c-TRAK TN: A clinical trial utilising ctDNA mutation tracking to detect minimal residual disease and trigger intervention in patients with moderate and high risk early stage triple negative breast cancer

PROTOCOL
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The c-TRAK TN trial has been endorsed by Cancer Research UK's Clinical Research Committee (CRC)

The c-TRAK TN trial is part of the National Institute for Health Research Clinical Research Network Trial Portfolio

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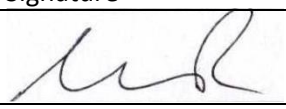
The Trial Management Group (TMG) will be constituted to include members of the Protocol Development Group including the Chief Investigator, ICR-CTSU Scientific Lead, Co-investigators and identified collaborators, key ICR-CTSU staff and a lay representative. Principal Investigators and other key trial personnel will be invited to join the TMG as appropriate to ensure representation from a range of sites and professional groups. A copy of the current membership of the TMG can be obtained from the c-TRAK TN Trial Manager at ICR-CTSU.

Protocol Authorised by:

Name & Role	Signature	Date
Prof Nicholas Turner (Chief Investigator)		16/06/2020

This protocol describes the c-TRAK TN trial and provides information about procedures for entering participants into this trial. The protocol should not be used as a guide for the treatment of patients outside of this trial.

Every care was taken in the preparation of this protocol, but corrections or amendments may be necessary. Protocol amendments will be circulated to participating sites as they occur, but sites entering patients for the first time are advised to contact ICR-CTSU to confirm they have the current version.

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TRIAL SUMMARY

PROTOCOL TITLE	**c-TRAK TN:** A clinical trial utilising **c**tDNA mutation **t**racking to detect minimal **r**esidual disease **a**nd trigger intervention in patients with high ris**k** early stage triple negative breast cancer
TARGET DISEASE	Moderate to high risk early stage triple negative breast cancer
TRIAL DESIGN	The design of the current component of the c-TRAK TN trial is a multi-centre, single-arm phase II trial that uses ctDNA surveillance to direct intervention. The trial consists of tissue screening, ctDNA surveillance and a therapeutic trial. If suitability is confirmed following tissue screening, patients will undergo serial ctDNA surveillance, with allocation to treatment with pembrolizumab in the therapeutic trial triggered by the detection of minimal residual disease (indicated by a ctDNA positive result) on or before their 12 month ctDNA surveillance assessment. *Prior to the implementation of protocol v6.0 the randomised component of c-TRAK TN was in place whereby ctDNA surveillance was blinded and the detection of minimal residual disease (indicated by a ctDNA positive result) on or before the 12 month ctDNA surveillance assessment triggered randomisation to treatment with pembrolizumab or observation (on a 2:1 ratio). In protocol version 6.0 randomisation was removed with all patients that have a positive ctDNA result being allocated to treatment intervention, following recommendations made by the c-TRAK TN IDMC. See section 1.2.1 for further details.* *Following the implementation of protocol v6.0 whereby patients transfer to the non-randomised component of the trial, all patients in ctDNA surveillance should re-consent before their next ctDNA blood test. Patients who do not re-consent to protocol v6.0, will need to stop ctDNA surveillance and continue standard follow up.*
PRIMARY OBJECTIVES	• To assess whether digital PCR ctDNA assays, as a non-invasive biomarker on serial blood samples, can predict which patients are at highest risk of relapse and identify patients with minimal residual disease not visible on imaging. • To assess whether pembrolizumab results in a sustained clearance of ctDNA in patients who have completed treatment for moderate or high risk early stage triple negative breast cancer, but have minimal residual disease (MRD) detected by serially assessed ctDNA analysis.
SECONDARY OBJECTIVES	• To assess time to first positive ctDNA detection from entry into the ctDNA surveillance.

- To assess the rate of detection of overt metastatic disease at the time of first ctDNA detection.
- To assess the median lead-time between ctDNA detection and relapse in the pembrolizumab treatment and observation groups.
- To assess sustained clearance of ctDNA in the observation group.
- Safety and tolerability of pembrolizumab.
- To assess the proportion of patients allocated to receive pembrolizumab who start pembrolizumab.

NB. Secondary objectives relating to the observation group will be evaluated in the patients randomised to the observation group prior to the implementation of protocol v6.0.

EXPLORATORY OBJECTIVES

- To explore differences in time between ctDNA detection and disease relapse, and disease free survival, between patients in the pembrolizumab and the observation groups.
- To explore predictors of sustained ctDNA clearance on pembrolizumab.
- To explore potential predictors of relapse and ctDNA detection, and alternative definitions of ctDNA clearance.
- To assess the association between ctDNA clearance and time to recurrence in the pembrolizumab group.

NB. Exploratory objectives relating to the observation group will be evaluated in the patients randomised to the observation group prior to the implementation of protocol v6.0.

TRIAL POPULATION

Patients with moderate or high-risk early stage triple negative breast cancer, with no evidence of distant metastatic disease, who have completed standard therapy (surgery and neoadjuvant/adjuvant chemotherapy).

RECRUITMENT TARGET

It is anticipated that 75% of patients tested will have mutations detected in their archival tumour tissue and be eligible to commence ctDNA surveillance. c-TRAK TN therefore aims to sequence archival tumour tissue from approximately 200 patients in order to recruit approximately 150 patients into ctDNA surveillance.

TISSUE SCREENING & ctDNA SURVEILLANCE COMPONENT

Patients meeting the eligibility criteria and with tumour tissue in which mutations are detected for which a ctDNA assay can be developed, will start ctDNA surveillance once they have completed standard adjuvant chemotherapy, with the exception of capecitabine, patients may start ctDNA surveillance prior to, and during, adjuvant radiotherapy. Blood samples for ctDNA analysis will be collected every 3 months for up to two years from starting ctDNA surveillance.

DNA extracted from plasma will be analysed for the presence of ctDNA, and assayed as positive (ctDNA detected) or negative (ctDNA not detected). Patients with a positive ctDNA result, within 12 months of starting ctDNA surveillance, will be allocated to treatment with pembrolizumab in the therapeutic trial.

Patients without a positive ctDNA result within 12 months of starting ctDNA surveillance will not be eligible for treatment but will continue to have exploratory ctDNA surveillance every 3 months up to 2 years from starting ctDNA surveillance.

THERAPEUTIC TRIAL

All patients with a new ctDNA positive result will be allocated pembrolizumab treatment. Following consent, patients in the observation group will be allocated to pembrolizumab at the next positive ctDNA time point. See section 1.2.1 for further details.

Pembrolizumab treatment group: 200mg pembrolizumab treatment given intravenously every 3 weeks for up to a maximum of 12 months (or until unacceptable toxicity or withdrawal of the patient's consent for any reason).

Prior to the implementation of protocol v6.0 during the randomised component of the trial, ctDNA surveillance was blinded and patients with a positive ctDNA result were randomised to treatment with pembrolizumab or observation (on a 2:1 ratio). In protocol version 6.0 randomisation was removed with all patients allocated to treatment intervention, following recommendations made by the c-TRAK TN IDMC. See section 1.2.1 for further details.

PRIMARY ENDPOINTS

1) Positive ctDNA detection by 12 and 24 months from start of ctDNA surveillance.
2) Absence of detectable ctDNA or disease recurrence 6 months (24 weeks) after commencing pembrolizumab.

SECONDARY ENDPOINTS

1) Time to ctDNA detection - defined as time from entry into the ctDNA surveillance to first positive ctDNA detection.
2) Detection of overt metastatic disease at the time of first ctDNA detection in patients allocated to pembrolizumab.
3) Lead-time between ctDNA detection and disease recurrence in the pembrolizumab treatment and observation group.
4) Absence of detectable ctDNA or disease recurrence after 6 months in the observation group.
5) Safety and tolerability of pembrolizumab

6) Commencement of treatment in patients allocated to receive pembrolizumab.

NB. Secondary endpoints relating to the observation group will be analysed in the patients randomised to the observation group prior to the implementation of protocol v6.0.

EXPLORATORY ENDPOINTS

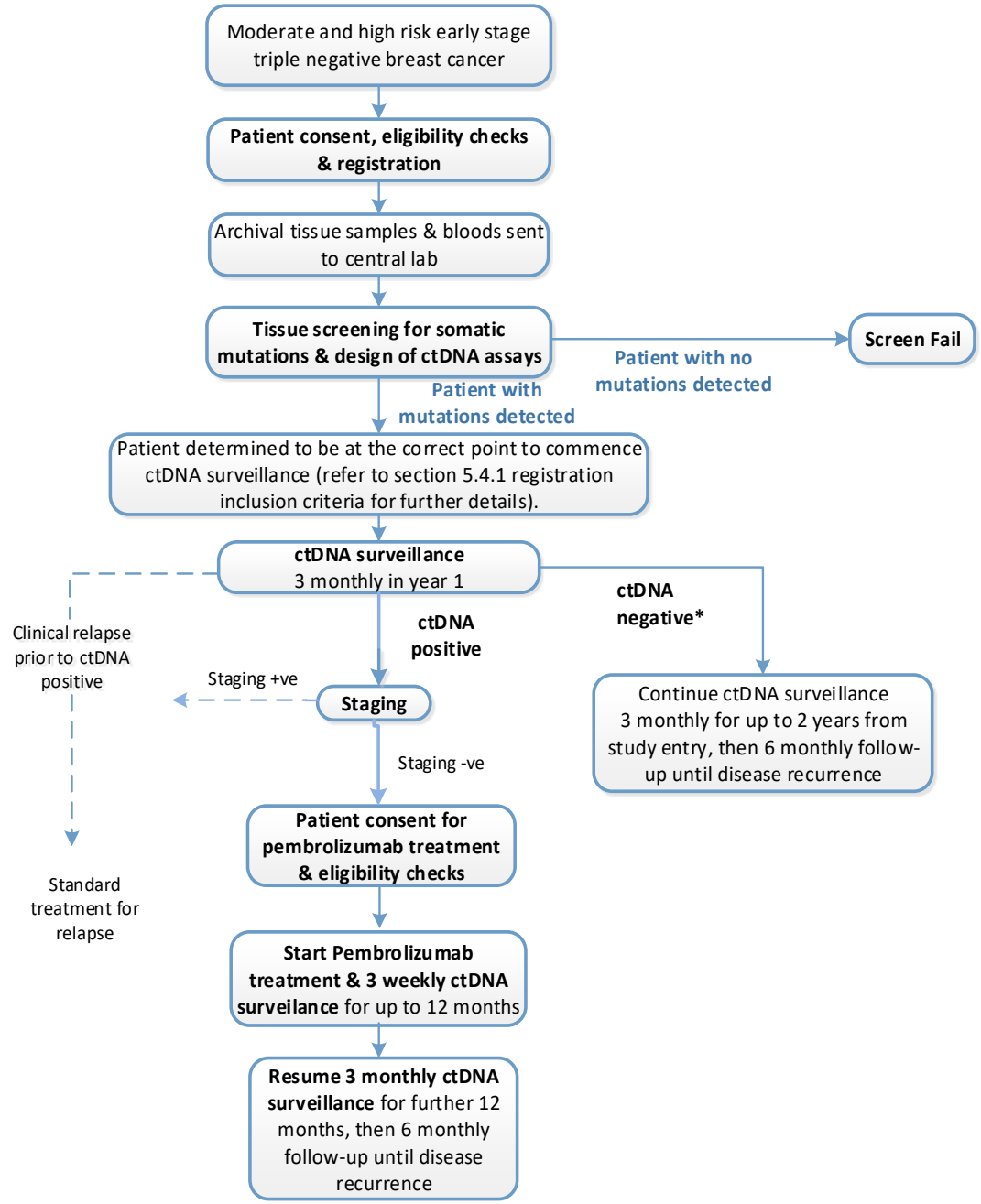
1) Descriptive differences in time between ctDNA detection and disease recurrence, and disease free survival, between patients in the pembrolizumab and the observation groups.
2) To explore predictors of sustained ctDNA clearance on pembrolizumab.
3) To explore potential predictors of relapse and ctDNA detection, and alternative definitions of ctDNA clearance.
4) Association between ctDNA clearance and time to recurrence in the pembrolizumab group.

NB. Exploratory endpoints relating to the observation group will be analysed in the patients randomised to the observation group prior to the implementation of protocol v6.0.

FOLLOW UP

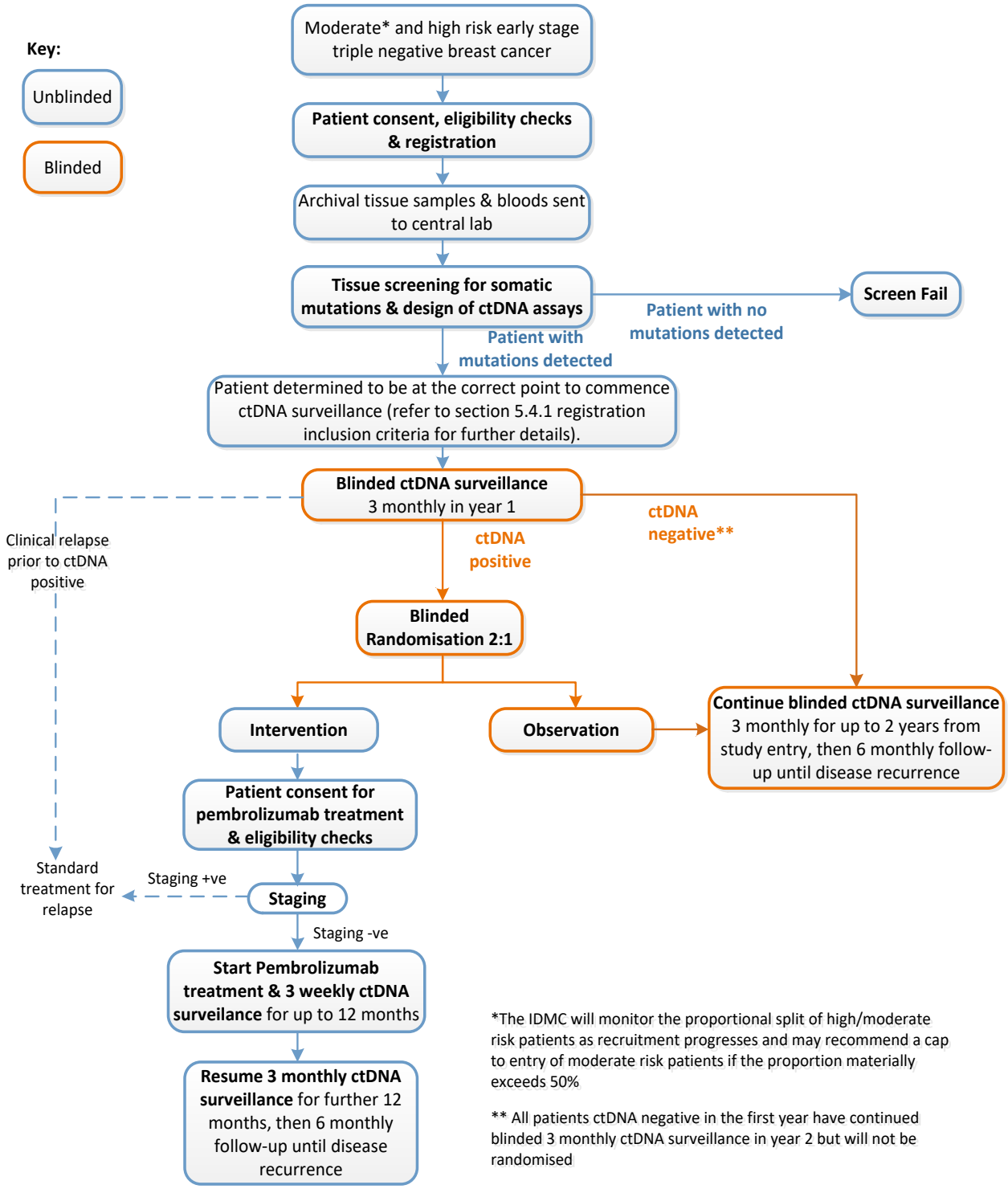
Following completion of 2 years of ctDNA surveillance or pembrolizumab treatment, patients should be followed up every 6 months until disease recurrence unless the patient specifically withdraws consent for follow up or until centres are advised that no further follow up is required.

CURRENT TRIAL SCHEMA – NON-RANDOMISED TRIAL COMPONENT (protocol v6.0 and subsequent versions)



* All patients ctDNA negative in the first year have continued 3 monthly ctDNA surveillance in year 2 but will not be allocated to receive pembrolizumab treatment

PREVIOUS TRIAL SCHEMA: RANDOMISED TRIAL COMPONENT (superseded in protocol v6.0)



*The IDMC will monitor the proportional split of high/moderate risk patients as recruitment progresses and may recommend a cap to entry of moderate risk patients if the proportion materially exceeds 50%

** All patients ctDNA negative in the first year have continued blinded 3 monthly ctDNA surveillance in year 2 but will not be randomised

1. INTRODUCTION

1.1. Background

Breast cancer is the most common cancer in women in the UK, with over 53,000 women diagnosed annually (2013 figures)[1]. Adjuvant therapies given alongside surgery and radiotherapy for women with primary breast cancer have substantially improved survival outcomes in the last two decades. However, due to its high incidence, breast cancer remains the second most common cause of cancer death in women with 11,400 deaths in 2014[1] .

Individual subtypes of breast cancer have different profiles in terms of risk of relapse and death. Triple negative breast cancers (TNBC) represent 10-15% of breast cancers and express neither hormone receptors (ER or progesterone receptor, PgR) nor are *HER2* amplified. TNBC is highly proliferative, aggressive and has a disproportionately high risk of metastasis and death. Poor outcomes are compounded by a lack of suitable targeted agents. Chemotherapy is the only systemic treatment available and although some patients with primary TNBC respond well to chemotherapy, those who do not achieve a pathological complete response to neoadjuvant chemotherapy have a markedly poor prognosis[2].

There is a critical clinical need to develop tests that can better identify and predict future risk of recurrence. In patient groups at high risk of relapse, such as TNBC, the focus would be on predicting who will relapse after standard therapy to guide the need for further therapy to prevent relapse, and conversely in lower risk groups (e.g. small, low grade ER+, HER2-ve) to identify those who are cured by surgery and do not need costly and toxic adjuvant therapy.

Circulating tumour DNA (ctDNA) represents a “liquid biopsy” alternative to invasive tumour biopsies, allowing for sensitive and specific serial sampling to be performed during the course of treatment[3]. Circulating DNA fragments carrying tumour specific sequence alterations (ctDNA) are found in the cell-free fraction of blood [4]. ctDNA can be detected in the plasma of patients with advanced cancer and can be used to track progress of disease [3, 5]. Detection of ctDNA requires highly sensitive assays due to the small proportion of ctDNA compared to the total circulating DNA [5]. Advances in sequencing technologies have enabled the rapid identification of somatic genomic alterations in individual tumours, and these can be used to design personalised assays for the monitoring of ctDNA [4]. Studies have shown the feasibility of using ctDNA to monitor tumour dynamics and in a proof-of-concept trial ctDNA was detected in 97% of metastatic breast cancers and provided an earlier measure in treatment response compared to Ca15-3 and circulating tumour cells[4, 5].

Recent work has shown that ctDNA analysis can be used to predict who is at risk of relapse [6]. In patients who have completed treatment for early breast cancer the subsequent detection of ctDNA is highly predictive for future relapse. A retrospective study of 20 primary breast cancers showed serial monitoring of ctDNA provided an average lead-time of 11 months over clinical detection of metastasis and was found to be a predictor of poor disease-free and overall survival[3].

In a prospective cohort of 55 early breast cancer patients detection of ctDNA in plasma after completion of apparently curative treatment, either at a single postsurgical time point or with serial follow-up plasma samples, predicted metastatic relapse with high accuracy. [Figure 1, hazard ratio, 13.6 (confidence interval, 4.5 to 41.2; log-rank $P < 0.001$) or 25.7 (from analysis using ctDNA status as a time-dependent covariate,

confidence interval, 8.3 to 79.8; Mantel-Byar test P < 0.0001), respectively]. Mutation tracking in serial samples increased sensitivity for the prediction of relapse, with a median lead time of 7.1 months over clinical relapse [6], data updated with 31.7 months median follow-up. Detection of ctDNA predicted a high risk of death from breast cancer (overall survival single post-surgical time point HR 84.7 confidence interval 9.8 to 730.4 p<0.001; serial follow samples HR confidence interval 6.1 to 366.1, P<0.001)[6].

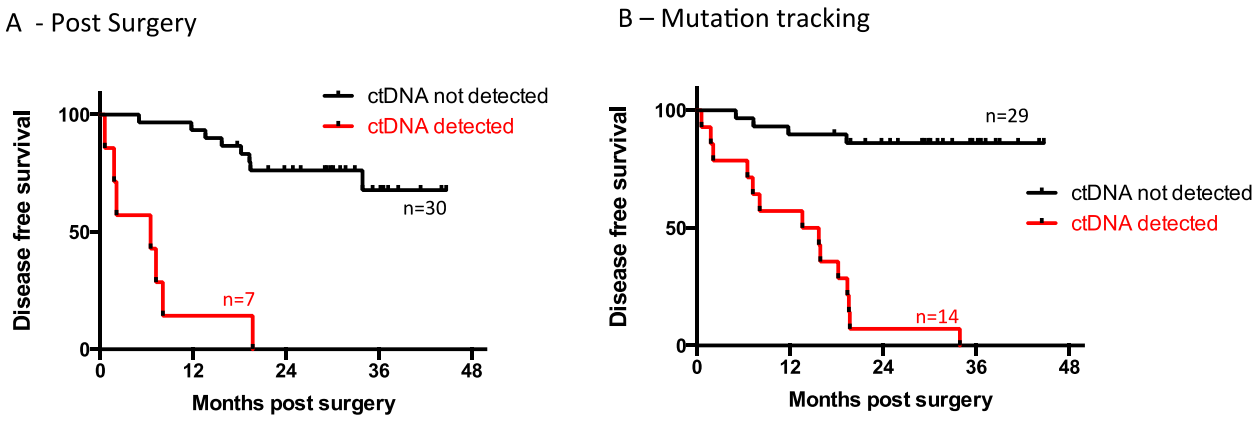


Figure 1. A. Disease-free survival according to the detection of ctDNA in the first postsurgical plasma sample [HR, 13.6 (95% CI, 4.5 to 41.2) P<0.0001]. P value determined by log-rank test. B. Disease-free survival according to the detection of ctDNA in serial post-surgery samples [HR 25.7, 95% CI (8.3 to 79.8), P < 0.0001]; P value determined by Mantel-Byar test.

In primary TNBC, despite treatment with surgery, chemotherapy and radiotherapy the risk of subsequent metastatic relapse is highest in the first two years. Identifying who is at a high risk of relapse would allow further therapy to be tailored to prevent or delay the onset of recurrence.

Immunotherapy is showing great promise in cancer treatment. Programmed Death 1 (PD-1) antibodies represent a promising novel immune therapy in the treatment for TNBC and the identification of a TNBC subtype characterised by the elevated expression of immune genes suggests that patients may benefit from such immune-based therapies[7]. PD-1 is a key immune checkpoint receptor that prevents overstimulation of immune responses and contributes to the maintenance of immune tolerance to self-antigens. It is expressed by activated T cells and mediates immunosuppression in peripheral tissues via interaction with its ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC), which are expressed by tumour cells, stromal cells or both[8, 9]. Inhibition of the PD-1 and PD-L1 interaction can enhance T cell responses and therefore cause detrimental immune responses and prevent autoimmunity. Most human cancers express PD-L1[10] and Topilian et al showed a 36% response rate in tumours expressing PD-L1 and no response in PD-L1 negative tumours when treated with an anti-PD-1 antibody[8].

The PD-1 ligand, PD-L1, is not detected in normal breast tissue, but has been reported to be expressed in about half of all breast cancers, particularly in hormone receptor (HR)-negative and high grade, proliferative tumours[11]. In addition, the presence of regulatory T cells, tumour PD-L1 expression, and PD-1–positive tumour infiltrating lymphocytes (TILs) have been associated with high histologic grade, ER negativity, and prominent tumour lymphocytic infiltration[12]. In an independent study, PD-L1 was found expressed in 23% of breast cancer specimens and it was again associated with age, tumour size, primary tumour classification, tumour grade, lymph node status, absence of ER expression, and high expression of the proliferation marker Ki-67[13]. A recent publication reported that PD-L1 messenger ribonucleic acid (mRNA) is expressed in nearly 60% of breast tumours, independently of HR status, and is positively correlated with PD-L1 protein expression

and increased TILs[14]. Another study mining the Cancer Genome Atlas (TCGA) RNA sequencing data showed that PD-L1 gene expression is significantly higher in TNBCs compared to non-TNBCs, and is associated with Phosphatase and Tensin Homolog (PTEN) loss; in the same study, PD-L1 was found expressed in 20% of TNBCs[15]. These studies demonstrate that TNBCs are characterized by PD-L1 positivity and presence of TILs, and thus suggest that PD-1 immune checkpoint inhibition is a therapeutic strategy worthy of further investigation for the treatment of this aggressive breast cancer subtype.

1.2. Trial Rationale

The purpose of the c-TRAK TN trial is two-fold. Firstly to assess the potential of digital PCR ctDNA assays to detect minimal residual disease (MRD) to predict relapse in patients who have completed treatment for moderate or high-risk early stage TNBC. Secondly, to assess in these patients, the potential effectiveness of further adjuvant therapy with pembrolizumab, assessed as the ability to result in sustained clearance of ctDNA.

It has been demonstrated that ctDNA mutation tracking has the key features that a test would require to identify patients that may require further adjuvant therapy; high sensitivity (80%) and specificity (100%) and a high predictive value for relapse HR, 13.6 (95% CI, 4.5 to 41.2) P<0.0001 (Figure 1). Garcia-Murillas *et al* have demonstrated that serial sampling is required to robustly identify ctDNA and have established a platform to design personalised digital PCR ctDNA assays[6].

1.2.1. Rationale for study design

Selection of patients for treatment on the basis of a positive ctDNA test

Although assays of MRD are standard in the management of haematological cancers, this will be one of the first studies to assess whether ctDNA assays have clinical utility in guiding further therapy. Patients recruited into the c-TRAK TN study will be of moderate or high risk of future relapse. The inclusion criteria, on which risk for relapse are based, are similar to many previous adjuvant therapy studies for breast cancer (for example, BEATRICE NCT00528567 [16]) that have randomised all patients unselected to treatment or placebo. However, in these studies many patients never relapse, are consequently treated unnecessarily, and do not contribute to the primary endpoint of the study. In c-TRAK TN we will use assays of ctDNA to further select from within this population a set of patients who are at very high risk of relapse (i.e. those with a positive ctDNA test).

Prior to the implementation of protocol v6.0 during the original randomised component of the trial, this high risk population was randomised between intervention with medicinal product or observation. The standard of care for these patients is observation, and no treatment will be withdrawn as a result of ctDNA testing.

In protocol version 6.0, following recommendations made by the c-TRAK TN IDMC (further detail provided in the paragraph below), the trial design changed into the non-randomised component; randomisation was removed and all patients with a positive ctDNA result will be allocated to intervention with pembrolizumab. Patients who were previously randomised to observation and are continuing with ctDNA surveillance, will be asked to re-consent and subsequently be allocated to pembrolizumab at the next positive ctDNA test.

Rationale for the change in study design – June 2020 (protocol v6.0)

Study progress to date has provided data enhancing the evidence that ctDNA presence in the blood of patients treated for moderate and high risk TNBC is a definitive positive predictor of disease recurrence. In addition to this, data from external sources and emerging data from this trial suggest that instances of overt metastatic disease in these patients presents more frequently than initially predicted at study onset, that

ctDNA detection is associated with near inevitability of disease recurrence in triple negative breast cancer, and that ctDNA does not spontaneously clear from the blood.

There is also new evidence for immuno-oncology therapy in TNBC; in 2019, the FDA granted accelerated approval for atezolizumab in combination with nab-paclitaxel in the treatment of patients with unresectable locally advanced or metastatic triple negative breast cancer, based on the findings from the phase III double-blind Impassion132 trial (NCT02425891) [17], further reinforcing the potential benefit of the interventional treatment arm within the study.

Taking this new data into account, the c-TRAK TN Independent Data Monitoring Committee proposed that the observation arm of the study was no longer required and should be closed. Following implementation of protocol v6.0, any patient with a subsequent ctDNA positive result detected in the blood (including those that had already been randomised to observation and remain in ctDNA surveillance) will be given the option to commence pembrolizumab treatment. This recommendation was endorsed by the ICR-CTSU Breast Systemic Therapies Trial Steering Committee and protocol version 6.0 was amended to close randomisation within the trial, with all patients allocated to treatment intervention.

Novel primary endpoint of sustained clearance of ctDNA

Even in patients with triple negative breast cancer, the most aggressive of breast cancer subtypes, the risk of relapse after treatment of primary breast cancer is relative low. The BEATRICE study [16] recently reported an invasive disease free survival relapse at 3 years occurred in only 16% of patients in the control group. Consequently phase III studies are large, and costly, with BEATRICE recruiting 2591 patients.

In the c-TRAK TN study we propose a novel primary endpoint of sustained clearance of ctDNA, defined as 'Absence of detectable ctDNA or disease recurrence 6 months (24 weeks) after commencing pembrolizumab'. This endpoint is chosen as a surrogate endpoint of treatment efficacy, which is anticipated to correlate with long-term outcome. A very similar primary endpoint (comparison of circulating tumour cell (CTC) detection rate at week 18 between trastuzumab treatment arm and observational arm) has been used in other studies in the adjuvant setting (TREAT-CTC, NCT01548677: Efficacy Study of Herceptin to Treat HER2-negative CTC Breast Cancer).

Use ctDNA diagnostics for detection of MRD

In the proposed c-TRAK TN study we will personalise each ctDNA assay to the mutations identified in each patients' tumour, and the assays will therefore not be CE marked. The detection of MRD in haematological malignancies is routinely done with non-CE marked assays, as the assays are designed to the rearrangements identified in an individual patients' leukaemia. All assays will be manufactured at the c-TRAK TN central laboratory and used on the premises of manufacture, in accordance with the Guidance on the In Vitro Diagnostic Medical Devices Directive 98/79/EC (August 2013). All assays will be subject to strict validation prior to use.

Rationale for protocol amendments to reduce the time between surgery and the start of ctDNA surveillance – May 2019 (protocol v5.0)

In protocol version 5.0 the permitted time from surgery to registration in the trial was reduced, to reflect the risk of very early relapse of patients with triple negative breast cancer [18], and to maximize the opportunity for patients to be able to start ctDNA surveillance prior to clinical relapse. In addition, the trial was amended to allow patients to start ctDNA surveillance before or during standard adjuvant radiotherapy, to reduce the time from surgery to starting ctDNA surveillance. In some patients this may result in patients starting pembrolizumab concurrent with adjuvant radiotherapy, similar to other trials in the post-neoadjuvant

therapy setting for example where adjuvant trastuzumab-emtansine is given concurrent with adjuvant radiotherapy [19]. In addition, the trial was amended to allow patients who have completed neoadjuvant chemotherapy, and are then receiving adjuvant capecitabine, to start ctDNA surveillance after 3 months capecitabine [18], as otherwise patients have a long delay between surgery and the start of ctDNA surveillance (refer to section 5.4.1 for details).

1.2.2. **Pembrolizumab**

Pembrolizumab is an immune stimulatory anti-PD1 antibody with high levels of activity in multiple tumour types, licensed for the treatment of metastatic melanoma and non-small cell lung cancer. Formerly known as lambrolizumab and MK-3475, pembrolizumab is a potent and highly selective humanised 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. The variable region sequences a very-high-affinity mouse antihuman PD-1 antibody (dissociation constant, 28 pM) and is grafted into a human IgG4 immunoglobulin with a stabilising S228P Fc alteration. The IgG4 immunoglobulin subtype does not engage Fc receptors or activate complement, thus avoiding cytotoxic effects of the antibody when it binds to the T cells that it is intended to activate[20].

Immunotherapy trials to date have shown results that improve survival but also have a prolonged duration of response leading to improved overall survival [8, 21-24]. In the first in-human phase I dose escalation study of pembrolizumab in solid tumours (KEYNOTE-001), the 1 year overall survival rate in 411 melanoma patients was 71%[25]. Long-term efficacy data of 655 patients showed the overall response rate (ORR) was 34%, with 6% complete response rate and the duration of response ranged from 6 to 98 weeks. With the continuing emerging data of improved ORR and a prolonged duration of response in melanoma patients, NICE approved pembrolizumab in October 2015, for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilumumab and, if BRAF V600 mutation positive, previous failed treatment with a BRAF inhibitor (vemurafenib and dabrafenib) or MEK inhibitor (trametinib).

1.2.3. **Pembrolizumab known benefits and risks**

Pembrolizumab is generally well tolerated over a spectrum of tumour types and demonstrates a favourable safety profile in comparison to chemotherapy. Safety data from over 2700 patients has reported the most common adverse events of all grades were diarrhoea, cough, pruritis, arthralgia, rash, pyrexia and back pain (Pembrolizumab Investigator Brochure, Merck, Sharp & Dohme). Common grade 3-4 drug related adverse events were anaemia, raised alanine aminotransferase and aspartate aminotransferase.

Immune toxicities are well known among the immunotherapy drugs. Immune-mediated adverse reactions of all grades included hypothyroidism (8.4%), hyperthyroidism (3.4%), pneumonitis (3.4%), colitis (1.8%), severe skin reactions (1.4%), hepatitis (0.7%), hypophysitis (0.6%), diabetes mellitus (0.2%) and nephritis (0.1%). Other immune-mediated adverse reactions of clinical significance were reported in less than 1% of patients included uveitis, myositis, Guillian-Barre syndrome and pancreatitis. Events have been reported to occur from as early as the first dose to several months after the last dose of treatment and early recognition can allow initiation of symptomatic treatment preventing delays and discontinuation of treatment. Complications requiring steroids or immunosuppression are commonly reversible.

The first trial to report clinical activity of an immune check point inhibitor in TNBC was a phase 1b trial (KEYNOTE-012) showing that single agent pembrolizumab given at 10mg/kg IV once every 2 weeks (Q2W) was well tolerated and effective[26]. Twenty-seven heavily pre-treated recurrent or metastatic TNBC positive for PD-L1 were treated showing 18.5% of evaluable patients responded with one complete response, 4 partial

responses and 7 stable disease responses. Patients with a LDH greater than twice the upper limit of normal, which represents rapidly progressive disease had no response to pembrolizumab. Three patients remained on pembrolizumab for at least 11 months, which supports previous suggestions of the durability of pembrolizumab. Previous studies have mentioned the use of PD-L1 as a biomarker for response. However this study lacked correlation between the degree of PD-L1 positivity and response, questioning the use of PD-L1 as an appropriate biomarker [26].

In KEYNOTE-012, the main toxicities of any grade included arthralgia (18.8%), fatigue (18.8%), myalgia (18.8%) and nausea (15.6%). There were five grade 3 treatment related adverse events: anaemia, aseptic meningitis (successfully treated with pembrolizumab interruption and steroids for 6 weeks, with reintroduction of pembrolizumab at a reduced dose), lymphopenia, headache and pyrexia. The patient who had aseptic meningitis had a prolonged partial response and remained on treatment for more than 17 months. There was one treatment related death due to disseminated intravascular coagulation in a patient who had with rapidly progressive disease having had three previous lines of treatment. There were a total of three immune related toxicities; grade 3 colitis (reported 40 days after the last pembrolizumab dose and after starting capecitabine), grade 3 hepatitis and grade 2 hypothyroidism[26]. Pembrolizumab may be given safely in combination with radiotherapy with no apparent increase in adverse effects [27-29]. Pembrolizumab has been given concurrently with adjuvant radiotherapy in completed and ongoing phase III trials (NCT03725059, NCT03036488).

1.2.4. **Rationale for pembrolizumab dose selection/regimen**

A fixed dose regimen will simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. It will reduce complexity in the logistical chain at treatment facilities and reduce wastage. A population pharmacokinetic (PK) model, which characterised the influence of body weight and other patient covariates on exposure, has been developed. The PK profile of pembrolizumab is consistent with that of other humanised monoclonal antibodies, which typically have a low clearance and a limited volume of distribution. Since the anti-tumor effect of pembrolizumab is driven through reactivation of adaptive immune response by blocking PD-1 expressed on T-cells, but not direct binding to cancer cells, once the PD-1 on T-cells are fully saturated by pembrolizumab, the shape of the exposure-response relationship among indications is expected to be similar. This is supported by exposure-response analysis in multiple indications. A flat exposure response relationship was demonstrated between pembrolizumab exposure (or dose) and efficacy or safety within the dose range of 2 to 10 mg/kg or 200 mg to 10 mg/kg (exposure at 2 mg/kg Q3W is similar to exposure at 200 mg Q3W). The similarity in efficacy between the tested dose regimens is further supported by comparisons of overall response rate (ORR)/survival outcomes for the tested dose regimens in the melanoma and NSCLC indications. A fixed dose regimen of pembrolizumab is now standard in ongoing pembrolizumab trials. A fixed dose of pembrolizumab 200mg every 3 weeks has been selected for the c-TRAK TN trial.

1.2.5. ***Blinding in the c-TRAK TN trial (prior to protocol v6.0)***

Prior to the implementation of protocol v6.0 during the randomised component of the trial, ctDNA analysis and randomisation were performed independently of the recruiting site in order to keep the treating team and patient blinded to the ctDNA result. The ctDNA analysis was performed centrally by the c-TRAK TN central laboratory. Randomisation to the pembrolizumab treatment/observation groups was performed centrally by the ICR-CTSU, triggered by notification from the central laboratory of a positive ctDNA result. If the patient

was allocated to the pembrolizumab treatment group, the Principal Investigator (or designated investigator) was notified by the ICR-CTSU, in order to confirm continued patient consent and perform eligibility checks to start pembrolizumab treatment. Such patients (and their treating team) were therefore 'unblinded' to a positive ctDNA result. If the patient is allocated to the observation group, the treating team and patient were not informed that randomisation had taken place, in order to maintain the blind to a positive ctDNA result and the patient would continue with blinded ctDNA surveillance for up to two years from start of ctDNA surveillance. By keeping both the treating team and patient blind to ctDNA test results the study aimed to avoid unnecessary anxiety in otherwise asymptomatic patients and avoid pressures to restart treatment. Given the uncertainty of the clinical implication of a positive ctDNA result at the time of trial initiation, knowledge of such may have caused concern if no treatment was offered and bias would be introduced if such patients were followed up differently. A similar study in patients treated for ovarian cancer in which both patients and investigators were blinded to the results of CA125 measurements done every 3 months during routine follow-up, showed no evidence of a survival benefit with early treatment of relapse on the basis of a raised CA125 concentration alone[30]. This supported the need for blinding of ctDNA surveillance results in the c-TRAK trial.

Following the implementation of protocol v6.0 whereby patients transfer to the non-randomised component of the trial, all ctDNA results will be open, and all ctDNA positive patients will be allocated to pembrolizumab intervention.

1.3. Description of Population

Patients with moderate or high risk early stage triple negative breast cancer who have completed neoadjuvant chemotherapy and surgery, or have completed primary surgery and are due to receive/are receiving adjuvant chemotherapy, will be invited to participate. Where radiotherapy is indicated, patients will be eligible for trial entry before/during radiotherapy. All patient groups should ideally be registered as early as possible in their treatment pathway to allow more time for tissue screening to be completed.

2. TRIAL OBJECTIVES

2.1. Primary Objectives

- To assess whether digital PCR ctDNA assays, as a non-invasive biomarker on serial blood samples, can predict which patients are at highest risk of relapse and identify patients with minimal residual disease not visible on imaging.
- To assess whether pembrolizumab results in a sustained clearance of ctDNA in patients who have completed treatment for moderate or high risk early stage triple negative breast cancer, but have minimal residual disease (MRD) detected by serially assessed ctDNA analysis.

2.2. Secondary Objectives

- To assess time to first positive ctDNA detection from entry into the ctDNA surveillance.
- To assess the rate of detection of overt metastatic disease at the time of first ctDNA detection.
- To assess the median lead-time between ctDNA detection and relapse in the pembrolizumab treatment and observation groups.
- To assess sustained clearance of ctDNA in the observation group.
- Safety and tolerability of pembrolizumab.
- To assess the proportion of patients allocated to receive pembrolizumab who start pembrolizumab.

NB. Secondary objectives relating to the observation group will be evaluated in the patients randomised to the observation group prior to the implementation of protocol v6.0.

2.3. Exploratory Objectives

- To explore differences in time between ctDNA detection and disease relapse, and disease free survival, between patients in the pembrolizumab and the observation groups.
- To explore predictors of sustained ctDNA clearance on pembrolizumab.
- To explore potential predictors of relapse and ctDNA detection, and alternative definitions of ctDNA clearance.
- To assess the association between ctDNA clearance and time to recurrence in the pembrolizumab group.

NB. Exploratory objectives relating to the observation group will be evaluated in the patients randomised to the observation group prior to the implementation of protocol v6.0.

3. TRIAL DESIGN

c-TRAK TN is a multi-centre, single-arm, phase II trial that uses ctDNA surveillance to direct intervention, in patients with moderate or high-risk early stage triple negative breast cancer, with no evidence of distant metastatic disease, who have completed standard therapy (surgery and neoadjuvant/adjuvant chemotherapy). Eligible patients may be enrolled straight after surgery or before/during adjuvant chemotherapy, or before/ during adjuvant radiotherapy, if indicated.

The trial consists of tissue screening, ctDNA surveillance and a therapeutic trial. If suitability is confirmed following tissue screening, patients will undergo serial ctDNA surveillance, with allocation to treatment with

pembrolizumab in the therapeutic trial triggered by the detection of minimal residual disease (indicated by a ctDNA positive result) on or before their 12 month ctDNA surveillance assessment.

Prior to the implementation of protocol v6.0 during the randomised component of the trial, ctDNA surveillance was blinded and the detection of minimal residual disease (indicated by a ctDNA positive result) on or before the 12 month ctDNA surveillance assessment triggered randomisation to treatment with pembrolizumab or observation (on a 2:1 ratio).

Following the implementation of protocol v6.0, patients will be asked to transfer to the non-randomised component of the trial, all patients who were previously randomised to observation and remain in ctDNA surveillance will transition to the non-randomised component of the trial following re-consent, and be allocated to pembrolizumab at the next positive ctDNA result.

On 19th March 2020, conditions were put in place to temporarily suspend ctDNA surveillance due to the COVID-19 outbreak. When trial activities are able to resume, the 12 month active surveillance period may be extended by a proportional amount of time to counteract the loss of surveillance samples for each patient during the suspension. For example, if ctDNA surveillance is halted for a duration of time which means that a patient misses **one** ctDNA surveillance sample, active surveillance will be extended beyond the original 12 months to include **one** of the designated exploratory samples (planned to be collected between months 12 and 24). Patients who are ctDNA positive in this extended period will be assessed in the protocol as if they were within in the originally planned 12 month window, and allocated to treatment.

3.1. Tissue Screening and ctDNA Surveillance

Entry into the tissue screening part of the trial will require mandatory provision of tissue from two archival tumour samples and provision of blood samples for germline DNA analysis and additional ctDNA analysis. For patients who have had tumours previously sequenced outside of the c-TRAK TN trial (for example, but not limited to, in the Genomics England Limited 100,000 genome initiative (GEL)), the time required for tissue screening may be significantly reduced if the report confirming the results of the tumour sequencing and the mutation(s) detected can be provided to the c-TRAK TN central laboratory. Patients will be asked to permit access to the report at the time of consent for trial entry. Please refer to section 7.1.2 for further details of the trial requirements for patients with prior sequencing information available.

The tissue and blood samples will be sent to the c-TRAK TN central laboratory for mutation analysis. If trackable mutations are detected in the patient's tumour tissue, ctDNA assays will be personalised and developed for the mutations found in each individual patient's cancer, and the ICR-CTSU will inform the treating team that ctDNA surveillance can be initiated.

If no mutations can be detected in the patient's tumour tissue, or if it is not possible to develop a ctDNA assay, this will be considered a screen failure, and the ICR-CTSU will inform the treating team that ctDNA surveillance should **not** be initiated. Patients with a mutation analysis screen failure will not be entered into ctDNA surveillance and will not be followed up within the c-TRAK TN trial.

Multifocal tumours where tissue screening reveals multiple mutations can be included **where deemed eligible by the Chief Investigator** or, if unavailable, the designated TMG member. This will be assessed on a case by case basis and the ICR-CTSU will inform the treating team whether or not ctDNA surveillance can be initiated.

Patients with tumour tissue in which mutations are detected and for which a ctDNA assay can be developed should start ctDNA surveillance once they have completed standard treatment, including adjuvant chemotherapy, **except when receiving adjuvant capecitabine**. If receiving capecitabine, patients should

commence ctDNA surveillance 3 months (+/- 2 weeks) after starting capecitabine and treatment can continue while ctDNA surveillance is ongoing. Patients who have stopped capecitabine prior to completing 3 months of treatment for any reason other than disease recurrence are also permitted to start ctDNA surveillance. Patients may start ctDNA surveillance prior to, and during, adjuvant radiotherapy.

Blood samples for ctDNA analysis should be collected every 3 months for up to two years from starting ctDNA surveillance and sent to the c-TRAK TN central laboratory for processing and analysis. DNA extracted from plasma will be analysed for the presence of ctDNA, and assayed as positive (ctDNA detected) or negative (ctDNA not detected). All positive ctDNA results within 12 months of a patient starting ctDNA surveillance will be notified to the ICR-CTSU in order for allocation to the therapeutic trial to be performed independently of the recruiting site.

If after the first 12 months of ctDNA surveillance, patients have not had a positive ctDNA result they will continue to have ctDNA surveillance every 3 months up to 2 years from starting ctDNA surveillance. All ctDNA blood samples collected in the second year of surveillance are exploratory, to confirm the optimal duration of ctDNA surveillance, and whether in subsequent studies two years of active surveillance as opposed to one year should be adopted. These samples will not be analysed in real-time. The exception to this is for patients who have missed blood samples within the first 12 months as a result of ctDNA surveillance suspension. For these patients real-time ctDNA testing, and allocation to trial treatment, may be extended beyond the original 12 months for a proportional amount of time to counteract the loss of surveillance samples for each patient.

Approximately 50% of patients entered into ctDNA surveillance will have moderate risk disease. The IDMC will monitor the proportional split of high/moderate risk patients as recruitment progresses and may recommend a cap to entry of moderate risk patients if the proportion materially exceeds 50%.

3.2. Therapeutic Trial

Patients who have positive detection of ctDNA on or before their 12 month ctDNA surveillance assessment will be allocated to pembrolizumab treatment within the therapeutic trial centrally by ICR-CTSU.

Prior to the implementation of protocol v6.0 during the randomised component of the trial, patients who had positive detection of ctDNA on or before their 12 month ctDNA surveillance assessment were randomised centrally by ICR-CTSU in a 2:1 ratio to pembrolizumab treatment or observation (continued blinded ctDNA surveillance).

All patients randomised to observation prior to implementation of protocol v6.0 who remain in ctDNA surveillance, will transition to the non-randomised trial component following re-consent, and be allocated to pembrolizumab at the next positive ctDNA result.

3.2.1. Pembrolizumab treatment group

If a patient is allocated to the pembrolizumab treatment group, the treating team will be informed by ICR-CTSU. If the patient is receiving adjuvant capecitabine or radiotherapy, they will be offered the option to join the therapeutic trial either by switching treatment from capecitabine to pembrolizumab, or commencing pembrolizumab in combination with radiotherapy. If the patient confirms their consent to receive trial treatment (including switching their treatment from capecitabine to pembrolizumab) staging scans should be done to detect for evidence of macroscopic disease, prior to commencing pembrolizumab. If macroscopic disease is detected, the patient will not receive pembrolizumab treatment and will instead receive standard treatment for recurrent disease outside of the c-TRAK TN trial. If there is no evidence of macroscopic disease,

the patient should commence treatment with pembrolizumab treatment (200mg given intravenously every 3 weeks) for up to a maximum of 12 months or until unacceptable toxicity or withdrawal of the patient's consent for any reason (see section 11.6). No further imaging will be performed after commencing pembrolizumab unless clinically indicated, in which case local procedure should be followed. Patients who are diagnosed with disease recurrence must stop pembrolizumab, and should receive standard treatment for recurrent disease outside of the c-TRAK TN trial. Patients who present with isolated lymphadenopathy may continue pembrolizumab until relapse is confirmed, and should be managed as per section 13.1.

Patients who are allocated to pembrolizumab but do not wish to commence pembrolizumab treatment (including switching treatment from capecitabine to pembrolizumab), will not enter the therapeutic component of the c-TRAK TN trial and if agreed should instead continue with 3 monthly ctDNA surveillance. Blood samples for ctDNA analysis should continue to be collected during and after pembrolizumab treatment as described in section 11. The results of these ctDNA analyses will not be reported to the treating team or patient.

3.2.2. *Observation group (continued blinded ctDNA surveillance – prior to protocol v6.0)*

Prior to the implementation of protocol v6.0 during the randomised component of the trial, if a patient was randomised to the observation group, the treating team and patient were ***not*** *informed that randomisation had taken place in order to remain blinded to the positive ctDNA result the patient would continue to have blood samples collected for ctDNA analysis as described in section 8.*

Following implementation of protocol v6.0, all patients who were previously randomised to observation and remain in ctDNA surveillance will transition to the non-randomised component of the trial following re-consent, and be allocated to pembrolizumab at the next positive ctDNA result.

3.3. Follow-up

3.3.1. Patients who receive pembrolizumab treatment

A safety assessment should be conducted for each patient approximately 30 days after the last administration of pembrolizumab treatment or before initiation of a new anti-cancer treatment, whichever comes first (see section 11.4). SAEs that occur within 90 days of the end of treatment, and before initiation of a new anti-cancer treatment, should also be reported, followed and recorded. Any SAEs that occur more than 90 days after the last dose of pembrolizumab that, in the opinion of the Principal Investigator (or designated investigator), are related to pembrolizumab should be reported to ICR-CTSU if the Principal Investigator (or designated investigator) becomes aware of them.

Patients should be followed up every 6 months (+/- 1 month) from start of ctDNA surveillance until disease recurrence (see section 13), unless the patient specifically withdraws consent for follow up (see section 12) or until centres are advised that no further follow up is required.

3.3.2. Patients who do not receive pembrolizumab treatment

Following completion of 2 years of ctDNA surveillance, patients should be followed up every 6 months (+/- 1 month) from the start of ctDNA surveillance, until disease recurrence (see section 13) unless the patient

specifically withdraws consent for follow up (see section 12) or until centres are advised that no further follow up is required.

3.3.3. Long term follow up

Patients who remain disease free after completing ctDNA surveillance may transfer to a separate umbrella protocol (subject to funding and the appropriate approvals) for further follow-up for disease recurrence. The protocol will be observational and no treatments (investigational or standard treatments) will be administered. Patients will be asked to consent to their full name, date of birth, hospital number, postcode and NHS number or equivalent, being collected at trial entry and to allow the ICR-CTSU access to their routinely collected NHS data and national health records to allow future linkage in order to follow up on patient health status.

4. TRIAL ENDPOINTS

4.1. Primary Endpoints

1) Positive ctDNA detection by 12 and 24 months from start of ctDNA surveillance.
2) Absence of detectable ctDNA or disease recurrence 6 months (24 weeks) after commencing pembrolizumab.

4.2. Secondary Endpoints

1) Time to ctDNA detection - defined as time from entry into the ctDNA surveillance to first positive ctDNA detection.
2) Detection of overt metastatic disease at the time of first ctDNA detection in patients allocated to pembrolizumab.
3) Lead-time between ctDNA detection and disease recurrence in the pembrolizumab treatment and observation group.
4) Absence of detectable ctDNA or disease recurrence after 6 months in the observation group.
5) Safety and tolerability of pembrolizumab.
6) Commencement of treatment in patients allocated to receive pembrolizumab.

NB. Secondary endpoints relating to the observation group will be analysed in the patients randomised to the observation group prior to the implementation of protocol v6.0.

4.3. Exploratory Endpoints

1) Descriptive differences in time between ctDNA detection and disease recurrence, and disease free survival, between patients in the pembrolizumab and the observation groups.
2) To explore predictors of sustained ctDNA clearance on pembrolizumab.
3) To explore potential predictors of relapse and ctDNA detection, and alternative definitions of ctDNA clearance.
4) Association between ctDNA clearance and time to recurrence in the pembrolizumab group.

NB. Exploratory endpoints relating to the observation group will be analysed in the patients randomised to the observation group prior to the implementation of protocol v6.0.

5. PATIENT SELECTION FOR REGISTRATION

Registration in the trial includes entry into tissue screening and if eligibility is confirmed, subsequent entry into ctDNA surveillance with the potential to be treated with pembrolizumab.

Patients should have completed any neoadjuvant treatment and surgery prior to registration, and must be registered before or on the day of the 3rd cycle of adjuvant chemotherapy (except capecitabine). Patients receiving adjuvant capecitabine must register before starting capecitabine**.** Patients may enrol before or during adjuvant radiotherapy, if indicated. Patients should be registered as soon as possible in order to ensure tissue screening is complete and screening for ctDNA surveillance can commence at the earliest opportunity. Please refer to specific windows for registration in inclusion criteria in section 5.4.1.

5.1. Number of participants

It is anticipated that 75% of patients tested will have mutations detected in their archival tumour tissue and be eligible to commence ctDNA screening. c-TRAK TN therefore aims to sequence archival tumour tissue from approximately 200 patients in order to recruit approximately 150 patients into ctDNA surveillance.

5.2. Source of participants

Participants will be recruited from approximately 15 participating sites in the UK, selected based on expertise and experience in immunotherapy and with proven track records of delivering early phase clinical trials. Potential participants will be identified in oncology clinics or at Multi-Disciplinary Team (MDT) meetings.

5.3. Procedure for obtaining informed consent for registration

5.3.1. Patients with tumour sequencing to be conducted within c-TRAK TN

The Principal Investigator (or designated investigator) must ensure that each trial patient is fully informed about the nature and objectives of the trial and possible risks associated with participation.

Prior to registration, patients should be given the current ethics approved **c-TRAK TN patient information sheet for registration** for their consideration. Patients should only be asked to consent to the trial after they have had sufficient time to consider the trial, and the opportunity to ask any further questions.

No protocol required assessments, unless they are performed routinely as part of standard patient care, should be conducted until the **c-TRAK TN consent form for registration** has been signed and dated by both the patient and the investigator.

Confirmation of the patient's consent and the informed consent process should be documented in the patient's medical notes. A copy of the signed consent form(s) should be provided to the patient and the original retained in the investigator site file, which must be made available for verification by ICR-CTSU trial staff or for regulatory inspection at any time.

5.3.2. Patients with tumour sequencing previously conducted outside of c-TRAK TN

The same consent procedure as described in section 5.3.1 should be followed for patients with prior tumour sequencing conducted outside of the c-TRAK TN trial. In addition, the study name in which the prior tumour sequencing was conducted should be entered on the consent form and the patient requested to initial the form in the appropriate box to confirm whether or not access to the report is permitted.

5.4. Eligibility criteria for patient registration

Patients will be considered eligible for registration into c-TRAK TN if they fulfil all the inclusion and none of the exclusion criteria listed below.

5.4.1. Inclusion Criteria

1) Signed Informed Consent Form for Registration.
2) Male or female patients ages 16 years or older.
3) ECOG performance status 0, 1 or 2.
4) Histologically proven primary triple negative breast cancer as defined as oestrogen receptor (ER) negative, progesterone receptor (PgR) negative (if available, otherwise PgR unknown), (as defined by Allred score 0/8 or 2/8 or stain in <1% of cancer cells) and HER2 negative (immunohistochemistry 0/1+ or negative by *in situ* hybridization) as determined by local laboratory.
5) Availability of tissue from two archival tumour tissue samples (either from diagnostic biopsy and/or primary surgery). If only one tumour sample is available, the site should inform the ICR-CTSU who will discuss eligibility with the Chief Investigator (or designated TMG member). Patients who have tumours previously sequenced outside the c-TRAK TN trial must provide one archival tumour tissue sample and the report that confirms the mutations detected.
6) Patients with moderate or high risk early stage triple negative breast cancer according to the following risk of relapse criteria:

   Neoadjuvant chemotherapy (no adjuvant chemotherapy planned)

   High risk criteria - Residual microscopic or macroscopic invasive cancer in the axillary nodes after chemotherapy

   Moderate risk criteria - Residual invasive cancer in the breast, and axillary lymph node negative after chemotherapy

   Adjuvant chemotherapy

   High risk criteria - Tumour size >50mm and node positive OR ≥4 nodes positive regardless of primary tumour size.

   Moderate risk criteria - Tumour size >20mm AND/OR involved axillary macroscopic lymph node.

   Both neoadjuvant and adjuvant chemotherapy

   Patients who have received both neoadjuvant chemotherapy and further adjuvant chemotherapy must fulfil only the adjuvant chemotherapy risk criteria to be eligible. They can fulfil the criteria on either clinical staging prior to neoadjuvant chemotherapy or pathological staging at surgery.
7) Patients must be registered according to the following criteria for timing of registration:

   Neoadjuvant chemotherapy (no adjuvant chemotherapy planned):

   Patients must be registered within 6 weeks of surgery. Patients may be registered before or during radiotherapy and **should be registered as early as possible.**

Adjuvant chemotherapy (no neoadjuvant chemotherapy received):

Patients must be registered before, or on the day of, the 3rd cycle of adjuvant chemotherapy and **should be registered as early as possible.**

Both neoadjuvant and adjuvant chemotherapy

Patients must be registered within 6 weeks of surgery. Patients may be registered before or during radiotherapy. **Patients must register before starting capecitabine.**

8) Consent to provide research blood samples.
9) Patients with bilateral tumours can be included if both are triple negative and if two archival tissues samples can be provided per tumour.
10) Patients must have had surgery achieving clear margins (as per local guidelines).
11) Female and male patients of reproductive potential must be willing to use an adequate method of contraception for the first year of the trial and, if allocated to pembrolizumab, for the duration of treatment through to 120 days after the last dose of pembrolizumab (see appendix 2). ***Note***: *Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the patient.*
12) Patients must be willing to have frequent blood tests (every 3 months for 2 years in ctDNA surveillance and 3 weekly if subsequently allocated pembrolizumab) and receive a 12 month course of pembrolizumab on ctDNA detection.
13) No evidence of distant metastatic disease or local recurrence on staging scans conducted at any time since initial diagnosis.

5.4.2. Exclusion Criteria

1) Any concurrent or planned treatment for the current diagnosis of breast cancer other than surgery, loco regional adjuvant radiotherapy, standard neoadjuvant or adjuvant chemotherapy, or a bisphosphonate/denosumab.
2) Prior treatment with a PDL1, PD1, or other immunomodulatory therapy.
3) Prior diagnosis of cancer (including prior diagnosis of breast cancer) in the previous 5 years, other than for basal cell carcinoma of the skin or cervical carcinoma in situ.
4) Patients previously entered into a therapeutic trial during or after neoadjuvant chemotherapy where experimental therapy is continued post-surgery (see protocol section 15).
5) Treatment with an unlicensed or investigational product within 4 weeks of trial entry.
6) Active autoimmune disease requiring systemic therapy in the last two 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) is not considered a form of such systemic treatment.
7) Diagnosis of immunodeficiency or receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of pembrolizumab.
8) Known history of active Tuberculosis Bacillus (TB).

9) Known history of Human Immunodeficiency Virus (HIV).
10) Known active Hepatitis B or Hepatitis C.
11) Known history of, or any evidence of active, non-infectious pneumonitis.
12) Active infection requiring systemic therapy.
13) Previous solid organ or allogenic stem cell transplantation.
14) Females who are pregnant or breastfeeding.
15) Presence of any systemic illness incompatible with participation in the clinical trial or inability to provide written informed consent.
16) A pathological complete response (pCR) to neoadjuvant chemotherapy

6. PROCEDURE FOR PATIENT REGISTRATION

Patient registration into the trial should take place only once patient consent has been obtained and eligibility has been confirmed, according to eligibility criteria for patient registration (see section 5.4). A **Registration Eligibility Checklist** (signed by the Principal Investigator or designated Co-investigator) must be completed prior to completing the **Registration Form**, and both must be completed prior to registration. Written confirmation that an investigator has assessed eligibility should also be documented in the patient's medical records.

Participants must be registered centrally with the trials unit (ICR-CTSU) before any tissue samples are sent to the c-TRAK TN central laboratory and before any research blood samples are collected.

Patients should be registered by telephoning ICR-CTSU on:

+44 (0)20 8643 7150

09.00-17.00 (UK time) Monday to Friday

The following information will be required at registration:

- Name of hospital, consultant and person registering patient
- Confirmation that patient has given written informed consent for trial participation
- Confirmation that patient is eligible for the trial by completion of the Registration Eligibility Checklist
- Patient's full name, hospital number, date of birth, postcode and NHS/CHI number
- Status of neoadjuvant and/or adjuvant treatment

The caller will be given the patient's unique Registration Number.

ICR-CTSU will send confirmation of a patient's registration into the trial to the main contacts at the recruiting site.

7. BIOLOGICAL SAMPLE PROCEDURES AT REGISTRATION

All c-TRAK TN trial samples should be collected, processed, labelled, stored and shipped as detailed in the c-TRAK TN Investigator Laboratory Manual. All samples must be labelled with the patient's unique trial identifier (patient Registration Number or Trial ID, as applicable), date of birth and date of sample collection to enable cross-referencing.

DNA will be extracted from tissue samples for mutation analysis. In patients with mutations detected, plasma collected at registration and subsequent ctDNA surveillance time-points will be analysed for each mutation, and reported in the laboratory database as positive (ctDNA detected) or negative (ctDNA not detected). DNA will be extracted from the plasma and mutations and copy number events analysed by digital PCR and/or DNA sequencing.

Receipt of the initial ctDNA surveillance blood samples and archival tissue samples within the required timeframes (detailed in section 7.1.1) is crucial for the c-TRAK TN trial as patient entry into the therapeutic component is dependent on ctDNA analysis results. The ICR-CTSU will track the receipt of samples at the c-TRAK central laboratory.

Samples labelled in an anonymised fashion may be shared with external institutions, including those outside the UK and EU, for future and exploratory research.

7.1. Tissue screening procedure following registration

Two archival tumour tissue samples are required to verify identification of mutations in the tumour for ctDNA screening **and** should be provided for each patient who is registered to the trial from either:

- diagnostic biopsy
- primary surgery

Two samples from the same diagnostic procedure/surgery may be supplied. For example a tissue sample from the primary breast and involved axillary lymph node. Where available, a post-chemotherapy surgical sample should be included as one sample, but not if the sample contains only isolated tumour cells/low tumour cellularity. If there is bilateral disease two samples from each site should be obtained.

If only one tumour tissue sample is available, the site should inform the ICR-CTSU who will discuss eligibility with the Chief Investigator or designated TMG member. The patient should not be registered in the trial until the ICR-CTSU confirms eligibility to the site. One sample may be sufficient should the patient have prior mutational analysis.

Tissue samples should be sent to the c-TRAK TN central laboratory as soon as possible, and ideally within 1 week, after the patient has consented and has been registered in the trial.

7.1.1. Patients with tumour sequencing to be conducted within c-TRAK TN

Following registration, the recruiting site must send the patient's archival tumour tissue samples to the c-TRAK TN central laboratory for mutation analysis. The provision of tissue is mandatory in order to assess eligibility to commence ctDNA surveillance. Blood samples for germline DNA analysis and additional ctDNA analysis should also be provided at this time (see section 7.2.1).

The mutation analysis is expected to take approximately 6 weeks to complete, from the time the lab receive both the tissue samples and additional blood samples. A delay in providing these samples will result in a delay to the mutation analysis and subsequent feedback of results.

The mutation analysis will be conducted with assays that are not CE marked, and in-house exemption will apply. Once the mutation analysis results are available, the ICR-CTSU will inform the treating team whether or not the patient is eligible for ctDNA surveillance. If the patient is confirmed as eligible, then the procedures for ctDNA surveillance in section 8 should be followed.

7.1.2. Patients with tumour sequencing previously conducted outside of c-TRAK TN

Patients who have had tumours previously sequenced outside of the c-TRAK TN trial (for example, via Genomics England limited 100,000 genome initiative (GEL)) may be eligible for entry into ctDNA surveillance provided that:

- The sequencing has been carried out in a laboratory that operates to Good Clinical Laboratory Practice (GCLP) or appropriate clinical laboratory standards, such as Clinical Laboratory Improvement Amendments (CLIA) or ISO.
- The patient meets all eligibility criteria for registration in the trial as described in section 5.4.
- The report which confirms the results of the tumour sequencing including the mutation(s) detected, is approved and validated by the c-TRAK TN Chief Investigator or a designated TMG member.

Following registration, a copy of the patient's tumour sequencing report and the laboratory accreditation where the sequencing was performed should be sent to the ICR-CTSU for onward review. In addition, one archival tumour tissue sample and four blood samples should be sent to the c-TRAK TN central laboratory to confirm the mutation status and perform additional ctDNA analysis (see section 7.2.1.).

Once the mutation analysis of the archival tissue and blood sample is complete, the ICR-CTSU will inform the treating team if ctDNA surveillance can proceed. Only those mutations validated by in-house mutation analysis will be used to generate ctDNA assays. If it is confirmed that ctDNA surveillance can proceed, then the procedures for ctDNA surveillance in section 8 should be followed.

7.2. ctDNA surveillance procedures

The trial assessments that should be performed during and after ctDNA surveillance are detailed in protocol section 8.

7.2.1. Blood sample collection for tissue screening

4 x 10 ml blood samples should be collected in ctDNA preservation tubes at registration for each patient entered into the trial. **This sample is required to determine if mutations are present in the tumour tissue (somatic mutation) and not in the germline DNA. It will also be used for additional ctDNA analysis.**

7.2.2. Blood sample collection during ctDNA surveillance

At registration, an additional sample for ctDNA surveillance should be taken. This will be analysed and used for surveillance for patients who at the time have completed standard therapy **as defined in section 3.1.**

The baseline blood sample for ctDNA surveillance should be taken within **4 weeks** of notification from the ICR-CTSU that the patient has a trackable mutation and so can commence ctDNA surveillance, or within **4**

weeks of completing adjuvant chemotherapy, whichever occurs later. However, for patients receiving adjuvant capecitabine, the first ctDNA surveillance sample should be taken 3 months +/- 2 weeks after the patient commenced capecitabine, or after the patient has discontinued capecitabine permanently within the first 3 months if treatment was stopped for reasons other than disease progression. Patients may start ctDNA surveillance prior to, and during, adjuvant radiotherapy.

Subsequent blood samples should be taken every 3 months (+/-2 weeks) for up to 2 years from starting ctDNA surveillance or until disease recurrence, unless the patient is allocated to the pembrolizumab treatment group (see sections 9 and 10).

During ctDNA surveillance, at each visit 40ml (4 x 10ml) blood should be collected in the ctDNA preservative tubes provided. Further details regarding the collection of blood samples for ctDNA analysis are given in the c-TRAK TN Investigator laboratory manual.

If a blood sample is of insufficient quality for analysis, ICR-CTSU will contact the treating team to request that a further blood sample is collected and sent to the c-TRAK TN central laboratory. In such a case, the patient should be invited to attend the clinic as soon as possible in order to provide another blood sample.

7.2.3. ctDNA surveillance results

ctDNA blood samples should be sent to the c-TRAK TN central laboratory for mutation analysis. The results from the ctDNA analyses performed during surveillance will not be provided to the treating team or patient.

Should a patient's blood sample be ctDNA positive within 12 months of starting surveillance, or at registration for patients that have completed standard therapy at the time of registration **as defined in section 3.1**, the c-TRAK TN central laboratory will notify ICR-CTSU and the patient will be allocated to pembrolizumab treatment within the therapeutic trial (see section 9).

8. TRIAL ASSESSMENTS PRIOR TO, AND FOLLOWING, REGISTRATION

8.1. Assessments for registration

The schedule of assessments for registration (section 8.2) shows all required trial assessments in table form. All blood and tissue samples should be collected according to the instructions provided in the c-TRAK TN Investigator Laboratory Manual.

8.1.1. Screening assessments prior to registration

The following assessments should be conducted within 28 days prior to registration. Only those procedures required as part of standard patient care should be conducted prior to obtaining written informed consent from the patient for registration in the c-TRAK TN trial:

- Inclusion / exclusion criteria
- Demographics and medical history
- Assessment of concomitant medications
- ECOG performance status

8.1.2. Assessments immediately following registration

The following assessments should be conducted immediately following patient registration:

- Research blood samples:
    - Germline DNA and additional ctDNA analysis: 4 x 10ml collected in ctDNA preservation tubes
    - Provision of two archival tumour tissue samples (see section 7.1.)

Blood and tissue samples should be sent to the c-TRAK TN central laboratory as soon as possible, and ideally within one week, after the patient has been registered in the trial.

8.1.3. Assessments during ctDNA surveillance

The following assessments should be conducted for all patients confirmed by ICR-CTSU as eligible for ctDNA surveillance for up to 2 years, except if a patient is allocated to the pembrolizumab treatment group (see sections 9 and 10):

- Research blood samples should be obtained every 3 months (+/- 2 weeks) from start of ctDNA surveillance:
    - ctDNA analysis: 4 x 10 ml collected in ctDNA preservation tubes
- Disease recurrence - patients should be followed-up in line with standard practice every 6 months (+/- 1 month) from the start of ctDNA surveillance

Note: The baseline blood sample for ctDNA surveillance should be taken **within 4 weeks** of notification from the ICR-CTSU that the patient is eligible for ctDNA surveillance or **within 4 weeks** of completing standard adjuvant treatment, including adjuvant chemotherapy, whichever occurs later, except for patients receiving adjuvant capecitabine. In this case, the first blood sample should be taken 3 months +/- 2 weeks after the patient commenced capecitabine treatment. If the patient discontinues capecitabine permanently within the first 3 months for reasons other than disease recurrence, the first ctDNA surveillance blood sample should be provided within **4 weeks** of stopping treatment. Patients may start ctDNA surveillance prior to, and during, adjuvant radiotherapy.

8.1.4. Follow up post ctDNA surveillance

Following completion of ctDNA surveillance, patients who were not allocated to the pembrolizumab treatment group should continue to be followed up in line with standard practice at 6 monthly intervals (+/- 1 month) until disease recurrence, unless the patient specifically withdraws consent for follow up (see section 12) or until the ICR-CTSU confirms to the recruiting sites that no further follow up is required. This can be done by a telephone call with the patient.

8.2. Schedule of assessments for registration and ctDNA surveillance

Assessments & procedures	Screening for registration	Registration	ctDNA surveillance (months)									Follow up
	Screening Day-28 to Day -1		0	3 ± 2w	6 ± 2w	9 ± 2w	12 ±2w	15 ± 2w	18 ± 2w	21 ± 2w	24 ± 2w	Every 6 months thereafter until disease recurrence +/- 1 month
Study Assessments												
Written informed consent[1]	X											
Inclusion/exclusion criteria	X											
Demographics and medical history	X											
Concomitant medication review	X											
Eligibility confirmation	X											
ECOG performance status	X											
Disease recurrence[2]					X		X		X		X	X
Tumour Tissue & Research Blood Collection												
Additional ctDNA blood sample[3]		X										
Blood sample for ctDNA analysis[4]			X[5]	X	X	X	X	X	X	X	X	
Archival tumour tissue samples[6]		X										

1. Written informed consent should be obtained from the patient for registration in the c-TRAK TN trial prior to conducting any protocol required assessments and procedures (unless required as part of standard patient care).
2. Patients should be followed-up in line with standard practice every 6 months (+/- 1 month) from start of ctDNA surveillance. During follow up post ctDNA surveillance this may be done by a telephone call to the patients. A recurrence tumour tissue sample should be provided at relapse for each patient who has tissue available from a biopsy taken routinely as part of standard patient care.
3. At trial registration, 4 x 10ml blood samples collected in ctDNA preservation tubes for germline DNA and additional ctDNA analysis.
4. 4 x 10ml of blood collected in ctDNA preservation tubes every 3 months (+/- 2 weeks) for up to 2 years from starting ctDNA surveillance. Patients must have completed standard treatment including adjuvant chemotherapy, except capecitabine, before starting ctDNA surveillance. Patients may start ctDNA surveillance prior to, and during, adjuvant radiotherapy.
5. The first blood sample for ctDNA surveillance should be taken within 4 weeks of notification from the ICR-CTSU that the patient is eligible for ctDNA surveillance or within 4 weeks of completing standard adjuvant treatment, including adjuvant chemotherapy, whichever occurs later, except for patients receiving adjuvant capecitabine. In this case, the first blood sample should be taken 3 months +/- 2 weeks after the patient commenced capecitabine treatment. If the patient discontinues capecitabine permanently within the first 3 months for reasons other than disease recurrence, the first ctDNA surveillance blood sample should be provided within 4 weeks of stopping treatment. Patients may start ctDNA surveillance prior to, and during, adjuvant radiotherapy.
6. Two archival tumour tissue samples either from diagnostic biopsy or primary surgery.

9. RANDOMISATION (PRIOR TO PROTOCOL V6.0) AND TREATMENT ALLOCATION

Prior to the implementation of protocol v6.0 during the original randomised component of the trial, The ICR-CTSU would perform the randomisation independently of the treating team. Each randomised patient was assigned a unique Trial ID.

Where a patient was randomised and allocated to the pembrolizumab treatment group, the ICR-CTSU notified the relevant staff members including the Principal Investigator at the recruiting site and would confirm the patient's unique Trial ID. Where a patient was randomised to the observation group (continued blinded ctDNA surveillance), the treating team and patient were not informed and patients would continue with 3 monthly blinded ctDNA surveillance for up to 2 years (from start of ctDNA surveillance) in an identical manner to those who have remained ctDNA negative.

Following the implementation of protocol v6.0 whereby patients transfer to the non-randomised component of the trial, a patient is allocated to the pembrolizumab treatment intervention and the ICR-CTSU will notify the relevant staff members (including the Principal Investigator) at the recruiting site and will confirm the patient's unique Trial ID.

Patients not receiving adjuvant capecitabine should be invited to confirm consent for the therapeutic part of c-TRAK TN, i.e. pembrolizumab treatment (see section 9.1).

If a patient is receiving adjuvant capecitabine when allocated to pembrolizumab treatment, the same process of informing the site is followed. The patient should be asked if they agree to switch their treatment from capecitabine to pembrolizumab if they are found to be eligible for the therapeutic part of the c-TRAK TN trial (see section 9). If the patient agrees in principle to switch their treatment, they should be invited to confirm consent for the therapeutic part of c-TRAK TN, i.e. pembrolizumab treatment.

Note: Patients who are allocated to pembrolizumab but do not wish to switch current treatment from capecitabine, will not be able to join the therapeutic component of the c-TRAK TN trial and should instead be treated as per the protocol for patients continuing on ctDNA surveillance.

If consent is obtained, the eligibility checks and pre-treatment assessments can then be carried out (see section 11.1). Once these checks have been completed the ICR-CTSU should be informed following the procedure in section 9.3.

9.1. Procedure for obtaining informed consent for pembrolizumab treatment

Patient consent for starting pembrolizumab treatment must be obtained following confirmation from ICR-CTSU that the patient has been allocated to the pembrolizumab treatment group.

The Principal Investigator (or designated investigator) must ensure that each patient is fully informed about the nature and objectives of the trial and possible risks associated with pembrolizumab treatment.

The patient should be given the current ethics approved **c-TRAK TN patient information sheet for pembrolizumab treatment** for their consideration. Patients should only be asked to confirm consent to the trial after they have had sufficient time to consider the implications of a positive ctDNA result, the rationale for accepting pembrolizumab treatment and had the opportunity to ask any further questions.

No further protocol required assessments, other than those required as part of the ctDNA surveillance and standard patient care, should be conducted until the **c-TRAK TN consent form for pembrolizumab treatment** has been signed and dated by both the patient and the investigator at the recruiting site.

Confirmation of the patient's consent and the informed consent process should be documented in the patient's medical notes. A copy of the signed consent form should be provided to the patient and the original retained in the investigator site file, which must be made available for verification by ICR-CTSU trial staff or for regulatory inspection at any time.

9.2. Eligibility criteria for pembrolizumab treatment group

Patients allocated to the pembrolizumab treatment group will be considered eligible to start pembrolizumab treatment only if they fulfil all the inclusion and none of the exclusion criteria listed below. Eligibility should be checked and pre-treatment assessments carried out once allocation to the pembrolizumab treatment group has been confirmed by ICR-CTSU and patient consent to start treatment has been obtained.

9.2.1. Inclusion Criteria

1) Signed Informed Consent Form for pembrolizumab treatment
2) ECOG performance status 0, 1 or 2
3) Adequate organ function as defined by:
    a. Bone marrow function: ANC ≥ 1.5 x 10^9/L; platelets >100 x 10^9/L; haemoglobin >9g/dL
    b. Hepatic function: aspartate aminotransferase(AST) and alanine aminotransferase (ALT) ≤2.5 x upper limit of normal ULM+N); bilirubin ≤1.5 x ULN; albumin ≥ 2.gmg/dL
    c. Renal function: serum creatinine ≤ 1.5 x ULN OR estimated GFR (using Cockcroft-Gault)), ≥ 60mL/min for patient with creatinine levels > 1.5 x ULN
    d. Coagulation: INR ≤ 1.5 x ULN; APTT ≤ 1.5 x ULN (unless patient receiving anticoagulation therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulant)
4) Women of childbearing potential should have a negative urine or serum pregnancy test within 72 hours prior to receiving the first dose of trial medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
5) Female and male patients of reproductive potential must be willing to use an adequate method of contraception (appendix 2), starting with the first dose of pembrolizumab through 120 days after the last dose of pembrolizumab. ***Note****: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the patient.*
6) Patients willing to receive a 12 month course of pembrolizumab and have frequent blood tests (every 3 weeks during treatment and every three months for a year following completion of pembrolizumab treatment).
7) No evidence of distant metastatic disease or local recurrence on staging scans conducted at any time since initial diagnosis.

9.2.2. Exclusion Criteria

1) Any concurrent or planned anti-cancer treatment since completing standard therapy for early stage triple negative breast cancer other than loco regional adjuvant radiotherapy or standard adjuvant chemotherapy, or a permitted bisphosphonate/denosumab.

2) Diagnosis of an additional cancer since enrolment in the trial other than basal cell carcinoma of the skin or cervical carcinoma in situ.

3) Prior chemotherapy, targeted small molecule therapy or radiation therapy within 4 weeks prior to first administration of pembrolizumab, with the exception of planned loco regional adjuvant radiotherapy.

4) Prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to first administration of pembrolizumab.

5) Has not recovered (≤ Grade 1 or patient's baseline) from adverse events due to a previously administered therapy. **Note**: Patients with ≤ grade 2 neuropathy or alopecia of any grade are an exception to this criterion and may qualify for the trial. If patient received major surgery, they must have recovered adequately from the toxicity and/or complications from the surgery prior to allocation.

6) Treatment with an unlicensed or investigational product within 4 weeks prior to first administration of pembrolizumab.

7) Active autoimmune disease requiring systemic therapy in the last 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) is not considered a form of such systemic treatment.

8) Diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first administration of pembrolizumab.

9) Known history of active Tuberculosis Bacillus (TB).

10) Known history of Human Immunodeficiency Virus (HIV).

11) Known active Hepatitis B or Hepatitis C.

12) Known history of, or any evidence of active, non-infectious pneumonitis.

13) Active infection requiring systemic therapy.

14) Previous solid organ or allogenic stem cell transplantation.

15) Received a live vaccine within 30 days of planned start of pembrolizumab (seasonal flu vaccines are generally inactivated and are permitted; however intranasal influenza vaccines (e.g. Flu-Mist) are live attenuated vaccines and are not permitted).

16) Presence of any systemic illness incompatible with participation in the clinical trial or inability to provide written informed consent.

17) Females who are pregnant or breastfeeding.

9.3. Procedure for confirming patient eligibility to start pembrolizumab treatment

If the patient is confirmed eligible to start pembrolizumab treatment, then treatment should commence within a maximum of 4 weeks of the date when the recruiting site was first notified about the treatment allocation. For patients who are switching treatment from capecitabine to pembrolizumab, a washout period of 4 weeks must be observed prior to starting trial treatment. This period may be extended if delays are

encountered for practical reasons including those caused by the COVID 19 pandemic and the patient remains eligible per protocol, please discuss such cases with the ICR-CTSU trial team.

Patients who are ineligible to start treatment due to macroscopic disease identified on pre-treatment imaging will not receive treatment within the c-TRAK TN trial and will instead be treated as per local guidelines for metastatic disease/recurrence. No further blood samples for ctDNA analysis should be collected.

Patients who do not meet the eligibility criteria (for reasons other than disease recurrence) or do not wish to receive pembrolizumab treatment, should continue to have bloods taken for ctDNA surveillance every 3 months for up to 2 years from starting ctDNA surveillance. Patients should then be followed up for disease recurrence detection as described in section 11.5.

The **pre-treatment eligibility checklist** must be completed for all patients allocated to pembrolizumab treatment to confirm patient consent and eligibility and the planned start date of treatment (where applicable). The pre-treatment eligibility checklist should be completed within 4 weeks of when the recruiting site was first notified about the treatment allocation. Written confirmation that eligibility has been assessed by an investigator and confirmation of the patient's consent and the informed consent process should be documented in the patient's medical records.

For patients confirmed eligible, pembrolizumab treatment should commence as soon as possible and within a maximum of 4 weeks from when the recruiting site was first notified about the treatment allocation. As stated above, for patients receiving capecitabine a 4 week washout is required. The trial assessments that should be performed before, during and after pembrolizumab treatment are detailed in section 11.

10. BIOLOGICAL SAMPLE PROCEDURES FOR PEMBROLIZUMAB TREATMENT GROUP

10.1.1. ctDNA blood sample collection during pembrolizumab treatment

Patients receiving pembrolizumab treatment will have blood samples for ctDNA analysis taken pre-dose on Day 1 of each 3 week cycle (-72 hours). Note that for patients receiving pembrolizumab treatment a reduced volume of 20ml (2 x 10ml) blood will be taken.

Following completion of pembrolizumab treatment, blood samples for ctDNA surveillance (2x 10ml) should be taken every 3 months (+/- 2 weeks) from start of ctDNA surveillance for a further 12 months or until disease recurrence (see section 11.5). For patients who have clinical recurrence whilst on pembrolizumab see section 13.

ctDNA blood samples should be sent to the c-TRAK TN central laboratory for mutation analysis. The results of any ctDNA analyses performed during and after pembrolizumab treatment will not be provided to the treating team or patient. If a blood sample is of insufficient quality for analysis, ICR-CTSU will contact the treating team to request that a further blood sample is collected and sent to the c-TRAK TN central laboratory. In such a case, the patient should be invited to attend the clinic as soon as possible in order to provide another blood sample.

Further details regarding the collection of blood samples for ctDNA analysis are given in the c-TRAK TN Investigator Laboratory Manual.

10.1.2. Blood sample collection for PBMC isolation

Only patients allocated to pembrolizumab treatment will be asked to provide blood for PBMC (Peripheral Blood Mononuclear Cell) isolation. The sites will be geographically determined in order for the sample to be couriered to the central c-TRAK TN laboratory within an appropriate timeframe for processing, eligible PBMC sites will be confirmed on a case by case basis. Where possible, blood for PBMC isolation should be collected in the morning to allow for prompt shipment and processing within the recommended timeframe.

Patients receiving pembrolizumab treatment at the designated PBMC sites will have blood samples for PBMC isolation taken on day 1 of cycle 1 and day 1 of cycle 3.

11. ASSESSMENTS FOR PEMBROLIZUMAB TREATMENT GROUP

The schedule of assessments for the pembrolizumab treatment group shows all required trial assessments in table form (see section 11.7). All blood and tissue samples should be collected according to the instructions provided in the c-TRAK TN Investigator Laboratory Manual.

The patient should start pembrolizumab treatment as soon as possible and within a maximum of 4 weeks of allocation to the pembrolizumab treatment group being confirmed by the ICR-CTSU.

11.1. Pre-treatment assessments

The following pre-treatment assessments should be conducted once **written informed consent to start pembrolizumab has been obtained** from the patient and after the treatment eligibility checklist has been completed by the principal investigator (or designated investigator) and submitted to ICR-CTSU. **The pre-treatment assessments should be done prior to the first dose of pembrolizumab treatment as below.**

Assessments to be conducted within 4 weeks prior to starting treatment:

- Imaging to assess for macroscopic disease (using same modality as diagnosis). For example patients screened with CT and bone scan at diagnosis should have both repeated, or patients screened with FDG PET-CT at diagnosis should have a FDG PET-CT repeated. Patients who did not have imaging at diagnosis should have imaging in line with local guidelines for high risk disease
- ECG
- Demographics and baseline conditions

Assessments to be conducted within 72 hours prior to the first dose of pembrolizumab:

- Safety blood tests: clotting (INR or PT/ aPTT), haematology (ANC, platelets, haemoglobin), biochemistry; hepatic function (bilirubin, ALT, AST, ALP and GGT), renal function (serum creatinine or estimated GFR (using Cockcroft-Gault)), thyroid function (T3, T4, TSH), glucose (random)
- Urinalysis
- Female patients of childbearing potential should have a negative urine or serum pregnancy test. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required
- Concomitant medication review
- Full physical examination (ECOG performance status, vital signs, height and weight)

- Research blood samples for ctDNA analysis:
    - 2 x 10ml collected in ctDNA preservation tubes
- Research blood sample for PBMC (peripheral blood mononuclear cell) isolation (PBMC sites only)
    - 3 x 10ml collected in heparin tubes, to be couriered as soon as possible after collection, as per the c-TRAK TN Investigator Laboratory Manual

11.2. Assessments during pembrolizumab treatment

Treatment with pembrolizumab will continue for a maximum of 12 months. Pembrolizumab should be administered on Day 1 of each cycle after all assessments have been completed as detailed below and in the schedule of assessments for pembrolizumab treatment. Pembrolizumab treatment may be administered up to 72 hours before or after the scheduled Day 1 of each cycle. Refer to section 17 for further information regarding dosing interruptions.

Patients should be followed-up for disease recurrence in line with standard practice every 6 months (+/- 1 month to coincide with a treatment cycle) from the start of ctDNA surveillance.

11.3. Prior to each cycle (cycle 2 onwards)

Please see section 11.1 for assessments required prior to the first dose of pembrolizumab (i.e. cycle 1 only). The following assessments should be conducted **within 72 hours prior to each cycle (from cycle 2 onwards) of pembrolizumab**:

- Concomitant medication review
- Review of adverse events
- Full physical examination (including weight at cycles 4, 8, 12 and 16 only, ECOG performance status, vital signs)
- Safety blood tests: clotting (INR or PT/ aPTT), haematology (ANC, platelets, haemoglobin), biochemistry; hepatic function (bilirubin, ALT, AST, ALP and GGT), renal function (serum creatinine or estimated GFR (using Cockcroft-Gault)), thyroid function (T3, T4 TSH), glucose (random)
- Urinalysis
- ECG (only if clinically indicated)
- Research blood samples for ctDNA analysis:
    - 2 x 10ml collected in ctDNA preservation tubes taken pre-dose on each cycle
- Research blood samples for PBMC isolation pre-dose **ONLY on cycle 3** (PBMC sites only)
    - 3 x 10ml collected in heparin tubes, to be couriered same day as per the c-TRAK TN Investigator Laboratory Manual.

11.4. Assessments at discontinuation of pembrolizumab treatment

On the **day of the last administration of pembrolizumab treatment** (or at the next planned visit if treatment discontinuation occurs between cycles), the following assessments should be conducted:

- Concomitant medication review
- Review of adverse events
- Full physical examination, ECOG performance status, vital signs
- Safety Blood tests: clotting (INR or PT/ aPTT), haematology (ANC, platelets, haemoglobin), biochemistry (hepatic function (bilirubin, ALT, AST, ALP and GGT), renal function (serum creatinine or estimated GFR (using Cockcroft-Gault)), thyroid function (T3, T4 TSH), glucose (random)
- Urinalysis
- Research blood samples for ctDNA analysis (at treatment discontinuation only):
    - 2 x 10ml collected in ctDNA preservation tubes

Approximately **30 days after the last administration of pembrolizumab treatment or before initiation of a new anti-cancer treatment, whichever comes first,** the following assessments should be conducted:

- Review of adverse events
- Safety Blood tests: clotting (INR or PT/ aPTT), haematology (ANC, platelets, haemoglobin), biochemistry (hepatic function (bilirubin, ALT, AST, ALP and GGT), renal function (serum creatinine or estimated GFR (using Cockcroft-Gault)), thyroid function (T3, T4 TSH), glucose (random)

11.5. Follow-up assessments post pembrolizumab treatment

For 12 months after the completion of pembrolizumab treatment, the following assessments should be conducted:

- Research blood samples for ctDNA analysis taken every 3 months from start of ctDNA surveillance:
    - 2 x 10ml collected in ctDNA preservation tubes at each visit
- Disease recurrence (every 6 months, +/- 1 month, from start of ctDNA surveillance)

Following this, all patients should continue to be followed up in line with standard practice at 6 monthly intervals (+/- 1 month) until disease recurrence unless the patient specifically withdraws consent for follow up (see section 12) or until the ICR-CTSU confirms to the recruiting sites that no further follow up is required. This may be done by a telephone call with the patient. A recurrence tumour tissue sample should be provided at relapse for each patient who has tissue available from a biopsy taken routinely as part of standard patient care.

Patients who stop pembrolizumab before completing 12 months of therapy, and without recurrence, should have research blood samples taken 3 monthly for a further 12 months, calculated from the start of ctDNA surveillance.

11.6. Discontinuation from pembrolizumab treatment

A patient may discontinue from pembrolizumab treatment at any time at their own request, or they may be discontinued at the discretion of the Principal Investigator (or designated investigator). Reasons for discontinuation may include:

- Disease recurrence
- Unacceptable toxicity

- Pregnancy

Patients who discontinue treatment for reasons other than disease recurrence should continue to be followed up as described in section 11.5. Patients who discontinue pembrolizumab treatment due to disease recurrence should be assessed as described in section 13 and then treated for disease recurrence as per local guidelines, no further blood samples for ctDNA analysis should be collected for these patients, and no further follow up is required.

11.7. Schedule of assessments for pembrolizumab treatment group

	Pre-treatment	Pembrolizumab Treatment Cycles					End of Treatment	Post-Treatment		
	Screening Day -28 to Day-1	C1		C2	C3	C4-C17	Discontinuation (max 12 months)	Safety Follow-up	Follow Up Visits	
Procedures & Assessments:		D1 -72 hrs	D1	D1[1] - 72hrs	D1[1] - 72hrs	D1[1] - 72hrs	At time of Discontinuation	30 days post discontinuation[2]	Every 3 months from the start of ctDNA surveillance for 12 months following treatment discontinuation[3]	Every 6 months from start of ctDNA surveillance until disease recurrence (+/- 1 month)
Administrative Procedures										
Written informed consent[4]	X									
Inclusion/exclusion criteria	X									
Demographics and medical history	X									
Concomitant medication review	X	X		X	X	X	X			
Eligibility confirmation to ICR-CTSU	X									
Clinical Procedures/Assessments										
Full physical examination[5]	X	X		X	X	X	X			
Imaging[6]	X									
Review adverse events				X	X	X	X	X		
Pembrolizumab administration[1]			X	X	X	X				
Disease recurrence[7]										X
Laboratory Procedures/Assessments: analysis performed by LOCAL laboratory										
Pregnancy test – urine or serum β-HCG[8]	X									
Clotting[9]	X	X		X	X	X	X	X		
Haematology[9]	X	X		X	X	X	X	X		
Biochemistry[9]	X	X		X	X	X	X	X		
Thyroid function[9]	X	X		X	X	X	X	X		
Hepatic function[9]	X	X		X	X	X	X	X		
Renal function[9]	X	X		X	X	X	X	X		
Glucose[9]	X	X		X	X	X	X			
ECG	X									
Urinalysis	X	X		X	X	X	X			
Research Blood Collection										
Blood sample for ctDNA analysis[10]		X		X	X	X	X		X	
Blood sample for PBMC isolation[11]		X			X					

1. Day 1 of each treatment cycle is the day of pembrolizumab treatment administration. All assessments, including sample collection, should be performed within 72 hours prior to day 1. Each cycle should be 21 days in length. Day 1 of each cycle should be planned for 21 days (+/- 72 hours) from day 1 of the previous cycle.
2. SAEs that occur within 90 days of the end of treatment and before initiation of a new anti-cancer treatment, whichever comes first, should be reported, followed and recorded.
3. Follow up bloods for patients who complete pembrolizumab or discontinue prematurely should be taken every 3 months in line with the start of ctDNA surveillance. For example if a patient goes on to receive treatment due to a positive ctDNA result at the 12 month ctDNA surveillance blood sample and then completes the full 12 months of pembrolizumab treatment, bloods will be taken at month 27, 30, 33, 36 and 39 from the start of ctDNA surveillance, and if a patient discontinues at month 7 bloods would be taken at month 21, 24, 27, 30 and 33 from start of surveillance
4. Written informed consent must be obtained from the patient prior to pre-treatment assessments being conducted.
5. Full physical examination includes height (prior to first dose of pembrolizumab only), weight (required prior to first dose of pembrolizumab and cycles 4, 8, 12 and 16 only), ECOG performance status and vital signs (heart rate, blood pressure).
6. Consent for imaging is included in registration PIS and ICF following implementation of protocol v6.0 onwards. Patients should undergo imaging immediately upon confirmation of a ctDNA positive result. The same investigations conducted as part of staging for the original primary breast cancer should be repeated. Patients who did not have imaging at diagnosis should have imaging in line with local guidelines for high risk disease.
7. Patients should be followed-up in line with standard practice every 6 months (+/- 1 month) from start of ctDNA surveillance. During post treatment follow up this can be conducted as a telephone call to the patient. Patients should be asked about relapse of breast cancer or other change in medical history. A recurrence tumour tissue sample should be provided at relapse for each patient who has tissue available from a biopsy taken routinely as part of standard patient care.
8. Pregnancy test should be done within 72hrs of first administration of pembrolizumab treatment.
9. With the exception of the end of treatment assessments, safety blood tests should be conducted within 72 hours prior to each cycle of pembrolizumab.
10. During pembrolizumab treatment, 2 x 10ml of blood should be collected in ctDNA preservation tubes on day 1 (pre-dose) of each cycle and until completion or withdrawal of pembrolizumab treatment. For 1 year after the administration of the last dose of pembrolizumab, 2 x 10ml of blood should be then collected every 3 months (+/- 2 weeks).
11. Research blood sample only at PBMC sites. 3 X 10ml heparin tubes, to be couriered to lab same day.

12. DISCONTINUATION FROM FOLLOW-UP

If a patient withdraws from further follow-up a trial deviation form should be submitted to ICR-CTSU stating whether the patient simply no longer wishes to attend trial follow up visits or whether the patient has withdrawn consent for any further information to be sent to the ICR-CTSU.

Should a patient withdraw consent for their samples to be used in c-TRAK TN, blood samples will be destroyed and tissue samples returned to the site for archiving following receipt of written confirmation from the site to ICR-CTSU.

13. PROCEDURE AT DISEASE RECURRENCE

Disease recurrence should be reported to ICR-CTSU on the appropriate eCRF and confirmed in writing via email (c-trak-tn-icrctsu@icr.ac.uk) as soon as possible. Patients should be treated as per local guidelines for metastatic disease/recurrence. Prior to commencement of any treatment for metastatic disease/recurrence, a ctDNA blood sample should be collected. In addition, a recurrence tumour tissue sample should be provided for each patient who has tissue available from a biopsy taken routinely as part of standard patient care (see section 13.2). Following this no further blood samples for ctDNA analysis should be collected and no further follow up is required.

For patients being treated with pembrolizumab, there is the possibility of development of lymphadenopathy that may not be indicative of disease recurrence. See below for guidance on continuing pembrolizumab treatment.

13.1. Development of lymphadenopathy on pembrolizumab treatment

Immunotherapeutic agents such as pembrolizumab may produce anti-tumour effects by potentiating endogenous cancer-specific immune responses. Immunotherapeutic agents may also induce lymphadenopathy. In addition, response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest as a clinical response after an initial increase in tumour burden or even the appearance of new lesions.

If new lymphadenopathy develops on treatment, staging imaging should be conducted to examine for evidence of disease recurrence according to local guidelines. If there is radiographic evidence of isolated lymphadenopathy patients should remain on treatment if they are clinically stable as defined by the following criteria:

1. Absence of signs and symptoms (including worsening of laboratory values) indicating disease recurrence
2. No decline in ECOG performance status
3. Absence of potential recurrent disease in sites other than lymphadenopathy

Repeat imaging should be done ≥ 4 weeks later. If lymphadenopathy remains isolated and has not progressed the patient may continue on pembrolizumab. If lymphadenopathy has progressed, or the patient has developed alternative potential sites of disease, appropriate investigations should be conducted to determine if recurrence has occurred. If recurrence is subsequently confirmed the patient must stop pembrolizumab.

The decision to continue pembrolizumab after first radiologic evidence of isolated lymphadenopathy is at the

investigator's discretion based on the clinical status of the patient.

13.2. Sample collection following recurrence

13.2.1. Tumour tissue

A recurrence tumour tissue sample should be provided for each patient who has tissue available from a biopsy taken routinely as part of standard patient care. The tissue sample should be sent to the c-TRAK TN central laboratory as soon as it is available. DNA will be extracted for mutation analysis and comparison with the primary tumour.

13.2.2. ctDNA blood sample

ctDNA blood samples should be provided prior to the patient commencing treatment for the recurrence. 20ml (2 x 10ml) blood should be obtained and send to the c-TRAK TN central laboratory.

14. LIFE STYLE GUIDELINES

Female participants must be surgically sterile or be post-menopausal, or must agree to use effective contraception from registration until 120 days after the last dose of pembrolizumab.

Male participants must be surgically sterile or must agree to use effective contraception after starting the first dose of pembrolizumab through 120 days after the last dose of pembrolizumab.

Accepted methods of contraception are defined in Appendix 2.

15. PARTICIPATION IN OTHER CLINICAL TRIALS

Patients previously entered into a therapeutic trial during or after neoadjuvant chemotherapy are only eligible if no experimental therapy is continued post-surgery. Patients involved in clinical trials involving experimental drugs prior to primary standard treatment (i.e. window of opportunity trials) can be considered for entry into c-TRAK TN. Patients who enter a window of opportunity study after completion of standard chemotherapy may not be eligible, due the potential of the window study treatment to effect ctDNA surveillance in c-TRAK TN. Entry of such patients into c-TRAK TN will be considered on a case by case basis by the TMG.

c-TRAK TN patients will not be permitted to participate in any other trials of investigational medicinal products until completion of follow-up at the time of disease recurrence.

16. c-TRAK TN SCREENING LOG

All participating sites will be required to keep a log of all patients with early stage triple negative breast cancer receiving neoadjuvant chemotherapy and those with confirmed moderate/high risk disease that are potentially eligible for this trial. The information collected on the log will include:

- Date patient identified
- Screening outcome (patient approached/accepted participation/declined participation)

- Reasons for not approaching patient / patient declining participation (if available)
- Registration number (if applicable)

This information will be used by the TMG to monitor recruitment activity. No patient identifiable data should be sent to ICR-CTSU.

17. TRIAL TREATMENT

Pembrolizumab is the investigational medicinal product within c-TRAK TN and is being used outside the licensed indication and will be given as a fixed dose regimen which differs to the standard dose recommended for pembrolizumab. A fixed dose regimen of pembrolizumab is now standard in ongoing pembrolizumab trials. Patients who become ctDNA positive within 12 months of starting ctDNA analysis will be allocated to pembrolizumab treatment.

Prior to the implementation of protocol v6.0 during the randomised component of the trial, patients who become ctDNA positive within 12 months of starting ctDNA analysis were randomised to treatment with pembrolizumab or observation (on a 2:1 ratio).

In protocol version 6.0 randomisation within the trial was removed, all patients with a positive ctDNA result will be allocated to treatment intervention. Any patients already in the observation group, will transition to the non-randomised trial component following re-consent, and be allocated to pembrolizumab at the time of the next positive ctDNA result.

17.1. Dose and schedule

Pembrolizumab will be given at a dose of 200mg as a 30 minute IV infusion (infusion window -5 to +10 minutes) on day 1 of each 3 week cycle. Pembrolizumab should be administered after all pre-treatment assessments have been completed (see section 11.1). Treatment may be administered up to 72 hours before or after the scheduled day 1 of each cycle. Refer to the c-TRAK TN Pharmacy Guidance Notes for specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

17.2. Prescription and dispensing

Pembrolizumab will be supplied in non-patient-specific vials for use within the c-TRAK TN trial only. Pembrolizumab should be labelled, stored and dispensed in accordance with the c-TRAK TN Pharmacy Guidance Notes.

17.3. Patient cards

Small wallet sized cards will be produced by ICR-CTSU on request by the recruiting site. Each card will state:

- the name of the recruiting site
- that the patient is participating in the c-TRAK TN trial
- that the patient is taking pembrolizumab (where applicable)
- an emergency contact number at site

17.4. Duration of treatment

Treatment will continue for a maximum of 12 months. Patients may withdraw from pembrolizumab before this time if they experience unacceptable toxicities or their Principal Investigator (or designated investigator) believes further treatment is no longer appropriate or if the patient withdraws consent for any reason.

17.5. Supportive care

Patients should receive appropriate supportive care measures as deemed necessary by the Principal Investigator (or designated investigator). Suggested supportive care measures for the management of adverse events (AEs) with potential immunologic aetiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous 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 AE, attempts should be made to rule out other causes such as 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. It may be necessary to perform conditional procedures, such as bronchoscopy, endoscopy, or skin photography, as part of the AE evaluation.

17.6. Immune-mediated adverse reactions

Immune-mediated adverse reactions occur in patients treated with pembrolizumab. Most adverse reactions are reversible and managed with interruptions of pembrolizumab, administration of corticosteroids and/or supportive care.

For suspected immune-mediated adverse reactions, ensure adequate evaluation to confirm aetiology or exclude other causes. Based on the severity of the reaction withhold pembrolizumab and administer corticosteroids as per guidelines in the table in section 17.16. Upon improvement to grade 1 or less, initiate corticosteroid taper and continue to taper over at least 1 month. Restart pembrolizumab if the adverse reaction remains grade 1 or less. If another episode of a severe adverse reaction occurs, permanently discontinue pembrolizumab.

Centres should monitor signs or symptoms that might indicate development of these adverse reactions and treat as per the guidance in section 17.16.

Treatment with pembrolizumab may increase the risk of solid organ transplant rejection. Patients who have had a solid organ transplant are excluded from c-TRAK TN.

17.7. Management of infusion reactions

Severe infusion reactions, including hypersensitivity and anaphylaxis have been reported in patients receiving pembrolizumab.

Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Monitor for signs and symptoms including rigors, chills, wheezing, pruritus, flushing, rash, hypotension, hypoxaemia and fever.

Table 2 below shows treatment guidelines for patients who experience an infusion reaction associated with administration of pembrolizumab.

Table 1. ***Treatment guidelines for infusion reaction associated with administration of pembrolizumab***

NCI CTCAE v4.0 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 patient is deemed medically stable in the opinion of the investigator	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., oxygen, IV fluids, antihistamines, non-steroidal anti-inflammatory drugs); prophylactic medications indicated for ≤ 24 hours	Stop Infusion and monitor symptoms. Additional appropriate medical therapy may include but is not limited to: Oxygen, IV fluids, antihistamines, non-steroidal anti-inflammatory drugs Increase monitoring of vital signs as medically indicated until the patient 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 patient should be pre-medicated for the next scheduled dose. Patients who develop grade 2 toxicity despite adequate premedication should be permanently discontinued from further pembrolizumab administration.	Patient may be pre-medicated 1.5 hours (± 30 min) prior to infusion of pembrolizumab with: Chloraphenamine 10mg po Paracetamol 500-1000mg po
Grades 3 or 4 **Grade 3:** Prolonged (i.e. not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g. renal impairment, pulmonary infiltrates) **Grade 4:** Life-threatening; pressor or	Stop infusion. Additional appropriate medical therapy may include but is not limited to: IV fluids, antihistamines, corticosteroids, oxygen, adrenaline. Increase monitoring of vital signs as medically indicated until the patient is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. Patient should be permanently discontinued from further pembrolizumab administration.	No subsequent dosing

NCI CTCAE v4.0 Grade	Treatment	Premedication at subsequent dosing
ventilatory support indicated		
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

17.8. Common adverse reactions related to pembrolizumab

The most serious adverse reactions reported in patients treated with pembrolizumab are immune-related reactions and severe infusion-related reactions (see sections 17.6/ 17.7). The adverse reactions listed in the table below are common, occurring in at least 1 out of 100 patients treated with pembrolizumab and the majority of those reported were grade 1 or 2 severity. Most reactions are reversible and managed without interruption of pembrolizumab. For the most up to date information on anticipated adverse reactions and their frequency, please refer to the latest version of the pembrolizumab Investigator Brochure

Table 2: ***Very common and common adverse reactions to pembrolizumab***

System Organ Class	Very Common and Common Adverse Reaction
Blood and Lymphatic system disorders	Anaemia
Endocrine disorders	Hyperthyroidism, hypothyroidism
Gastrointestinal disorders	Diarrhoea, nausea, colitis, vomiting, abdominal pain, constipation, dry mouth
General disorders and administration site conditions	Fatigue, asthenia, oedema, pyrexia, influenza-like illness, chills
Immune system disorders	Infusion related reactions
Investigations	Raised ALT, AST, ALP, creatinine
Metabolism and nutritional disorders	Decreased appetite
Musculoskeletal and connective tissue disorders	Arthralgia, myositis, musculoskeletal pain, pain in extremity, arthritis
Nervous system disorders	Headache, dizziness, dysgeusia
Respiratory, thoracic and mediastinal disorders	Pneumonitis, dyspnea, cough
Skin and subcutaneous tissue disorders	Rash, pruritus, severe skin reactions, vitiligo, dry skin, erythema

17.9. Significant rare reactions related to pembrolizumab

There are some rare adverse events that have been reported in patients treated with pembrolizumab. They occur in <1 in every 100 patients. For the most up to date information on anticipated adverse reactions and their frequency, please refer to the latest version of the pembrolizumab Investigator Brochure.

Table 3: ***Significant rare reactions to pembrolizumab***

System Organ Class	Rare Adverse Reaction
Blood and Lymphatic system disorders	Neutropenia, lymphopenia
Musculoskeletal and connective tissue disorders	Polymyalgia rheumatic, tendonitis
Immune system disorders	Sarcoidosis
Infections and infestations	Encephalitis Pneumonia

17.10. Embryofoetal toxicity

Based on its mechanism of action, pembrolizumab can cause foetal harm if administered to a pregnant woman. Patients who are pregnant or breastfeeding are excluded from entering the trial. To be eligible for the trial, female and male patients of reproductive potential must be willing to use an adequate method of contraception for the first year of the trial and, if allocated to pembrolizumab, for the duration of treatment through to 120 days after the last dose of pembrolizumab (see appendix 2).

If a trial patient or a trial participants' partner becomes pregnant while receiving pembrolizumab, treatment should be permanently discontinued immediately. All pregnancies during treatment or up to 120 days after receiving pembrolizumab, should be reported to ICR-CTSU immediately using the pregnancy reporting form (see section 18.8).

17.11. Concomitant therapy and non-permissible medications

Medications or vaccinations specifically prohibited in the exclusion criteria (sections 5.4.2 and 9.2.2) and below are not allowed during the administration of pembrolizumab treatment. If there is a clinical indication for any medication or vaccination specifically prohibited during the trial, discontinuation from pembrolizumab may be required and this should be discussed with the Chief Investigator (or designated TMG member). The decision to continue the patient on trial therapy requires the mutual agreement of the Chief Investigator (or designated TMG member, if the Chief Investigator is unavailable), Principal Investigator (or designated investigator) and the patient.

17.11.1. Acceptable concomitant medications

All treatments that the investigator considers necessary for a patient'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 eCRF including all prescription, over-the-counter (OTC), and IV medications and fluids.

All concomitant medications received within 28 days before commencing treatment with pembrolizumab and 30 days after the last dose of pembrolizumab should be recorded.

17.11.2. Prohibited concomitant medications

Patients are prohibited from receiving the following therapies:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy (except planned loco regional adjuvant radiotherapy)
- Live vaccines within 30 days prior to commencing pembrolizumab and during pembrolizumab treatment. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella, herpes zoster, yellow fever, rabies, BCG, and typhoid (oral) vaccines. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed. However, intranasal influenza vaccines are live attenuated vaccines, and are not allowed.
- After commencing pembrolizumab: Glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Chief Investigator (or designated TMG member).

Patients who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management may need to be discontinued from trial treatment (see section 17.11). Patients may receive other medications that the investigator deems to be medically necessary.

17.12. Dose modifications

AEs (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic aetiology. These AEs may occur shortly after the start of treatment or several months after the last dose of treatment. Pembrolizumab should be withheld for drug-related toxicities, and severe or life-threatening AEs, as per section 17.16 below. Supportive care guidelines, including use of corticosteroids, is also found in section 17.16.

17.13. Missed doses

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

17.14. Overdoses

For this trial, an overdose will be defined as ≥1000 mg (5 times the dose) of pembrolizumab. No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, the patient should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated. Following investigations into the cause and effect of the overdose, the patient may continue on trial treatment if effects are minor and the patient and treating clinician agree to continue with pembrolizumab treatment.

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

If a dose of pembrolizumab meeting the protocol definition of overdose is taken without any associated

clinical symptoms or abnormal laboratory results, the overdose should be 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 ICR-CTSU (see section 18.1.5.).

17.15. Discontinuation and subsequent therapy

In cases where toxicity does not resolve to grade 0-1 within 12 weeks after the last infusion, pembrolizumab should be discontinued. Patients with a laboratory adverse event still at grade 2 after 12 weeks post last infusion may restart pembrolizumab if asymptomatic and only after consultation with the Chief Investigator.

17.16. Pembrolizumab Dose Modification Guidelines for Drug-Related AE

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed if AE has been reduced to Grade 1 or 0 and corticosteroid has been appropriately tapered to <10mg prednisolone (or equivalent) per day within 12 weeks of the last pembrolizumab infusion. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks.
3. Patients with a laboratory adverse event still at grade 2 after 12 weeks post last infusion may restart pembrolizumab if asymptomatic and only after consultation with the chief investigator.
4. For severe and life-threatening Immune Related AEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	Toxicity grade or conditions (CTCAE v4.0)	Action taken to pembrolizumab	Immune related AE management with corticosteroid and/or other therapies	Monitor and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg/day prednisone or equivalent) • Administer additional anti-inflammatory measures as needed. • Add prophylactic antibiotics for opportunistic infections	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 (for > 3 days) or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg/day prednisone or equivalent)	• Monitor participants for signs and symptoms of enterocolitis (i.e. diarrhoea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (i.e. peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with 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
	Grade 4	Permanently discontinue		
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg/day prednisone or equivalent)	• Monitor with liver function tests (consider weekly or more frequently) until liver enzyme value returned to baseline or is stable

	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg/day prednisone or equivalent)	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold until patient is clinically and metabolically stable	• Initiate insulin replacement therapy for participants with T1DM and for grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes. • Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide. • For patients with grade 3 or 4 endocrinopathy that improves to grade 2 or less and is controlled with hormone replacement, continuation of pembrolizumab may be considered.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated. • Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency) • Replacement of appropriate hormones may be required as the corticosteroid dose is tapered. • For patients with grade 3 or 4 endocrinopathy that improves to grade 2 or less and is controlled with hormone replacement continuation of pembrolizumab may be considered.
	Grade 3 or 4	Withhold or permanently discontinue[a]		
Hyperthyroidism	Grade 3	Withhold	• Treat with non-selective beta-blockers (e.g., propranolol) or thionamides as appropriate • Replacement of appropriate hormones may be required as the corticosteroid dose is tapered.	• Monitor for signs and symptoms of thyroid disorders. • Any grade 2 hyperthyroidism may also be treated as recommended. • For patients with grade 3 or 4 endocrinopathy that improves to grade 2 or less and is controlled with hormone replacement continuation of pembrolizumab may be considered.
	Grade 4	Discontinue[a]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (e.g. levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders. • For patients with grade 3 or 4 endocrinopathy that improves to grade 2 or less and is controlled with hormone replacement continuation of pembrolizumab may be considered.
	Grade 2	Withhold		• Monitor changes of renal function

Nephritis[b] and Renal dysfunction	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (prednisone 1-2 mg/kg/day or equivalent)	
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
Infusion Reaction[c]	Grade 2	Withhold		• Permanently discontinue if toxicity develops despite adequate premedication
	Grade 3 or 4	Permanently discontinue		
Hepatitis	Grade 2 - 4	Withhold	• For grade 2, administer corticosteroids (prednisone 0.5-1mg/kg/day or equivalent) • For grade 3 / 4, administer corticosteroids (prednisone 1-2 mg/kg/day or equivalent)	• Withhold or discontinue pembrolizumab based on severity of liver enzyme elevations.
Skin reactions including: • Dermatitis exfoliative • Erythema multiform • Exfoliative rash • Bullous pemphigoid • Stevens-Johnson syndrome • grade ≥ 3 pruritus and rash (generalised or maculopapular)	Grade 2 or 3	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg/day or equivalent)	• Monitor patients for suspected severe skin reactions and exclude other causes. • Refer the patient to dermatology for assessment and treatment. • If SJS or TEN is confirmed, permanently discontinue pembrolizumab.
	Grade 4	Permanent discontinue		
All other immune-related AEs[d] May include:	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes • Pembrolizumab may only be restarted after grade 3 reactions following discussion with the ICR-CTSU.
	Grade 3	Withhold or discontinue based on		

• Cytokine release syndrome • Arthritis • Uveitis • Myositis • Guillain-Barre syndrome • Myasthenia gravis • Vasculitis • Pancreatitis • Haemolytic anaemia		the type of event. Events that require discontinuation include and not limited to: Guillain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

Note: Permanently discontinue pembrolizumab for any severe or grade 3 (grade 2 for pneumonitis) drug-related AE that recurs or any life-threatening event.

a. Withholding or permanently discontinuing pembrolizumab is at the discretion of the investigator or treating physician.
b. Renal function can be calculated using Serum creatinine OR estimated GFR (using Cockcroft-Gault).
c. For infusion reactions: 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 patient should be pre-medicated for the next scheduled dose; Refer to Table 2 – Infusion Treatment Guidelines for further management details.
d. Patients with intolerable or persistent grade 2 drug-related AE may hold pembrolizumab at investigator's discretion. Permanently discontinue pembrolizumab for persistent grade 2 adverse reactions for which pembrolizumab has been on hold for ≥12 weeks.

17.17. Supply and distribution

Pembrolizumab is manufactured and provided free of charge by Merck, Sharp & Dohme to participating sites.

Mawdsleys will distribute pembrolizumab to participating sites.

No drug will be distributed to participating centres unless ICR-CTSU is satisfied that the required approvals and agreements and initiation procedures are complete.

17.18. Formulation, packaging, storage conditions and labelling

Pembrolizumab is provided as a liquid solution (100 mg/vial) in Type I glass vials, intended for single use only.

Pembrolizumab vials should be stored in original containers to protect from light exposure. The drug is stored as a liquid solution under refrigerated conditions (2 to 8 °C).

Please refer to the current approved pembrolizumab Investigator Brochure and the c-TRAK TN Pharmacy Guidance Notes for further information and guidance on the preparation, storage and stability of pembrolizumab within c-TRAK TN.

Merck Sharp & Dohme will label the pembrolizumab vials in accordance with the MHRA approved c-TRAK TN label. Pharmacies should add a dispensing label to the infusion bag at the time of reconstitution. Please refer to the Pharmacy c-TRAK TN Pharmacy Guidance Notes for more information.

17.19. Pharmacy responsibilities and drug accountability

The trial drug must not be used outside the context of the c-TRAK TN protocol.

Records must be kept of all deliveries, dispensing and destruction in accordance with the c-TRAK TN Pharmacy Guidance Notes. These records may be requested by ICR-CTSU during the trial to monitor supply and usage of stock. Account must be given of any discrepancies and certificates of delivery and return must be signed.

18. PHARMACOVIGILANCE

18.1. Definitions

18.1.1. Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or clinical trial patient administered an investigational medicinal product; the event does not necessarily have a causal relationship with the treatment or usage.

18.1.2. Serious Adverse Event (SAE)

An SAE is any untoward medical occurrence that occurs after the commencement of trial treatment and within 90 days of the last administration or 30 days following cessation of treatment if the patient initiates new anti-cancer therapy, whichever is earlier, and:

- results in death,
- is life-threatening
- requires hospitalisation or prolongation of existing inpatients´ hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect

Important adverse events that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above, may also be considered serious.

In addition, adverse events meeting either of the criteria listed below, are reportable to the ICR-CTSU in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by ICR-CTSU for collection purposes.

- Is a new cancer (that is not a condition of the study)
- Is associated with an overdose

Recurrence of the indicated disease and death due to recurrence of the indicated disease are not considered SAEs.

Pregnancy whilst participating in a trial is not itself considered an SAE but should be followed up for congenital anomalies or birth defects.

18.1.3. Serious Adverse Reaction (SAR)

A serious adverse reaction is an SAE that is suspected as having a causal relationship to the investigational medicinal product, as assessed by the investigator responsible for the care of the patient. A suspected causal relationship is defined as possibly, probably or definitely related (see definitions of causality table below).

Definitions of causality

Relationship	Description
Unrelated	There is no evidence of any causal relationship with the trial drug
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the 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
Definitely	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

18.1.4. Suspected Unexpected Serious Adverse Reaction (SUSAR)

A serious adverse reaction, the nature or severity of which is not consistent with the reference safety information provided in the applicable Investigator's Brochure (IB), and is assessed as unexpected by the Chief Investigator.

18.1.5. Events of Clinical Interest (ECI)

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 ICR-CTSU.

Events of clinical interest for this trial include:

1. An overdose of pembrolizumab, as defined in section 17.14, that is not associated with clinical symptoms or abnormal laboratory results.
2. An elevated AST or ALT lab value that is ≥3 times the ULN and an elevated total bilirubin lab value that is ≥2 times the ULN and, at the same time, an alkaline phosphatase lab value that is ≥2 times the ULN, 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.

18.2. Reporting Adverse Events to ICR-CTSU

Any toxicity, sign or symptom that occurs after commencement of pembrolizumab treatment and within 90 days of the last administration of pembrolizumab, or 30 days following cessation of treatment if the patient initiates new anti-cancer therapy, whichever is earlier, and which is not unequivocally due to recurrence of disease, should be considered an AE.

All AEs must be reported on the relevant CRF and submitted to ICR-CTSU.

The severity of AEs should be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.0. For each AE, the highest grade observed since the last visit should be reported.

Abnormal laboratory findings that meet the criteria for grade 2 or above toxicity as defined by NCI CTCAE v4.0 are considered to be clinically significant and should be reported as AEs. Whenever one or more toxicity/sign/symptom corresponds to a disease or a well-defined syndrome only the main disease/syndrome should be reported.

18.3. Reporting of Serious Adverse Events to ICR-CTSU

Any SAE that occurs after the commencement of pembrolizumab treatment and up to 90 days following the last dose of pembrolizumab, or 30 days following cessation of treatment if the patient initiates new anti-cancer therapy, whichever is earlier, must be reported.

Any SAEs that occur more than 90 days after the last dose of pembrolizumab that, in the opinion of the Principal Investigator (or designated investigator), are related to pembrolizumab should be reported to ICR-CTSU if the Principal Investigator (or designated investigator) becomes aware of them.

All SAEs should be reported to ICR-CTSU within 24 hours of the Principal Investigator (or designated investigator) becoming aware of the event, by completing the c-TRAK TN SAE form and email to:

The ICR-CTSU safety team

Email: sae-icr@icr.ac.uk

Copying in the c-TRAK TN trial team

Email: c-trak-tn-icrctsu@icr.ac.uk

As much information as possible, including the Investigator's assessment of causality, must be reported to ICR-CTSU in the first instance. Additional follow up information should be reported as soon as it is available.

All SAE forms must be completed, signed and dated by the Principal Investigator or designated investigator.

All reported SAEs and follow up information will be forwarded to Merck, Sharp & Dohme upon receipt at ICR-CTSU.

18.4. Review of Serious Adverse Events

The Chief Investigator (or designated representative) will assess all reported SAEs for causality and expectedness (NB. The Chief Investigator cannot down-grade the Principal Investigator's assessment of causality).

SAEs assessed as having a causal relationship to pembrolizumab and as being unexpected (SUSARs) will undergo expedited reporting to the relevant authorities and all other interested parties by ICR-CTSU (see below).

Sites should respond as soon as possible to requests from the Chief Investigator or designated representative (via ICR-CTSU) for further information that may be required for final assessment of an SAE.

18.5. Expedited reporting of SUSARs

If an SAE is identified as being a SUSAR by the Chief Investigator, and is fatal or life threatening, it will be reported by ICR-CTSU to the MHRA, the REC, the Sponsor, Merck, Sharp & Dohme and all other interested parties within 7 days of being notified of the event.

If an SAE is identified as a SUSAR by the Chief Investigator, and is not fatal or life threatening, it will be reported by ICR-CTSU to the MHRA, the REC, the Sponsor and Merck, Sharp & Dohme within 15 days of ICR-CTSU being notified of the event.

ICR-CTSU will report any additional relevant information to the MHRA, REC, the Sponsor and Merck, Sharp & Dohme as soon as possible, or within 8 days of the initial report of a fatal/life threatening SUSAR.

The Principal Investigators at all actively recruiting sites will be informed of any SUSARs occurring within the trial at appropriate intervals.

18.6. Follow up of Serious Adverse Events

SAEs should be followed up until clinical recovery is complete or until disease has stabilised. SAE outcomes should be reported to ICR-CTSU using the relevant section of the SAE form as soon as the Principal Investigator or designee becomes aware of the outcome.

18.7. Annual reporting of Serious Adverse Reactions

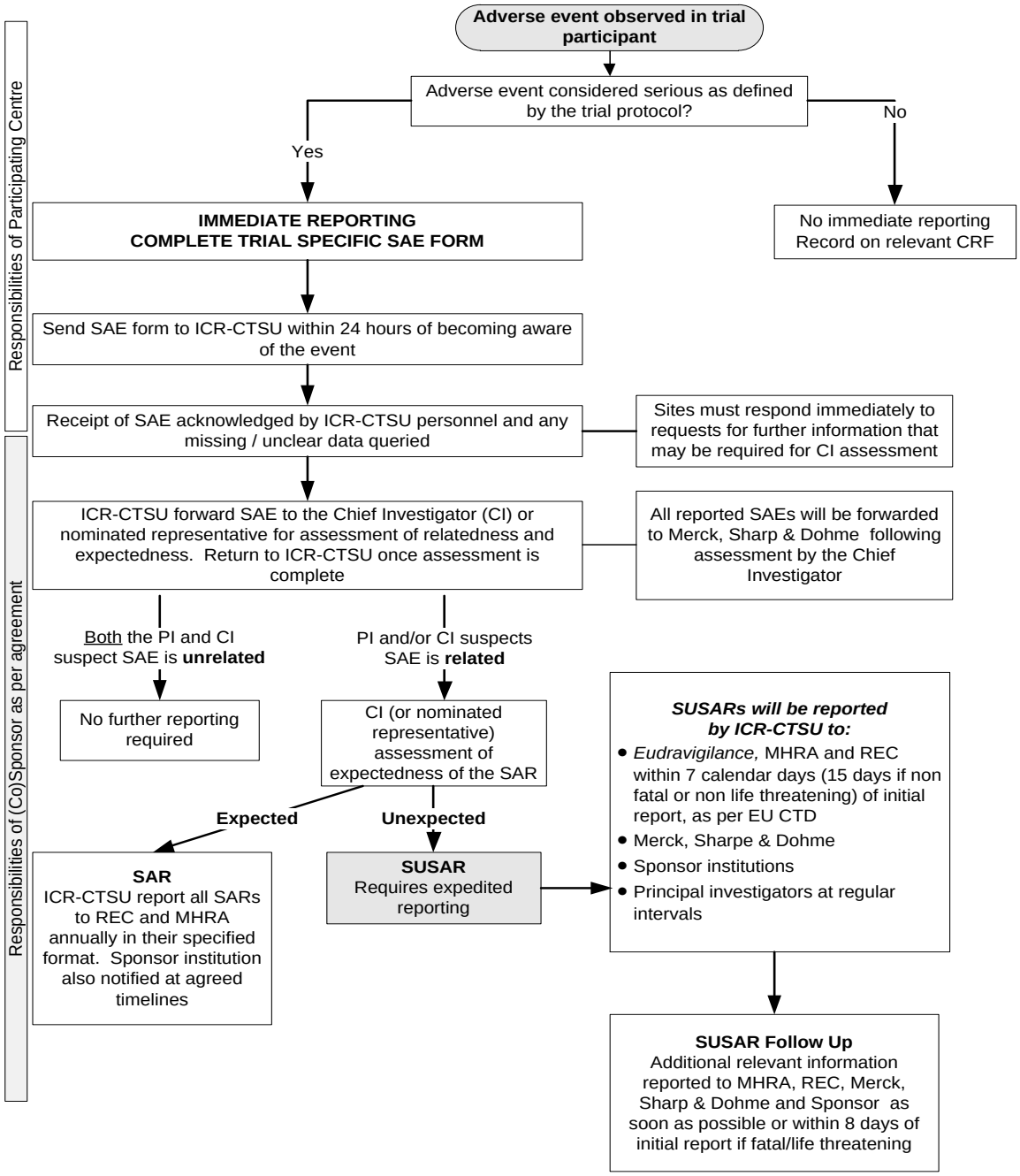
An annual report will be provided to the MHRA and the main REC by ICR-CTSU and copied to the Sponsor at the end of the reporting year.

18.8. Reporting pregnancies

Patients who become pregnant during ctDNA surveillance should stop ctDNA surveillance and be followed up as per protocol. Pregnancy during the ctDNA surveillance period should be reported to the ICR-CTSU via the deviation form, the pregnancy reporting form should not be completed.

If any trial patient or a trial participants' partner becomes pregnant while receiving trial drug or up to 120 days after receiving pembrolizumab, this should be reported to ICR-CTSU immediately using the pregnancy reporting form. Participants who become pregnant should discontinue from pembrolizumab immediately. Pregnancies should be followed up until conclusion and all follow-up information should be reported to ICR-CTSU. If the outcome of the pregnancy meets the definition of serious (i.e. congenital abnormality) this should be reported to ICR-CTSU following the serious adverse event reporting procedures described above. All reported pregnancies and follow up information (whether related to an SAE or not) will be forwarded to Merck, Sharp & Dohme upon receipt at ICR-CTSU.

Figure 2: Flow diagram for SAE reporting, and action following report



NB. All SAEs should continue to be followed up as specified above

19. STATISTICAL CONSIDERATIONS

19.1. Statistical design and sample size justification

19.1.1. Screening component

This consists of a single cohort who will have ctDNA surveillance every 3 months for 2 years. Patients who are found to be ctDNA positive within the first 12 months of ctDNA surveillance will enter the therapeutic trial. The size of the screening cohort has been chosen to anticipate the required number of patients eligible for the therapeutic trial (assuming 30% patients have a positive ctDNA during the first 12 months) and will provide further descriptive information relating to ctDNA incidence and lead time over detection of overt clinical relapse. The trial aims to recruit 150 patients into ctDNA surveillance. It is anticipated that approximately 75% of patients initially enrolled will be eligible for ctDNA surveillance, therefore approximately 200 patients will be recruited in total. The Independent Data Monitoring Committee (IDMC) will monitor the ctDNA positivity rate observed throughout the course of the trial and make recommendations regarding any increase or decrease in the overall size of the screening cohort based on this. All patients who enter the screening cohort will have the opportunity to enter the intervention phase if they become ctDNA positive within the first 12 months, regardless of the overall number of patients currently recruited to the therapeutic component of the trial.

If the incidence assumptions are correct and approximately 30% of patients have a positive ctDNA during the first 12 months of surveillance then 150 patients will enable the incidence of ctDNA positivity to be estimated with a two-sided 95% confidence interval of +/-7.3%.

In the original randomised trial design (prior to the implementation of protocol v6.0) the planned exploratory analyses were detailed as follows: Exploratory analysis will be performed comparing time to macroscopic relapse between two groups, 1) patients who remain ctDNA negative within the first 12 months and do not enter the therapeutic trial, and 2) patients who become ctDNA positive and are randomised to the observation group in therapeutic trial.

Subsequent to the removal of the randomised component (implementation of protocol v6.0) the above analyses will be amended to censor any patient who had been previously randomised to the observation group at the date they subsequently started pembrolizumab.

Under the original randomised study design (prior to protocol v6.0), of 150 patients screened, approximately 70% will remain ctDNA negative and 10% will be allocated to the observation group in the therapeutic trial (with the remaining 20% allocated to pembrolizumab in the therapeutic trial). Therefore assuming a 7:1 allocation ratio between groups 1) and 2) described above, a hazard ratio of 3.02 (assuming a reduction in 2 year relapse free rate from 80% to 51%) can be detected with 80% power, alpha 5% (two sided). We note that the HR demonstrated in retrospective testing for the ctDNA assay was 13.6, and the trial will be well powered for prospective validation.

19.1.2. Therapeutic component

Under the original randomised study design (prior to protocol v6.0), those patients allocated via randomisation to observation provide a reference control rather than a formal randomised comparator group. The statistical considerations underpinning the therapeutic intervention evaluation are those of a single group trial with patients treated with pembrolizumab in both the randomised and non-randomised

trial components combined. The original design hypothesised frequencies of ctDNA+ and allocation to pembrolizumab as per the text and table outlined below. The revision in the design to close the randomised component has the potential to increase the number of patients commencing pembrolizumab. No redefinition of the criteria have been undertaken since the success criteria are determined by the number starting treatment and not the overall proportion available to receive treatment.

The rate of ctDNA positivity is prone to stochastic effects, and as such a range of effect sizes is presented. All patients in the screening cohort who are observed to have a positive ctDNA will be entered into the therapeutic component, therefore the eventual number entering that phase will only be defined as the trial progresses. The most likely incidence, on which the primary calculations were made, is for a 30% positivity rate during the first 12 months. Calculations use an A'Hern single-stage design within pembrolizumab treated patients, alpha=0.1, power=90%, unacceptable ctDNA clearance rate p0 (of 0.3) and minimum required level of efficacy p1 of approximately 0.5. The number of clearances to conclude the required level of success and width of the 95% confidence interval which could be detected around a proportion with ctDNA clearance of approximately 0.5 are given for each hypothesised rate of ctDNA positivity.

Rate of ctDNA positivity	No. ctDNA positive (of 150 in ctDNA surveillance phase)	No. randomised to receive pembrolizumab	p0	p1	No. successes required	Width of 95% confidence interval detectable around proportion of 0.5
45%	68	45	0.30	0.49	18	+/- 0.14
30%	45	30	0.30	0.54	13	+/- 0.17
24%	36	24	0.30	0.57	10	+/- 0.19

In prior research no patients with ctDNA detected at baseline spontaneously cleared ctDNA thus it is anticipated that such clearance would demonstrate activity of the intervention. The reference group in the randomised component of the trial will however provide further evidence to support that assertion. The number of patients randomised to the observation group (prior to the implementation of protocol v6.0) who have spontaneous clearance will be recorded and will be presented together with an associated 95% confidence interval to illustrate the likely theoretical upper bound of such an occurrence. Patients who subsequently receive pembrolizumab after transitioning to the non-randomised component of the trial will be excluded from this analysis if they start treatment within 24 weeks of becoming ctDNA positive and are therefore not assessable for the ctDNA clearance endpoint.

19.2. Treatment allocation

Following the implementation of protocol v6.0 whereby patients transfer to the non-randomised component of the trial, all participants will be allocated to receive pembrolizumab treatment in the therapeutic trial centrally by ICR-CTSU upon confirmation of a positive ctDNA result.

Prior to the implementation of protocol v6.0 during the randomised component of the trial, in the therapeutic component, participants were randomised between pembrolizumab treatment and observation (continued ctDNA surveillance) on a 2:1 basis. Treatment allocation is via a minimisation algorithm, balancing for time from trial entry to randomisation (0 months i.e. ctDNA detected in baseline screening sample vs. 3-12 months) and adjuvant capecitabine status (i.e. whether or not a patient received adjuvant capecitabine).Minimisation is a widely recognised method of ensuring balance between treatment groups for several prognostic factors in clinical trials with smaller sample sizes. Treatment allocation to the next participant enrolled depends on

the characteristics of the patients already involved, thus minimising the imbalance across the prognostic factors. To make the allocation of treatment more unpredictable, a random element was incorporated in the algorithm.

19.3. Endpoint definitions

19.3.1. Primary Endpoints

ctDNA surveillance

- **Positive ctDNA detection by 12 and 24 months from start of ctDNA surveillance**. This will be calculated as the proportion of patients with ctDNA positivity by each time point from those with assessment performed at that time point. Proportions will be presented with associated exact 95% confidence intervals.

Therapeutic component

- **Absence of detectable ctDNA or disease recurrence 6 months (24 weeks) after commencing pembrolizumab.** This will be calculated as the proportion of patients without either detectable ctDNA or disease recurrence 6 months after commencing pembrolizumab. Proportions will be presented with associated exact 95% confidence intervals.

19.3.2. Secondary Endpoints

Secondary endpoints relating to the observation group will be analysed in the patients randomised to the observation group prior to the implementation of protocol v6.0.

ctDNA surveillance

1) **Time to ctDNA detection.** This will be calculated as the time from entry into the ctDNA surveillance to first positive ctDNA detection. Statistical techniques for time to event data will be used to summarise data and display the pattern of ctDNA detection graphically.

Therapeutic component

1) **Detection of overt metastatic disease at the time of first ctDNA detection in patients allocated to pembrolizumab.** This will be calculated as the proportion of patients with metastatic disease from CT scan at the same time point as first positive ctDNA detection, and presented with associated exact 95% confidence interval.
2) **Lead-time between ctDNA detection and recurrence in the pembrolizumab treatment and observation groups.** This will be calculated as the time between therapeutic trial entry and first confirmed detection of recurrent disease. Recurrence will include loco-regional and distant recurrence, but will exclude new primary cancers. Statistical techniques for time to event data will be used to summarise data and display time between ctDNA detection and recurrence graphically.
3) **Absence of detectable ctDNA or disease recurrence after 6 months in the observation group.** This will be calculated as the proportion of patients without either detectable ctDNA or disease recurrence 6 months after randomisation to the observation group. Proportions will be presented with associated exact 95% confidence intervals.
4) **Safety and tolerability of pembrolizumab in this patient group.** Safety will be assessed throughout the treatment period using the NCI CTCAE v4.0 and summarised in tabular format. Reported toxicities

will be coded using Med DRA (current version). The proportion of patients reporting a dose reduction/delay during pembrolizumab will be presented.

5) **Commencement of treatment in patients allocated to receive pembrolizumab.** This will be calculated as the proportion of patients allocated to receive pembrolizumab who start this therapy. The proportion will be presented with associated exact 95% confidence interval.

19.3.3. Exploratory Endpoints

Exploratory endpoints relating to the observation group will be analysed in the patients randomised to the observation group prior to the implementation of protocol v6.0.

1) **Descriptive differences in time between ctDNA detection and disease recurrence, and disease free survival, between patients in the pembrolizumab treatment and observation groups.** The times from first ctDNA detection to disease recurrence, and to disease free survival event (i.e. including new breast primary cancer or breast cancer death) will be calculated using statistical techniques for time to event data and summarised for each randomised treatment group. This will be calculated and presented separately for the pembrolizumab and observation randomised groups, with patients included in their randomised group regardless of treatment actually received. If recurrence is confirmed after initial continuation of treatment for development of isolated lymphadenopathy, the date of first scan will be used in analyses as date of recurrence.

2) **To explore predictors of sustained ctDNA clearance on pembrolizumab.** The proportion of patients with absence of detectable ctDNA or disease recurrence 12 months after commencing pembrolizumab will be tabulated by clinical and biological factors of interest. Consideration will be given to the use of logistic regression to investigate relationships between factors considered and ctDNA clearance in univariate and multivariable models.

3) **To explore potential predictors of relapse and ctDNA detection, and alternative definitions of ctDNA clearance.** Lead time will be calculated as described previously. Statistical techniques for time to event data will be used to summarise lead time data and display graphical according to clinical and biological factors of interest. Alternative definitions of the proportion of patients without either detectable ctDNA or disease recurrence at 12 months, and other time points, after randomisation to the observation group. Proportions will be presented with associated exact 95% confidence intervals.

4) **Association between ctDNA clearance and time to recurrence in the pembrolizumab group.** The times from first ctDNA detection to recurrence event will be calculated using statistical techniques for time to event data and summarised separately for patients with and without sustained ctDNA clearance. If numbers allow, time to recurrence will be formally compared between those with and without ctDNA clearance using standard time to event methods.

19.4. Statistical Analysis Plan

In the therapeutic component of the trial, analyses will be performed using the intention to treat (ITT) population meaning patients who do not meet the eligibility criteria (for reasons other than disease recurrence) or do not wish to receive pembrolizumab treatment will still be included in the analysis of the primary endpoint (ctDNA clearance). These patients should continue to have bloods taken for ctDNA surveillance every 3 months for up to 2 years from starting ctDNA surveillance. Patients will then be followed up until disease recurrence detection. Sensitivity analyses will also be performed using a per-protocol

population including only patients who start pembrolizumab treatment. If large differences are observed between ITT and per-protocol analyses, both results will be presented and reasons for failure to start pembrolizumab (ineligibility, patient choice) further explored.

All analysis will be repeated in the subset of patients who enter into the study within the recruitment time windows of protocol version 5.0, and the subset of patients who enter the study outside the recruitment time windows of protocol version 5.0. Further details of analysis methods will be specified in a Statistical Analysis Plan in accordance with ICR-CTSU Standard Operating Procedures.

The Statistical Analysis Plan will include a section to detail how data impacted by strategies introduced to mitigate risks relating to the COVID-19 pandemic will be handled.

19.5. Interim analyses and stopping rules

No formal interim analysis of efficacy will be performed however the IDMC will meet regularly (at least annually) to assess safety and tolerability and also to review the emerging data on ctDNA incidence to inform the anticipated overall size of the therapeutic trial (i.e. to ensure processes in place for sufficient drug supply etc.) and to determine the relevant success criteria for the above A'Hern design required.

20. TRIAL MANAGEMENT

20.1. Trial Management Group (TMG)

A Trial Management Group (TMG) will be set up and will include the Chief Investigator, ICR-CTSU Scientific Lead, Co-investigators and identified collaborators, the Trial Statistician and Trial Manager. Principal Investigators and key trial personnel will be invited to join the TMG as appropriate to ensure representation from a range of sites and professional groups. Where possible, membership will include a lay/consumer representative. The TMG will meet at regular intervals, and at least annually. Notwithstanding the legal obligations of the Sponsor and Chief Investigator, the TMG have operational responsibility for the conduct of the trial. The Committee's terms of reference, roles and responsibilities will be defined in a charter issued by ICR-CTSU.

20.2. Trial Steering Committee (TSC)

The trial will be monitored by the generic ICR-CTSU Breast Systemic Therapy Trials Steering Committee (TSC). The TSC will meet at regular intervals, and at least annually. The TSC will provide expert independent oversight of the trial on behalf of the Sponsor and funder. The Committee's terms of reference, roles and responsibilities will be defined in charter issued by ICR-CTSU and based on MRC Good Clinical Practice (MRC GCP).

20.3. Independent Data Monitoring Committee (IDMC)

An Independent Data Monitoring Committee (IDMC) will be set up to monitor the progress of the trial and will comprise a Chairman and at least two further members with clinical or statistical expertise (at least one member must be a statistician). Membership of the IDMC will be proposed by the TMG and approved by the TSC.

The IDMC will meet in confidence at regular intervals, and at least annually. A summary of findings and any recommendations will be produced following each meeting. This summary will be submitted to the TMG and TSC, and if required, the main REC and the MHRA.

The IDMC will reserve the right to release any data on outcomes or side-effects through the TSC to the TMG (and if appropriate to participants) if it determines at any stage that the combined evidence from this and other studies justifies it.

The Committee's terms of reference, roles and responsibilities will be defined in a charter issued by ICR-CTSU.

21. RESEARCH GOVERNANCE

21.1. Sponsor responsibilities

The Sponsor of the c-TRAK TN trial is the Institute of Cancer Research (ICR).

21.2. Participating site responsibilities

Responsibilities delegated to participating sites are defined in an agreement between the Sponsor and the individual site.

21.3. Merck Sharp & Dohme responsibilities

Merck Sharp & Dohme (MSD) on behalf of the Sponsor will manufacture, package, and label trial drug in accordance with Good Manufacturing Practice and all applicable local legislation. Responsibilities are defined in an agreement between MSD and the Sponsor.

21.4. Mawdsleys responsibilities

Mawdsleys on behalf of the Sponsor will distribute the trial drug to sites in accordance with Good Manufacturing Practice and all applicable local legislation. Responsibilities are defined in an agreement between Mawdsleys and the Sponsor.

22. TRIAL ADMINISTRATION & LOGISTICS

22.1. Site activation

Before recruitment can commence at a site, the site agreement must have been signed by all required signatories, the required trial documentation (as specified by ICR-CTSU) must be in place and a site initiation must have taken place. Site initiation may be by teleconference or by on site visit if requested by the Principal Investigator or if deemed appropriate by ICR-CTSU. ICR-CTSU will provide the final confirmation that recruitment can commence at a site.

22.2. Data acquisition

Electronic Case Report Forms (eCRFs) will be used for the collection of trial data from source data. ICR-CTSU will provide guidance to sites to aid the completion of the eCRFs. The Trial Management Group reserves the right to amend or add to the eCRF template as appropriate. Such changes do not constitute a protocol amendment, and revised or additional forms should be used by sites in accordance with the guidelines provided by ICR-CTSU.

22.3. Central data monitoring

Once data has been entered on the eCRF by the site personnel, ICR-CTSU will review it for compliance with the protocol, and for inconsistent or missing data. Should any missing data or data anomalies be found, queries will be raised for resolution by the site.

Any systematic inconsistencies identified through central data monitoring may trigger an on-site monitoring visit.

22.4. On-site monitoring

If a monitoring visit is required, ICR-CTSU will contact the site to arrange the visit. Once a date has been confirmed, the site should ensure that full patient notes of participants selected for source data verification are available for monitoring.

ICR-CTSU staff conducting on-site monitoring will review essential documentation and carry out source data verification to confirm compliance with the protocol. If any problems are detected during the course of the monitoring visit, ICR-CTSU will work with the Principal Investigator or delegated individual to resolve issues and determine appropriate action.

22.5. Completion of the trial and definition of Trial End Date

The trial end date is deemed to be the date of last data capture.

22.6. Archiving

Essential trial documents should be retained according to local policy and for a sufficient period for possible inspection by the regulatory authorities (at least 5 years after the date of last data capture). Documents should be securely stored and access restricted to authorised personnel.

23. PATIENT PROTECTION AND ETHICAL CONSIDERATIONS

23.1. Trial approvals

This trial has been formally assessed for risk by the Sponsor.

ICR-CTSU, on behalf of the Sponsor, will ensure that the trial has received ethics approval from a research ethics committee for multi-centre trials, regulatory approval from the MHRA and the relevant NHS permissions. Before recruiting patients, the Principal Investigator at each site is responsible for gaining local approvals.

23.2. Trial conduct

This trial will be conducted according to the approved protocol and its amendments, supplementary guidance and manuals supplied by the Sponsor and in accordance with The Medicines for Human Use (Clinical Trials) Regulations 2004 as amended, the Research Governance Framework for Health and Social Care and the principles of GCP.

23.3. Informed consent

Patients should be asked to sign the current ethics approved c-TRAK TN consent form at trial entry and prior to starting treatment after receiving both verbal and written information about the trial, having been given sufficient time to consider this information. All consent forms must be countersigned by the Principal Investigator or a designated individual. A signature log of delegated responsibilities, listing the designated individuals and the circumstances under which they may countersign consent forms, must be maintained at the participating site. This log, together with original copies of all signed patient consent forms, should be retained in the Site Investigator File and must be available for inspection. The current ethics approved c-TRAK TN patient information sheets should be provided in addition to any standard patient information sheets that are provided by the site and used in routine practice.

23.4. Patient confidentiality

Patients will be asked to consent to their full name being collected at trial entry in addition to their date of birth, hospital number, postcode and NHS number or equivalent to allow linkage with routinely collected NHS data and ensure accuracy in handling biological samples.

Each investigator should keep a separate log of all participants' Registration Numbers, Trial IDs, names, addresses and hospital numbers. The investigator must retain trial documents (e.g. participants' written consent forms) in strict confidence. The investigator must ensure the patients' confidentiality is maintained at all times.

Representatives of ICR-CTSU and the regulatory authorities will require access to patients' hospital notes for quality assurance purposes. ICR-CTSU will maintain the confidentiality of participants at all times and will not reproduce or disclose any information by which participants could be identified.

23.5. Data protection

ICR-CTSU will comply with all applicable data protection laws.

23.6. Insurance and liability

Indemnity to meet the potential legal liability of investigators participating in this trial is provided by the usual NHS indemnity arrangements.

24. FINANCIAL MATTERS

This trial is investigator designed and led, has been endorsed by Clinical Research Committee (CRC) of Cancer Research UK, and meets the criteria for R&D support as outlined in the Statement of Partnership on Non-Commercial R&D in the NHS in England.

The trial is part of the National Institute for Health Research Clinical Research Network (NIHR CRN) portfolio by virtue of its endorsement by CRC. NIHR CRN resources should therefore be made available for the trial to cover UK specific research costs.

The Sponsor has received Investigator Initiated Research grants (IIR) from Merck Sharp & Dohme and ICR NIHR Biomedical Research Centre for the conduct of this trial.

25. PUBLICATION POLICY

The main trial results will be published in a peer-reviewed journal, on behalf of all collaborators. The manuscript will be prepared by a writing group, consisting of members of the TMG. Participating clinicians may be selected to join the writing group on the basis of intellectual and time input. All participating clinicians will be acknowledged in the publication.

Patient participation will be acknowledged in any publication resulting from the c-TRAK TN trial. Results of the trial will be sent to treating clinicians to relay to patients who participated in the trial.

Any presentations and publications relating to the trial must be authorised by the TMG. Authorship of any secondary publications e.g. those relating to sub-studies, will reflect intellectual and time input into these studies.

No investigator may present or attempt to publish data relating to the c-TRAK TN trial without prior permission from the TMG.

26. ASSOCIATED STUDIES

26.1. Translational studies

26.1.1. PDL1

Archival tissue may be used to assess PD-L1 assessment by IHC (subject to funding availability). PD-L1 can be shed from tumour and released into the blood, which would be a less invasive component to measure PD-L1 protein biomarker. Analyses may include exploratory analysis of time from ctDNA detection and disease recurrence according to tumour PDL1 expression and prediction of sustained ctDNA clearance on pembrolizumab by PD-L1 expression.

26.1.2. Nucleic acid extractions and analyses

Nucleic acids including DNA and RNA will be extracted from tissue samples, and DNA will be extracted from normal buffy coat and plasma. DNA samples will be subjected to digital PCR or sequencing analysis or other molecular techniques to identify mutations relevant to cancer biology.

Exploratory analyses may include identification of biomarkers that predict sustained ctDNA clearance on pembrolizumab, and predictor relapse and ctDNA detection.

26.1.3. Protein analysis

Tissue sections from the trial will be analysed by immunohistochemistry, immunofluorescence**,** or other techniques for analysis of proteins. Tissue samples may be processed for analysis of proteins using alternative techniques.

Exploratory analyses may include identification of biomarkers that predict sustained ctDNA clearance on pembrolizumab, and predictor relapse and ctDNA detection.

26.1.4. Assessment of intra-tumoural heterogeneity and clonality

Exploratory assessment will be made on whether a targetable mutation is apparently clonal (likely present in all cancer cells in the tumour) or sub-clonal (present in only a subset of cancer cells in the body). Evidence of sub-clonality may include discordance in the mutation between ctDNA surveillance blood sample and tissue biopsy, or evidence that the mutation has reduced allele frequency compared to other mutations present in the ctDNA.

26.1.5. Assessment of isolated PBMC

Blood samples for PBMC isolation will be collected from patients at designated (geographically determined) PBMC sites. These samples will be used for exploratory research on predictors of sustained ctDNA clearance on pembrolizumab. PBMC will be isolated from the blood tests, and live PBMC cells frozen in the central laboratory. PBMC will be grown to isolate cytotoxic T cells, and establish if patient cytotoxic T cells can recognise tissue mutations. PBMC isolated on treatment (cycle 3) will be used to explore whether cytotoxic T cells expand on pembrolizumab therapy, and whether expansion of cytotoxic T cells predicts for sustained ctDNA clearance.

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A1. ECOG performance status

Grade	*Performance Status*
0	Able to carry out all normal activity without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out light work.
2	Ambulatory and capable of all self-care but unable to carry out any work; up and about more than 50% of waking hours.
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours.
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair.

A2. Contraception

Male patients 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 patient will be considered of non-reproductive potential if:

1. They are 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. Have a congenital or acquired condition that prevents childbearing.

Female and male patients of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, from trial registration for the ctDNA surveillance phase of the study and, if allocated to pembrolizumab treatment, whilst receiving trial drug and for 120 days after the last dose of trial 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 patient's male partner
  - contraceptive rod implanted into the skin
- Combination method (requires use of two of the following):
  - diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
  - cervical cap with spermicide (nulliparous women only)
  - contraceptive sponge (nulliparous women only)
  - male condom or female condom (cannot be used together)
  - hormonal contraceptive: oral contraceptive pill (oestrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the patient's preferred and usual lifestyle. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

Patients should be informed that taking the trial medication may involve unknown risks to the foetus (unborn baby) if pregnancy were to occur during the trial. In order to participate in the trial patients of childbearing potential must adhere to the contraception requirement (described above) from registration, throughout ctDNA surveillance and, if allocated to receive pembrolizumab treatment, whilst receiving trial medication up to 120 days after the last dose of trial therapy. If there is any question that a patient of childbearing potential will not reliably comply with the requirements for contraception, that patient should not be entered into the trial.

A3. Pembrolizumab use in pregnancy and nursing women

Use in pregnancy

If a patient inadvertently becomes pregnant while on treatment with pembrolizumab, the patient will immediately be discontinued from trial treatment. The site will contact the patient at least monthly and document the patient's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Chief Investigator or TMG without delay.

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, patients who are breast-feeding are not eligible for enrollment.

A4. GLOSSARY

AE	Adverse Event
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
AUC	Area Under Curve
CI	Chief Investigator
CIS	Carcinoma In Situ
CRF	Case Report Form
ctDNA	Circulating Tumour DNA
DCF	Data Capture Form
DFS	Disease Free Survival
eCRF	Electronic Case Report Form
EORTC	European Organisation for Research and Treatment of Cancer
FBC	Full Blood Count
G-CSF	Growth Colony-Stimulating Factors
GFR	Glomerular Filtration Rate
HR	Hazard Ratio
IB	Investigator's Brochure
ICR	The Institute Of Cancer Research
ICR-CTSU	The Institute Of Cancer Research Clinical Trials and Statistics Unit
IDMC	Independent Data Monitoring Committee
LFT	Liver Function Test
MDT	Multi-disciplinary team
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
PI	Principal Investigator
PIS	Patient Information Sheet
PBMC	Peripheral Blood Mononuclear Cell
QoL	Quality of Life
R&D	Research and Development
RCT	Randomised Controlled Trial
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction

SMPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee
U+E	Urea & Electrolytes
ULN	Upper Limit of Normal
WBC	White Blood Cell
WHO	World Health Organisation