-Kuehne
Study Statistician

Date: _____

SIGNATURE PAGE 4 (PRINCIPAL INVESTIGATOR)

The signature of the below constitutes agreement of this protocol by the signatory and provides the necessary assurance that this study will be conducted at his/her investigational site according to all stipulations of the protocol including all statements regarding confidentiality.

Study Title: A Phase II, Two-Stage, Trial of Pembrolizumab in Cancer of unknown primary (CUP) - CUPem

Protocol Number: C/37/2017

Address of Institution: _____

_____

_____

Signed: _____

Print Name and Title: _____

Date: _____

13 APPENDICES

Appendix A: Guidelines for Evaluation of Objective Tumour Response Using RECIST 1.1 (Response Evaluation Criteria in Solid Tumours)

Guidance Document Version 2.0 date 17th November 2014

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumours (RECIST) guideline (version 1.1) [Eur J Ca 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumour lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

Definition of Disease Parameters

Measurable disease Must be accurately measured in a least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:
10mm by CT scan (CT scan slice thickness no greater than 5mm; when CT scans have slice thickness >5mm, the minimum size should be twice the slice thickness).
10mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measureable).
20mm by chest X-ray.

Note: Tumour lesions situated in a previously irradiated area or in an area subjected to other loco-regional therapy are usually not considered measureable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

Malignant lymph nodes Criteria for lymph nodes given as ≥15mm short axis for target lesions and 10mm to <15mm for non-target lesions. Nodes under 10mm to be considered non-pathological.

Non-measurable disease All other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with 10 to <15 mm short axis), as well as truly non-measurable lesions. Lesions considered truly non-measurable include; leptomeningeal disease, ascites, pleural/pericardial effusions, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measureable by reproducible imaging techniques.

Note: Lytic bone lesions or mixed lytic-blastic lesions with identifiable soft tissue components that can be evaluated by cross-sectional imaging techniques such as CT or MRI can be considered measurable if the soft tissue component meets the definition of measurability described above.

'Cystic lesions' thought to represent cystic metastases can be considered measurable if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions

and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and be representative of all involved organs, as well as their suitability for reproducible repeated measurements. All measurements should be recorded in metric notation using calipers if clinically assessed.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters, which will be used as reference to further characterize any objective tumour regression in the measurable dimension of the disease. If lymph nodes are to be included in the sum, only the short axis will contribute.

Non-target lesions All lesions (or sites of disease) not identified as target lesions, including pathological lymph nodes and all non-measurable lesions, should be identified as non-target lesions and be recorded at baseline. Measurements of these lesions are not required and they should be followed as 'present', 'absent' or in rare cases, 'unequivocal progression'.

Methods of Measurement
All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

CT/MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. MRI is also acceptable in certain situations (e.g. for body scans but not for lung).

Chest x-ray Lesions on chest x-ray may be considered measurable lesions if they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Clinical lesions Clinical lesions will only be considered measurable when they are superficial and ≥10mm in diameter as assessed using calipers. For the case of skin lesions, documentation by colour photography, including a ruler to estimate the size of the lesion, is recommended.

Ultrasound (US) should not be used to measure tumour lesions.

Tumour markers Tumour markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [JNCI 96:487-488, 2004; J Clin Oncol 17, 3461-3467, 1999; J Clin Oncol 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression

criteria which are to be integrated with objective tumour assessment for use in first-line trials in ovarian cancer [JNCI 92:1534-1535, 2000].

Cytology, Histology Can be used in rare cases (e.g. for evaluation of residual masses to differentiate between Partial Response and Complete Response or evaluation of new or enlarging effusions to differentiate between Progressive Disease and Response/Stable Disease).

Endoscopy, Laparoscopy Use of endoscopy and laparoscopy is not advised. However, they can be used to confirm complete pathological response.

Response Criteria
Evaluation of Target Lesions
Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this may include the baseline sum). The sum must also demonstrate an absolute increase of at least 5mm.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.
Evaluation of Non-Target Lesions
Complete Response (CR): Disappearance of all non-target lesions and normalization of tumour marker levels. All lymph nodes must be non-pathological in size (<10 mm short axis)

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumour marker level above the normal limits

Progressive Disease (PD): Unequivocal progression of existing non-target lesions.
When patient has measurable disease – to achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumour burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status.

When patient has none-measurable disease – there is no measurable disease assessment to factor into the interpretation of an increase in non-measurable disease burden. Because worsening in non-target disease cannot be easily quantified, a useful test that can be applied is to consider if the increase in overall disease burden based on change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease. Examples include an increase in a

pleural effusion from ‘trace’ to ‘large’ or an increase in lymphangitic disease from localised to widespread.

Evaluation of Best Overall Response
Summary of overall response status calculation at each time point, for patients who have measurable disease at baseline.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	>4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	>4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	documented at least once >4 wks. from baseline**
Not all evaluated	Non-PD	No	Not evaluated	
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomised trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as “symptomatic deterioration.” Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR/PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, (taking as reference for PD the smallest measurements recorded on study).

Duration of stable disease: SD is measured from the start of the treatment (in randomised trials, from the date of randomization) until the criteria for disease progression are met, taking as reference the smallest sum on study (if baseline sum is the smallest, this is the reference for calculation of PD). The clinical relevance of the duration of SD varies for different studies and diseases. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study. If the proportion of patients achieving stable disease for a minimum period of time is an endpoint of importance in a particular trial, the protocol should specify the minimal time interval required between two measurements for determination of stable disease.

Response Review

For trials where the objective response (CR and PR) is the primary endpoint it is recommended that all responses be reviewed by an expert(s) independent of the study. Simultaneous review of the patients' files and radiological images is the best approach.

Appendix B: Immune Related RECIST (iRECIST)

Immune-related RECIST is RECIST 1.1 adapted to account for the unique tumour response

The guidelines in this protocol for response criteria for use in trials testing immunotherapeutics is adapted From *Lancet Oncol* 2017; 18: e143–52; www.thelancet.com/oncology Vol 18 March 2017

RECIST 1.1 will be adapted to account for the unique tumour response characteristics seen with treatment of pembrolizumab. Immunotherapeutic agents such as pembrolizumab may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest a clinical response after an initial increase in tumour burden or even the appearance of new lesions. Standard RECIST 1.1 may thus, not provide an accurate response assessment of immunotherapeutic agents such as pembrolizumab.

Based on an analysis of patients with melanoma enrolled in KEYNOTE-001, 7% of evaluable patients experienced delayed or early tumour pseudo-progression. Of note, patients who had progressive disease by RECIST 1.1 but not by immune-related response criteria had longer OS than patients with progressive disease by both criteria. Additionally, the data suggest that RECIST 1.1 may underestimate the benefit of pembrolizumab in approximately 15% of patients. These findings support the need to potentially apply a modification to RECIST 1.1 that takes into account the unique patterns of atypical response in immunotherapy and enable treatment beyond initial radiographic progression.

When feasible, subjects should not be discontinued until progression is confirmed by the local site investigator/radiology assessment. This allowance to continue treatment despite initial radiologic PD takes into account the observation that some subjects can have a transient tumour flare in the first few months after the start of immunotherapy, and then experience subsequent disease response. Subjects that are deemed clinically unstable are not required to have repeat tumour imaging for confirmation of PD.

Tumour flare includes any of the following scenarios:

Worsening of existing target lesion(s)

Worsening of existing non-target lesion(s)

Development of new lesion(s)

In subjects who have shown initial evidence of radiological PD by RECIST 1.1 it is at the discretion of the PI whether to continue a subject on study treatment until repeat imaging is obtained. This clinical judgment decision by the site investigator should be based on the subject's overall clinical condition, including performance status, clinical symptoms, and laboratory data. Subjects may receive study treatment and tumour assessment should be repeated ≥4 weeks later to confirm PD by irRECIST per site assessment.

Clinical stability is defined as the following:

1) Absence of symptoms and signs indicating clinically significant progression of disease, including worsening of laboratory values,

2) No decline in ECOG performance status,

3) Absence of rapid progression of disease, and

4) Absence of progressive tumour at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention.

Any subject deemed clinically unstable should be discontinued from trial treatment

In determining whether or not the tumour burden has increased or decreased per irRECIST, the local site investigator should consider all target and non-target lesions as well as any incremental new lesion(s).

In scenarios where PD is ***not*** confirmed at repeat imaging if ALL of the following occur by irRECIST:
Target lesion sum of diameters is <20% or <5 mm absolute increase compared to nadir,
Non-target disease resulting in initial PD is qualitatively stable or improved,
New lesion resulting in initial PD is qualitatively stable or improved,
No incremental new lesion(s) since last evaluation, and
No incremental new non-target lesion progression since last evaluation.
If repeat imaging does not confirm PD by irRECIST as assessed by the local site investigator and the subject continues to be clinically stable, treatment may continue and follow the regular imaging schedule.

Scenarios where PD is confirmed at repeat imaging if ANY of the following occur by irRECIST:
Target lesion sum of diameters remains ≥20% and at least 5 mm absolute increase compared to nadir,
Non-target disease resulting in initial PD is qualitatively worse,
New lesion resulting in initial PD is qualitatively worse,
Additional new lesion(s) since last evaluation,
Additional new non-target lesion progression since last evaluation.
If repeat imaging confirms PD due to any of the scenarios listed above, subjects will be discontinued from study therapy.

NOTE: If a subject has confirmed radiographic progression (i.e., 2 scans at least 4 weeks apart demonstrating progressive disease) per irRECIST, but the subject is achieving a clinically meaningful benefit and there is no further increase in the tumour burden at the confirmatory tumour imaging, an exception to continue treatment may be considered following consultation with the Sponsor. In this case, if treatment is continued, tumour imaging should continue to be performed following the study intervals.
Adapted From *Lancet Oncol* 2017; 18: e143–52; www.thelancet.com/oncology Vol 18 March 2017:-

	RECIST 1.1	iRECIST
Definitions of measurable and non-measurable disease; numbers and site of target disease	Measurable lesions are ≥10 mm in diameter (≥15 mm for nodal lesions); maximum of five lesions (two per organ); all other disease is considered non-target (must be ≥10 mm in short axis for nodal disease)	No change from RECIST 1.1; however, new lesions are assessed as per RECIST 1.1 but are recorded separately on the case report form (but not included in the sum of lesions for target lesions identified at baseline)
Complete response, partial response, or stable disease	Cannot have met criteria for progression before complete response, partial response, or stable disease	Can have had iUPD (one or more instances), but not iCPD, before iCR, iPR, or iSD
Confirmation of complete response or partial response	Only required for non-randomised trials	As per RECIST 1.1
Confirmation of stable disease	Not required	As per RECIST 1.1
New lesions	Result in progression; recorded but not measured	Results in iUPD but iCPD is only assigned on the basis of this category if at next assessment additional new lesions appear or an increase in size of new lesions is seen (≥5 mm for sum of new lesion target or any increase in new lesion non-target); the appearance of new lesions when none have previously been recorded, can also confirm iCPD
Independent blinded review and central collection of scans	Recommended in some circumstances—eg, in some trials with progression-based endpoints planned for marketing approval	Collection of scans (but not independent review) recommended for all trials
Confirmation of progression	Not required (unless equivocal)	Required
Consideration of clinical status	Not included in assessment	Clinical stability is considered when deciding whether treatment is continued after iUPD

"i" indicates immune responses assigned using iRECIST. RECIST=Response Evaluation Criteria in Solid Tumours. iUPD=unconfirmed progression. iCPD=confirmed progression. iCR=complete response. iPR=partial response. iSD=stable disease.

Table 1: **Comparison of RECIST 1.1 and iRECIST**

Adapted from *Lancet Oncol* 2017; 18: e143–52; www.thelancet.com/oncology Vol 18 March 2017:-

	Timepoint response with no previous iUPD in any category	Timepoint response with previous iUPD in any category*
Target lesions: iCR; non-target lesions: iCR; new lesions: no	iCR	iCR
Target lesions: iCR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iPR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iSD; non-target lesions: non-iCR/non-iUPD; new lesions: no	iSD	iSD
Target lesions: iUPD with no change, or with a decrease from last timepoint; non-target lesions: iUPD with no change, or decrease from last timepoint; new lesions: yes	Not applicable	New lesions confirm iCPD if new lesions were previously identified and they have increased in size (≥5 mm in sum of measures for new lesion target or any increase for new lesion non-target) or number; if no change is seen in new lesions (size or number) from last timepoint, assignment remains iUPD
Target lesions: iSD, iPR, iCR; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in the size of non-target disease (does not need to meet RECIST 1.1 criteria for unequivocal progression)
Target lesions: iUPD; non-target lesions: non-iCR/non-iUPD, or iCR; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in sum of measures ≥5 mm; otherwise, assignment remains iUPD
Target lesions: iUPD; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed based on a further increase in previously identified target lesion iUPD in sum of measures ≥5 mm or non-target lesion iUPD (previous assessment need not have shown unequivocal progression)
Target lesions: iUPD; non-target lesions: iUPD; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in previously identified target lesion iUPD sum of measures ≥5 mm, previously identified non-target lesion iUPD (does not need to be unequivocal), or an increase in the size or number of new lesions previously identified
Target lesions: non-iUPD or progression; non-target lesions: non-iUPD or progression; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of an increase in the size or number of new lesions previously identified

Target lesions, non-target lesions, and new lesions defined according to RECIST 1.1 principles; if no pseudoprogression occurs, RECIST 1.1 and iRECIST categories for complete response, partial response, and stable disease would be the same. *Previously identified in assessment immediately before this timepoint. "i" indicates immune responses assigned using iRECIST. iCR=complete response. iPR=partial response. iSD=stable disease. iUPD=unconfirmed progression. non-iCR/non-iUPD=criteria for neither CR nor PD have been met. iCPD=confirmed progression. RECIST=Response Evaluation Criteria in Solid Tumours.

Table 2: **Assignment of timepoint response using iRECIST**

Adapted From *Lancet Oncol* 2017; 18: e143–52; www.thelancet.com/oncology Vol 18 March 2017:-

	Timepoint response 1	Timepoint response 2	Timepoint response 3	Timepoint response 4	Timepoint response 5
Example 1	iCR	iCR, iPR, iUPD, or NE	iCR, iPR, iUPD, or NE	iUPD	iCPD
Example 2	iUPD	iPR, iSD, or NE	iCR	iCR, iUPD, or NE	iCR, iPR, iSD, iUPD, iCPD, or NE
Example 3	iUPD	iPR	iPR, iSD, iUPD, or NE	iPR, iSD, iUPD, NE, or iCPD	iPR, iSD, iUPD, NE, or iCPD
Example 4	iUPD	iSD or NE	iPR	iPR, iSD, iUPD, or NE	iPR, iSD, iUPD, iCPD, or NE
Example 5	iUPD	iSD	iSD, iUPD, or NE	iSD, iUPD, iCPD, or NE	iSD, iUPD, iCPD, or NE
Example 6	iUPD	iCPD	Any	Any	Any
Example 7	iUPD	iUPD (no iCPD)	iCPD	Any	Any
Example 8	iUPD	NE	NE	NE	NE

Eight examples are presented for patients with target disease at baseline, but many more scenarios exist following the same principles. Table assumes a randomised study in which confirmation response or partial response is not required. For patients with non-target disease only at baseline, only iCR or non-complete response or non-progression of disease can be assigned at each time in the table for ease of presentation). "i" indicates immune responses assigned using iRECIST. iBOR=best overall response. iCR=complete response. iPR=partial response. NE=not evaluable. iUPD progression. iCPD=confirmed progression. iSD=stable disease. RECIST=Response Evaluation Criteria in Solid Tumours.

Table 3: **Scenarios of assignments of best overall response using iRECIST**

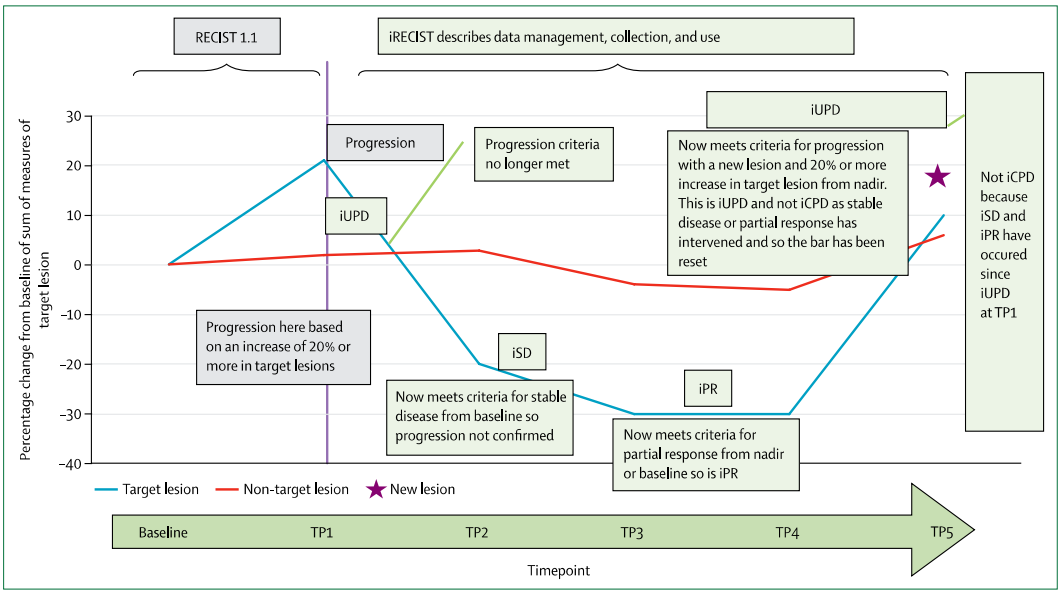


Figure 2: **RECIST 1.1 and iRECIST: an example of assessment**
Prefix "i" indicates immune responses assigned using iRECIST; others without "i" are confirmed by RECIST 1.1. RECIST=Response Evaluation Criteria in Solid Tumours. iCR=complete response. iCPD=complete progression. iPR=partial response. iSD=stable disease. iUPD=unconfirmed progression. TP=timepoint.

Appendix C: NHS 100,000 Genomes Project

The NHS 100,000 Genomes Project is a programme of whole genome sequencing (WGS) as part of the UK Government's Life Sciences Strategy. The principal objective of the 100,000 Genomes Project is to sequence 100,000 genomes from patients with cancer, rare disorders, and infectious disease, and to link the sequence data to a standardised, extensible account of diagnosis, treatment, and outcomes. The Project is designed to produce new capability and capacity for genomic medicine that will transform the NHS. It will also produce new capability for clinical genomics research. As part of the proposal a secure infrastructure will be established for the protection and analysis of clinical and genomic data. This will be made available for approved academic and industrial research purposes, including those of the contributing clinical organisations from the NHS. To identify and enrol participants we have created NHS Genomic Medicine Centres (GMCs). NHS England has commissioned a number of these centres to harness the capability and capacity of the NHS across England to contribute to the Project from 2015.

The goals of the cancer WGS programme are to:

- Use WGS to identify novel driver mutations for cancer and to understand its evolutionary genetic architecture through primary and secondary malignant disease (by multiple biopsy and WGS).
- Partner stratified healthcare programmes and outcome studies with patients from the NHS in England, to enable understanding of WGS
- Benefits in defining predictors of therapeutic response to cancer therapies.
- To use multi-omic approaches including transcriptomics, proteomics and epigenetics to additional biological insights into cancer.
- To utilise WGS to identify new pathways for cancer therapies.

For tumour DNA, The project will sequence at 75X coverage. However, in some cancers, greater depth of coverage may be required. WGS will be performed using the same technology and QC approaches outlined for the rare diseases programme. In addition, the frequency of single nucleotide variations (SNVs) potentially caused by DNA alterations created during the FFPE process, (such as G to A, or A to G transitions) due to PCR amplification, will be monitored.

A major component in cancer annotation is analysing the consequence of larger scale genomic changes, such as structural variants, copy number aberrations, loss of heterozygosity and other chromosomal mutational events. Since some will not be unique to the tumour sample, a paired approach to the analysis of the two samples will be important to ensure the origin of the variant is clear. Regarding the annotation of individual sequence variants, the consequence of copy number and structural variants will be assessed for their impact on genes implicated in cancer.
CUP is included in the 100K genome project from April 2017. There is a separate consent process and form for this process in every hospital partaking in the UK. Any data generated from the 100k project is stored centrally by NSHE and anonymised clinical annotation datasets are linked to the genomic or multi-omic data generated. A project application is made where patients are receiving therapies routinely or in a trial for access to the data so that a researcher can interrogate important scientific questions of interest, to that disease or therapy.

Further detailed information can be obtained from
https://www.genomicsengland.co.uk/the-100000-genomes-project/

Appendix D: Evaluating Adverse Events

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
	Grade 4	**Life threatening consequences; urgent intervention indicated.**
	Grade 5	**Death related to AE**
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of MSD product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a MSD product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours and to MSD within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours to the Sponsor and to MSD within 2 working days..	

	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause MSD product to be discontinued?	
Relationship to MSD Product	Did MSD product cause the adverse event? The determination of the likelihood that MSD product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between MSD product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely MSD product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to MSD product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of MSD product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors

Relationship to MSD Product (continued)	**The following components are to be used to assess the relationship between the test drug and the AE: (continued)**	
	Dechallenge	Was MSD product discontinued or dose/exposure/frequency reduced? If yes, did the AE resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the AE resulted in death or permanent disability; (2) the AE resolved/improved despite continuation of the Sponsor's product; or (3) the trial is a single-dose drug trial); or (4) Sponsor's product(s) is/are only used one time.)
	Rechallenge	Was the subject re-exposed to MSD product in this study? If yes, did the AE recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial AE resulted in death or permanent disability, or (2) the trial is a single-dose drug trial); or (3) Sponsor's product(s) is/are used only one time). NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY MSD PRODUCT, OR IF REEXPOSURE TO MSD PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT, THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE SPONSOR AS PER DOSE MODIFICATION GUIDELINES IN THE PROTOCOL.
	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the AE consistent with previous knowledge regarding MSD product or drug class pharmacology or toxicology?
The assessment of relationship will be reported on the case report forms /worksheets by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.		
Record one of the following	**Use the following scale of criteria as guidance (not all criteria must be present to be indicative of MSD product relationship).**	
Yes, there is a reasonable possibility of MSD product relationship.	There is evidence of exposure to MSD product. The temporal sequence of the AE onset relative to the administration of MSD product is reasonable. The AE is more likely explained by MSD product than by another cause.	
No, there is not a reasonable possibility of MSD product relationship	Subject did not receive the MSD product OR temporal sequence of the AE onset relative to administration of MSD product is not reasonable OR the AE is more likely explained by another cause than the MSD product. (Also entered for a subject with overdose without an associated AE.)	

V4.0 CTCAE Grading	**Grade 1**	**Mild; asymptomatic or mid symptoms; clinical or diagnostic observations only; intervention not indicated.**
	Grade 2	**Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.**
	Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalization or prolongation or hospitalization indicated; disabling; limiting self-care ADL.**
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Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of MSD product that:	
	†**Results in death**; or	
	†**Is life threatening; or** places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.); or	
	†**Results in a persistent or significant disability/incapacity** (substantial disruption of one's ability to conduct normal life functions); or	
	†**Results in or prolongs an existing inpatient hospitalization** (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization for an elective procedure to treat a pre-existing condition that has not worsened is not a serious adverse event. A pre-existing condition is a clinical condition that is diagnosed prior to the use of a MSD product and is documented in the patient's medical history.); or	
	†**Is a congenital anomaly/birth defect** (in offspring of subject taking the product regardless of time to diagnosis);or	
	Is a new **cancer** (that is not a condition of the study) **(**although not serious per ICH definition, is reportable to the Sponsor within 24 hours and to MSD within 2 working days to meet certain local requirements); **or**	
	Is an overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event for collection purposes. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours to the Sponsor and to MSD within 2 working days..	
	Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	

Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units	
Action taken	Did the adverse event cause MSD product to be discontinued?	
Relationship to MSD Product	Did MSD product cause the adverse event? The determination of the likelihood that MSD product caused the adverse event will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information. **The following components are to be used to assess the relationship between MSD product and the AE**; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely MSD product caused the adverse event (AE):	
	Exposure	Is there evidence that the subject was actually exposed to MSD product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?
	Time Course	Did the AE follow in a reasonable temporal sequence from administration of MSD product? Is the time of onset of the AE compatible with a drug-induced effect (applies to trials with investigational medicinal product)?
	Likely Cause	Is the AE not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors