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

Statistical Analysis Plan

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Sponsor	University of Birmingham
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KEY PERSONNEL INVOLVED IN THE PREPARATION OF THE STATISTICAL ANALYSIS PLAN:

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DOCUMENT CONTROL SHEET

STATISTICAL ANALYSIS PLAN VERSION:	REASON FOR UPDATE:
1.0, 23-Mar-2021	Creation from Protocol Version 1.0 dated 05-Oct-2018
2.0 02-Dec-2021	Clarify the Per Protocol population in section 5.1 DEFINITION(S) OF POPULATIONS FOR ANALYSIS Clarified definitions of non-progression and confirmed objective response according to RECIST 1.1 in section 8.1 DEFINITION AND CALCULATION OF OUTCOME MEASURE (for PROGRESSION-FREE SURVIVAL TIME) and section 8.2 DESCRIPTIVE ANALYSES (for OR rate) Update the SAP to indicate that final analysis should be performed when the ongoing patients reach week 27 primary outcome measure in section 1 SUMMARY OF THE TRIAL and section 2.2 FINAL ANALYSES Add a subgroup analysis for MHC Class II expression using the 5% cut off and a subgroup analysis for liver metastasis (yes/no) in section 8.6 SUBGROUP ANALYSIS

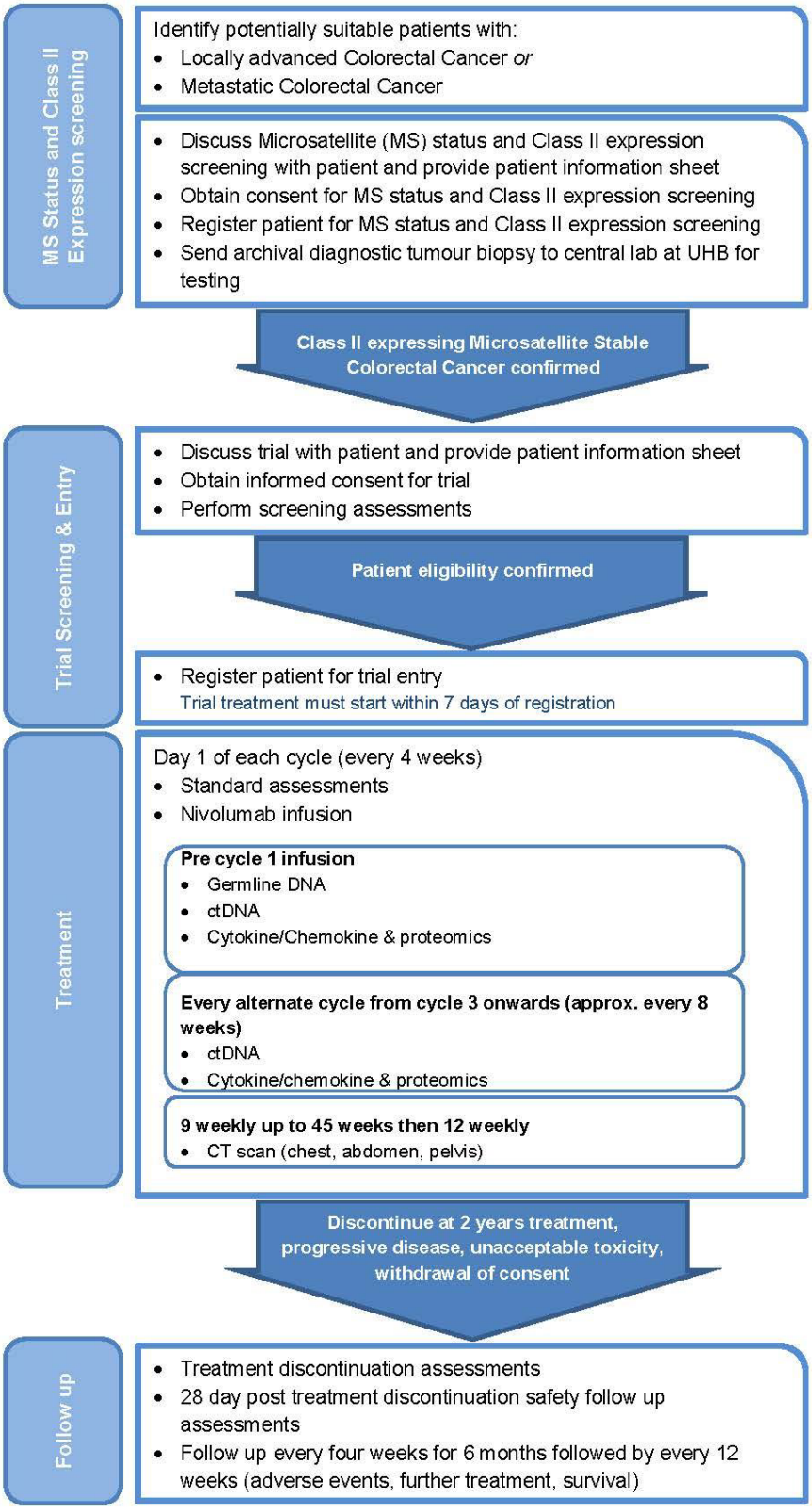
TRIAL SYNOPSIS

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 of nivolumab in patients with class II expressing 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 objectives:** To discover possible biomarker for the precision 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 (DCB) **Secondary outcomes:** • Objective response (OR) • Best percentage change in sum of target lesion diameters (PCSD) • Time to maximal response (TTMR) • Progression-free survival time (PFS) • Overall survival time (OS)
Patient Population	Patients with locally advanced or metastatic MSS CRC with class II expression
Sample Size	36 patients

Key Inclusion Criteria	• 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 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	• 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. • Female patients that are either pregnant or breast feeding.
Trial Treatment	Nivolumab intravenous infusion, 480 mg flat dose over 60 minutes, every 4 weeks
Trial Duration	18 months recruitment, up to two years treatment
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 alternative 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 Tel: 0121 414 6754 Email: ANICCA-ClassII@trials.bham.ac.uk
Sponsor	University of Birmingham

Figure 1. Trial Schema



1. INTRODUCTION

PURPOSE OF THE STATISTICAL ANALYSIS PLAN

This Statistical Analysis Plan (SAP) provides guidelines for the analysis and presentation of results for the ANICCA-Class II trial. This plan, along with all other documents relating to the analysis of this trial, will be stored in the 'Statistical Documentation' section of the Trial Master File. The statistical analysis will be carried out by the Trial Statistician in collaboration with the Lead Statistician.

SUMMARY OF THE TRIAL

Originally, a single arm phase II trial in which 36 microsatellite stable colorectal cancer (MSS CRC) patients with Class II expression were to be recruited to receive nivolumab infusions every four weeks for up to two years, until progressive disease is identified or unacceptable toxicities occur. The trial was planned to continue until all patients have been followed up for a minimum of 18 months after registration. Following the trial closed to recruitment on 06-Sep-2021 as suggested by Trial Steering Committee (TSC), Trial Management Group (TMG) agreed that the final analysis should be performed when the primary outcome measure at week 27 is obtained for the ongoing patients. The trial will use a Bayesian analysis to estimate the durable clinical benefit rate and other clinically relevant signal of effects in patients treated with the single agent nivolumab to justify further investigation in subsequent studies.

2. TIMING AND REPORTING OF INTERIM AND FINAL ANALYSES

2.1 INTERIM ANALYSES

The Bayesian adaptive trial design allows interim analysis of the primary outcome at any point in the trial but a formal interim analysis is planned after 18 per protocol patients (defined in section 5.1) have been recruited into the single-arm trial and reached their third scan at approximately 27 weeks since treatment start date. Early stopping for efficacy will not be considered. Decisions to stop recruitment early for futility will be based on the posterior probability distributions for all outcome measures at that time. The decision criteria at interim analysis for the primary outcome measure is specified in section 8.4. Secondary outcome measures will also be considered and contribute to this decision. Decision to stop early will be made in consultation with the TSC. Recruitment will not be suspended whilst interim analyses are being performed and TSC is reviewing the data.

Informal interim analysis will be conducted at least annually for the purposes of the TSC. The TSC report will include (when appropriate) but is not limited to: recruitment, CRF return rates, data quality, protocol deviations, patient withdrawals, patient characteristics, treatment compliance, primary and secondary outcome measures and adverse events. The TSC may consider closing the trial early or request more frequent meetings if data quality is deemed unacceptable or if there are safety concerns. An emergency meeting may also be convened if a safety issue is identified.

2.2 FINAL ANALYSES

Originally, the final analysis would be performed after 36 per protocol patients had reached their third scan at approximately 27 weeks since treatment start date and after a minimum follow-up of 18 months since registration date. At the final analysis, the trial would recommend further research if the efficacy criteria based on the primary

outcome measure (see section 8.4) was met. Secondary outcome measures would also be considered and contributed to this decision.

As suggested by TSC, the trial closed to recruitment on 06-Sep-2021 based on the responses of the first 18 patients. Some patients were still on treatment at the interim analysis. It was agreed by TMG that the final analysis will be performed when the ongoing patients have their week 27 primary outcome measure available.

We will seek to conduct and distribute for publication the final analysis within 12 months of the final protocol assessment of the final patient.

3. RECRUITMENT

3.1 RECRUITMENT

Total number of patients screened will be provided. Number of patients recruited to the main trial will be reported by month with an average monthly recruitment rate and by site. The following information will be provided (if relevant to the purpose of analysis):

- date first site opened for recruitment
- date first patient recruited
- date of the snapshot on which analysis is based
- date trial closed (when relevant)
- number of sites open to recruitment and in set up

Data sources: Screening Registration Form and Trial Entry Form.

3.2 RANDOMISATION

This is a single arm trial so no randomisation is required for this trial design.

3.3 INELIGIBLE PATIENTS

Ineligible patients: defined as patients recruited to the main trial who are subsequently found to not meet the eligibility criteria of the trial. The number (and proportion) of ineligible patients and reasons for their ineligibility will be reported. There are two types of ineligible patients:

- Inadmissible ineligibles:

Patients whose reasons for ineligibility enhance the possibility of toxicity (and hence withdrawal) or could affect any component of the pharmacokinetics of the drug (i.e. absorption, distribution, metabolism and excretion) are called inadmissible ineligibles. As this is an early phase II trial looking for signals of drug activity, these patients will be excluded from all analyses and if possible replaced by additional patient recruits.

- Admissible ineligibles:

Patients whose reasons for ineligibility are deemed not likely to influence the evaluability of treatment effect are called admissible ineligibles and they will be included in the analysis.

3.4 WITHDRAWAL OF CONSENT

Patients are entitled to withdraw consent from the trial at any time. There are three possible levels of withdrawal:

• 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)

The number of patients who have withdrawn consent will be reported, together with

- type of withdrawal and reason for their withdrawal (if stated)
- timing of their withdrawal from the date they registered and/or started treatment
- the number of cycles of treatment received before withdrawal
- which category of population for analysis that the patient belongs to, as defined in section 5.1.

Patients who withdraw from trial treatment before completing 1 cycle of protocol treatment are defined as 'non-treated' patients and are excluded from the per protocol population for analysis (see Section 5.1).

4. DATA QUALITY

4.1 RETURN RATE FOR EACH CRF FORM

This trial will utilise Electronic Case Report Forms (eCRFs) to collect all required study data. The remotely entered data will be reviewed by the trial co-ordinator (or delegate) and queries raised where appropriate. Data quality will be measured by the following key indicators:

CRFs return rates (except the ad-hoc forms): Return rates for each CRF type will be calculated as a proportion of the number of each type of CRF expected at that time point. They will be reported by site.

Completion rates for the outcome variables will be investigated at least annually in accordance with the current version of the statistical data validation plan. Any missing data or forms will be brought to the attention of the trial coordinator for resolution.

4.2 LENGTH OF PATIENT FOLLOW-UP

Patients are expected to be followed up every 4 weeks for 6 months and then every 12 weeks until disease progression or end of follow-up, whichever is earlier. Patients lost to follow-up will not be excluded from the analysis but will be censored at the appropriate date. The number of alive patients lost to follow-up will be reported. The median length of follow up for alive patients will be reported and a reverse Kaplan Meier will be carried out if sufficient number of patients have been recruited.

Quality of patient follow-up is assessed using the following measures:

- For those being followed up for response
  - Individual's length of time from last CT/MRI scan to date of data snapshot
  - median, minimum and maximum for follow-up time specified above
- For those being followed up for disease progression
  - Individual's length of time from date last seen progression-free to date of data snapshot
  - median, minimum and maximum for follow-up time specified above
- For those being followed up for death
  - Individual's length of time from date last seen alive to the date of data snapshot
  - median, minimum and maximum for follow-up time specified above

5. TRIAL POPULATION

5.1 DEFINITION(S) OF POPULATIONS FOR ANALYSIS

Intention-to-treat population (ITT): All patients registered for the main trial (including all ineligible and non-treated patients). Non-treated patients are defined as patients who did not receive/complete 1 cycle of the protocol treatment. Inadmissible ineligible and non-treated patients will be replaced by new recruits if possible. Numbers in the ITT population will be reported in the recruitment section and be used as the population to report AEs and SAEs but no analysis of efficacy outcome measures will be undertaken on this population.

Per-Protocol population (PP): All eligible and admissible ineligible patients registered for the main trial who have received at least 1 cycle (4 weeks) of protocol treatment. This is the primary population for analysis of primary and secondary outcome measures. If they fail to reach their third CT/MRI scan, for instance, disease progression/death or withdrawal (from all trial data collection) before week 27, the patient will be included in the PP population and considered as a "non-responder".

5.2 BASELINE PATIENT CHARACTERISTICS

Descriptive analysis of baseline characteristics for PP population will be presented. Mean, median, minimum, maximum and interquartile ranges will be presented for continuous variables and number and percentage for categorical variables.

6. TREATMENT RECEIVED

Descriptive statistics of treatment received by PP patients will be reported, including:

- Time from registration to first treatment
- Number and % of patients started, by cycle/week
- Number of and size of dose reductions, by cycle/week, plus reasons
- Number of and size of treatment delays, by cycle/week, plus reasons
- Number of and size of treatment interruptions, by cycle/week, plus reasons
- Number and % of patient completed, by cycle/week
- (Relative) dose intensity, by cycle/week

Swimmer plots will be used to graphically represent the treatment received by each patient and will include details of the CT/MRI scans in relation to treatment.

7. TOXICITY AND SAFETY ANALYSIS

Descriptive statistics of adverse events experienced by ITT patients will be reported including:

- Number of AEs by grade (including AEs involved in SAEs) - overall and by type
- Number of adverse reactions (ARs) by grade - overall and by type
- Number of patients experiencing at least one AE by highest grade reported - overall and by type
- Number of patients experiencing at least one AR by highest grade reported - overall and by type
- Number of Serious Adverse Events (SAEs) and Serious Adverse Reactions (SARs)
- Line listings of SAE details (provided by Trial Coordinator)

Incidence of AEs over time may be reported if relevant.

In addition to adverse event data, other abnormal clinical data will be collected for each patient depending on the clinical requirements, including clinical chemistry, haematological, thyroid function and cortisol tests, physical exam, vital signs and ECOG performance status. No hypothesis testing will be conducted using this data, but descriptive summaries will be reported as appropriate.

8. ANALYSIS

8.1 DEFINITION AND CALCULATION OF OUTCOME MEASURE

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/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. If a patient fails to reach their third CT/MRI scan due to disease progression/death or withdrawal (from all trial data collection) before week 27, they will be treated as “non-DCB”.

SECONDARY OUTCOME MEASURES:

- **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), Not Evaluable (NE), 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.

- **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.

- **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.

- **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 a RECIST outcome that was not progression.

- **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.

8.2 DESCRIPTIVE ANALYSES

Summary statistics will be calculated on the population as described below:

- DCB rate will be calculated as the proportion of PP patients who have experienced a DCB
- OR rate will be calculated as the proportion of PP patients who have experienced a confirmed CR or PR as their best overall response
- Waterfall plots will be used to graphically represent the PCDS for each patient ranked in descending order. Means, medians, inter-quartile ranges and ranges will be used to summarise the outcome measure.
- For TTMR, PFS and OS, Kaplan-Meier curves will be used to graphically represent the outcome measures. Median survival time and survival rates at landmarks will be reported.

8.3 BAYESIAN ANALYSIS OF OUTCOME MEASURE

The trial will use a Bayesian analysis to determine a clinically relevant signal of effect of nivolumab in MSS CRC population. For the primary outcome measure, 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 binary outcome measures will use a Beta-Binomial conjugate analysis whilst the time-to-event outcome measures will use an exponential-inverse-gamma conjugate analysis (Thall et al 2005). The primary analysis will be based on minimally-informative priors but, where appropriate, secondary analysis with informative priors may be carried out to incorporate other relevant information external to the data.

Posterior probability distributions will be plotted. Summary statistics from the posterior probability distribution will be reported and displayed, in particular the mean/median (representing point estimates), standard deviation (representing the standard error) and 95% credible intervals.

8.4 DECISION CRITERIA

Formal decision-making is planned at one interim analysis and at the final analysis. Decision-making for early stopping for futility and final go/no go for efficacy warranty of further research will be based on the posterior probability distribution for the primary outcome measure. 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)**. The efficacy criteria will not be considered at interim analyses. A decision to stop early will be made in consultation with the TSC.

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

8.5 SAMPLE SIZE AND OPERATING CHARACTERISTICS

Target sample size is 36 per protocol (PP) patients for the final analysis and 18 PP patients for interim analysis. With an expected prevalence of around 10% this will require screening of approximately 360 patients. Inadmissible and non-treatment patients might be replaced by new recruits. In addition, there may be Per Protocol (PP) patients whose responses are not evaluable for the outcome measures. The TMG agreed that whether or not increasing target recruitment is needed should be assessed at the interim analysis (18 PP patients).

Operating characteristics were evaluated for the decision criteria specified in Section 8.4. At this design stage, a non-informative Beta(1,1) prior was used. The current standard treatment in these patients is likely to give a DCB rate of 30%; therefore 30% has been chosen as the critical threshold value. Sample size was chosen to minimise the chance of false positive conclusions (in particular making a GO decision when the true DCB rate is less than 30%) and maximise the chance of true positive conclusions (in particular, making a GO decision when the true DCB rate is greater than 30%). Operating characteristics of the design with an interim sample size of 18 and final sample size of 36 satisfy the above-mentioned requirements. The operating characteristics that are calculated using exact binomial probabilities are given in table below. More specifically, the table below shows that there is a 9% chance of incorrectly recommending further research when the true DCB rate is 20% (i.e., chance of false positive conclusions that is equivalent to a type I error rate) and 91% chance of correctly recommending further research when the true DCB rate is 40% (i.e., chance of true positive conclusions that is equivalent to power).

Table 2: Operating characteristics of the trial design with decision criteria specified in Section 8.4

	True DCB=10%	True DCB=20%	True DCB=30%	True DCB=40%	True DCB=50%
P(STOP early)	0.734	0.271	0.060	0.008	0.001
P(STOP at final)	0.266	0.641	0.409	0.084	0.005
P(GO at final)	0.001	0.088	0.531	0.908	0.994

8.6 SUBGROUP ANALYSIS

MHC class II (%) and liver metastasis are considered important factors associated with the outcome. A subgroup analysis for MHC Class II expression using 5% cut off (≥ 5% vs < 5%) will be conducted. This will be used to investigate if a patient with higher MHC Class II expression will be more likely to response to the PD-1 inhibitor nivolumab.

Liver is regarded as the metastatic site with the worst prognosis. It is hypothesised that outcome for patients with and without liver metastasis will be different. A subgroup analysis for liver metastasis (yes/no) will also be performed to investigate the association of liver metastasis and the efficacy of nivolumab.

These will be exploratory, descriptive analyses (without a formal testing between groups) due to small sample size.

9. STATISTICAL SOFTWARE

Statistical calculations will be performed using Stata or R. The software including version number used to conduct the analysis will be documented on all reports and publications.

10. STORAGE AND ARCHIVING

Data snapshots, statistical programmes and report used in interim and final analysis will be stored here:

S:\Stats\Shared\Trials Work\EDD\ANNICA-CLASSII\

In subfolders named according to the purpose and date.

11. REFERENCES

[1] PF Thall, LH Wooten and NM Tannir. Monitoring event times in early phase clinical trials: some practical issues. *Clin Trials* (2005) 2; 467