A phase II trial assessing nivolumab in class II expressing microsatellite stable colorectal cancer

Protocol

Version 5.0, 20-May-2020

Sponsor	University of Birmingham
Sponsor Protocol Number	RG_17-215
CRCTU Number	CR2008
ISRCTN	40245896
IRAS ID	237804
EudraCT Number:	2018-000318-39

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ANICCA-Class II Trial Office
Cancer Research UK Clinical Trials Unit (CRCTU)
Institute of Cancer and Genomic Sciences
University of Birmingham
B15 2TT

Tel: 0121 414 6754
Email: ANICCA-ClassII@trials.bham.ac.uk

Patient Registration

https://www.cancertrials.bham.ac.uk/ANICCAClassIILive

Serious Adverse Event Reporting

Email: reg@trials.bham.ac.uk

TRIAL MANAGEMENT GROUP

Chief Investigator	Professor Gary Middleton Institute of Cancer & Genomic Sciences, University of Birmingham
Clinical Coordinators	Professor John Bridgewater University College London
	Dr Paul Ross Guy's Hospital
	Dr Kathryn Herring Cancer Research UK Clinical Trials Unit (CRCTU), University of Birmingham
Lead Statistician	Professor Lucinda Billingham Biostatistics Team, CRCTU, University of Birmingham
Trial Histopathologist	Dr Phillipe Taniere The Queen Elizabeth Hospital, Birmingham
Trial Management Team Leader	Dr Joshua Savage Early Drug Development Team, CRCTU, University of Birmingham
Trial Coordinator	Ms Jessica Crook Early Drug Development Team, CRCTU, University of Birmingham
Trial Statistician	Ms Wenyu Liu Biostatistics Team, CRCTU, University of Birmingham
Patient Representatives	Mr Len Evans Mrs Susan Ann Smith

SIGNATURE PAGE

ANICCA-Class II Trial Protocol version 5.0, 20-May-2020

This protocol has been approved by:

Name:	Professor Gary Middleton	Trial Role:	Chief Investigator
Signature:		Date:	

This protocol describes the ANICCA-Class II trial and provides information about procedures for patients taking part in the ANICCA-Class II trial. The protocol should not be used as a guide for treatment of patients not taking part in the ANICCA-Class II trial.

Protocol written using template CRCTU-PRT-QCD-001 version 1.0

AMENDMENTS

The following amendments and/or administrative changes have been made to this protocol since the implementation of the first approved version:

Amendment number	Date of amendment	Protocol version number	Type of amendment	Summary of amendment
1	02-Apr-2019	3.0	Substantial	• Change to collect only month and year for date of birth. • Clarification of follow-up period for pregnancies.
2	14-Nov-2019	4.0	Substantial	• Addition of ISRCTN • Section 5.2: eRDC webpage amended • Addition of wording for new biopsy if archival biopsy is not sufficient or available • Change of PI at Beatson Cancer Centre
3	20 May 2020	5.0	Substantial	• Change in Class II expression requirements for eligibility • Addition of wording to allow treatment beyond progression if there is clinical benefit • Removal of fax number throughout • Clarification of pregnancy test requirements • Changes to assessment time points within the schedule of events to accommodate treatment dose changes. • Change to treatment dose and schedule from 2 to 4 weekly. • Addition of exploratory objective.

TRIAL SYNOPSIS

Title	ANICCA-Class II: A phase II trial assessing nivolumab in class II expressing microsatellite stable colorectal cancer (MSS CRC)
Trial Design	An open-label, single arm, phase II, multicentre clinical trial to determine the rate of durable clinical benefit (DCB) of nivolumab in patients with class II expressing microsatellite stable colorectal cancer (MSS CRC).
Objectives	**Primary objective:** To detect the rate of durable clinical benefit in patients with class II expressing MSS CRC treated with single agent nivolumab, to justify further investigation in subsequent studies. **Secondary objectives:** To evaluate the benefit of single agent nivolumab to patients with class II expressing MSS CRC in terms of other clinical outcomes relating to tumour response and progression-free and overall survival time. **Exploratory objective:** To discover possible biomarkers for the prediction of a response to treatment with nivolumab. To investigate whether CD8+ and PD-1 T cells are supplementary biomarkers of response to treatment with nivolumab.
Outcome Measures	**Primary outcome:** • Durable clinical benefit **Secondary outcomes:** • Objective response • Best percentage change in sum of target lesion diameters (PCSD) • Time to maximal response • Progression-free survival time • Overall survival time
Patient Population	Patients with locally advanced or metastatic MSS CRC with class II expression
Sample Size	36 patients

Key Inclusion Criteria (refer to section 4.1)	• Histologically confirmed locally advanced or metastatic MSS CRC with class II expression
	• Patients must have completed all standard of care therapy that the treating oncologist deems appropriate. Trial treatment as first line therapy is permitted if the patient has declined standard of care therapy.
	• Age ≥ 18 years
	• Eastern Cooperative Oncology Group (ECOG) performance status 0-2
	• CT scan of chest, abdomen, pelvis within 28 days of registration demonstrating uni-dimensionally measurable disease as per RECIST version 1.1
	• Adequate haematological function:
	  ○ Platelet count ≥100 x 10^9 /L
	  ○ Neutrophils ≥1.5 x 10^9/L
	  ○ Haemoglobin ≥ 90 g/L
	• Adequate renal function

	○ Creatinine clearance <1.5 x Upper Limit of Normal (ULN) and >30 ml/min (as per institutional standard). • Adequate hepatic function: ○ Serum bilirubin ≤1.5 x upper limit of normal (ULN) ○ Serum AST or ALT ≤2.5 x ULN or <5 x ULN in the presence of liver metastases • Written informed consent
Key Exclusion Criteria (refer to section 4.2)	• Prior treatment with PD1/PDL1 inhibitors. • Untreated symptomatic brain or leptomeningeal metastatic disease. • Administration of chemotherapy, radioactive or biological cancer therapy within 4 weeks prior to the first dose of trial therapy. • Patient has not recovered to NCI Common Terminology Criteria for Adverse Events (CTCAE) grade 1 or better from the Adverse Event (AE) due to cancer therapeutics administered more than 4 weeks earlier. • Active autoimmune disease that has required systemic treatment in past 2 years. • Diagnosis of immunodeficiency or receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment. • At risk of bowel obstruction or bowel perforation. • History of tuberculosis, non-infectious pneumonitis, active pneumonitis • Known history of other malignancy, unless confirmation of potentially curative therapy with no evidence of disease for 3 years. • Positive for HIV, Hepatitis B or C. • Has had a live vaccine within 30 days of prior to first dose of trial treatment.
Trial Treatment	Nivolumab intravenous infusion, 480mg flat dose over 60 minutes, every 4 weeks
Trial Duration	18 months recruitment, up to two years treatment, all patients will be followed up for a minimum of 18 months after registration.
Sample Collection	• Germline DNA cycle 1, day 1 pre-infusion • ctDNA, Cytokine/Chemokine Panel and proteomics cycle 1, day 1 pre-infusion followed by every alternate cycle from cycle 3 onwards e.g. cycle 3, 5, 7 etc. (approx. every 8 weeks)
ANICCA-Class II Trial Office Contact Details	Cancer Research UK Clinical Trials Unit (CRCTU), Institute of Cancer & Genomic Sciences, University of Birmingham, Edgbaston, Birmingham. B15 2TT ☎ 0121 414 6754 ✉ ANICCA-Class II@trials.bham.ac.uk
Sponsor	University of Birmingham

TRIAL SCHEMA

Figure 1 Trial Schema

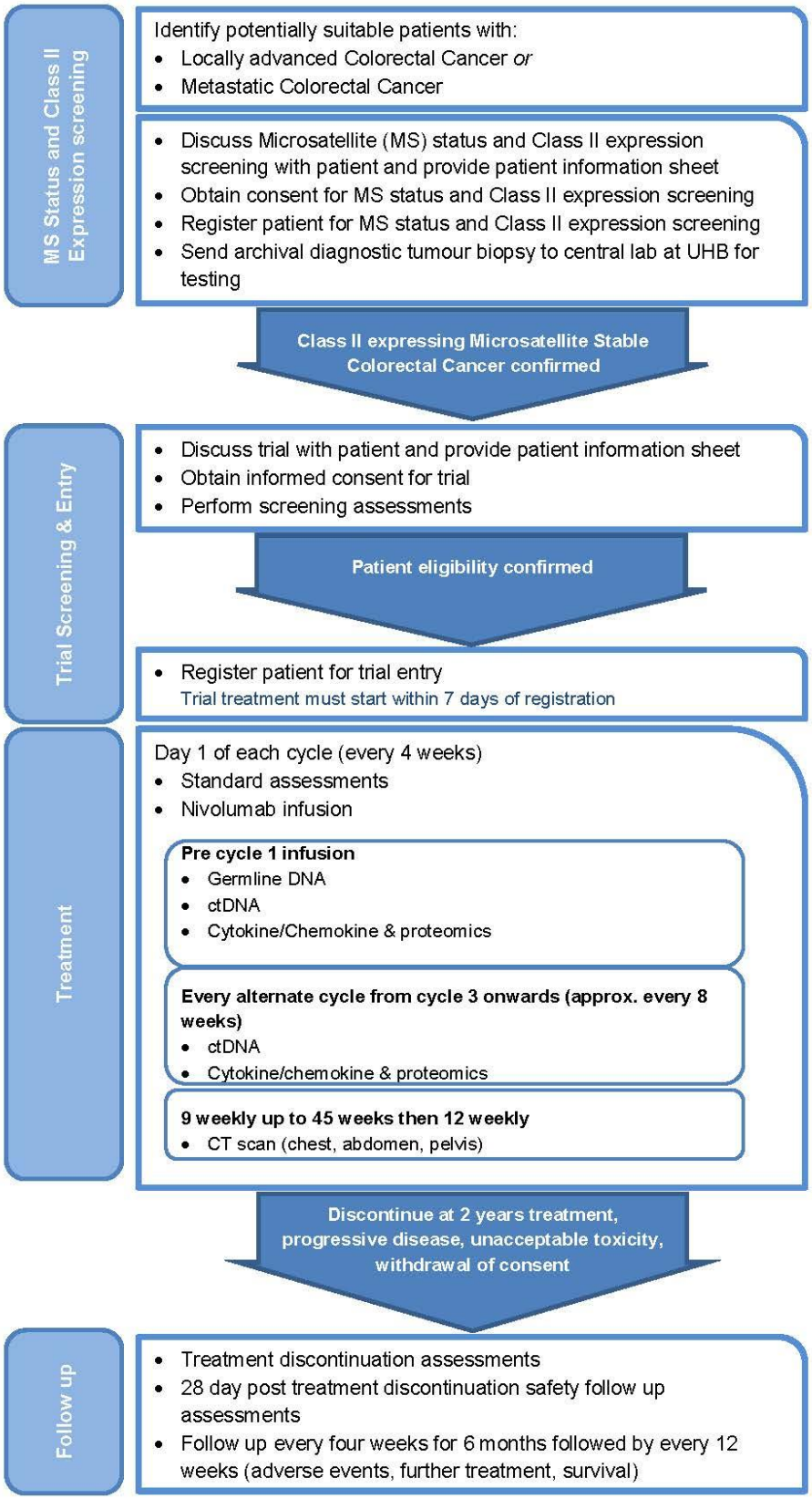
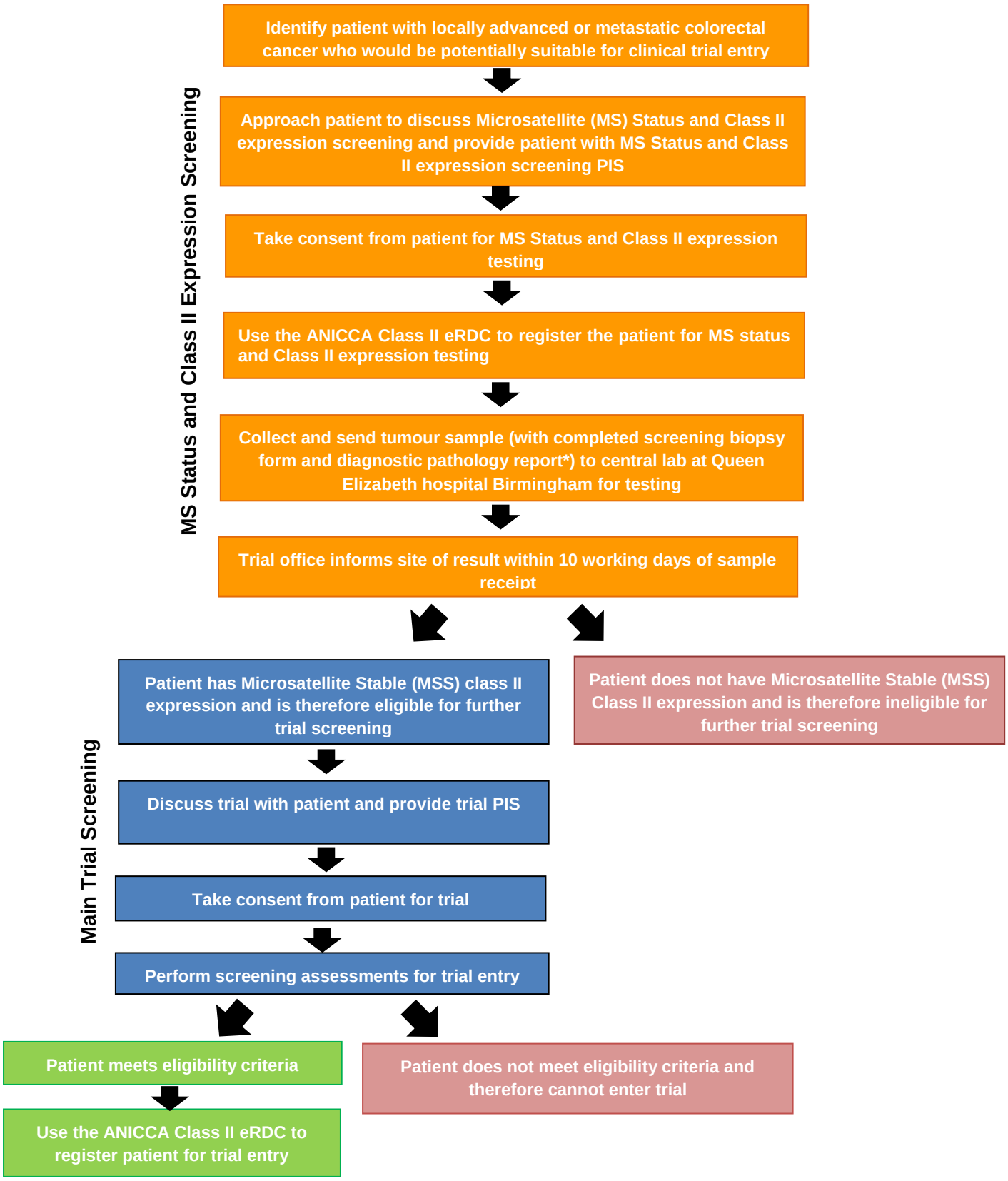


Figure 2 Screening Schema



* If MS status is determined locally central testing is not required. Please ensure the pathology report which is sent with the biopsy sample confirms patient is Microsatellite Stable (MSS) and details RAS mutation testing results.

SCHEDULE OF EVENTS

Table 1 Schedule of Events

	Microsatellite Status and Class II Expression Screening	Screening		TRIAL ENTRY	Treatment						Follow up	
		Within 28 days of registration	Within 7 days of registration		Day 1, cycle 1	Day 1, Every cycle (every 4 weeks) +/- 3 days	Every alternate cycle from cycle 3 onwards (approx. every 8 weeks) +/- 3 days	Every 9 weeks	Every 12 weeks	Treatment discontinuation visit (end of treatment)	Safety follow up visit (28 days post treatment discontinuation)[21]	Every 4 weeks for 6 months then every 12 weeks
Informed consent[1]	x	x										
Archival diagnostic tumour biopsy sample for Class II and MS testing[2]	x											
Demographics/Medical History/Prior Medications[3]		x										
Vital Signs[4]			x		x	x				x	x	
Physical Examination (including weight)			x		x	x				x	x	
ECOG performance status[5]			x		x	x				x	x	
Review Concomitant Medications[6]			x		x	x				x	x	
Review Adverse Events[7]			x		x	x				x	x	x
Full blood count (FBC)[8]			x		x	x				x	x	
Comprehensive serum chemistry panel[8]			x		x	x				x	x	
Urinalysis			x							x		
Coagulation parameters[9]		x								x		
HIV, Hepatitis B and C[10]		x										
Pregnancy Test –Serum β-HCG[11]			x									
Pregnancy Test – Urine[12]					x	x						
Renal function – GFR[13]			x								x	
Thyroid function and cortisol[14]			x			x				x	x	
Cytokine/Chemokine Panel and proteomics[15]					x		x			x		
Germline DNA[16]					x							
ctDNA[17]					x		x			x		
CT scan with contrast (chest, abdomen, pelvis)[18]		x						x (up to 45 weeks)	x (after 45 weeks)			x (see notes)
Nivolumab administration[19]					x	x						
Survival assessment[20]										x	x	x

Notes

1. Written informed consent must be obtained prior to testing patient's biopsy for MSS/Class II status and then further informed consent prior to any protocol specific procedure and within 28 days to starting treatment.
2. The minimum requirement for a biopsy sample must be five slides of stained sections from formaldehyde fixed paraffin embedded (FFPE) tissue, or preferably representative blocks of tumour tissue. Cytology samples will also be accepted if a formalin fixed clot is available. For patients who have already had their MS status determined as MSS ensure pathology report confirming this is sent with their tumour biopsy sample. RAS testing results need to be provided on the pathology report sent with the biopsy sample.
3. Includes smoking and alcohol history, any relevant significant medical conditions (other than colorectal cancer) and autoimmune conditions, history of treatment for the primary diagnosis, prior systemic treatment, radiation treatment and surgical treatment, including best response to prior treatments where applicable. Date of last prior cancer treatment must be documented and must not be within 4 weeks of registration.
4. Vital signs to include: temperature, pulse, respiratory rate and blood pressure (average of 3 BP readings). Do not need repeating on day 1 of cycle 1 if performed within 7 days of intended IMP administration date.
5. For ECOG performance status criteria see Appendix 1. Do not need repeating on day 1 of cycle 1 if performed within 7 days of intended IMP administration date.
6. Concomitant medications taken within four weeks prior to registration should be recorded at baseline. Do not need repeating on day 1 of cycle 1 if performed within 7 days of intended IMP administration date.
7. Adverse Events (AEs) and laboratory safety measurements will be graded per NCI Common Terminology Criteria for Adverse Events (CTCAE) version v4.03 (see Appendix 2). All adverse events, whether gradable by CTCAE or not, will also be evaluated for seriousness. In addition to AE review at each cycle, extra safety monitoring will be performed at monthly intervals up to 6 months after treatment discontinuation. During follow-up, AE review can be performed via telephone call. If at the 6 month follow up review there are any toxicities at grade 2 or higher that the investigator considers to be related to the trial treatment then these should be followed up until they have reduced to grade 1 or less. Do not need repeating on day 1 of cycle 1 if performed within 7 days of intended IMP administration date.
8. Routine laboratory tests (e.g., Full blood count (FBC); comprehensive serum chemistry panel; urinalysis) will be performed by the local trial site laboratory or their contract laboratory. FBC to include: white blood cells, haematocrit, haemoglobin, platelets, neutrophils, lymphocytes, red blood cells, monocytes, basophils, eosinophils, absolute neutrophil count and serum chemistry to include: sodium, potassium, urea nitrogen, creatinine, magnesium, calcium (total), bilirubin (total), direct bilirubin, albumin, alkaline phosphatase (ALP), aspartate transferase (AST), alanine transferase (ALT), glucose, lactate dehydrogenase (LDH), phosphate, total protein, C reactive protein (CRP), Carcinoembryonic antigen (*CEA*). Routine laboratory tests may be performed up to 3 days prior to treatment administration visit. Do not need repeating on day 1 of cycle 1 if performed within 7 days of intended IMP administration date.
9. Prothrombin (PT) / International Normalised Ratio (INR) and partial thromboplastin (aPTT) should be collected at screening and at Treatment Discontinuation Visit. Coagulation parameters should be determined throughout the trial when clinically indicated.
10. Testing will be performed by the local laboratory at screening. Include Hepatitis C Virus (HCV) RNA (qualitative), Hepatitis B surface Antigen (HBsAg), and Human Immunodeficiency Virus (HIV) 1/2 antibodies.
11. Women of a child bearing potential must have a negative serum pregnancy test prior to registration, this must be performed within 7 days of patient registration.

12. Women of a child bearing potential must have a negative serum or urine pregnancy test (Minimum sensitivity 25 IU/L or equivalent units of HCG) this should be performed before the first IMP administration and then at every cycle before IMP administration (approx.. 4 weeks) i.e before cycle 1, cycle 2, cycle 3 etc.
13. To be eligible for trial entry patients must have adequate renal function as defined by Creatinine clearance <1.5 x ULN and creatinine clearance >30 ml/min (calculated as per institutional standard).
14. Analysis of cortisol, T3, T4 and Thyroid Stimulating Hormone (TSH) will be performed by the local site laboratory. During treatment, testing will be performed at every cycle (approx. every 4 weeks), at Treatment Discontinuation Visit and at Safety Follow up Visit (28 days post treatment discontinuation).
15. Collected before start of infusion at cycle 1 followed by every alternate cycle from cycle 3 onwards e.g. cycle 3, 5, 7 etc. and at Treatment Discontinuation Visit. Processing and storage of blood samples are to be carried out as described in ANICCA-Class II Laboratory Manual.
16. Collected before start of infusion at cycle 1.
17. Collected before start of infusion at cycle 1, followed by every alternate cycle from cycle 3 onwards e.g. cycle 3, 5, 7 etc. and at Treatment Discontinuation Visit. Processing and storage of blood samples are to be carried out as described in ANICCA- Class II Laboratory Manual.
18. Tumour imaging by CT with contrast (chest, abdomen, pelvis) will be performed within 28 days prior to registration. The same imaging technique has to be used in a patient throughout the trial. Scans will be reported by RECIST 1.1 (Appendix 3). Tumour imaging will be performed every 9 weeks from treatment commencement up to 45 weeks and following this every 12 weeks until disease progression. Patients may initially show evidence of disease progression as a response to immunotherapy therefore patients may be allowed to continue treatment beyond RECIST defined disease progression if they are clinically stable and tolerating therapy. Trial treatment must then be discontinued upon further progression defined as the presence of any new lesion or an additional 10% increase in existing tumour burden from time of the initial progression .Following treatment discontinuation if patient has stopped trial treatment without progressive disease they should continue to be scanned according to this schedule until progressive disease is identified. NOTE. In this situation, it is important if treatment is discontinued earlier than 45 weeks from commencement of treatment, scans should continue to be performed every 9 weeks until this time point or disease progression. If disease progression has not been identified at 45 week scan then scans should continue to be performed every 12 weeks until disease progression or end of follow up. If patient discontinues without progressive disease after 45 weeks from commencement of treatment they should have scans every 12 weeks until disease progression. Response status will be assessed by the trial site.
19. First administration of nivolumab should be given within 7 days of registration.
20. Follow up will be completed every 4 weekly for 6 months then 12 weekly thereafter to obtain further treatment details and overall survival status.
21. For patients who begin another cancer therapy within 28 days after discontinuation of study therapy, the Safety Follow-Up Visit should occur prior to the patient receiving another cancer therapy.

ABBREVIATIONS

AE	Adverse Event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
AR	Adverse Reaction
AST	Aspartate transaminase
CD4+	Cluster of differentiation 4
CD8+	Cluster of differentiation 8
CIITA	Class II major histocompatibility complex transactivator
CIRC	Co-ordinated Immune Response Cluster
CR	Complete Response
CRCTU	Cancer Research UK Clinical Trials Unit
CRC	Colorectal cancer
CRF	Case Report Form
ctDNA	Circulating Tumour DNA
CT	Computerised Tomography
CTCAE	NCI Common Terminology Criteria for Adverse Events
CTLA4	Cytotoxic T-Lymphocyte Associated Protein 4
CTLs	Cytotoxic T Lymphocytes
DCB	Durable Clinical Benefit
DILI	Drug Induced Liver Injury
DLT	Dose Limiting Toxicity
DNA	Deoxyribonucleic Acid
ECOG	Eastern Cooperative Oncology Group
EORTC	European Organisation for Research and Treatment of Cancer
eRDC	Electronic Remote Data Capture
FBC	Full Blood Count
FFPE	Formalin fixed paraffin embedded
FSH	Follicle-Stimulating Hormone
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GP	General Practitioner
Hb	Haemoglobin
HBsAg	Hepatitis B antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HRA	Health Research Authority
HuMAb	Human Monoclonal Antibody
IB	Investigator Brochure
IFN-γ	Interferon gamma
IgG4	Immunoglobulin G4
IHC	Immunohistochemistry
IMP	Investigational Medicinal Product
INR	International Normalised Ratio
irAE	Immune-related Adverse Event
ISF	Investigator Site File
IV	Intravenous
KCO	Transfer coefficient measure
LMWH	Low Molecular Weight Heparin
LVEF	Left Ventricular Ejection Fraction
MDT	Multi-Disciplinary Team
MHC	Major histocompatibility complex

MHRA	Medicines and Healthcare products Regulatory Agency
MSI	Microsatellite instability
MS	Microsatellite
MSS	Microsatellite stable
MTD	Maximum Tolerated Dose
NSCLC	Non-Small Cell Lung Cancer
NHS	National Health Service
NYHA	New York Heart Association
OR	Objective Response
ORR	Objective Response Rate
OTC	Over The Counter
PD	Progressive Disease
PD-1	Programmed cell Death protein 1
PDL-1	Programmed Death Ligand 1
PFS	Progression Free Survival
PO	By mouth
PPK	Population Pharmacokinetics
PR	Partial Response
PS	Performance Status
PT	Prothrombin
QTc	Corrected QT interval
REC	Research Ethics Committee
RECIST	Response Evaluation Criteria in Solid Tumours
RNA	Ribonucleic acid
RCC	Renal Cell Cancer
RT	Radiotherapy
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SD	Stable Disease
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
T3	Total Triiodothyronine
T4	Free Thyroxine
TBX21	T-box transcription factor 21
TH1	T Helper 1 cells
TMG	Trial Management Group
TNO	Trial Number
TSH	Thyroid Stimulating Hormone
ULN	Upper Limit of Normal

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1 BACKGROUND AND RATIONALE

1.1 BACKGROUND

Immuno-oncology is transforming the care of certain patients with cancer. Not all patients respond to these therapies however, and in some common cancers checkpoint blockade has failed to make any real impact. In 2014 there were over 41,000 new cases of colorectal cancer (CRC) in the UK and nearly 16,000 deaths from the disease, making it the second commonest cause of cancer death (Cancer Research UK Cancer Statistics Key Facts). 15% of patients with CRC develop it as a result of deficient mismatch repair (microsatellite instability – MSI): this cohort of patients respond well to PD-1/PD-L1 blockade as these tumours harbour a very high number of mutations thus increasing the likelihood of the presence of immunogenic neo-epitopes which elicit an immune response[1]. The majority of CRC patients, particularly those with metastatic disease (around 95%), do not display this hyper-mutator phenotype (microsatellite stable (MSS) CRC) and in these patients the results of PD-1/PD-L1 blockade have been disappointing[1].

It is now recognised that not all MSS CRC are immunologically impoverished. We recently described a 28-gene T helper cell 1 (Th1)-centric immune metagene, the co-ordinated immune response cluster (CIRC) [2], which readily distinguished MSI CRC (with high CIRC expression) from MSS cancers (with significantly lower CIRC expression). However, around 10-15% of MSS patients have CIRC expression similar to MSI-high patients, thus suggesting the existence of a group of MSI-like MSS CRC patients and this was not a group with an unusually high mutational burden.

The CIRC is highly significantly correlated with key discrete immune cell subset signatures, in particular the Th1 cell and cytotoxic cell signatures[3], in spite of little overlap in the gene sets[4], underlying its validity as a measure of the strength of microenvironmental immunity. We have also shown that TH1 CIRC highly correlates with the immunoscorecomputerised data. Th1 cells recognise peptides expressed on cancer cells in association with MHC class II molecules. Th1 cells maintain CD8+ T cell number, the function of antigen-specific cytotoxic T lymphocytes (CTLs) and enhance intra-tumoral CTL trafficking[5-7]. They are major mediators of immunological tumour cell death[8,9] and adoptive transfer approaches involving autologous CD4+ T cells have resulted in durable responses[10,11]. Class II expression is prognostic in CRC and associated with the presence/absence of metastases[12]. Importantly, the majority of immunogenic mutated neo-epitopes are class II restricted and recognised by CD4+ cells[13].

The CIRC contains 9 separate class II genes. We asked whether high class II expression might be a surrogate for high CIRC expression which characterises the MSI-like MSS samples by analysing the relationship between CIRC expression and mean HLA DR/DP/DQ expression (Figure 3). As expected, mean expression of these HLA genes is highly correlated with the CIRC (Pearson = 0.856) and revealed that a high proportion of the top 10% and top 15% of MSS high CIRC-expressing patients are in the top 10 or 15% of class II gene expression. Thus high class II expression correlates very closely with CIRC expression.

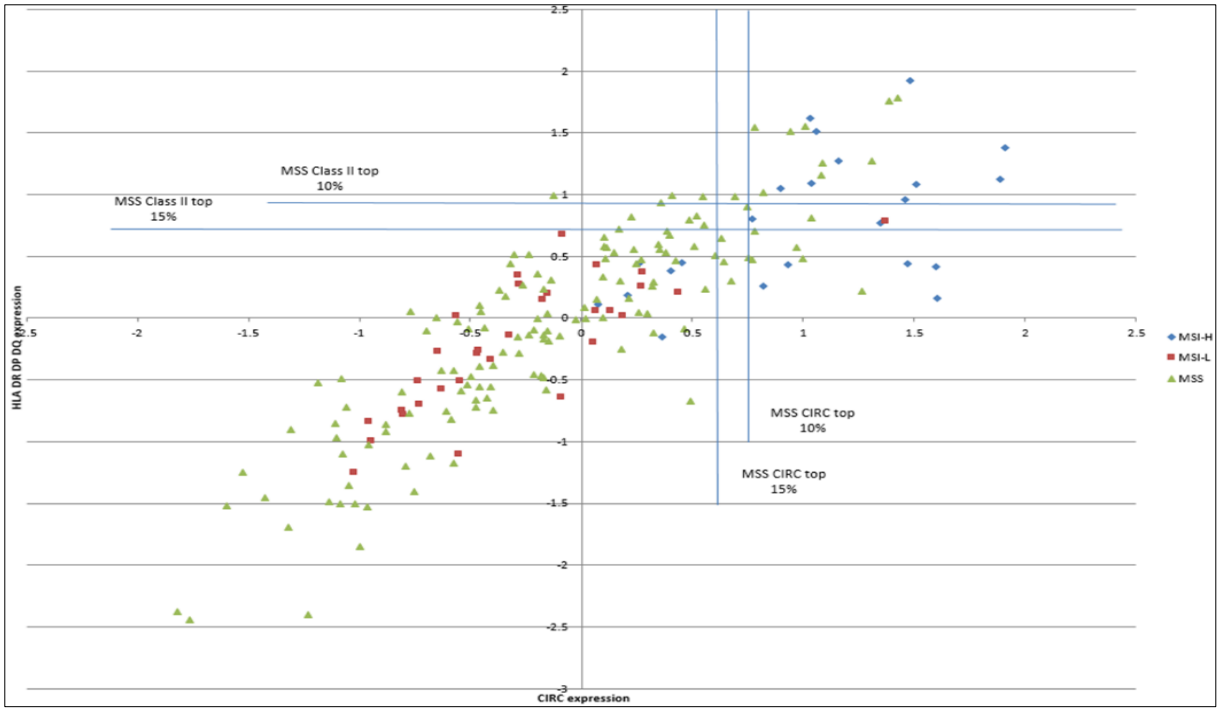

Figure 3 Relationship between CIRC expression and mean HLA DR/DP/DQ expression in the TCGA

The pivotal role of class II expression in driving microenvironmental immunological reactivity was demonstrated in studies where the master class II transcriptional activator (CIITA) was transfected into poorly immunogenic class II negative cancer cell lines (but which expressed class I)[14,15]. Unlike parental cells, most CIITA transfectants failed to grow. Immunological memory was established in animals receiving CIITA transfectants; parental cell re-challenge was rejected. CIITA transfected cancer cells developed robust antigen processing function and tumours formed by CIITA transfected cells were massively infiltrated by both CD4+ and CD8+ T cells. In melanoma tumour membrane class II negative patients had lower response rate, progression free survival (PFS) and overall survival (OS) when treated with PD-1/PD-L1 blockade than Class II positive patients: Class I expression and T cell density were not significantly predictive[16]. Furthermore, there is in vitro data that PD-L1 blockade enhances Th1-mediated cytotoxicity only against cancer cells that express high levels of class II expression[17]. Recently, class II expression on Hodgkin Reed-Sternberg cells, has emerged as a key predictive biomarker of response to checkpoint blockade in Hodgkin's disease[18].

It was originally planned that the criteria for nivolumab treatment in the trial would be >50% cancer cell staining for class II, as these MSS cancers immune-copied MSI cancers.

With positivity defined as >50% cancer cell staining, a 10% positivity rate estimate was obtained from a large series of primary resections. Earlier data, again showing a strong class II positive rate (i.e. >50% cancer cell positivity) of around 10% in primary cancers, explored the prognostic impact of class II staining. In a Cox proportional model which included stage and microsatellite status the HR for strong DR expression versus none was 0.42 and 5 year survival in MSS patients with high class II expression was around 80%[19]. Thus, it is likely that the proportion of strong class II positive patients presenting with metastatic disease will be less than 10% simply due their good prognosis. This would be analogous to MSI cancers where these account for around 15% primary cases but only 3-4% metastatic cases because of their much better prognosis.

In the most comprehensive analysis of the impact of MHC expression on outcome with checkpoint blockade in melanoma[20], the expression of MHC class II was generally low with

around 30% of samples in 2 clinical trial datasets expressing >1% melanoma cell class II staining. This staining was concentrated at the invasive inflammatory margin consistent with local class II induction by IFNγ produced by Th1 cells, the chief producers of IFNγ along with NK cells, and the expression of an IFNγ signatures was positively correlated with class II expression and the level of tumour-infiltrating T cells. Whereas class I levels had no predictive impact on outcome with single agent nivolumab (but was predictive for outcome with ipilimumab), >1% class II expression predicted for greater degrees of disease control: using the 1% threshold only 13% with high expression had PD as BOR compared with 46% with low expression. Class II expression using this threshold was not predictive of outcome with CTLA4 blockade or dual nivolumab/ipilimumab, an important finding when considering the use of combination therapy in patients selected for checkpoint blockade therapy on the basis of class II expression: indeed, recent data suggests that the MHC-II+ phenotype drives immune escape via TIL up-regulation of LAG3[21]. >1% expression was associated with better survival in those treated with nivolumab (HR=0.11, CI 0.02-0.83)[20]. Finally, as expected a number of IFNγ signatures were also associated with improved outcome with nivolumab but not with ipilimumab. Thus baseline levels of IFNγ and of class II induced on tumour cells by IFNγ predicted for significantly improved outcome with nivolumab and a threshold of >1% was sufficient to dichotomise patients by class II expression. Efficacy of anti-PD1 therapy was dependant on low level pre-existing IFNγ driven inflammation related to the recognition of neoantigen presented on cancer cells in association with class II by CD4+ T cells. Checkpoint blockade enables these CD4+ cells to produce more IFNγ and institute an amplification loop through the up-regulation of more class II (thus enhancing further antigen recognition and T cell activation and potentially naïve T cell priming[22] and CXCL9/10 induction on tumour cells to facilitate T cell trafficking. Finally, the first study demonstrating the predictive power of class II expression in determining outcome with anti-PD1 therapy also used a low class II threshold (5%). The institution of a positive amplification loop will only be possible if some of the cancer cells can be induced to express class II by IFNγ, have a collection of microenvironmentally annotated organoids and even in the tumours with totally absent class II on whole sections the minimum inducible population is 25% and can be greater than 50% and in those with low (2-3%) staining the majority of the cells can be induced (unpublished data).

Thus, tumours stain with any degree of class II cancer cell positivity be considered for nivolumab therapy. It is important to note that the data we have generated on class II positivity rates in metastatic patients is itself an important outcome: it is now little surprise that the response rate in MSS CRC is so low, given that only approximately 10% of the cancers in the metastatic setting have class II staining indicative of a total lack of IFNγ the cancers which as mentioned can be induced to express class II by IFNγ.

1.2 TRIAL RATIONALE

1.2.1 RATIONALE FOR DESIGN

As a proof of concept trial, a single arm phase II design is adopted. MS status and Class II expression screening will be undertaken for 360 patients with the aim of identifying 36 patients with class II MSS CRC, assuming class II MSS rate of 10% in the population. The trial will use a Bayesian design to determine clinically relevant signal of effect in this population.

1.2.2 RATIONALE FOR PATIENT POPULATION

The majority of patients with locally advanced or metastatic CRC have tumours which exhibit microsatellite stability– MSS CRC and in these patients the results of PD-1/PD-L1 blockade have been disappointing. A proportion of these patients with class II expression demonstrate similarities to MSI CRC in terms of immunologically favourable microenvironment. These patients may receive more clinical benefit from PD-1 blockade than those with lower or

negative class II expression. Clinical trials in melanoma support this hypothesis where tumour membrane class II negative patients had lower response rate, PFS and OS when treated with PD-1/PD-L1 blockade than Class II positive patients.

1.2.3 CHOICE OF TREATMENT

Nivolumab is a human immunoglobulin G4 (IgG4) monoclonal antibody (HuMAb), which binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2. The PD-1 receptor is a negative regulator of T-cell activity that has been shown to be involved in the control of T-cell immune responses. Engagement of PD-1 with the ligands PD-L1 and PD-L2, which are expressed on antigen presenting cells and may be expressed by tumours or other cells in the tumour microenvironment, results in inhibition of T-cell proliferation and cytokine secretion. Nivolumab potentiates T-cell responses, including anti-tumour responses, through blockade of PD-1 binding to PD-L1 and PD-L2 ligands. In the pooled dataset of nivolumab 3 mg/kg as monotherapy across tumour types (n = 2578) with minimum follow-up ranging from 2.3 to 28 months, the most frequent adverse reactions (≥ 10%) were fatigue (30%), rash (17%), pruritus (13%), diarrhoea (13%), and nausea (12%). The majority of adverse reactions were mild to moderate (Grade 1 or 2).

The Checkmate 142 trial investigated nivolumab treatment in patients with MSI CRC that were intolerant of or progressed on one or more lines of previous therapy. The trial showed good responses in heavily pre-treated patients. PFS at 12 months was 48% with an overall response rate of 31%. Grade 3-4 treatment related adverse events occurred in 20% of patients, making it a well-tolerated treatment[23]. Nivolumab has now been licenced by the FDA for use in MSI-CRC.

1.2.4 RATIONALE FOR DOSE

Based on modelling of dose/exposure efficacy and safety relationships, there are no clinically significant differences in efficacy and safety between a nivolumab dose of 240 mg every 2 weeks or 3 mg/kg every 2 weeks. Additionally, based on these relationships, there were no clinically significant differences between a nivolumab dose of 480 mg every 4 weeks or 3 mg/kg every 2 weeks in adjuvant treatment of melanoma, advanced melanoma and advanced RCC[24]. The treatment schedule for the ANICCA Class II trial was previously 240mg every 2 weeks however as there is no clinically significant differences in efficacy and safety between a nivolumab dose of 240 mg every 2 weeks and a 480mg dose every 4 weeks, a decision was made to change the dosing of nivolumab to 480mg every 4 weeks. Treatment every 4 weeks is less demanding on patients. Following consultation with participating sites and Bristol Myers Squibb the nivolumab treatment schedule for with trial will now be 480 mg every 4 weeks.

1.2.5 RATIONALE FOR INFUSION TIME

With reference to the nivolumab Summary of Product Characteristics the recommended dose for Nivolumab is 240mg every 2 weeks over 30 minutes or 480mg every 4 weeks over 60 minutes. It has been decided that the does should be 480mg over 60 minutes every 4 weeks. This would reduce the number of visits patients would have to attend clincis thorught out the trial which would ease the burden on them in the treatment of Melanoma and Renal Cell Carcinoma

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2 AIMS, OBJECTIVES AND OUTCOME MEASURES

2.1 OBJECTIVES

2.1.1 PRIMARY OBJECTIVE

Detect the rate of durable clinical benefit in patients with class II expressing MSS CRC treated with single agent nivolumab, to justify further investigation in subsequent studies.

2.1.2 SECONDARY OBJECTIVES

Evaluate the benefit of single agent nivolumab to patients with class II expressing MSS CRC in terms of other clinical outcomes relating to tumour response and progression-free and overall survival time.

2.1.3 EXPLORATORY OBJECTIVE

To discover possible biomarkers for the prediction of a response to treatment with nivolumab.

To investigate whether CD8+ and PD-1 T cells are supplementary biomarkers of response to treatment with nivolumab.

2.2 OUTCOME MEASURES

2.2.1 PRIMARY OUTCOME MEASURE

- Durable clinical benefit

2.2.2 SECONDARY OUTCOME MEASURE

- Objective response
- Best percentage change in sum of target lesion diameters (PCSD)
- Time to maximal response
- Progression-free survival time
- Overall survival time

3 TRIAL DESIGN

This is an open label, multi-centre, single arm phase II trial. Recruitment will take place over an 18 month period in which 36 MSS CRC patients with Class II expression will be registered to receive 4 weekly nivolumab infusions for up to two years, until progressive disease is identified or unacceptable toxicities occur. The trial will continue until all patients have been followed up for a minimum of 18 months after registration.

4 ELIGIBILITY

4.1 INCLUSION CRITERIA

- Histologically confirmed locally advanced or metastatic MSS CRC with class II expression (greater than 1% cancer cell positivity for class II expression on immunohistochemistry).
- Eastern Cooperative Oncology Group (ECOG) performance status 0-2 (APPENDIX 1)
- Age ≥ 18 years
- Patients must have completed all standard of care therapy that the treating oncologist deems appropriate. Trial treatment as first line therapy is permitted if the patient has declined standard of care therapy.
- CT scan of chest, abdomen, pelvis within 28 days of registration demonstrating uni-dimensionally measurable disease as per RECIST version 1.1 (APPENDIX 3).
- Demonstrate adequate haematological function:
  - Platelet count ≥100 x 10^9 /L
  - Neutrophils ≥1.5 x 10^9/L
  - Haemoglobin ≥ 90 g/L
- Demonstrate adequate hepatic function:
  - Serum bilirubin ≤1.5 x upper limit of normal (ULN)
  - Serum AST or ALT ≤2.5 x ULN or <5 x ULN in the presence of liver metastases
- Demonstrate adequate renal function
  - Creatinine clearance <1.5 times ULN and >30ml/min (as per institutional standard).
- Provision of signed and dated, written informed consent prior to any trial specific procedures, sampling and analyses.
- Negative pregnancy test (female patients of reproductive potential). (Serum Test must be negative)
- Patients must agree to the use of contraception as detailed in section 7.8

4.2 EXCLUSION CRITERIA

- Previous treatment with PD1/PDL1 inhibitors.
- Untreated symptomatic brain or leptomeningeal metastatic disease.
- Medical or psychiatric conditions compromising informed consent.
- Any medical condition which, in the opinion of the Investigator, would compromise the ability of the patient to participate in the trial or which would jeopardise compliance with the protocol.
- Administration of chemotherapy, radioactive or biological cancer therapy within 4 weeks prior to the first dose of trial therapy Patient has not recovered to CTCAE grade 1 or better from the Adverse Event (AE) due to cancer therapeutics administered more than 4 weeks earlier.
- Active autoimmune disease that has required systemic treatment in past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g. thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
- Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment. Replacement therapy (e.g. thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

- Patient has risk factors for bowel obstruction or bowel perforation (examples include but not limited to a history of acute diverticulitis, intra-abdominal abscess and abdominal carcinomatosis).
- Patient has a known history of other malignancy, unless the patient has undergone potentially curative therapy with no evidence of that disease for 3 years.
- Has a history of non-infectious pneumonitis requiring steroids or has active pneumonitis.
- Female patients that are either pregnant or breast feeding.
- Male and female patients (of childbearing age) not willing to use adequate contraception.
- Patient previously had a severe hypersensitivity reaction to treatment with another monoclonal antibody.
- Patient is positive for Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies), active Hepatitis B (HBsAg reactive) or Hepatitis C (HCV RNA (qualitative) is detected); patients with negative Hepatitis C antibody testing may not need RNA testing.
- Known history of tuberculosis.
- Patient has an active infection requiring therapy.
- Has received a live vaccine within 30 days prior to the first dose of trial treatment.
- Patient is, at the time of signing informed consent, a regular user (including “recreational use”) of any illicit drugs or had a recent history (within the last year) of substance abuse (including alcohol).

5 INFORMED CONSENT AND SCREENING

Patients attending the oncology clinic with histologically confirmed locally advanced or metastatic CRC and who in the Investigator's opinion would be potentially suitable for trial entry will be approached to obtain consent for screening; this will be conducted as a two-stage process. Please refer to Figure 2 for an overview of the screening process for the trial. Full details are provided below.

5.1 INFORMED CONSENT

It is the responsibility of the Investigator to obtain written informed consent for each patient prior to performing any trial related procedure. Patient Information Sheets (PIS) are provided to facilitate this process. Investigators must ensure that they adequately explain to the patient the aim, screening process, trial treatment, assessment schedule, anticipated benefits and potential hazards of taking part in the trial. The Investigator should also stress that the patient is completely free to refuse to take part or withdraw from the trial at any time without their medical care being affected. The patient should be given ample time (e.g. 24 hours) to read the Patient Information Sheet and to discuss their participation with others outside of the site research team. The patient must be given an opportunity to ask questions which should be answered to their satisfaction. The right of the patient to refuse to participate in the trial without giving a reason must be respected.

If the patient expresses an interest in participating in the trial they should be asked to sign and date the latest version of the applicable Informed Consent Forms (ICF). The Investigator must then sign and date the form at the same time. A copy of Informed Consent Forms should be given to the patient, a copy should be filed in the hospital notes, and the original placed in the Investigator Site File (ISF). Once the patient is entered into screening, the patient's trial number should be entered on Informed Consent Forms maintained in the ISF. In addition, if the patient has given explicit consent a copy of the signed Informed Consent Forms must be sent in the post to the ANICCA-Class II Trial Office for review.

Details of the informed consent discussions should be recorded in the patient's medical notes. This should include date of, and information regarding, the initial discussion, the date consent was given, the name of the trial and the version number and date of the Patient Information Sheet and Informed Consent Form that was provided. Throughout the trial, the patient should have the opportunity to ask questions about the trial and any new information that may be relevant to the patient's continued participation should be shared with them in a timely manner. On occasions it may be necessary to re-consent the patient in which case the process above should be followed and the patient's right to withdraw from the trial respected.

There are two types of PIS and ICF for the ANICCA-Class II Trial:

1. Microsatellite (MS) Status and Class II Expression Screening PIS and ICF. This provides information regarding the testing of histological specimens for MS status (if not established locally) and Class II expression.
2. Main Trial PIS and ICF

Electronic and paper copies of the PIS and ICF are available from the ANICCA-Class II Trial Office and should be printed or photocopied onto the headed paper of the local institution.

5.2 SCREENING

5.2.1 STAGE 1: MS STATUS AND CLASS II EXPRESSION SCREENING

The patient should be provided with the MS status and Class II Expression Screening PIS at a clinic visit and, if willing to participate in stage 1 of the screening process, informed consent should be gained using MS status and Class II Expression ICF.

Patients are registered for screening with the Cancer Research UK Clinical Trials Unit (CRCTU) at the University of Birmingham. Informed consent must be obtained prior to registration as described in section 5.1. Screening registration should be performed by sites using the ANICCA online Remote Data Capture (eRDC) system which has been developed by CRCTU.

https://www.cancertrials.bham.ac.uk/ANICCAClassIILive

At screening registration the patient will be given a unique Trial Number (TNO). The TNO will be used to identify the patient throughout the whole trial and should be recorded on any correspondence with the ANICCA-Class II Trial Office. The TNO should be documented on the original signed Informed Consent Form filed in the ISF. Additionally a copy should be sent to the ANICCA-Class II Trial Office for review. A copy of the screening registration report should be printed and filed in the ISF and in the patient's medical records.

In case of any problems with online screening registration, a paper Screening Registration Form should be completed. These details can be phoned through to the ANICCA-Class II Trial Office using the numbers below:

In case of any problems with online registration, details can be phoned through on:

☎ 0121 414 6754

09:00 to 17:00 GMT, Monday to Friday

The patient's archival tumour biopsy should be obtained, packaged and sent for central pathology testing as per the laboratory manual. The minimum requirement for a biopsy sample must be five slides of stained sections from formaldehyde fixed paraffin embedded (FFPE) tissue, or preferably representative blocks of tumour tissue. Cytology samples will also be accepted if a formalin fixed clot is available. If there is not enough tumour sample available to confirm class II expression, patients may need to have an additional biopsy. Samples should be sent to The Queen Elizabeth Hospital Birmingham (University Hospitals Birmingham NHS Foundation Trust), for central immunohistochemical testing at a nationally recognised Molecular Pathology Diagnostic Service for determination of MS status (if not established locally) and Class II status. A screening biopsy form should be completed and enclosed with the sample in addition to a copy of the patient's pathology report confirming CRC diagnosis, RAS Mutation test results and MSS status (if applicable).

The samples will be assessed by the central pathology team for MSS status (if applicable) and class II overexpression also referred to as class II expression. This is defined as more than 1% of tumour cells showing membranous staining. Each sample should have at least 50 viable neoplastic cells overall to be assessed. If the result obtained is equivocal, the sample analysis will be repeated and reviewed by another member of the central pathology team. The ANICCA-

Class II Trial Office will inform sites of patient's MSS (if applicable) and Class II status within 10 working days of sample receipt. MSS and class II expression must be confirmed for the patient to be eligible for the trial.

5.2.2 STAGE 2: TRIAL SCREENING

Once MSS with Class II expression is confirmed by the ANICCA-Class II Trial Office, the patient should then attend a clinic visit to be given the PIS for the trial and if the patient wishes to participate obtain informed consent using the trial ICF.

Trial treatment should commence within 28 days of consent, any deviation from this should be discussed with the ANICCA-Class II Trial Office in advance.

If the patient consents, the following screening tests should be undertaken locally up to 28 days prior to trial entry:

- Full medical history - To include smoking and alcohol history, any relevant significant medical conditions (other than colorectal cancer) and autoimmune conditions, history of treatment for the primary diagnosis, including prior systemic treatment, radiation treatment and surgical treatment including best response to prior treatments where applicable. Date of last prior cancer treatment must be documented. Radiographic studies performed prior to trial entry may also be requested for review.
- Coagulation parameters: Prothrombin (PT) / International Normalised Ratio (INR) and partial thromboplastin (aPTT)
- CT scan with contrast – chest, abdomen and pelvis within 28 days of planned treatment date (RECIST v1.1 reporting – Appendix 3).
- Hepatitis C Virus (HCV) RNA (qualitative), Hepatitis B Surface Antigen and Human Immunodeficiency Virus (HIV) 1 / 2 antibodies.

The following screening tests should be undertaken locally up to 7 days prior to trial entry:

- Physical examination including weight
- Vital signs - temperature, pulse, respiratory rate and blood pressure (average of 3 BP readings)
- ECOG performance status (see APPENDIX 1)
- Adverse events - Adverse Events (AEs) and laboratory safety measurements will be graded per NCI Common Terminology Criteria for Adverse Events (CTCAE) version v4.03 (see APPENDIX 2). All adverse events, whether gradable by CTCAE or not, will also be evaluated for seriousness
- Concomitant medications – taken within four weeks prior to registration.
- Full blood count (FBC) to include: white blood cells, haematocrit, haemoglobin, platelets, neutrophils, lymphocytes, red blood cells, monocytes, basophils, eosinophils, absolute neutrophil count
- Urinalysis
- Pregnancy test if applicable (serum β-HCG)
- Comprehensive serum chemistry panel to include: sodium, potassium, urea, nitrogen, creatinine, magnesium, calcium (total), bilirubin (total), direct bilirubin, albumin, alkaline phosphatase (ALP), aspartate transferase (AST), alanine transferase (ALT), glucose, lactate dehydrogenase (LDH), phosphate, total protein, C reactive protein (CRP), Carcinoembryonic antigen (CEA)
- Renal function - Patients must have adequate renal function as defined by Creatinine clearance <1.5 x ULN concurrent with creatinine clearance >50 ml/min (calculated as per institutional standard)

- Thyroid function and cortisol - analysis of cortisol, T3, T4 and Thyroid Stimulating Hormone (TSH)

5.2.3 SCREENING LOG

Investigators must keep a screening log of all patients who receive a Patient Information Sheet, all patients who consent to have tumour tissue sent for MS Status and Class II testing, and all patients that consent to the ANICCA-Class II trial. The following details of all patients screened for participation in this trial should be provided on the screening log:

- TNO (if registered for screening)
- Date screened
- Date of Birth (Month and year only)
- Sex
- Whether the patient gave written informed consent
- Whether the person was entered
- Reasons(s) patient was not entered

6 TRIAL ENTRY

6.1 PATIENT REGISTRATION

Once the investigator has confirmed the patient's eligibility, the patient should be entered onto the trial using the eRDC system as described in section 5.2.1.

During the trial entry process the patient's TNO and confirmation of their eligibility will need to be provided. Following successful trial entry, a copy of the trial entry report should be printed and filed in the ISF and in the patient's medical records. The TNO should be documented on the original signed Informed Consent Form filed in the ISF. A copy should also be sent to the ANICCA-Class II Trial Office for review. Additionally the eligibility form should be sent to the ANICCA-Class II Trial Office for review.

Once a patient has been entered into the trial their name should be added to the Patient Identification Log. With the patient's prior consent their General Practitioner (GP) should be informed that they are taking part in the trial. A GP Letter is provided for this purpose. Electronic and paper copies of the GP letter are available from the ANICCA-Class II Trial Office and should be printed or photocopied onto the headed paper of the local institution. A copy of the letter sent to the GP should be filed in the patient's medical notes.

7 TREATMENT DETAILS

7.1 TRIAL TREATMENT

7.1.1 DESCRIPTION OF INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

Nivolumab will be considered an IMP for the purposes of this trial.

Full details of the IMP are contained in the ANICCA-Class II Pharmacy Manual, which also lists the Pharmacist's' responsibilities, details of labelling, record keeping for prescribing, dispensing, and accountability of the IMP. The ANICCA-Class II Pharmacy Manual will be sent to the responsible Pharmacist.

7.1.2 INVESTIGATIONAL MEDICINAL PRODUCT SUPPLY AND LABELLING

Nivolumab will be provided as vials containing 10 mg/mL concentrate of drug substance in solution for infusion.

Nivolumab must be kept in a secure place under appropriate storage conditions (refer to pharmacy manual for full details).

7.1.3 NIVOLUMAB ADMINISTRATION

Nivolumab will be administered as a 60 minute IV infusion, with a window of -5 and +10 minutes, at a flat dose of 480mg. A dosing interval every 4 weeks (Q4W) will be employed. See the ANICCA-Class II Pharmacy Manual for further information about nivolumab.

Table 2 Dosing instructions for nivolumab

Drug	Dose/ Potency	Dose Frequency	Route of Administration	Regimen/ Treatment Period	Use
Nivolumab	480mg	Q4W	60 minute IV infusion	Until progressive disease, unacceptable toxicity, withdrawal of consent to treatment or two years of treatment	Experimental

7.1.4 DRUG ACCOUNTABILITY

The investigator must maintain adequate records documenting the use, loss, or other disposition, of the IMP for trial use. The ANICCA-Class II Trial Office will supply a drug accountability form which must be used for the IMP, or you may request to use your standard dispensing forms which should be approved prior to use by contacting the ANICCA-Class II ANICCA-Class II Trial Office. In either case, the forms must identify the IMP, including batch or code numbers and expiry dates, and account for its allocation on a patient-by-patient basis, including specific dates and quantities. The forms must be signed by the individual who dispenses the drug, and copies must be provided to the ANICCA-Class II Trial Office on request. The prescribed dose must also be recorded in the patient's medical records.

7.2 TREATMENT SCHEDULE

Patients should start nivolumab treatment within 7 days of registration. Patients will be administered nivolumab for a maximum of two years, or until disease progression, unacceptable toxicity or withdrawal of consent to treatment; each cycle being 28 days. Trial

treatment should be administered on day 1 of each cycle after all procedures/assessments have been completed as detailed in the schedule of events. Following the first cycle, trial treatment may be administered up to 3 days before or after the scheduled day 1 of each cycle due to administrative reasons. Trial treatment will be administered on an outpatient basis. At the first treatment visit the patient should be given a patient identification card and advised to carry it with them at all times while participating in the trial.

At the end of the treatment period, the IMP manufacturer Bristol-Myers Squibb Pharmaceuticals Ltd. will not continue to supply nivolumab to patients unless the Sponsor-Investigator chooses to extend their trial. The investigator is responsible in ensuring that the patient receives appropriate standard of care or other appropriate treatment in the independent medical judgement of the Investigator to treat the condition under study.

7.3 ASSESSMENTS

7.3.1 ON-TREATMENT

The following assessments should be performed on day 1 (or within 3 days prior) of each 28 day cycle:

If below assessments were performed within 7 days of IMP administration they do not need repeating on day 1 of cycle 1.

Day 1 of each 28 day cycle can be +/-3 days.

- Vital signs - temperature, pulse, respiratory rate and blood pressure (average of 3 BP readings).
- Physical Examination including weight
- ECOG performance status (see APPENDIX 1)
- Review AEs and Concomitant Medications - AEs and laboratory safety measurements will be graded per NCI Common Terminology Criteria for Adverse Events (CTCAE) version v4.03, (see APPENDIX 2). All AEs, whether gradable by CTCAE or not, will also be evaluated for seriousness
- pregnancy test (urine or serum β-HCG) within 24 hours of IMP administration.
- FBC to include: white blood cells, haematocrit, haemoglobin, platelets, neutrophils, lymphocytes, red blood cells, monocytes, basophils, eosinophils, absolute neutrophil count
- Comprehensive serum chemistry panel to include: sodium, potassium, urea, nitrogen, creatinine, magnesium, calcium (total), bilirubin (total), direct bilirubin, albumin, ALP, AST or ALT, glucose, LDH, phosphate, total protein, CRP, CEA
- Thyroid function and cortisol - analysis of cortisol, T3, T4 and TSH

In addition to assessments undertaken every 28 days, as detailed above, the following assessments should also be performed:

Every 9 weeks up to 45 weeks then every 12 weeks (within 3 days prior):

- CT Tumour imaging with contrast (chest, abdomen, pelvis) - scans will be reported by RECIST 1.1 (APPENDIX 3). The same imaging technique has to be used in a patient throughout the trial. Tumour imaging will be performed every 9 weeks from trial entry up to 45 weeks followed by every 12 weeks until disease progression. Response status will be assessed by the trial site. Patients may initially show evidence of disease progression as a response to immunotherapy therefore patients may be allowed to continue treatment beyond RECIST defined disease progression if they are clinically stable and tolerating therapy. Trial treatment must then be discontinued upon further

progression defined as the presence of any new lesion or an additional 10% increase in existing tumour burden from time of the initial progression.

7.3.2 TREATMENT DISCONTINUATION VISIT (END OF TREATMENT)

The following assessments should be performed on the day the patient is discontinued from trial treatment:

- Vital signs - temperature, pulse, respiratory rate and blood pressure (average of 3 BP readings)
- Physical Examination including weight
- ECOG performance status (see Appendix 1)
- FBC to include: white blood cells, haematocrit, haemoglobin, platelets, neutrophils, lymphocytes, red blood cells, monocytes, basophils, eosinophils, absolute neutrophil count
- Comprehensive serum chemistry panel to include: sodium, potassium, urea, nitrogen, creatinine, magnesium, calcium (total), bilirubin (total), direct bilirubin, albumin, ALP, AST or ALT, glucose, LDH, phosphate, total protein, CRP, CEA
- Urinalysis
- Coagulation parameters: Prothrombin (PT) / International Normalised Ratio (INR) and partial thromboplastin (aPTT)
- Thyroid function and cortisol - analysis of cortisol, T3, T4 and TSH
- Review Adverse Events and Concomitant Medications - Adverse Events (AEs) and laboratory safety measurements will be graded per NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03, (see APPENDIX 2). All adverse events, whether gradable by CTCAE or not, will also be evaluated for seriousness

7.3.3 SAFETY FOLLOW UP VISIT

The following assessments should be performed 28 days post discontinuation of trial treatment:

PLEASE NOTE: For patients who begin another cancer therapy before 28 days after discontinuation of study therapy, the Safety Follow-Up Visit should occur prior to the patient receiving another cancer therapy.

- Vital signs - temperature, pulse, respiratory rate and blood pressure (average of 3 BP readings)
- Physical Examination including weight
- ECOG performance status (see APPENDIX 1)
- FBC to include: white blood cells, haematocrit, haemoglobin, platelets, neutrophils, lymphocytes, red blood cells, monocytes, basophils, eosinophils, absolute neutrophil count
- Comprehensive serum chemistry panel to include: sodium, potassium, urea, nitrogen, creatinine, magnesium, calcium (total), bilirubin (total), direct bilirubin, albumin, ALP, AST or ALT, glucose, LDH, phosphate, total protein, CRP, CEA
- Renal function - GFR
- Thyroid function and cortisol - analysis of cortisol, T3, T4 and TSH
- Review Adverse Events and Concomitant Medications - Adverse Events (AEs) and laboratory safety measurements will be graded per NCI Common Terminology Criteria for Adverse Events (CTCAE) version v4.03, (see APPENDIX 2). All adverse events, whether gradable by CTCAE or not, will also be evaluated for seriousness

7.3.4 FOLLOW UP

The following assessments should be performed via telephone call (with the exception of CT scan):

- Review Adverse Events every four weeks for up to 6 months post treatment discontinuation - Adverse Events (AEs) will be graded per NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03, (see APPENDIX 2). All adverse events, whether gradable by CTCAE or not, will also be evaluated for seriousness. If at the 6 month review there are any toxicities at grade 2 or higher that the investigator considers to be related to the trial treatment then these should be followed up every 12 weeks until they have reduced to grade 1 or less.
- Further cancer treatment every four weeks up to 6 months and every 12 weeks thereafter.
- CT Tumour imaging with contrast (chest, abdomen, pelvis) if patient has stopped trial treatment without progressive disease they should continue to be scanned according to schedule until progressive disease is identified. NOTE. In this situation, it is important if treatment is discontinued earlier than 45 weeks from commencement of treatment, scans should continue to be performed every 9 weeks until this time point or disease progression. If disease progression has not been identified at 45 week scan then scans should continue to be performed every 12 weeks until disease progression or end of follow up. Scans will be reported by RECIST 1.1 (APPENDIX 3). Response status will be assessed by the trial site. Patients may initially show evidence of disease progression as a response to immunotherapy therefore patients may be allowed to continue treatment beyond RECIST defined disease progression if they are clinically stable and tolerating therapy. Trial treatment must then be discontinued upon further progression defined as the presence of any new lesion or an additional 10% increase in existing tumour burden from time of the initial progression.

7.4 SAMPLE COLLECTION

7.4.1 BLOOD COLLECTION

Blood collection for safety evaluation assumes priority over other procedures. Whenever possible, pre-treatment blood samples should be obtained by fresh peripheral venepuncture. If a patient does not have peripheral access, the sample may be collected from a central catheter immediately after an initial withdrawal of at least 10 ml of blood; or preferably, after a series of other blood sample collections from the central catheter.

7.4.2 TRANSLATIONAL SAMPLES

The following additional blood samples should be collected:

Cytokine/Chemokine Panel and proteomics

- Collected before start of first infusion followed by every alternate cycle from cycle 3 onwards e.g. cycle 3, 5, 7 etc. (approx. every 8 weeks) and at Treatment Discontinuation Visit.

Germline DNA

- Collected before start of infusion at cycle 1.

ctDNA

- Collected before start of first infusion followed by every alternate cycle from cycle 3 onwards e.g. cycle 3, 5, 7 etc (approx. every 8 weeks) and at Treatment Discontinuation Visit.

Processing and storage of blood samples are to be carried out as described in Laboratory Manual.

7.5 DOSE MODIFICATION AND TOXICITY MANAGEMENT

AEs associated with nivolumab exposure may represent an immunologic etiology. Immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of nivolumab treatment and may affect more than one body system simultaneously. Based on existing clinical trial data, most irAEs were reversible and could be managed with interruptions of nivolumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Based on the severity of irAEs, withhold or permanently discontinue nivolumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with nivolumab are provided in Table 3. For any general disorders, if the performance status is poor, the investigator should withhold or discontinue treatment.

Table 3 Dose modification and toxicity management guidelines for immune-related AEs associated with nivolumab

Immune-related AEs	Toxicity grade or conditions	Action taken to nivolumab[2, 3]	AE management with corticosteroid[1, 4] and/or other therapies	Monitoring and Supportive Care
Pneumonitis	Grade 2	Withhold	• *Administer corticosteroids (initial dose of 1-2mg/kg prednisone or equivalent) followed by taper*	• *Monitor subjects for signs and symptoms of pneumonitis* • *Evaluate subjects with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment* • *Add prophylactic antibiotics for opportunistic infections*
	Grade 3 or 4	Permanently discontinue		
Diarrhoea / colitis	Grade 2 or 3	Withhold	• *Administer corticosteroids (initial dose of 1-2mg/kg prednisone or equivalent).* • *Recommence*	• *Monitor subjects 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).* • *Subjects with ≥ Grade 2 diarrhoea suspecting colitis should consider GI consultation and performing endoscopy and cross sectional imaging to rule out colitis. Investigations should also be performed to exclude infective causes.* • *Subjects with diarrhoea/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 Total Bilirubin	Grade 2	Withhold	• *Administer corticosteroids (initial dose of 0.5- 1mg/kg prednisone or equivalent).*	• *Monitor with liver function tests (consider weekly or more frequently, withhold until liver enzyme value returned to baseline or is stable).*
	Grade 3 or 4	Permanently discontinue	• *Administer corticosteroids (initial dose of 1-2mg/kg prednisone or equivalent.*	

Immune-related AEs	Toxicity grade or conditions	Action taken to nivolumab[2, 3]	AE management with corticosteroid[1, 4] and/or other therapies	Monitoring and Supportive Care
Diabetes	Grade 3	Withhold	• *For symptomatic diabetes initiate insulin replacement therapy as appropriate.*	• *Monitor subjects blood sugar levels.*
	Grade 4	Permanently discontinue		
Hypophysitis	Symptomatic Grade 2 or 3	Withhold	• *Administer corticosteroids and initiate hormonal replacements as clinically indicated.*	• *Monitor for signs and symptoms of hypophysitis (including hypopituitarism).*
	Grade 4	Permanently discontinue		
Hyperthyroidism	Symptomatic Grade 2 or 3	Withhold	• *Treat with non-selective beta-blockers (e.g. propranolol) or thioamides) as appropriate.* • *For symptomatic hyperthyroidism initiate antithyroid therapy as appropriate.*	• *Monitor for signs and symptoms of thyroid disorders.*
	Grade 4	Permanently discontinue		
Hypothyroidism	Symptomatic Grade 2 or 3	Withhold	• *Initiate thyroid replacement hormones (e.g. levothyroxine or liothyronine) per standard of care.*	• *Monitor for signs and symptoms of thyroid disorders.*
	Grade 4	Permanently discontinue		
Adrenal insufficiency	Grade 2	Withhold	• *Administer corticosteroids and initiate hormonal replacements as clinically indicated.*	• *Monitor for signs and symptoms of adrenal insufficiency.*
	Grade 3 or 4	Permanently discontinue		
Creatinine elevation	Grade 2 or 3	Withhold	• *Administer corticosteroids (prednisone 1-2mg/kg or equivalent).*	• *Monitor changes of renal function, withhold until creatinine returns to baseline or is stable.*
	Grade 4	Permanently discontinue		
Rash	Grade 3	Withhold	• *If severe administer corticosteroids (1-2 mg/kg/day)*	• *Monitor for rash and other signs/symptoms of skin disorders.*

Immune-related AEs	Toxicity grade or conditions	Action taken to nivolumab[2, 3]	AE management with corticosteroid[1, 4] and/or other therapies	Monitoring and Supportive Care
	Grade 4	Permanently discontinue	*methylprednisolone or equivalent.*	
Stevens-Johnson Syndrome (SJS) or Toxic Epidermal Necrolysis	-	Permanently discontinue		
Myocarditis	Grade 3	Permanently discontinue		
All Other immune-related AEs	Grade 3	Withhold	• *Based on severity of AE administer corticosteroids*	• *Ensure adequate evaluation to confirm etiology or exclude other causes*
	Grade 4 or recurrent Grade 3; persistent Grade 2 or 3 despite treatment modification ; inability to reduce corticosteroid dose to 10 mg prednisone or equivalent per day	Permanently discontinue		

Footnotes: General Instructions for management of irAE

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 nivolumab has been withheld, nivolumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered.. For subjects with Grade 3 or 4 immune-related endocrinopathy, nivolumab may be resumed when AE improves to Grade 2 or lower and is controlled with hormonal replacement therapy.*
3. *For situation where nivolumab is withheld initially, nivolumab 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.*
4. *For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.*

Nivolumab may be interrupted for situations other than treatment-related AEs such as medical/surgical events or logistical reasons not related to trial therapy (e.g. elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should recommence trial therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the ANICCA-Class II Trial Office. The reason for interruption should be documented in the patient's medical notes and on the Case Report Form (CRF).

7.6 TREATMENT COMPLIANCE

All dose modifications, delays and omissions must be recorded on the CRF.

7.7 CONCOMITANT MEDICATION

7.7.1 ACCEPTABLE CONCOMITANT MEDICATIONS

Throughout the trial any concomitant medication or therapy deemed necessary to provide adequate supportive care may be prescribed and should be recorded within the CRF including all prescription, over-the-counter (OTC) and IV medications and fluids, with the exception of those detailed in section 7.7.2. The indication for the treatment should be recorded at the same time.

If changes occur during the trial period e.g. drug dosage, frequency, route date, this should also be documented in the CRF.

All concomitant medications received within 28 days prior to trial registration, during trial treatment and 28 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 28 days of the last dose of trial treatment only need to be recorded when reporting Serious Adverse Events (SAEs).

Antibiotics are allowed to be given to be treated for infections however these may decrease the efficacy of Nivolumab. Antibiotics should only be given if essential and ideally should not be given 28 days before treatment and 2 months after treatment has stopped.

7.7.2 PROHIBITED CONCOMITANT MEDICATIONS

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Anti-cancer systemic chemotherapy or biological therapy within 28 days prior to the first dose of trial treatment and while participating in the trial.
- Immunotherapy not specified in this protocol.
- Chemotherapy not specified in this protocol.
- Investigational agents other than nivolumab.
- Radiation therapy
    - Note: Palliative Radiation therapy may be allowed after consultation with the ANICCA-Class II Trial Office.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial and for 3 months after the last dose of therapy. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed.

- Glucocorticoids for any purpose other than to manage symptoms of an immune-related adverse event. The use of physiologic doses of corticosteroids may be approved after consultation with the ANICCA-Class II Trial Office.
- Whilst participating on the trial, patients may be given systemic steroids due to an acute medical situation but the steroids may only be given for a maximum of 2 weeks. During this period, trial treatment must stop and can be restarted when the steroid administration is complete. If the subject requires the continued use of steroid treatment for more than 2 weeks, they should be withdrawn from the trial.
- Any form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment and while participating on the trial. Replacement therapy (e.g. thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
- Any botanical preparation (e.g. herbal supplements or traditional Chinese medicines) intended to treat the disease under study or provide supportive care.

Subjects who, on the assessment by the Investigator, require the use of any of the aforementioned treatments for clinical management should be discontinued from trial treatment. Subjects may receive other medications that the Investigator deems to be medically necessary.

There are no prohibited therapies during the post-treatment follow-up phase.

7.8 CONTRACEPTION

7.8.1 WOMEN OF CHILDBEARING POTENTIAL

Women of childbearing potential are defined as women who have not had a hysterectomy and oophorectomy. Women with amenorrhea for <2 years will only be considered not to be of reproductive potential if they have prior documentation in the medical notes confirming post-menopausal status.

Adequate contraception should be used from the time of screening, during the trial and for at least 23 weeks after completion of treatment**.**

Acceptable methods of contraception include total abstinence (if this is the patient's usual and preferred lifestyle choice), tubal ligation, combined oral, transdermal or intra-vaginal hormonal contraceptives, medroxyprogesterone injections (e.g. Depo-provera), copper-banded intra-uterine devices, hormone impregnated intra-uterine systems and vasectomised partners. All methods of contraception (with the exception of total abstinence) should be used in combination with the use of a condom by their male sexual partner for intercourse.

7.8.2 MALES

Male patients with sexual partners who are pregnant or who could become pregnant (i.e. women of child-bearing potential) should use a condom during sexual intercourse during the trial and for at least 7 months after completion of treatment. Partners of male patients should also use effective contraception that is not a female condom.

Male patients are prohibited from giving sperm donation during the trial and for at least 7 months after completing treatment.

7.9 PROGRESSION AND DEATH

The ANICCA-Class II Trial Office should be notified immediately if a patient progresses or dies by completion of the progression form or the death form (see section 10.1).

7.10 TRIAL TREATMENT DISCONTINUATION AND WITHDRAWAL OF CONSENT

7.10.1 DISCONTINUATION OF INVESTIGATIONAL MEDICINAL PRODUCT

Patients should discontinue trial treatment in the following circumstances:

- Intolerable toxicity
- Confirmed disease progression
- Pregnancy
- Severe non-compliance to protocol
- Investigator decision, for example if the patient requires a prohibited concomitant medication
- Completion of two years treatment

The ANICCA-Class II Trial Office should be notified of treatment discontinuation immediately via completion a Treatment Discontinuation Form.

7.10.2 WITHDRAWAL OF CONSENT

Patients may withdraw consent at any time without reason. For the purposes of this trial three types of withdrawal are defined:

- The patient would like to withdraw from trial treatment, but is willing to be followed up in accordance with the schedule of assessments (i.e. the patient has agreed that data can be collected and used in the trial analysis)
- The patient would like to withdraw from trial treatment and does not wish to attend trial visits in accordance with the schedule of assessments but is willing to be followed up at standard clinic visits (i.e. the patient has agreed that data can be collected at standard clinic visits and used in the trial analysis)
- The patient would like to withdraw from trial treatment and is not willing to be followed up for the purposes of the trial at any further visits (i.e. only data collected prior to the withdrawal of consent can be used in the trial analysis)

A patient that decides to withdraw from nivolumab treatment should always be asked about the presence of any AEs.

The details of withdrawal (date, reason (if patient willing to provide) and type of withdrawal) should be clearly documented in the source data. A Withdrawal Form should be completed to notify the ANICCA-Class II Trial Office of the patient's withdrawal from the trial.

8 TRANSLATIONAL RESEARCH

Exploratory outcomes will be based on the identification of possible biomarkers predictive of response to nivolumab. Previous studies looking at Neoadjuvant immune-checkpoint blockade in resectable colon cancer identified that the levels of PD-1 and CD-8+ were predictive of patient response to checkpoint blockade therapy such as nivolumab[25]. During class II expression screening, if class II expression (>1%) is observed subsequent immunohistochemistry staining for CD-8+ and PD-1 will be carried out to look for supplementary biomarkers of response to nivolumab. This will be performed by the Molecular Pathology Diagnostic Service at the Queen Elizabeth Hospital Birmingham. Secondly we will analyse blood samples to identify possible biomarkers of response. This will be a purely discovery analysis and new putative biomarkers would require prospective validation. Sample analysis will be performed at the University of Birmingham or affiliated academic institutions either during or upon completion of the trial.

9 ADVERSE EVENT REPORTING

The collection and reporting of AEs will be in accordance with the Medicines for Human Use Clinical Trials Regulations 2004 and its subsequent amendments. Definitions of different types of AE are listed in APPENDIX 4. The Investigator should assess the seriousness and causality (relatedness) of all AEs experienced by the patient (this should be documented in the source data) with reference to the summary of product characteristics (SPC).

9.1 REPORTING REQUIREMENTS

9.1.1 ADVERSE EVENTS

All medical occurrences which meet the definition of an AE (see Appendix 5 for definition) should be reported.

9.1.2 LABORATORY TEST ABNORMALITIES

All laboratory test results captured as part of the trial should be recorded following institutional procedures. Test results that constitute SAEs (see Appendix 5 for definition) should be documented and reported to the ANICCA-Class II Trial Office as such.

The following laboratory abnormalities should be documented and reported appropriately:

- any laboratory test result that is clinically significant or meets the definition of an SAE
- any laboratory abnormality that required the participant to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (e.g., anemia versus low hemoglobin value).

9.1.3 DRUG INDUCED LIVER INJURY (DILI) EVENTS

DILI events will be reported and captured as part of the safety reporting profile and those meeting the criteria below will require expedited reporting to the ANICCA-Class II Trial Office as SAEs.

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. Potential drug induced liver injury is defined as:

1. AT (ALT or AST) elevation > 3 times upper limit of normal (ULN)
2. Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)
3. No other immediately apparent possible causes of AT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

9.1.4 SERIOUS ADVERSE EVENTS

Investigators should report AEs that meet the definition of an SAE (see APPENDIX 4 for definition) as described in section

9.1.5 MONITORING PREGNANCIES FOR POTENTIAL SERIOUS ADVERSE EVENTS

It is important to monitor the outcome of pregnancies of patients and/or their partners in order to provide SAE data on congenital anomalies or birth defects.

In the event that a patient or their partner becomes pregnant during the SAE reporting period, please complete a Pregnancy Notification Form (providing the patient's details) and return to the ANICCA-Class II Trial Office as soon as possible and ideally no later than 24 hours from first becoming aware. The patient should be given a release of medical information form or the patient should be asked to give this to their partner. If the patient/partner is happy to provide information on the outcome of their pregnancy they should sign the release of medical information form. Once consent has been obtained provide details of the outcome of the pregnancy on a follow-up Pregnancy Notification Form and if necessary also complete an SAE Form. Follow-up information will be collected for up to 10 years.

9.1.6 REPORTING PERIOD

Details of all AEs will be documented and reported from the date patient consented to main trial until 6 months after treatment discontinuation. If at the 6 month review there are any toxicities at grade 2 or higher that the investigator considers to be related to the trial treatment then these should be followed up until they have reduced to grade 1 or less.

SAEs that are judged to be at least possibly related to IMP must still be reported in an expedited manner irrespective of how long after IMP administration the reaction occurred.

9.2 REPORTING PROCEDURE

9.2.1 REPORTING PROCEDURES FOR SITE

9.2.1.1 ADVERSE EVENTS

AEs should be reported on an AE Monitoring Form (and where applicable on an SAE Form). An AE Monitoring Form should be completed at each visit.

AEs will be reviewed using the CTCAE, version v4.03 (see APPENDIX 2). For each AE, the highest grade observed since the last visit should be recorded.

9.2.1.2 SERIOUS ADVERSE EVENTS

For more detailed instructions on SAE reporting refer to the SAE Form Completion Guidelines contained in the ISF.

AEs defined as serious and which require reporting as an SAE should be reported on an SAE Form. When completing the form, the Investigator will be asked to define the causality and the severity of the AE, which should be documented using the CTCAE version 4.03.

On becoming aware that a patient has experienced an SAE, the Investigator (or their delegate) must complete, date and sign an SAE Form. The form should be emailed to the ANICCA-Class II Trial Office using one of the numbers listed below as soon as possible and no later than 24 hours after first becoming aware of the event:

To report an SAE, email the SAE Formto:

reg@trials.bham.ac.uk

On receipt the ANICCA-Class II Trial Office will allocate each SAE a unique reference number. This number will be emailed back to the site as proof of receipt. If confirmation of receipt is not received within 1 working day please contact the ANICCA-Class II Trial Office. The SAE

reference number should be quoted on all correspondence and follow-up reports regarding the SAE.

For SAE Forms completed by someone other than the Investigator, the Investigator will be required to countersign the original SAE Form to confirm agreement with the causality and severity assessments. The form should then be returned to the Trials Office in the post and a copy kept in the ISF. Investigators should also report SAEs to their own Trust in accordance with local practice.

9.2.1.3 PROVISION OF FOLLOW-UP INFORMATION

Patients should be followed up until resolution or stabilisation of the event. Follow-up information should be provided on a new SAE Form (refer to the SAE Form Completion Guidelines for further information).

9.2.2 ANICCA-CLASS II TRIAL OFFICE

On receipt of an SAE Form, seriousness and causality will be determined independently by a Clinical Coordinator. An SAE judged by the Investigator or Clinical Coordinator to have a reasonable causal relationship with the trial medication will be regarded as a Serious Adverse Reaction (SAR). The Clinical Coordinator will also assess all SARs for expectedness. If the event meets the definition of a SAR that is unexpected (i.e. is not defined in the Reference Safety Information) it will be classified as a Suspected Unexpected Serious Adverse Reaction (SUSAR).

9.2.3 REPORTING TO THE COMPETENT AUTHORITY AND RESEARCH ETHICS COMMITTEE

9.2.3.1 SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTIONS

The ANICCA-Class II Trial Office will report a minimal data set of all individual events categorised as a fatal or life threatening SUSAR to the Medicines and Healthcare products Regulatory Agency (MHRA) and Research Ethics Committee (REC) within 7 days. Detailed follow-up information will be provided within an additional 8 days.

All other events categorised as SUSARs will be reported within 15 days.

9.2.3.2 SERIOUS ADVERSE REACTIONS

The ANICCA-Class II Trial Office will report details of all SARs (including SUSARs) to the MHRA and REC annually from the date of the Clinical Trial Authorisation, in the form of a Development Safety Update Report.

9.2.3.3 ADVERSE EVENTS

Details of all AEs will be reported to the MHRA on request.

9.2.3.1 OTHER SAFETY ISSUES IDENTIFIED DURING THE COURSE OF THE TRIAL

The MHRA and REC will be notified immediately if a significant safety issue is identified during the course of the trial.

9.2.4 INVESTIGATORS

Details of all SUSARs and any other safety issue which arises during the course of the trial will be reported to Principal Investigators. A copy of any such correspondence should be filed in the ISF.

9.2.5 TRIAL STEERING COMMITTEE

The Trial Steering Committee will review all SAEs.

9.2.6 MANUFACTURER OF INVESTIGATIONAL MEDICINAL PRODUCT

All SAEs will be reported to Bristol-Myers Squibb Pharmaceuticals Ltd. (IMP manufacturer) within an agreed timeframe from first awareness of the event at the ANICCA-II Trial Office.

10 DATA HANDLING AND RECORD KEEPING

10.1 DATA COLLECTION

The Case Report Form (CRF) will comprise the forms detailed below in Table 4.

Table 4 Case Report Form

Form	Summary of data recorded	Schedule for submission
Screening Biopsy Form	Patient details, details of patient's CRC (with copy of diagnostic biopsy report) and biopsy sample provided for MS status and Class II expression testing.	Must be sent with biopsy sample
Eligibility Checklist	Confirmation of patient eligibility.	Email at point of registration Original to be posted within one week of registration.
Screening Registration Form	Patient details	Within one week of registration
Medical History Form	Patient's medical history including smoking and alcohol status and relevant significant medical and autoimmune conditions	Within one week of registration
Previous Cancer Treatment Form	Any treatment patient has previously received for their CRC, best response for each treatment and reasons for stopping each treatment	Within one week of registration
Adverse Event Monitoring Form	Any adverse events patient suffers during screening, treatment and follow up	Baseline AE form – within one week of registration, Treatment AE form – within one week of treatment visit, Follow up AE form – within one week of visit
Concomitant Medication Log	Any concomitant medication patient is taking during screening, baseline and follow up	Baseline concomitant medication log – within one week of registration, Treatment concomitant medication log – within one week of treatment visit, Follow up concomitant medication log – within one week of visit
Treatment Form	Pre-treatment assessment data, details of nivolumab delivery including date given,	Within one week of treatment visit

	dose administered, details of dose reduction/delay/interruption if necessary. Details of translational blood sample collection.	
Laboratory Tests Form	Details of clinical chemistry and haematological assessments.	Within one week of registration and then within one week of treatment visit.
Response Evaluation Form-Baseline	Details of target/non-target lesions at baseline.	Within one week of registration
Response Evaluation Form	Target/non-target tumour assessment during treatment and follow up according to RECIST 1.1.	Treatment response evaluation form – within one week of assessment, Follow up response evaluation form – within one week of assessment.
Treatment Discontinuation Form	Date treatment was discontinued and reason for discontinuation	Immediately upon discontinuation of treatment
Trial Entry Form	Confirmation of entry into the main trial	Within one week of entry into main trial
End of Treatment Form	End of treatment assessment data, details of translational blood sample collection.	Within one week of end of treatment visit
Safety Follow up Visit Form	Assessments performed at 28 day post discontinuation safety visit	Within one week of visit
Follow up Form	Date contact was last made with patient and any further treatment they have received for their CRC	Within one week of contact
Death Form	Date and cause of death	Immediately upon notification of patient's death
Deviation Form	Completed in the event of a deviation from the protocol	Immediately upon discovering deviation
Withdrawal Form	Used to notify the Trials Office of patient withdrawal from the trial	Immediately upon patient withdrawal
Pregnancy Notification Form	Dates related to pregnancy and outcome	Within 24 hours of becoming aware of pregnancy and outcome
Serious Adverse Event Form	Completed in the event of patient experiencing a serious adverse event (refer to APPENDIX 4 for definition)	Within 24 hours of awareness of event

This trial will use an electronic remote data capture (eRDC) system which will be used for completion of the CRF. Access to the eRDC system will be granted to individuals via the ANICCA-Class II Trial Office. The eligibility checklist, SAE reporting and Notification of Pregnancy will be paper-based.

The CRF must be completed by the Investigator or an authorised member of the site research team (as delegated on the Site Signature and Delegation Log) within the timeframe listed above. The exceptions to this are the SAE Form and Eligibility Checklist, which must be co-signed by the Investigator. SAE Forms should be emailed to the ANICCA-Class II Trial Office as soon as possible and no later than 24 hours after first becoming aware of the event.

Entries on the paper based forms should be made in ballpoint pen, in blue or black ink, and must be legible. Any errors should be crossed out with a single stroke, the correction inserted and the change initialled and dated. If it is not obvious why a change has been made, an explanation should be written next to the change.

Data reported on each form should be consistent with the source data or the discrepancies should be explained. If information is not known, this must be clearly indicated on the form. All missing and ambiguous data will be queried. All sections are to be completed before returning. In all cases it remains the responsibility of the Investigator to ensure that the CRF has been completed correctly and that the data are accurate.

The format and content in the CRF may be amended by the ANICCA-Class II Trial Office, as appropriate, throughout the duration of the trial. Whilst this will not constitute a protocol amendment, sites will be notified of new versions of the form when they are available in the eRDC system, and new versions of the form must be implemented by participating sites immediately on receipt.

10.2 ARCHIVING

It is the responsibility of the Principal Investigator to ensure all essential trial documentation and source records (e.g. signed Informed Consent Forms, ISF, Pharmacy File, patients' hospital notes, copies of CRFs etc.) at their site are securely retained for at least 25 years after the end of the trial. Do not destroy any documents without prior approval from the CRCTU Archivist.

11 QUALITY MANAGEMENT

11.1 SITE SET-UP AND INITIATION

All sites will be required to sign a Clinical Trial Site Agreement prior to participation. In addition all participating Investigators will be asked to sign the necessary agreements and registration forms; and supply a current CV to the ANICCA-Class II Trial Office. All members of the site research team will also be required to sign the Site Signature and Delegation Log, which should be returned to the ANICCA-Class II Trial Office. Prior to commencing recruitment all sites will undergo a process of initiation. Key members of the site research team will be required to attend either a meeting or a teleconference covering aspects of the trial design, protocol procedures, AE reporting, collection and reporting of data and record keeping. Sites will be provided with an Investigator Site File and a Pharmacy File containing essential documentation, instructions, and other documentation required for the conduct of the trial. The ANICCA-II Trial Office must be informed immediately of any change in the site research team.

11.2 ON-SITE MONITORING

Monitoring will be carried out as required following a risk assessment and as documented in the ANICCA-Class II Trial Quality Management Plan. Additional on-site monitoring visits may be triggered; for example by poor CRF return, poor data quality, low SAE reporting rates, an excessive number of patient withdrawals or deviations. If a monitoring visit is required, the ANICCA-Class II Trial Office will contact the site to arrange a date for the proposed visit and will provide the site with written confirmation. Investigators will allow the ANICCA-Class II trial staff access to source documents as requested.

11.3 CENTRAL MONITORING

Where a patient has given explicit consent sites are requested to send in copies of signed Informed Consent Forms for in-house review.

Trials staff will be in regular contact with the site research team to check on progress and address any queries that they may have. Trials staff will check incoming Case Report Forms for compliance with the protocol, data consistency, missing data and timing. Sites will be sent Data Clarification Forms requesting missing data or clarification of inconsistencies or discrepancies. For CRF on eRDC, data clarifications will be raised on this system. Sites are expected to answer the data clarifications in a timely manner.

Sites may be suspended from further recruitment in the event of serious and persistent non-compliance with the protocol and/or GCP, and/or poor recruitment. Any major problems identified during monitoring may be reported to the ANICCA-Class II TMG or TSC, and the relevant regulatory bodies. This includes reporting serious breaches of GCP and/or the trial protocol to the REC and the MHRA.

11.4 AUDIT AND INSPECTION

The Investigator will permit trial-related monitoring, audits, ethical review, and regulatory inspection(s) at their site, providing direct access to source data/documents.

Sites are also requested to notify the ANICCA-Class II Trial Office of any MHRA inspections.

11.5 NOTIFICATION OF SERIOUS BREACHES

In accordance with Regulation 29A of the Medicines for Human Use (Clinical Trials) Regulations 2004 and its amendments the Sponsor of the trial is responsible for notifying the licensing authority in writing of any serious breach of:

- The conditions and principles of GCP in connection with that trial or;

- The protocol relating to that trial, within 7 days of becoming aware of that breach

For the purposes of this regulation, a “serious breach” is a breach which is likely to effect to a significant degree:

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

Sites are therefore requested to notify the ANICCA-Class II Trial Office of a suspected trial-related serious breach of GCP and/or the trial protocol. Where the ANICCA-Class II Trial Office is investigating whether or not a serious breach has occurred sites are also requested to cooperate with the ANICCA-Class II Trial Office in providing sufficient information to report the breach to the MHRA where required and in undertaking any corrective and/or preventive action.

12 END OF TRIAL DEFINITION

The end of trial will be six months after the last data capture. This will allow sufficient time for the completion of protocol procedures, data collection and data input. The ANICCA-Class II Trial Office will notify the MHRA and REC that the trial has ended and will provide them with a summary of the clinical trial report within 12 months of the end of trial.

13 STATISTICAL CONSIDERATIONS

13.1 DEFINITION OF OUTCOME MEASURES

13.1.1 PRIMARY OUTCOME MEASURE

Durable clinical benefit (DCB)

A patient will be defined as experiencing DCB if they remain free of disease progression at their third trial specific CT scan or MRI scan since treatment start date (i.e. at approximately 27 weeks) or at any CT/MRI scan after 27 weeks that shows the patient remains free of disease progression. While either CT or MRI may be used, as per RECIST 1.1, CT is the preferred imaging technique in this trial.

13.1.2 SECONDARY OUTCOME MEASURES

13.1.2.1 OBJECTIVE RESPONSE (OR)

Patients will have CT/MRI scans every 9 weeks from treatment commencement up to 45 weeks, then every 12 weeks, until disease progression. On each occasion, overall tumour burden will be assessed using RECIST version 1.1 (Eisenhauer *et al.* 2009). Best overall response is the best response recorded over the whole period of assessment and could be complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD) or inevaluable for response (for which reasons such as early death due to disease or early death due to toxicity will be specified). Objective response is the occurrence of CR or PR as the best overall response. Objective response will be based on responses confirmed using the subsequent 9 or 12-weekly scan but objective response based on unconfirmed responses will also be reported.

13.1.2.2 BEST PERCENTAGE CHANGE IN SUM OF TARGET LESION DIAMETERS (PCSD)

At each evaluation, the longest diameters of all selected target lesions will be measured and summed and the percentage change from the baseline measurement will be calculated. The best percentage change is the one that reflects either the greatest decrease or the least increase over the whole period of assessment.

13.1.2.3 TIME TO MAXIMAL RESPONSE (TTMR)

This is defined as the time from commencement of trial treatment to the date of CT/MRI scan that first records objective response as per RECIST version 1.1. Objective response is the occurrence of CR or PR as the best overall response. TTMR is evaluable for patients who achieve objective response.

13.1.2.4 PROGRESSION-FREE SURVIVAL TIME (PFS)

This is defined as the time from commencement of trial treatment to the date of CT/MRI scan when progressive disease first recorded or date of death without previously recorded progression. Patients who are alive with no recorded progression at the time of analysis will be censored at the date of the CT/MRI scan when they were last recorded with an evaluable measure that was not progression.

13.1.2.5 OVERALL SURVIVAL TIME (OS)

This is defined as the time from commencement of trial treatment to the date of death. Patients who are alive at the time of analysis will be censored at the date last seen alive.

13.2 ANALYSIS OF OUTCOME MEASURES

The trial will use a Bayesian analysis to determine a clinically relevant signal of effect in this population. A true DCB rate of at least 30% would provide sufficient proof of concept to warrant further research (efficacy criteria). The statistical analysis plan is to estimate a posterior

probability distribution for the true DCB rate given the observed trial data and a minimally-informative prior. As well as providing estimates for the true DCB rate, the analysis will calculate the probability that the true DCB rate is ≥ 30%. In terms of secondary outcome measures, posterior probability distributions will be estimated for the true OR rate, median PCSD, median TTMR, median PFS and median OS. The statistical analysis plan will provide details of the selection of subjects to be included in the analysis in addition to procedures for accounting for missing, unused or spurious data.

13.3 PLANNED INTERIM ANALYSES

At the interim analysis, the trial will be recommended to stop early if p(true DCB rate<30%)>90%, i.e. if there is a high chance that the true signal in the targeted group falls below a clinically relevant threshold value (futility criteria). Secondary outcome measures will also be considered and contribute to this decision. The efficacy criteria will not be considered at interim analyses. A decision to stop early will be made in consultation with the TSC.

13.4 PLANNED FINAL ANALYSES

At the final analysis, the trial will recommend further research if p(true DCB rate≥30%) >50% (efficacy criteria). Secondary outcome measures will also be considered and contribute to this decision.

13.5 SAMPLE SIZE

Target sample size is 36 patients with an interim analysis at 18. With an expected MHC class II prevalence of around 10% in locally advanced or metastatic MSS CRC this will require screening of approximately 360 patients. It is projected that each site will enrol approximately four patients for trial treatment.

The operating characteristics for this design show that there is a 9% chance of incorrectly recommending further research when the true DCB rate is 20% (equivalent to a type I error rate) and 91% chance of correctly recommending further research when the true DCB rate is 40% (equivalent to power).

14 TRIAL ORGANISATIONAL STRUCTURE

14.1 SPONSOR

The trial is sponsored by The University of Birmingham.

14.2 COORDINATING CENTRE

The trial is being conducted under the auspices of the CRCTU, University of Birmingham according to their local procedures.

14.3 TRIAL MANAGEMENT GROUP (TMG)

A TMG will be established and as a minimum will include the Chief Investigator, Co-Investigators, the Lead and Trial Statistician, Trial Management Team Leader and Trial Coordinator. Other key personnel may be invited to join the TMG as appropriate to ensure representation from a range of professional groups.

Notwithstanding the legal obligations of the Sponsor and Chief Investigator, the TMG will be responsible for the day-to-day running and management of the trial and will meet by teleconference or in person as required.

14.4 TRIAL STEERING COMMITTEE (TSC)

The role of the TSC is to provide overall supervision for the trial on behalf of the Trial Sponsor (University of Birmingham) and to ensure that the trial is conducted to the rigorous standards set out in the GCP standards. In particular, the TSC will concentrate on progress of the trial, adherence to the protocol, patient safety, evidence on main efficacy outcome measures and the consideration of new information of relevance to the research question. The safety and well-being of the trial participants are the most important considerations and should prevail over the interests of science and society. The TSC will provide advice, through its Chair, to the Chief Investigator and the University of Birmingham on all appropriate aspects of the trial. Membership of the TSC includes an independent Chair and two other independent members, who do not sit on the TMG. The TSC will be asked to comment in detail on substantial changes to the protocol. The TSC will meet as often as required, at least once per year during recruitment.

14.5 FINANCE

This is an investigator-initiated and investigator-led trial funded by Bristol-Myers Squibb Pharmaceuticals Ltd. Per-patient payments will be made to NHS Trusts for research related costs upon receipt and validation of a completed CRF.

Nivolumab will be provided free of charge by Bristol-Myers Squibb Pharmaceuticals Ltd.

15 ETHICAL CONSIDERATIONS

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects, adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964, amended at the 48th World Medical Association General Assembly, Somerset West, Republic of South Africa, October 1996 (website: http://www.wma.net/en/30publications/10policies/b3/index.html) (APPENDIX 5).

The trial will be conducted in accordance with the UK Policy Framework for Health and Social Care Research, the applicable UK Statutory Instruments, (which include the Medicines for Human Use Clinical Trials 2004 and subsequent amendments, the General Data Protection Regulation 2016/679 and the Data Protection Act 2018, the Human Tissue Act 2004) and Good Clinical Practice (GCP). This trial will be carried out under a Clinical Trial Authorisation in accordance with the Medicines for Human Use Clinical Trials regulations. The protocol will be submitted to and approved by REC and Health Research Authority (HRA) prior to circulation.

Before any patients are enrolled into the trial, the Principal Investigator at each site is required to obtain local R&D approval. Sites will not be permitted to enrol patients until written confirmation of R&D approval is received by the ANICCA-Class II Trial Office.

In the case of a substantial amendment to the protocol, approval will be sought by the Sponsor from the REC, MHRA and HRA prior to implementation.

It is the responsibility of the Principal Investigator to ensure that all amendments gain the necessary local approval. This does not affect the individual clinicians' responsibility to take immediate action if thought necessary to protect the health and interest of individual patients.

16 CONFIDENTIALITY AND DATA PROTECTION

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the General Data Protection Regulation 2016/679 and the Data Protection Act 2018. With the patient's consent, their date of birth (month and year only), National Health Service (NHS) number, or in Scotland the Community Health Index (CHI), hospital number will be collected at trial entry to allow tracing through national agencies (e.g. the Office of National Statistics and Cancer Registries) to assist with long-term follow up via other healthcare professionals (e.g. patient's GP). Patients will be identified using only their unique TNO and initials on the CRF and correspondence between the ANICCA-Class II Trial Office and the participating site. However, patients are asked to give permission for the ANICCA-Class II Trial Office to be sent a copy of their signed Informed Consent Forms, which will not be anonymised. This will be used to perform in-house monitoring of the consent process.

The Investigator must maintain documents not for submission to the ANICCA-Class II Trial Office (e.g. Patient Identification Logs) in strict confidence. In the case of specific issues and/or queries from the regulatory authorities, it will be necessary to have access to the complete trial records, provided that patient confidentiality is protected.

The ANICCA-Class II Trial Office will maintain the confidentiality of all patients' data and will not disclose information by which patients may be identified to any third party other than those directly involved in the treatment of the patient and organisations for which the patient has given explicit consent for data transfer. Representatives of the ANICCA-Class II trial team may

be required to have access to patient's notes for quality assurance purposes but patients should be reassured that their confidentiality will be respected at all times.

17 INSURANCE AND INDEMNITY

University of Birmingham employees are indemnified by the University insurers for negligent harm caused by the design or co-ordination of the clinical trials they undertake whilst in the University's employment.

In terms of liability at a site, NHS Trust and non-Trust hospitals have a duty to care for patients treated, whether or not the patient is taking part in a clinical trial. Compensation is therefore available via NHS indemnity in the event of clinical negligence having been proven.

The University of Birmingham cannot offer indemnity for non-negligent harm. The University of Birmingham is independent of any pharmaceutical company, and as such it is not covered by the Association of the British Pharmaceutical Industry (ABPI) guidelines for patient compensation.

18 PUBLICATION POLICY

Results of this trial will be submitted for publication in a peer reviewed journal. The manuscript will be prepared by the TMG and authorship will be determined by mutual agreement.

Any secondary publications and presentations prepared by Investigators must be reviewed by the TMG. Manuscripts must be submitted to the TMG in a timely fashion and in advance of being submitted for publication, to allow time for review and resolution of any outstanding issues. Authors must acknowledge that the trial was performed with the support of the University of Birmingham. Intellectual Property rights will be addressed in the Clinical Site Study Agreement between Sponsor and site.

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APPENDIX 1 EASTERN COOPERATIVE ONCOLOGY GROUP PERFORMANCE (ECOG) STATUS[26]

Table 5 ECOG

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

APPENDIX 2 COMMON TOXICITY CRITERIA GRADINGS

Toxicities will be recorded according to the CTCAE, version v4.03. The full CTCAE document is available on the National Cancer Institute website, the following address was correct when this version of the protocol was approved:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

APPENDIX 3 RECIST V1.1 CRITERIA

RECIST version 1.1 will be used in this trial for assessment of tumour response. While either CT or MRI may be used, as per RECIST 1.1, CT is the preferred imaging technique in this trial.

The following contains excerpts from the RECIST version 1.1 plus trial specific instructions. A free copy of the revised guidelines is available from http://www.eortc.be/Recist/documents/RECISTGuidelines.pdf

Measurability of Tumour Lesions at Baseline

Only patients with measurable disease at baseline should be included. Measurable disease is defined by the presence of at least one measurable lesion. At baseline, tumour lesions will be categorised as follows:

- Measurable
- Non-measurable

Measurable lesions are those that can be accurately measured in at least one dimension (longest diameter to be recorded) with a minimum size of 10 mm by CT scan (CT scan slice thickness no greater than 5 mm), 10 mm calliper measurement by clinical exam (lesions which cannot be accurately measured with callipers should be recorded as non-measurable) and 20 mm by chest X-ray. For malignant lymph nodes to be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by a CT scan (at baseline and during treatment, only the short axis will be measured and followed).

Non-measurable lesions are all other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to >15 mm short axis) and truly non-measurable lesions.

Lesions considered to be truly non-measurable include the following: bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, abdominal masses that are not confirmed and followed by imaging techniques, and cystic lesions.

Tumour lesions that are situated in a previously irradiated area are not considered measurable.

The term "evaluable" in reference to measurability is not recommended and will not be used because it does not provide additional meaning or accuracy.

All measurements should be recorded in metric notation using callipers (or a ruler) if clinically assessed. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of treatment.

Specifications by Methods of Measurements

The same method of assessment and the same technique should be used to characterise each identified and reported lesions at baseline, during treatment and at the post-treatment assessment. Image-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the anti-tumour effect of a treatment. CT is the best currently available and reproducible method for measuring target lesions selected for response assessment. Investigators should utilize the best available CT imaging technique available to them for determining response and PFS of patients participating in the ANICCA-II trial.

Tumour Response Evaluation

Baseline Documentation of "Target" and "Non-target" Lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as “target” lesions and recorded and measured at baseline.

Target lesions should be selected on the basis of their size (those with the longest diameter) and their suitability for accurate, reproducible, repeated measurements.

A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as the reference by which to characterise the objective tumour response.

All other lesions (or sites of disease) should be identified as “non-target” lesions and should also be recorded at baseline. Measurements of these lesions are not required but these lesions should be followed as ‘present’, ‘absent’ or in rare cases ‘unequivocal progression’ and recorded.

Response Criteria

Table 6 Evaluation of Target Lesions

Response Category	Description
Complete Response (CR)	Disappearance of all target lesions
Partial Response (PR)	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD
Progressive Disease (PD)	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions. In addition to this, the sum must also demonstrate an absolute increase of at least 5 mm. The appearance of one or more lesion is also considered progression.
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

Table 7 Evaluation of Non-target Lesions

Response Category	Description
Complete Response (CR)	Disappearance of all non-target lesions
Incomplete Response/ Stable Disease (SD)	Persistence of one or more non-target lesion(s)
Progressive Disease (PD)	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions [1]

Notes:

1. To achieve “unequivocal progression” on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumour burden has increased sufficiently to merit discontinuation of therapy.

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of treatment until disease progression In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Overall Responses for all Possible Combinations of Tumour Responses in Target and Non-target Lesions With or Without the Appearance of New Lesions

Table 8 Overall Response evaluation

Target Lesions	Non-target Lesions	New Lesions	Overall Response
Complete response (CR)	CR	No	CR
Complete response (CR)	Non-CR/non-PD	No	PR
Complete response (CR)	Not evaluated	No	PR
Partial response (PR)	Non-PD	No	PR
Stable disease (SD)	Non-PD	No	SD
Not all evaluated	Non-PD	No	Not evaluable (NE)
Progressive disease (PD)	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration." Every effort should be made to document the objective disease progression, even after discontinuation of treatment.

APPENDIX 4 DEFINITION OF ADVERSE EVENTS

Adverse Event (AE)

Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Comment:

An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory findings), symptom or disease temporally associated with the use of an investigational medicinal product, whether or not related to the investigational medicinal product.

Adverse Reaction (AR)

All untoward and unintended responses to an IMP related to any dose administered.

Comment:

An AE judged by either the reporting Investigator or Sponsor as having causal relationship to the IMP qualifies as an AR. The expression reasonable causal relationship means to convey in general that here is evidence or argument to suggest a causal relationship.

Serious Adverse Event (SAE)

Any untoward medical occurrence or effect that at any dose:

- Results in death (unrelated to cancer under study)
- Is life-threatening*
- Requires hospitalisation** or prolongation of existing in patients hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly/birth defect
- Or is otherwise considered medically significant by the Investigator***

For the purposes of the ANICCA-Class II trial the following are also regarded as serious adverse events:

- A suspected transmission of an infectious agent (eg, pathogenic or non-pathogenic) via the trial drug
- A potential drug-induced liver injury (DILI), meeting the defined criteria (see section 9.1.3)
- An overdose of the trial drug
- Pregnancy

Comments:

The term severe is often used to describe the intensity (severity) of a specific event. This is not the same as serious, which is based on patient's/event outcome or action criteria.

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

**Hospitalisation is defined as an unplanned, formal inpatient admission, even if the hospitalisation is a precautionary measure for continued observation. Thus hospitalisation for protocol treatment (e.g. line insertion), elective procedures (unless brought forward because of worsening symptoms) or for social reasons (e.g. respite care) are not regarded as an SAE.

*** Medical judgment should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should be considered serious. Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.

Serious Adverse Reaction (SAR)

An Adverse Reaction which also meets the definition of a Serious Adverse Event.

Suspected Unexpected Serious Adverse Reaction (SUSAR)

A SAR that is unexpected i.e. the nature, or severity of the event is not consistent with the applicable product information.

A SUSAR should meet the definition of an AR, UAR and SAR.

Unexpected Adverse Reaction (UAR)

An AR, the nature or severity of which is not consistent with the applicable product information.

Comments:

When the outcome of an AR is not consistent with the applicable product information the AR should be considered unexpected.

APPENDIX 5 WMA DECLARATION OF HELSINKI

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI (1886 Version)

Recommendations guiding physicians in biomedical research involving human subjects

Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964 and amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975, 35th World Medical Assembly, Venice, Italy, October 1983, 41st World Medical Assembly, Hong Kong, September 1989 and the 48th General Assembly, Somerset West, Republic of South Africa, October 1996

INTRODUCTION

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfilment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The Health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognised between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. BASIC PRINCIPLES

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.
2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the Investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.
3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The

responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.
4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.
5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.
6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.
8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.
9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.
10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.
11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation. Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.
12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. MEDICAL RESEARCH COMBINED WITH PROFESSIONAL CARE (Clinical Research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, re-establishing health or alleviating suffering.
2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.
3. In any medical study, every patient - including those of a control group, if any - should be assured of the best proven diagnostic and therapeutic method. This does not exclude the use of inert placebo in studies where no proven diagnostic or therapeutic method exists.
4. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).
6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. NON-THERAPEUTIC BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS (Non-Clinical Biomedical Research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.
2. The subject should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.
3. The Investigator or the investigating team should discontinue the research if in his/her or their judgement it may, if continued, be harmful to the individual.
4. In research on man, the interest of science and society should never take precedence over considerations related to the wellbeing of the subject.

APPENDIX 6 MANAGEMENT OF TOXICITIES

The ESMO guidelines for Management of Toxicities from immunotherapy[27] will be sent as an electronic copy to all participating sites. A hard copy will also be provided by the trials office in the investigator site file for sites to use.

The guidelines can also be found by clicking on the link below:

https://www.esmo.org/Guidelines/Supportive-and-Palliative-Care/Management-of-Toxicities-from-Immunotherapy