CANCER RESEARCH UK & UCL CANCER TRIALS CENTRE
UCL Cancer Institute

VEROnA

A window of opportunity study of vandetanib-eluting radiopaque beads (BTG-002814) in patients with resectable liver malignancies

Protocol Number:	BTG-002814-01

STATISTICAL ANALYSIS PLAN

Date finalised	**16 May 2019**
Version number	**1.0**
Prepared by	**Allan Hackshaw, Ricky Sharma, Laura Beaton**

Signed: Allan Hackshaw

Contents

1. Summary description of the trial

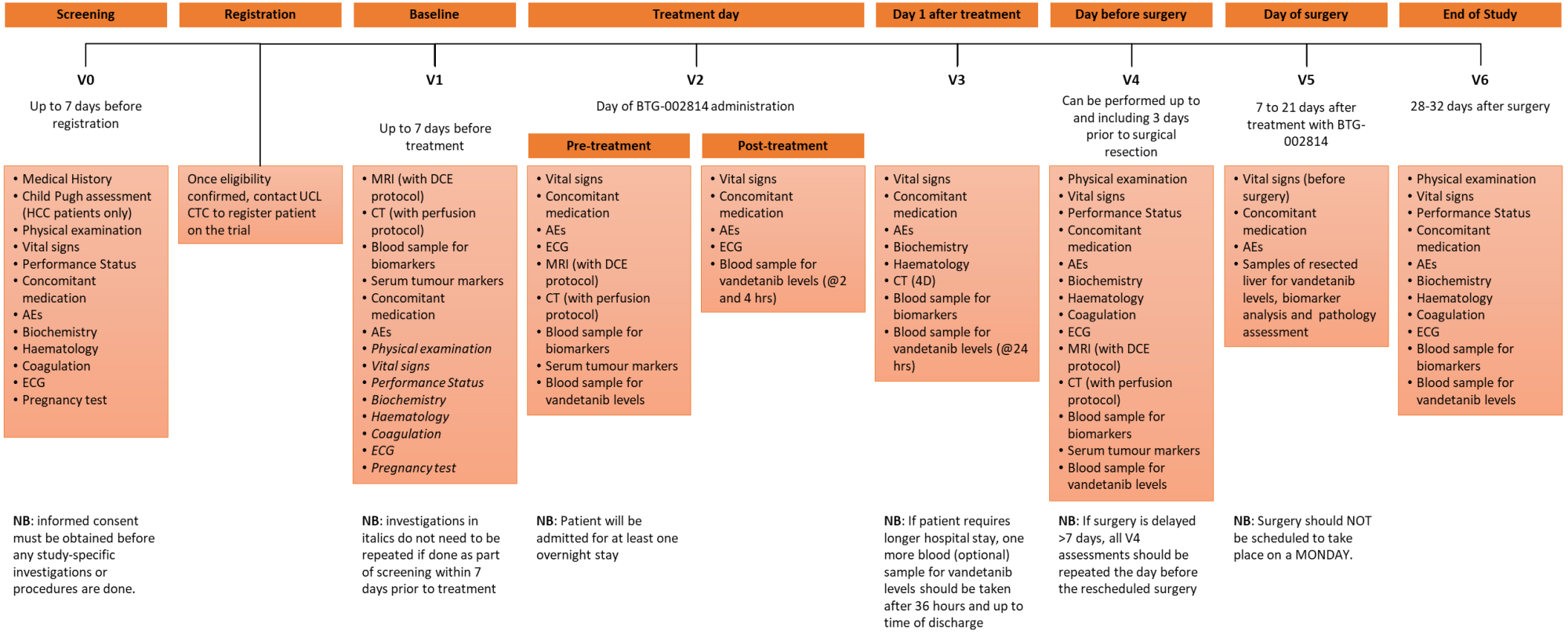
Sample size	A target of 12 patients
Intervention arms (what treatments were given)	BTG-002814 (vandetanib-eluting radiopaque embolic beads), given 7-21 days before surgery.
Primary trial objectives	Primary objectives: • To assess the safety and tolerability of treatment with BTG-002814 • Measure the plasma and resected liver tissue concentrations of vandetanib and the N-desmethyl metabolite following treatment with BTG-002814
Primary outcome measures	• Adverse events (AEs) related to treatment with BTG-002814 using the standardised grading criteria (National Cancer Institute- Common Terminology Criteria for Adverse Events- Version 4.0 (NCI-CTCAE v4.0)) • Concentration of vandetanib and N-desmethyl vandetanib in plasma and in resected liver tissue following treatment with BTG-002814.

SAP developed according to protocol version 6, 03 December 2018

The analyses will be performed using SAS (likely version 9.4).
Missing data in the analyses below will be indicated as such in tables (there will be no imputation).

Secondary objectives
• Evaluate the anatomical distribution of BTG-002814 on non-contrast enhanced imaging using 4D CT • Evaluate histopathological features in the surgical specimen following treatment with BTG-002814 • Assess changes in blood flow on dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) following treatment with BTG-002814
Secondary outcomes measure
• Distribution of BTG-002814 on non-contrast enhanced imaging of tumour vasculature and regions of interest using 4D CT • Evaluation of histopathological features in the surgical specimen (malignant and non-malignant liver tissue): tumour necrosis, viable tumour, vascular changes • Changes in blood flow on DCE-MRI following treatment with BTG-002814. The following parameters will be derived from DCE-MRI images: K_{trans}, K_{ep} and V_e
Exploratory Objectives and Outcomes
• Study blood biomarkers with the potential to identify patients likely to respond to treatment with BTG-002814 • Study tissue biomarkers to explore key immune, inflammatory and drug related mechanisms • Correlate the distribution of BTG-002814 on imaging with pathology by 3D modelling

Study Schema



2. Baseline characteristics

Table showing the number of patients (categorical variables) or the mean/median and range (for continuous variables), for:

- Gender, age, ethnicity, ECOG performance status.
- HCC or colorectal cancer
- For HCC, state whether histological or radiological diagnosis
- Location (liver segments) and characteristics of the tumour: number of liver lesions, sum of longest diameters (mm).
- Vital signs: blood pressure, weight, height, heart rate, ECG results (including QTc interval)
- Key baseline laboratory measures: Hemoglobin, absolute neutrophil count, platelets, serum bilirubin, serum creatinine, liver function tests, and others agreed by the investigators (from the screening case report form Visit 0).
- Child-Pugh score and serum alpha-fetoprotein level (HCC patients only)
- Tumour markers (colorectal cancer paitents only): CEA, CA19-9, CA125
- Previous systemic treatments for cancer

Provide in the text or CONSORT diagram:

- The number of patients who were ineligible, and the reasons why they were ineligible
- The number of patients who were recruited to the trial but withdrew later on, and the reasons (if available)
- List any possible major protocol violations or ineligibility criteria that would lead to the patient not being included in the analysis. This information is not in the main database but would be summarised in a table.

3. BTG-002814 treatment

Assessments on treatment day

Measurements	Mean/median, and range, and standard deviation
Before BTG-002814 treatment (absolute values)	
Blood pressure	
Heart rate	
ECG QTc interval (state method used for calculation)	
ECG results (descriptive)	
After BTG-002814 treatment (but use the change/difference from the pre-treatment levels)	
Blood pressure	
Heart rate	
ECG QTc interval	
ECG results (descriptive)	

Delivery of BTG-002814

Measurements	
Volume of drug delivered (mL)	Mean/median, and range
Full volume of drug delivered: yes or no	Percentage
Full volume of drug not delivered- reasons	Percentages
Stasis reached	
Complication	
State other reasons	
Total time between start of 1st infusion and end of the last infusion (minutes)	Mean/median, and range
Number of infusions over all patients	Mean/median, and range
Radiation dose-length product (mGy-cm)	Mean/median, and range
Any TACE-related problems: yes or no	Percentage
Different TACE-related problems	Percentages

4. Primary endpoints

4.1 Safety

All adverse events will have severity graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0
This will be based on combining all the AEs, SAEs and SUSARs, and any other source of adverse events (e.g. unscheduled bloods case report form).

For each type of event, for each patient, the maximum grade will be obtained.

4.1.1 Number of patients with each type of event (each patient only counted once on each row)

Event Term	Grade 1	Grade 2	Grade 3	Grade 4
Abdominal pain				
Anorexia				
Ascites				
Back pain				
Bronchospasm				
Cardiac disorders				
Constipation				
Cough				
Diarrhea				
Dry mouth				
Dyspepsia				
Dyspnea				
Electrocardiogram QT corrected interval prolonged				
Fatigue				
Flu like symptoms				
Gastritis				
etc				

- Several versions of the above table:

AEs post-TACE:
- AEs on the day after BTG-002814 treatment only
- AEs after BTG treatment and up to (but not including) the day of surgery
- AEs after BTG treatment and up to (but not including) the day of surgery, which are considered to be related to vandetanib
- AEs after BTG treatment and up to (but not including) the day of surgery, which are considered to be related to TACE

AEs post Surgery:
- AEs only occurring after surgery (up to the end of the 32-day follow up)
- All AEs occurring at any time from the delivery of BTG treatment

4.1.2 Number of events with each type of event (a patient can be counted more than once on each row if they experienced the same event several times)

Event Term	Grade 1	Grade 2	Grade 3	Grade 4
Abdominal pain				
Anorexia				
Ascites				
Back pain				
Bronchospasm				
Cardiac disorders				
Constipation				
Cough				
Diarrhea				
Dry mouth				
Dyspepsia				
Dyspnea				
Electrocardiogram QT corrected interval prolonged				
Fatigue				
Flu like symptoms				
Gastritis				
etc				

- Several versions of the above table:

AEs post-TACE:

- AEs on the day after BTG treatment only
- AEs after BTG-002814 treatment and up to (but not including) the day of surgery
- AEs after BTG-002814 treatment and up to (but not including) the day of surgery, which are considered to be related to vandetanib
- AEs after BTG-002814 treatment and up to (but not including) the day of surgery, which are considered to be related to TACE

AEs post Surgery:

- AEs only occurring after surgery (up to the end of the 32-day follow up)
- All AEs occurring at any time from the delivery of BTG treatment
- For any grade 3 or 4 event, look at time to the first occurrence of the grade 3/4 event (BTG treatment day is Day 0): do scatter plot of these

4.1.3 Examine which grade 3 or 4 AEs that occur any time after BTG-002814 delivery were already present at baseline in the patient (i.e. pre-treatment).

Descriptively, provide summary of which had got worse.

4.2 Drug concentrations

These analyses will be performed by BTG.

4.2.1 Plasma

For each patient, obtain the plasma time-concentration curve for:

- vandetanib
- N-desmethyl metabolite

Each plot would be indicated as to whether the patient had HCC or mCRC.

Time points are:

- Day of BTG treatment but before administration (0 hours)
- 2 hours post-BTG treatment
- 4 hours post-BTG treatment
- 24 hours post-BTG treatment
- 3 days to1 day prior to surgery
- 28-32 days post-surgery.

PK parameters such as Tmax (time taken to reach the highest concentration) and Cmax (the maximum concentration) could be obtained, and use summary statistics to describe these. Other standard PK parameters can be included (e.g. AUC to 32 days, and AUC to the end of the observation time period), as determined by the investigators, such as $T_{1/2}$, clearance CL/F, and volume of distribution (V_z/F).

4.2.2 Resected tissue

For each patient, provide a concentration-time plot for:

- vandetanib
- N-desmethyl metabolite

(concentration as the y-axis and time as the x-axis).
Each plot would be indicated as to whether the patient had HCC or mCRC.

This will be for:

- malignant tissue – three measurements for each patient:
  - Centre of tumour
  - Mid tumour
  - Edge of tumour
- non-malignant tissue
  - 1 cm from the edge of the tumour
- paired samples (difference, per patient, using malignant (based on the average of the 3 locations/samples) minus non-malignant values). Can be analysed using a t-test or non-parametric test
- Describe technical reasons for missing data points

5. Secondary objectives/outcomes (other blood, tissue and imaging markers)

5.1 Distribution of BTG-002814

The distribution of BTG-002814 within the tumour vasculature and regions of interest will be assessed on non-contrast enhanced imaging. The findings will be summarised descriptively, and HCC or mCRC will be indicated.

5.2 Histopathology of resected liver

An evaluation of histopathological features in both malignant and non-malignant liver tissue from the surgical specimen will be performed. The extent of tumour necrosis, viable tumour and any vascular changes observed will be summarised descriptively.

5.3 3D modelling

Associations between the distribution of BTG-002814 on imaging and within the pathological specimen will be described using 3D modelling, and HCC or mCRC will be indicated.

5.4 Blood flow following embolisation

Assessment of changes in blood flow will be assessed using DCE-MRI following treatment with BTG-002814. For individual patients, the following parameters will be derived from DCE-MRI scans at visits 1 and 2 (pre-treatment) and visit 4 (post-treatmemt), and HCC or mCRC will be indicated.

Dual-input single-compartment model

- Arterial flow (ml/min/100g)
- Portal venous flow (ml/min/100g)
- Arterial fraction (%)
- Mean transit time (seconds)
- Distribution volume (%)

Tofts model

- Volume transfer constant between plasma and extravascular extracellular space - K^{trans} (min^{-1})
- Volume transfer constant between extravascular extracellular space and plasma - K_{ep} (min^{-1})
- Volume of extravascular extracellular space per unit volume of tissue - V_e (%)

Semi-quantitative analysis

- Time-to-peak – TTP (seconds)
- Area under curve at 60 seconds (mmol/L.s)
- Peak Gadolinium concentrations – C_{peak} (mmol/L)
- Upslope - C_{peak}/TTP (mmol/L.s)

For each patient, perfusion parameters for visit 1 and 2 will be used to estimate intra-patient reproducibility. Visit 4 parameters from each patient will be used to measure potential changes following treatment with BTG-002814, in the context of the baseline reproducibility for each patient, and HCC or mCRC will be indicated.

The same principles will be applied to perfusion CT parameters shown below.

Maximum slope method:

- Arterial flow (mL/min/100ml)
- Portal flow (mL/min/100ml)
- Perfusion index

Patlak model:

- Blood volume (ml/ml tissue)
- Arterial perfusion (mL/min/100ml)
- Flow extraction product (ml/min/100ml)

For changes observed within each patient, data from DCE-MRI will be compared to changes observed in perfusion CT at the same timepoints to confirm the veracity of potential changes observed in blood flow.

5.5 Blood/serum biomarker analysis

The following serum biomarkers will be measured at baseline (before BTG treatment) and at several time points afterwards (i.e pre-treatment (visit 2), Day 1 after treatment (visit 3), pre-surgery (visit 4) and end of study (visit 6):

- Levels of blood biomarkers (cytokines, chemokines and growth factors relevant to cancer and inflammation).
- Levels of serum alpha-fetoprotein (AFP) in patients with HCC.
- Levels of serum CEA, CA19-9 and CA125 in patients with mCRC.

Time-concentration plots for each biomarker will be obtained for each patient. Each plot would be indicated as to whether the patient had HCC or mCRC.

Changes in levels (last recorded value minus the baseline value, for each patient) summarized as scatter plots, with mean/median and range of the change.

Non-parametric tests to be used for biomarkers that appear to show large changes.

6. Tumour pathology after surgery (of the resected tissue)

6.1 Tumour characteristics

Pathology outcome	Number of patients (%)
HCC patients	
T-stage	
N-stage	
M-stage	
Histologic grade (G1-4)	
Resection (R0, R1, R2)	
Distance to nearest resection margin Median (range)	
Tumour necrosis (%) Median (range)	
Viable tumour (%) Median (range)	
Vascular changes	
Colorectal cancer patients	
Resection margins (R0, R1, R2)	
Grade (G1, G2, G3, G4)	
Tumour necrosis (%) Median (range)	
Viable tumour (%) Median (range)	
Vascular changes	

6.2. Tumour size/measurement

- The longest diameter of each lesion (mm) is measured at baseline and again on the day before surgical resection.
- The sum of the diameters can be taken for each patient, using the same lesions.
- Produce a waterfall plot showing the change in the sum from baseline to pre-surgery.
- Change in tumour size can also be compared using a paired t-test or Mann-Whitney test, depending on whether Normally distributed or not.
- Waterfall plot obtained for all patients, then separately for HCC and mCRC.

7. Concomitant Medications

Patient ID	Drug name/type	Time (days) from day of BTG treatment to start of non-trial drug
1	A	
1	B	
1	C	
2	D	
2	E	
2	F	

8. Follow up and deaths

	All patients	HCC	mCRC
	n (%)	**n (%)**	**n (%)**
No. patients who died			
Cause of death			
No. patients who dropped out/withdrew from the trial			
Reasons for withdrawal			
Lost to follow up			

9. Other Safety Parameters

Other safety parameters such as ECG, vital signs and physical examinations will be summarised

For laboratory paramaters, observed values, changes from baseline, shifts in CTC grade and normal values/above and below normal values will be summarised with descriptive statistics. A listing of individual laboratory data will be provided with values outside normal ranges flagged.