Phase II Randomised, Double Blind, Placebo Controlled Trial of Neoadjuvant Artesunate in Stage II/III Colorectal Cancer (NeoART-M trial)

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Signature Page and Statement

The Chief Investigator (CI) and the Sponsor representative have discussed this protocol version. The investigators agree to perform the investigations and to abide by this protocol except in the case of a medical emergency or where departures from the protocol are mutually agreed in writing.

The investigators agree to conduct the trial in compliance with the approved protocol, Malaysian Good Clinical Practice and National Pharmaceutical Regulatory Agency (NPRA) Regulations for Clinical Trial Import License, the Malaysian Data Protection Act, local governance policy, the Sponsor's Standard Operating Procedure (SOPs), and other regulatory requirements as amended.

This protocol has been written in accordance with the Sponsor's procedure and is intended for use at Malaysian sites only.

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Acknowledgements and Protocol contributories

Professor Sanjeev Krishna and Professor Devinder Kumar (St George's, University of London) conceived the study; Professor Krishna, Professor Kumar, Professor Thomas Efferth (University of Mainz), Professor Peter Kremsner (University Hospital Tübingen), Dr Yolanda Augustin (St George's, University of London), Debbie Rolfe (St George's, University of London), and Nia Al-Samarrai (St George's Hospital) assisted with trial design. Dr Irina Chis Ster (St George's, University of London) provided statistical expertise in clinical trial design and is conducting the statistical analysis. All authors contributed to refinement of the study protocol and approved the final manuscript.

Contents

1. List of abbreviations

AE	Adverse Event
AR	Adverse Reaction
ATM/ATR	Ataxia telangiectasia mutated/Ataxia telangiectasia and Rad3-related protein
ATP	Adenosine triphosphate
AUC	Area under the curve
BMI	Body Mass Index
CA	Competent Authority
CADT	Centre for Affordable Diagnostics and Therapeutics
Cape-OX	Capecitabine-Oxaliplatin
CEA	Carcinoembryonic Antigen
CI	Chief Investigator
Chk1/Chk2	Checkpoint kinase 1/Checkpoint kinase 2
Cip/Kip	CDK interacting protein/Kinase inhibitory protein
CD31	Cluster of Differentiation 31
CRC	Colorectal Cancer
CRC	Clinical Research Centre
CRF	Case Report Form
CRM	Clinical Research Malaysia
CT	Computed tomography
CTA	Clinical Trial Authorisation
CTC	Common Toxicity Criteria
CTIMP	Clinical Trial of Investigational Medicinal Product
DHA	Dihydroartemisinin
DNA	Deoxyribonucleic acid
DDSB	DNA double–strand breaks
DDR	DNA damage response
DMC	Data Monitoring Committee
DSUR	Development Safety Update Report
EGFR	Epidermal Growth Factor Receptor
eGFR	Estimated Glomerular Filtration Rate
EUCTD	European Clinical Trials Directive
EudraCT	European Clinical Trials Database
JePEM	Jabatan Etika Penyelidikan Manusia USM
FAK	Focal Adhesion Kinase
FDT	First Definitive Treatment
5FU	Fluorouracil
FOLFIRI	Fluorouracil-Irinotecan
FOLFOX	Fluorouracil-Oxaliplatin
GCP	Good Clinical Practice
GCSF	Granulocyte colony-stimulating factor
GFR	Glomerular Filtration Rate
g/l	grams per litre
GP	General Practitioner
Hb	Haemoglobin

HIF-1α	Hypoxia induced factor - 1α
HTA	Human Tissue Authority
IB	Investigator Brochure
ICF	Informed Consent Form
ID	Identification
IIT	Investigator Initiated Trial
IMP	Investigational Medicinal Product
IP	Investigational Product
IP	Intellectual Property
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Trial Number
ITT	Intention to treat
KG	Kilogram
KRAS	Kirsten rat sarcoma viral oncogene homolog
LFT	Liver Function Test
MA	Marketing Authorisation
MAPK	Mitogen-activated protein kinase
MG	Milligram
ML	Millilitre
MMPs	Metalloproteinases
MMR	Mismatch Repair
MoU	Memorandum of Understanding
MOH	Ministry of Health
MOHE	Ministry of Higher Education
MRI	Magnetic Resonance Imaging
MS	Member State
NCAM	Neural cell adhesion molecule
NCI	National Cancer Institute
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHS	National Health Service
NIMP	Non- Investigational Medicinal Product
NoK	Next of Kin
NPRA	National Pharmaceutical Regulatory Agency
OD	Once daily
PBMC	Peripheral blood mononuclear cell
PDGF	Platelet-derived growth factor
PFS	Progression free Survival
PI	Principal Investigator
PIS	Participant Information Sheet
Plts	Platelets
PO	Oral administration
pRb	Retinoblastoma protein
QA	Quality Assurance
QC	Quality Control
QOL	Quality of Life
QP	Qualified Person
R&D	Research & Development

RCT	Randomised Control Trial
REC	Research Ethics Committee
ROS	Reactive Oxygen Species
RSI	Reference Safety Information
SAR	Serious Adverse Reaction
SAE	Serious Adverse Event
SDV	Source Document Verification
SGUL	St George's, University of London
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SSAR	Suspected Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
UM	University of Malaya
TF	Transferrin
TIMP	Tissue inhibitors of metalloproteinase
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee
TTP	Time to progression
ULN	Upper limit of normal
VEGF	Vascular endothelial growth factor
WCC	White Cell Count
WHO	World Health Organisation

2. Roles and Responsibilities

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emergency number: +60 (0) 17 2152964

Trial Management Group (TMG):

Membership will consist of personnel involved in daily operational issues in the management of the trial and will act upon advice/recommendations received by the sponsor and/or the Data Monitoring Committee.

Membership: Professor Sanjeev Krishna, Dr Yolanda Augustin, Dr Nafeesa Mat Ali, Professor Devinder Kumar, Sponsor's Metanoic Health Limited representative.

Trial Steering Committee (TSC):

Membership will consist of personnel directly involved in the trial and/or independent individuals. The TSC will concentrate on the progress of the trial in relation to protocol compliance and review of any participant safety considerations including the review of any recommendations made by the Data Monitoring Committee if relevant to the trial and to advise the Sponsor of any decisions.

Membership:
Chair – Mr Clive Whitehouse
Independent expert – Professor Vikram Mathews
Co-Investigator – Mr Nirooshan Rajendran, Professor Thi Thirumalaisamy P Velavan
Lay persons – Mr Duncan McNair, Mr Clive Whitehouse

Data Monitoring Committee (DMC):

An independent committee established by the Sponsor to assess at intervals the progress of the clinical trial, the safety data and the critical efficacy end points and to recommend the Trial Steering Committee whether to continue, modify or stop the trial.

Membership; Professor Jack Cuzick, Professor Ajit Lalvani, Dr Pan Pantziarka

3. Study Synopsis

Brief Title	NeoART - M Trial
Official title:	Phase II Randomised, Double Blind, Placebo Controlled Trial of Neoadjuvant Artesunate in Stage II/III Colorectal Cancer
Brief Summary	This is a Phase II randomized, double-blind, placebo-controlled trial of neoadjuvant artesunate given orally as a dose of 200 mg once a day for 14 days to patients with histologically confirmed Stage II/III colorectal cancer (CRC) awaiting surgical treatment with curative intent.
Sponsor reference number:	2023-MHL-001
Public database Trial identifier number	Clinicaltrials.gov NCT02633098
Study type & Phase	Phase II Clinical Trial of an Investigational Medicinal Product (CTIMP)
Study Design	Prospective, randomised, double blind, placebo controlled
Study Population	Patients with colorectal cancer suitable for surgical resection
Condition	Stage II/III colorectal cancer
Study Group/cohort (s)	Patients with Stage II/III colorectal cancer planned for curative surgery
	Inclusion criteria : 1. Aged 18 or over 2. Histologically proven single primary site colorectal adenocarcinoma or high grade dysplasia plus unequivocal radiological evidence of invasive cancer 3. Stage II/III colorectal cancer planned for surgical resection and no clinical indication for neoadjuvant preoperative chemotherapy/chemoradiation therapy 4. WHO performance status 0,1 or 2 5. Adequate full blood count: White Cell Count (WCC) >3.0 x 10^9 /l; Platelets >100 x 10^9/l; Haemoglobin (Hb) >80g/L 6. Adequate renal function :

	Glomerular Filtration Rate >30ml/min by Cockcroft-Gault formula 7. Adequate hepatobiliary function : Total bilirubin < 3 x Upper limit normal 8. Female participants of child bearing potential must have a negative pregnancy test < 72 hours prior to initiating study intervention and agree to avoid pregnancy using adequate, medically approved contraceptive precautions for up to 6 weeks after the last dose of study treatment intervention 9. Male participants with a partner of childbearing potential must agree to use adequate, medically approved contraceptive precautions during and for up to 6 weeks after the last dose of the study treatment intervention 10. Patient able and willing to provide written, informed consent for the study
	Exclusion criteria: 1. Contraindication to the use of artesunate due to hypersensitivity 2. Pregnancy or lactation 3. Male or female participants unwilling to use an effective method of birth control (either hormonal in the form of the contraceptive pill or barrier method of birth control accompanied by the use of a proprietary spermicidal foam/gel or film); or agreement of true abstinence from time consent is signed until 6 weeks after the last dose of study treatment intervention (i.e. withdrawal, calendar, ovulation, symptothermal and post ovulation methods are not considered acceptable methods) 4. History of immunosuppression 5. History of hearing or balance problems 6. Weight < 52 kg or > 110 kg 7. Other planned intervention, apart from standard of care 8. Any other malignant disease diagnosis within the preceding 2 years with the exception of non-melanomatous skin cancer and carcinoma in situ 9. Lactose intolerance
Target number of participants:	200 patients in total The study will be run in 3 countries with the following recruitment targets:

	1.NeoART - Malaysia: 120 patients 2. NeoART - UK: 34 patients 3. NeoART -Vietnam: 46 patients
Criteria for evaluation:	Primary outcome measure : The primary outcome measure is recurrence free survival 2 years after surgery.
	Secondary outcome measure(s) : • Recurrence free survival at 5 years • Overall survival at 2 and 5 years • Colon cancer specific death at 2 and 5 years • Artesunate drug related toxicity • Adverse events • Pathological assessment of tumour regression (involvement of lymph nodes ; serosa ; resection margin) • Quality of life (assessed using validated EuroQol EQ-5D, EORTC QLQ C-30 and EORTC QLQ CR29 quality of life tools) • Surgical morbidity/mortality • Predictive value of tumour biomarkers in terms of predicting response to artesunate therapy • Biomarker and molecular profiling studies
Sources of funding:	Funded by the Malaysian Federal Government, Selangor State Government, charitable and philanthropic donations provided financial support for the study.
Start date:	Open to recruitment in Malaysia: Q4 2024
Anticipated primary recruitment completion date:	Q4 2026
Sponsor	Metanoic Health Limited
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4. Primary Objective

The primary objective of the study is to determine if neoadjuvant oral artesunate improves progression free survival at 2 years in Stage II/III colorectal cancer compared to standard treatment with surgery alone +/- adjuvant post-operative chemotherapy where indicated.

4.1 Primary endpoint

The primary outcome measure for the comparison of the artesunate versus placebo group is recurrence free survival 2 years after surgery.

5. Secondary Objectives

- To assess the impact of neoadjuvant artesunate on recurrence free survival at 5 years post randomisation
- To assess the impact of neoadjuvant oral artesunate on overall survival at 2 and 5 years post-randomisation
- To assess the tolerability of neoadjuvant artesunate
- To assess the impact of artesunate treatment on patient quality of life
- To assess the nature and frequency of surgical complications following neoadjuvant artesunate treatment
- To assess the prognostic and predictive value of tumour biomarkers
- To conduct biomarker and molecular profiling studies

5.1 Secondary end points

- Recurrence free survival at 5 years
- Overall survival at 2 and 5 years
- Colon cancer specific death at 2 and 5 years
- Artesunate drug related toxicity
- Adverse events
- Pathological assessment of tumour regression (involvement of lymph nodes ; serosa ; resection margin)
- Patient Quality of Life (assessed using validated EuroQol EQ-5D, EORTC QLQ C-30, EORTC QLQ C-29 quality of life tools)
- Surgical morbidity/mortality
- Predictive value of tumour biomarkers in terms of predicting response to artesunate therapy
- Biomarker and molecular profiling studies

6. Background

6.1 Study disease

Colorectal cancer is the third most common cancer worldwide and represents a significant health care burden with an incidence of one million new cases per year (Ferlay *et al*. 2012). Each day in the UK, 110 new colorectal cancer cases are diagnosed, with over 50 % of new cases presenting with locally advanced disease and older patients being particularly at risk of death (UK CR., 2014). Current standard multimodality treatment with surgery, chemotherapy and radiotherapy has not thus far increased 5 year overall survival beyond 60%.

Cancer development in humans is a complex multistep process defined by 8 key hallmarks: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming energy metabolism and evading immune destruction (Hanahan&Weinberg., 2011).

The Quasar study and meta-analysis have shown that postoperative chemotherapy improves survival in Stage III colorectal cancer and some Stage II colorectal cancers with negative prognostic features (QUASAR., 2007; Price *et al.,* 2013). However for a significant number of patients the current treatment paradigm of surgery followed by adjuvant chemotherapy fails to eradicate locoregional disease and distant micrometastases, resulting in disease recurrence. Current adjuvant therapy in colorectal cancer includes combination chemotherapy with intravenous 5-fluorouracil or oral capecitabine in combination with oxalipatin (FOLFOX or Cape-OX) (Price *et al.,* 2013). In the metastatic colorectal cancer setting, current treatment paradigms include 5-FU based chemotherapy regimens combined with oxaliplatin (FOLFOX) or irinotecan (FOLFIRI) alongside biologically targeted agents such as the humanised monoclonal anti-vascular endothelial growth factor (VEGF) antibody bevacizumab and epidermal growth factor receptor (EGFR) antibodies cetuximab and panitumumab (Price et al., 2013). These regimens however carry significant toxicity profiles and remain unaffordable for the majority of patients in developing countries.

Neoadjuvant pre-operative chemotherapy has been shown to be more effective in a number of other cancer types supporting the theory that optimal neoadjuvant systemic therapy at the earliest opportunity may be more effective at eradicating distant metastases compared to adjuvant therapy delivered following the delay and immunological stress of surgery. Colorectal cancer metastases have a short doubling time (Tanaka *et al.,* 2004 ; Finlay *et al*., 1988) and it has been postulated that micrometastatic tumour growth may be further accelerated in the early post operative period by enhanced growth factor activity (Zeamari *et al*., 2004 ; Fahmy *et al.,* 2003 ; Schelfhout *et al.,* 2002). Neoadjuvant therapy also confers the potential advantage of primary tumour shrinkage, thereby reducing the risk of incomplete surgical excision and tumour shedding contaminating the surgical field during surgery (Nelson *et al.,* 2001). This may have particular relevance for transcoeliac spread with peritoneal metastases found in up to 50% of patients with locoregional relapse (Assehorn *et al*., 1999). Neoadjuvant preoperative therapy in colorectal cancer started within days of diagnostic and staging investigations provides an unique treatment paradigm that could potentially eradicate micrometastases not managed with current standard treatment regimes. Indeed, neoadjuvant combination chemotherapy +/- targeted anti-EGFR monoclonal antibody therapy with panitumumab forms the basis of the current FOxTROT trial (FoxTROT Collaborative Group., 2012). As previously mentioned, these regimens are, however, often associated with significant side effects and result in a delay in surgery whilst patients recover. A well tolerated, affordable, novel anticancer agent that could be given to patients whilst they wait for surgery, without causing a surgical delay due to treatment related toxicity, would therefore have a significant clinical impact on patient care.

6.2 Investigational Medicinal Product (IMP)

Artesunate

Artemisinins are a family of sesquiterpene trioxane anti-malarial agents derived from Sweet wormwood (Artemisia annua L) that have been used in traditional Chinese medicine for centuries to treat fevers. Artesunate, artemether and arteether are derivatives of artemisinin that are converted into their active metabolite dihydroartemisinin (DHA). Artesunate is approved for the treatment of uncomplicated and multidrug-resistant malaria and is on the WHO list of Essential Medicines (WHO., 2015). Artesunate has a hemisuccinate group which confers substantial water-solubility and high oral bioavailability and therefore a convenient oral route of administration. Artesunate has a good safety and tolerability profile, having been used to treat tens of millions of adults and children globally (Hien *et al.,* 1994 ; Kremsner *et al.,* 2004). It has a renal mode of excretion and is rapidly eliminated with a short metabolic half life of hours (WHOPAR., 2011).

6.3 Experimental lab work examining the anti-cancer effects of artemisinins.

Several reviews have examined the evidence for the anti-cancer effects of artemisinins in detail (Ho *et al*., 2014 ; Krishna *et al.,* 2008). Studies of artemisinins in in vitro experiments and animal models have demonstrated broad anti-cancer activity including pro-apoptotic, anti-proliferative, anti-angiogenesis and anti-metastatic effects (Efferth *et al*., 2001,2007 ; Anfonsso et al., 2006 ; Krishna et al., 2008). Artesunate has demonstrated cytotoxic effects against a number of cancer cell lines including colon, breast, leukaemia, melanoma, central nervous system, ovarian, prostate and renal cancer (Efferth *et al*., 2001, 2003). The active metabolite of artemisinin, dihydroartemisinin (DHA), has also displayed antineoplastic effects on breast, glioma, colon, lung, ovarian and pancreatic cancer cell lines (Singh & Lai., 2001 ; Kim *et al.,* 2006 ; Lu *et al*., 2011 ; Mu *et al.,* 2007 ; Chen *et al.,* 2009 ; Hooft van Huijsduijnen., 2013).

The exact underlying mechanisms remain to be elucidated but include actions on cell cycle proteins that control transit through cell cycle G1 phase restriction, induction of apoptosis, inhibition of NF-kB, antioangiogenic and antimetastatic effects. Iron-mediated endoperoxide bridge cleavage and the formation of toxic free radicals are thought to be key mechanisms underlying the anti-cancer effects of artesunate (Efferth *et al*., 2003, 2004). Cancer cells are highly proliferative, requiring a heavy iron load which acts as a cofactor in the synthesis of deoxyriboses prior to cell division (Daniels et al., 2012). Artemisinins induce cellular damage via the formation of reactive oxygen species (ROS) such as hydroxyl and superoxide anion radicals. When free iron is available, artemisinins are converted into a highly potent alkylating radical, capable of inducing direct oxidative damage in cancer cells (Efferth *et al*., 2003, 2004). Cancer cells frequently overexpress the transferrin (TF) receptor. Increased antitumor activity has been observed in human liver hepatocellular carcinoma and lung adenocarcinoma cell lines with increased TF receptor adducts (Yang *et al.,* 2014). Artemisinin–transferrin conjugates have been shown to possess higher anti-cancer efficacy than artemisinins alone (Lai *et al.,* 2009 ; Nakase et al., 2008, 2009). For example, a DHA–transferrin conjugate has demonstrated at least 280 times more potent anti-cancer activity in breast cancer cells compared to normal breast cells (Xie *et al*., 2009).

Artesunate has also been shown to induce oncosis in renal cancer cell lines (Jong *et al*., 2015). Increased levels of calpain-1 and calpain-2 expression and decreased levels of α-tubulin expression were exhibited in cell lines treated with artesunate. Calpains are a family of calcium-dependent cysteine proteases that exert proteolytic cleavage on a number of cellular substrates, including cytoskeletal proteins. In oncosis, ion pumps are affected by adenosine triphosphate (ATP) depletion, leading to the collapse of mitochondrial potential resulting in cytomembrane destruction and the accumulation of reactive oxygen species (ROS). Downregulation of cyclin D1, c-MYC, survivin, c-Met, EGFR, Src, FAK and α-tubulin expression were also observed.

Berdelle *et al*. (2011) showed that artesunate induced oxidative deoxyribonucleic acid (DNA) damage, sustained DNA double-strand breaks (DDSB) and activated Ataxia telangiectasia mutated/Ataxia telangiectasia and Rad-3 related protein (ATM/ATR) damage response in glioblastoma cell lines. Artesunate was shown to be a powerful inducer of oxidative DNA damage, which was dose dependent and paralleled by cell death via apoptosis and necrosis. Artesunate was also shown to provoke a DNA damage response (DDR) with phosphorylation of ATM/ATR, Chk1 and Chk2 proteins. Wang *et al.* (2015) showed that artesunate induced oxidative stress, DNA double-strand breaks (DSB), downregulated RAD51 foci and homologous recombination repair (HRR), thereby impairing DSB repair in ovarian cancer cell lines.

6.4 Artemisinins display effects on the cell cycle.

Cell growth and repair require progression through the cell cycle which is controlled by a series of cyclins and cyclin-dependent kinases. Inhibitors of cell division include the cip/kip family of inhibitory proteins such as p16, p21, p27 and p57. Recent studies have shown that artemisinins are capable of inducing cell cycle arrest via a number of pathways. Artesunate was shown to markedly impede the growth of colon carcinoma,

leukemia, small cell lung carcinoma and glioma cell lines by inducing cell cycle arrest at G2/M phase (Steinbruck *et al*., 2010) and inducing G1 cell cycle arrest in human breast cancer cell and nasopharyngeal cancer cell lines (Tin *et al*., 2012). Artesunate induced cell cycle arrest at the G2/M phase and induced oncosis-like cell death in renal cancer cell lines (Jong *et al.*, 2015). Decreased levels of cyclin B, cyclin D1 and transription factor E2F1 were also observed.

In human nasopharyngeal cancer cells, artemisinin upregulated p16 and p27 and suppressed the level of cyclin D1, cyclin E, CDK2, CDK4 and CDK6 (Wu *et al*., 2011). Artemisinins have been shown to induce G1 cell cycle arrest by deactivating the retinoblastoma protein (pRb), a mediator of cell cycle progression and disrupt transcription of the cyclin CDK4 promoter in prostate cancer lines (Willoughby *et al*., 2009). DHA was also found to inhibit cell cycle progression from G0/G1 into S phase in pancreatic cell lines via reduction of CDK2, CDK4 and CDK6, cyclin E and NF-κB. Levels of the p27 inhibitory protein were amplified (Chen *et al.,* 2010).

Liu *et al*. (2010) showed that artesunate caused dose dependent decreases in cell numbers in colorectal, breast and lung cancer cell lines via cytotoxic and cytostatic mechanisms. Artesunate was found to alter proteins that regulate G1 transit. Levels of CDK4 and cyclin proteins were reduced in a dose dependent manner along with concomitant reductions in pRb protein. Levels of the cyclin-dependent kinase p21 inhibitory protein were increased. Artesunate induced cytotoxicity was enhanced by a recovery phase which was associated with an easing of the G2 blockade and a concomitant increase in the sub-G1 (apoptotic) cell phase in all 3 cell lines.

Artesunate has been shown to induce radiosensitivity in cervical cancer cell lines by inducing apoptosis and G2/M cell cycle arrest (Luo *et al*., 2014). Radiosensitisation with artesunate has also been shown in glioblastoma cell lines by diminishing expression of the anti-apoptotic protein survivin (Reichert et al., 2012) and lung cancer cell lines by increasing nitrous oxide production and associated signal transduction pathways, to induce cell cycle arrest in G2/M phase (Zhao *et al.,* 2011).

6.5 Artemisinins induce apoptosis in cancer cells.

Apoptosis (programmed cell death) is maintained by a complex balance between a family of pro-apoptotic proteins (BAX, BAK, BAD, Bid) and anti-apoptotic proteins (Bcl-2 and Bcl-xl) (Hanahan&Weinberg., 2011). When cells detect DNA damage, tumour suppressor protein TP53 is upregulated, leading to increased levels of pro-apoptotic proteins and cytochrome c (caspase activator) leading to programmed cell death. Artemisinins have been shown to activate BAX induction of cytochrome c in human colon cancer cell lines resulting in apoptosis (Riganti *et al.,* 2009). Similar effects have been seen in human lung adenocarcinoma (Lu *et al.,* 2009) and prostate cancer cell lines (Nakase *et al.,* 2009). Artesunate has been shown to induce apoptosis in leukemic T cells and breast cancer cell lines via iron dependent ROS formation resulting in cytochrome c release and cleavage of procaspases-2, 3, 8 and 9 (Efferth *et al.,* 2007; Hamacher- Brady *et al.,* 2011).

Lu *et al*. (2015) showed that dihydroartemisinin (DHA) induced apoptosis is associated with inhibition of sarco/endoplasmic reticulum calcium ATPase activity in colorectal cancer. Artesunate was found to inhibit proliferation, enhance apoptosis and arrest the cell cycle at G0/G1. Intracellular calcium concentrations were also significantly increased after artesunate treatment. The expression of CAAT/enhancer binding protein homologous protein (CHOP) in a DHA-treated colorectal cancer cell line was upregulated, as was the expression of pro-apoptotic protein Bax with a reduction in levels of anti-apoptotic protein Bcl-2. An increase in Bid accompanied increased activation of caspase-3 in cancer cells exposed to DHA.

Li *et al*. (2007) showed that artesunate suppressed proliferation and promoted apoptosis of colorectal cancer cells in a dose-dependent manner. Immunohistochemical staining showed membranous translocation of regulator protein beta-catenin and inhibition of unrestricted activation of the Wnt/beta-catenin pathway. In vivo studies showed that artesunate significantly slowed the growth of colorectal human tumor xenografts.

Bioluminescent imaging showed that artesunate decreased the physiological activity of tumor xenografts and delayed the development of liver metastases.

Yang *et al.* (2014) showed that artesunate induced apoptotic cell death in cervical and hepatocellular cancer cell lines. Artesunate accumulated in lysosomes and activated lysosomal function via promotion of lysosomal V-ATPase assembly. Lysosomes function upstream of mitochondria in the production of reactive oxygen species (ROS). Lysosomal iron is required for lysosomal activation. Artesunate treatment induced mitochondrial ROS production. Artesunate-induced cell death was mediated by release of iron in the lysosomes, which resulted from the lysosomal degradation of ferritin, an iron storage protein, and is postulated to be one mechanism through which artesunate may have a cytotoxic effect on cancer cells.

Du *et al.* (2010) showed dose dependent pancreatic tumour regression in xenograft models treated with artesunate. Artesunate treated cells showed loss of mitochondrial membrane potential, undergoing caspase-independent and non-apoptotic cell death in the presence of reactive oxygen species (ROS). Artesunate has also been shown to induce dose-dependent DNA damage and apoptosis in embryonal rhabdomyosarcoma cell lines via the production of ROS and induction of the p38 mitogen-activated protein kinase (MAPK) pathway (Beccafico *et al.,* 2015).

6.6 Artemisinins display anti-angiogenic effects

Angiogenesis plays a vital role in cancer cell growth and development. Isolated tumor cells without an adequate nutrition and oxygen supply display a growth restriction of about 1–2 mm^3. Cancer cells require pro-angiogenic stimuli such as metalloproteinase (MMP) and vascular endothelial growth factor (VEGF) and a reduction in anti-angiogenic factors such thrombospondin and tissue inhibitor of metalloproteinase (TIMP) to maintain a viable blood supply required for growth (Hanahan & Weinberg., 2011). Under hypoxic conditions, hypoxia induced factor (HIF-1α) and nuclear factor-κB (NF-κB) are activated resulting in transcription of VEGF and angiogenesis. Artemisinins were shown to reduce the levels of HIF-1α and VEGF in mouse embryonic stem cells (Wartenberg *et al.,* 2003). In a mouse lung carcinoma model, administration of artemisinin resulted in a reduction in VEFG-C and lymphangiogenesis (Wang *et al.,* 2008). Artesunate also suppressed levels of VEGF and KDR/flk-1 resulting in reduced tumor growth in BALB/c nude mice implanted with human ovarian cancer cells (Chen *et al*., 2004), in Kaposi's sarcoma (KS-IMM) xenograft mice (Dell'Eva *et al.,* 2004) and in a rat glioma model (Wu et al., 2009). Artesunate was also shown to exert anti-angiogenic effects on renal cancer cells in vitro in a dose-dependent manner (Jong *et al.,* 2015). Following treatment with artesunate, renal cell tumours in subcutaneous xenografts models showed decreased levels of proliferation marker Ki-67 and reductions in mean microvessel density compared to controls.

6.7 Artemisinins block tissue invasion and metastasis

A key hallmark of cancer cells is their ability to detach from the primary tumour, degrade the extracellular matrix and metastasise through the bloodstream (Hanahan& Weinberg., 2011). Metalloproteinases (MMPs) play a critical role in tumour migration, invasion and metastases via degradation of the extracellular matrix. E-cadherin is an important cell adhesion molecule. One study in human melanoma cells, showed that artemisinin was able to reduce MMP2 levels thereby blocking cell migration (Buommino *et al.,* 2009). In human pancreatic and ovarian cancer cells lines, DHA suppressed the levels of MMP2, inhibiting NF-κB and metastases (Wang et al., 2011 ; Wu et al., 2012). Similarly, artesunate was shown to abrogate MMPs and NF-κB activity, thereby blocking metastases in non-small cell lung cancer lines. Artemisinins downregulated the levels of MMP2 and TIMP-2 whilst up-regulating E-cadherin in hepatocarcinoma cells lines (Weifeng et al., 2011). Artesunate has been shown to upregulate the expression of adhesion molecules integrin β1 and neural cell adhesion molecule (NCAM) in embryonal rhabdomyosarcoma cells, thereby reducing migration and invasion (Beccafico *et al*., 2015).

The Wnt/beta-catenin cell signalling pathway has been identified as a critical pathway in colorectal carcinogenesis (MacDonald *et al*., 2009 ; Clevers *et al*., 2006). Artesunate has been shown to inhibit the hyperactive Wnt/beta-catenin pathway in colorectal cancer cell lines, thereby attenuating cancer cell growth (Li *et al*., 2008). The anticancer effects of artesunate on three colorectal cancer cell lines were compared and the relationship between drug sensitivity and malignant tumour phenotype analysed. In poorly-differentiated colorectal cancer cell line – CLY, beta-catenin accumulation and loss of E-cadherin was seen in the nucleus; in moderately-differentiated Lovo cells these two proteins accumulated in the cytoplasm; and in well-differentiated HT-29 cells beta-catenin and E-cadherin localized to the cell membrane. In vitro and in vivo, poorly- or moderately-differentiated CLY and Lovo cells were more susceptible to artesunate treatment than well-differentiated HT-29. The response of CLY and Lovo to artesunate were associated with membranous translocation of beta-catenin and increased expression of E-cadherin, which indicated the inhibition of the hyperactive Wnt/β-catenin signaling pathway and the reversion of the epithelial to mesenchymal transition, respectively. Artesunate has also been shown to induce apoptosis via inhibition of the Wnt/β-catenin pathway in myelodysplastic syndrome (MDS) cells (Xu *et al*., 2015).

6.8 Clinical evidence of anti-tumour effects of artesunate

A number of case reports have reported antitumour effects of artesunate in small groups of patients. One case study in a patient with laryngeal squamous cell carcinoma described a 70% reduction in tumor size after two months of artesunate treatment (Singh & Verma., 2002). Another case study reported a slowing of disease progression in a pituitary macroadenoma patient treated with artemether for 12 months (Singh & Panwar., 2006). Artesunate was also found to control tumor growth and improve survival in metastatic uveal melanoma (Berger *et al*., 2005). One patient with stage IV uveal melanoma experienced objective regression of splenic and lung metastases following the addition of artesunate to dacarbazine and remained alive 47 months after first diagnosis. An open label pilot study of dihydroartemisinin in 10 patients with advanced cervical cancer reported that treatment was well tolerated and improved patient symptoms (Jansen *et al*., 2011). A randomized controlled trial of artesunate in metastatic non small cell lung cancer randomised 60 patients to a control arm treated with doublet chemotherapy [intravenous vinorelbine (25mg/m^2) on Day 1 and Day 8 and intravenous cisplatin (25mg/m^2) from Day 2-Day 4] and 60 patients treated with the same chemotherapy regime in addition to artesunate 120mg IV once daily from Day 1-Day 8 (Zhang *et al*., 2008). No statistically significant differences were seen in terms of the 1-year survival rate between the two treatment arms, although the disease control rate of the artesunate intervention group (88.2%) was significantly higher than that of the control group (72.7%) ($P<0.05$), and the artesunate arm's median time to progression (TTP) was significantly longer than that of the control arm (24 weeks vs 20 weeks) ($P<0.05$). No significant difference was found in toxicity between the two groups, including that of myelosuppression and gastrointestinal symptoms ($P>0.05$). A pharmaokinetic study of oral artesunate in 23 patients with metastatic breast cancer was also recently published (Ericcson *et al*., 2014). Survival data were not reported.

The first randomized placebo controlled double blind pilot study of artesunate in colorectal cancer randomized 23 patients with resectable colorectal cancer to receive either 14 daily doses of artesunate or placebo (Krishna *et al*., 2015). During a median follow up time of 42 months, 1 patient in the artesunate group developed recurrent colorectal cancer compared to 6 patients in the placebo group. Six patients receiving artesunate and 4 patients receiving placebo had measurable Carcinoma Embryonic Antigen (CEA) levels. Three patients in the placebo group had a rise in their CEA on rechecking their levels after the trial intervention whereas no patients in the artesunate group experienced a rise in their CEA levels ($p=0.03$). In one patient receiving artesunate there was a 75 % reduction in circulating CEA level after 2 weeks of artesunate treatment alone. Patients in the artesunate arm showed a high probability of a reduction in the ki67 proliferation index marker on tumour immunohistochemical analysis compared to those in the placebo arm. Artesunate was generally well tolerated apart from 2 cases of Grade 3 neutropenia and anaemia with one of the cases undergoing a spontaneous recovery of neutrophils and the other requiring granulocyte colony stimulating factor (GCSF). Both patients received a blood transfusion and went on to have surgery with no further complications.

6.9 Summary

Artesunate is a safe and effective antimalarial with evidence of anticancer properties across a range of cancer cell lines. Results from our pilot feasibility study in colorectal cancer patients showed that artesunate was safe and well tolerated (Krishna *et al*., 2015). These findings provided the basis for a Phase II clinical trial investigating the effects of neoadjuvant artesunate on progression free survival and overall survival (NeoART).

6.10 Other treatments

No other agents or treatments will be used in this study.

6.11 Study Rationale and risk/benefit analysis

Neoadjuvant pre-operative chemotherapy is effective in a number of other cancer types supporting the theory that optimal neoadjuvant systemic therapy at the earliest opportunity may be more effective at eradicating distant metastases compared to adjuvant therapy delivered following the delay and immunological stress of surgery. Colorectal cancer metastases have a short doubling time (Tanaka *et al*., 2004 ; Finlay *et al*., 1988) and it has been postulated that micrometastatic tumour growth may be further accelerated in the early post operative period by enhanced growth factor activity (Zeamari *et al*., 2004 ; Fahmy *et al*., 2003 ; Schelfhout *et al*., 2002). Neoadjuvant therapy also confers the potential advantage of primary tumour shrinkage, thereby reducing the risk of incomplete surgical excision and tumour shedding contaminating the surgical field during surgery (Nelson *et al*., 2001). This may have particular relevance for transcoeliac spread with peritoneal metastases found in up to 50% of patients with locoregional relapse (Assersohn *et al*., 1999). Neoadjuvant preoperative therapy in colorectal cancer started within days of diagnostic and staging investigations provides an unique treatment paradigm that could potentially eradicate micrometastases not managed with current standard treatment regimes.

Indeed, neoadjuvant combination chemotherapy with or wothout targeted anti- EGFR monoclonal antibody therapy with panitumumab forms the basis of the current FOxTROT trial (FOxTROT Collaborative Group., 2012). As previously mentioned however these regimens are associated with significant toxicities and surgery is often delayed whilst patients recover from these effects. These treatments are also expensive and remain economically out of reach for the majority of patients in developing countries.

There remains an urgent need to improve survival outcomes in patients with colorectal cancer globally using affordable interventions where possible. Artesunate has a good safety and tolerability profile, having been used to treat tens of millions of malaria patients globally. It is also relatively affordable at around £0.70 per daily dose. If neoadjuvant treatment with artesunate is found to reduce the risk of cancer recurrence and improve survival in operable colorectal cancer, this would be a practice- changing observation and would significantly improve care for millions of colorectal cancer patients worldwide.

6.12 Assessment & management of potential risk

Participants who develop CTCAE v5.0, Grade 3 or 4 serious adverse effects related to artesunate (or placebo) will be withdrawn from the study treatment but will continue in the study to enable an intention to treat analysis. The main possible risks associated with artesunate in this phase II trial are neutropenia and haemolytic anaemia. Patients should be monitored closely for these potential side effects and sites should take appropriate clinical action if such adverse events occur.

Defination of aanemie is a disorder characterized by a reduction in the amount of haemoglobin in 100 ml of blood. Signs and symptoms of anaemia may include pallor of the skin and mucous membranes, shortness of

breath, palpitations of the heart, soft systolic murmurs, lethargy, and fatigability.Patients developing anaemia as defines by CTCAE 5.0

- Grade 3 anaemia: Hgb <8.0 g/dL; <4.9 mmol/L;<80 g/L; transfusion indicated
- Grade 4 anaemia: Life-threatening consequences; urgent intervention indicated

The treatment for anaemia is blood transfusion.

Definition of neutropenia: A disorder characterized by an ANC <1000/mm3 and a single temperature of >38.3 degrees C (101 degrees F) or a sustained temperature of >=38 degrees C (100.4 degrees F) for more than one hour. Patients developing neutropenia as defines by CTCAE 5.0

- Grade 3 neutropenia : ANC <1000/mm3 with a single temperature of >38.3 degree C (101 degrees F) or a sustained temperature of >=38 degrees C (100.4. degrees F) for more than one hour
- Grade 4 neutropenia: Life-threatening consequences; urgent intervention indicated

Treatment for neutropenia is GCSF.

There will be a 24 hour emergency card provided to each participant when they receive their IMP dispensed from pharmacy. The 24 hour emergency card will contain the contact details of the local PI and site team in addition to a NeoART-M 24 hour emergency telephone number.

The IMP will be supplied double-blinded and IMP allocation will be performed by each site pharmacy or clinical resarch centre in accordance with the randomisation list provided by the sponsor's representative at the start of the study. In the case of a medical emergency each Pharmacy or CRC will have the ability to unblind treatment.

The participating sites all have appropriate experience and knowledge of the target patient population. All team members will have demonstrated experience in clinical research and Good Clinical Practice (GCP).

The study is categorised 'Somewhat higher than the risk of standard medical care'. This would therefore require that IMP storage and accountability. The CI and the CI delegate will construct a Trial Master File and ensure that all essential trial documents are stored safely and securely. The trial will be subject to monitoring in accordance with the Sponsor risk-based monitoring plan. The purpose of the monitoring is to ensure the safety, rights and well being of the trial participants are upheld and that any risk to the trial data is minimised.

7. Trial design

7.1 Overall Design

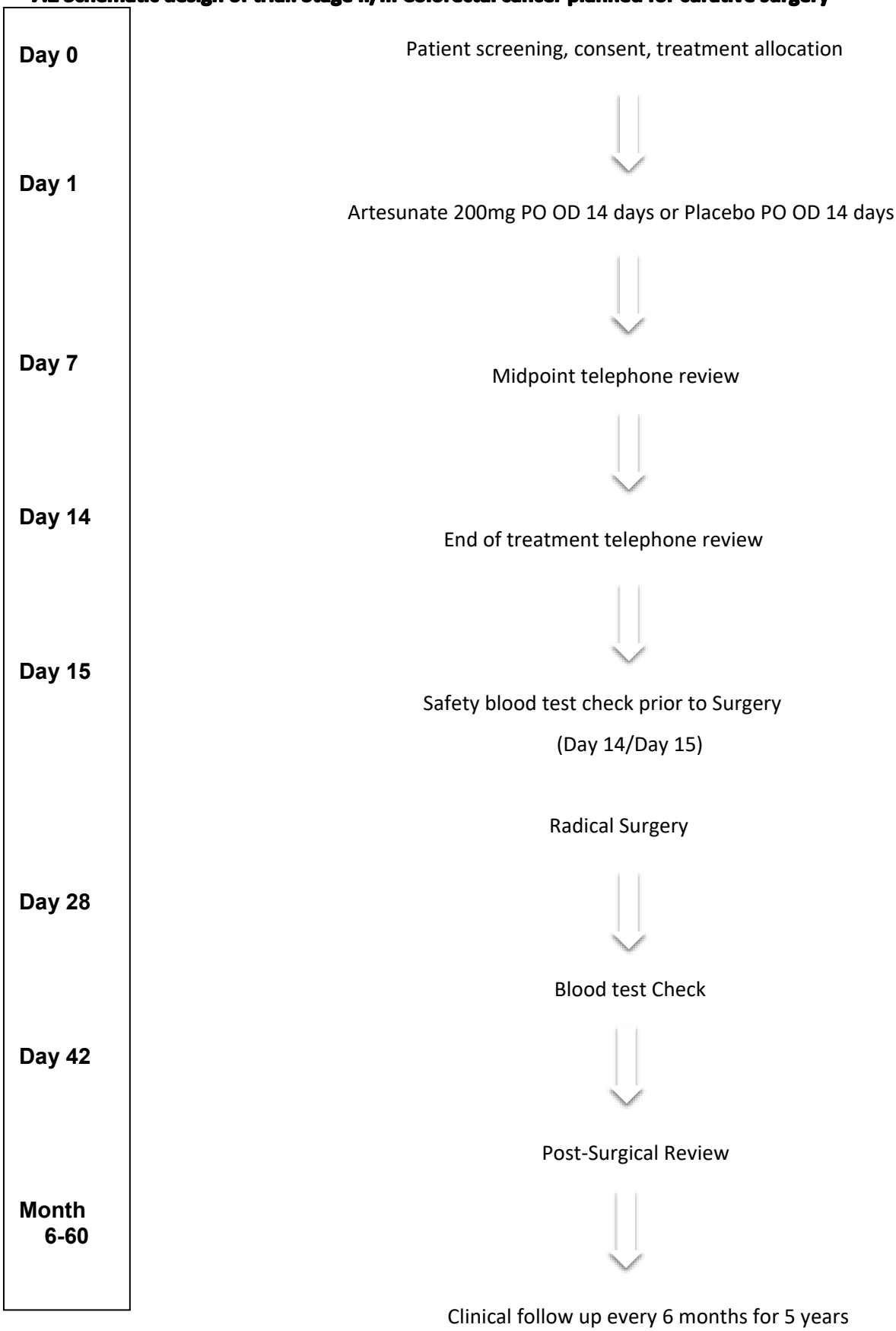
This study is a Phase II, randomised, double blind, placebo controlled, multi-centre trial. We aim to recruit 200 patients presenting with stage II/III colorectal cancer.. Patients who consent to enter the study will receive the trial intervention for 2 weeks prior to their planned surgery and then be followed up for 5 years following surgery.

A master randomisation list generated by the Sponsor representative was made available at the start of the study. The master randomisation list is further divided into blocks of 20 and provided to each study site with the IMP deliveries. The Research Pharmacy or CRC at each site will retain the randomisation list upon receipt in their Pharmacy Site File. Upon receipt of a NeoART prescription the next sequential allocation will indicate whether to dispense active artesunate or matched placebo tablets to a particular participant. At the time of

dispensing the tear off label identifying the product will be removed by the Research Pharmacy or CRC and added to the accountability log. This will ensure that the investigators and study participants remain blinded to the treatment allocation. In exceptional circumstances, when knowledge of the investigational drug is essential for medical treatment of the patient, treatment allocation can be unmasked by pharmacy or CRC centres by a study coordinator that is not involved in the study directly .

The Sponsor retain a copy of the master randomisation list in their respective study files.

7.2 Schematic design of trial: Stage II/III Colorectal cancer planned for curative surgery



8. IMP Dosage regimen and rationale

The dose of artesunate for the study will be 200 mg per oral (PO) once daily (OD) for fourteen days. There are no data on the most appropriate dose of artesunate in cancer in humans. This dose has been chosen by a consensus among medical experts experienced with using artesunate for the treatment of malaria and following published results from a pilot feasibility and safety study of neodajuvant oral artesunate in patients with colorectal cancer (Krishna *et al*. 2015).

The daily dose of artesunate used in combination treatments to treat uncomplicated Plasmodium falciparum malaria is 4 mg/kg, given over three days. When given as monotherapy for uncomplicated falciparum malaria the total dose is approximately 20 mg/kg, over seven to ten days. For curative courses in severe malaria, the total doses of artesunate (parenteral and oral combined) can reach 24 mg/kg. The daily dose of artesunate for a patient in this study will be between 2 mg/kg and 4 mg/kg, depending on the weight of the participant (ranging between 52-110 kg). At daily doses of 6 mg/kg the dose limiting side effect of artesunate is neutropenia. The study products do not contain porcine but contains produced from bovine that is safe for human consumption.

Metabolite

Artesunate is extensively hydrolysed by plasma esterases and possibly CYP2A6. Its main metabolite, DHA is metabolised through glucuronidation (WHOPAR., 2011).

Elimination

The plasma half-life of artesunate is 3-29 minutes whilst its active metabolite DHA has a plasma half-life of 40 to 95 minutes (WHOPAR., 2011).

If vomiting occurs during the course of treatment and the tablet is visible in vomit, the dose should not be retaken. The occurrence and frequency of any vomiting during the reporting period must be noted in the adverse events log and CRF.

The effect of food

The effect of food consumption with artesunate has been studied. When healthy volunteers consumed artesunate fixed dose combinations with a high fat meal, the Cmax and AUC (0-t) of artesunate decreased by 66% and 13% respectively, compared to fasting. The Cmax and AUC (0-t) of the active metabolite dihydroartemisinin (DHA) decreased by 48% and 5% respectively with a high-fat meal, compared to fasting (WHOPAR., 2011). Patients should take the study medication after a light meal.

Packaging and storage conditions

Study medication will be provided in bottles. One patient bottle will provide 28 tablets – sufficient for the duration of the study. The product labelling will be fully compliant with both Appendix E: Labelling Requirements of the Rules and guidance for Malaysian-Guideline-for-Application-of-CTIL-and-CTX-7.1-Edition and the protocol submitted and authorised by the NPRA.

Drug interactions with artesunate

There are no reports of negative drug interactions with artesunate to date.

Adverse events associated with artesunate

Artesunate is a safe drug that has been taken by millions of patients worldwide. In data taken from studies of malaria, serious side-effects are extremely rare.

The Summary of Product Characteristics for Artesunate provided to sites lists the potential side effects.

The reference safety information document provided to sites lists the adverse events considered by the Sponsor as being 'expected'.

Pregnancy

Artesunate is teratogenic in animal models and is contraindicated in the first trimester of pregnancy in humans. Pregnant women with malaria nevertheless are exposed to artesunate when no alternatives are available for treatment and studies published to date do not indicate an increased risk of teratogenicity. Pregnant women will not be recruited and participants will be required to use effective methods of medical contraception during the study. Women of child bearing age will be required to undergo a pregnancy test within 72 hours prior to commencing the study and must consent to adequate contraceptive methods from the time of consent and for up to 6 weeks following the end of treatment. Male participants with a partner of childbearing potential must agree to use adequate, medically approved contraceptive precautions from the time study consent is signed till up to 6 weeks after the last dose of study treatment intervention.Methods of contraception that are acceptable for the trial include the following:

The implant, the coil and male or female sterilisation will be acceptable for the trial. The injection and most forms of hormonal contraception will also be deemed acceptable for the trial if used in combination with condoms or other barrier methods. However, condoms alone won't be sufficient during the study.

IMPs and non-IMPs used in the trial

Patients entered into the study will be randomised to receive artesunate 200 mg (administered as 2 x 100mg tablets) or identical matching placebo once a day orally for 14 days.

Dosage modifications

No dosage modifications will be undertaken. Any Grade 3/4 reaction (according to Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0) which occurs in any patient and is deemed related to the IMP or placebo by the treating investigator will result in that patient's trial treatment being discontinued.

8.1 Source of IMPs including placebo

Bulk artesunate 100 mg and matching placebo tablets are manufactured by HCM- Medical

according to Good Manufacturing Practice (GMP). The matching placebo tablets contain lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, colloidal anhydrous silica and a magnesium stearate blend.

Artesunate and placebo tablets are packaged into bottles each containing 28 tablets of either artesunate or placebo. Package labelling was performed under controlled conditions to ensure no cross contamination. The bottles have a removable label affixed. The removable label will allow identification of each bottle to facilitate correct treatment allocation in accordance with the randomisation list held in the pharmacy or clinical research centre departments. The pharmacy department or research centre will remove the labels during the dispensing process and affix all of the labels to the IMP accountability records.

The named Qualified Person (QP) will issue a QP release certificate. The IMP will be delivered, accompanied by the QP batch release certificate.

Shipment of the IMP will be under temperature-controlled conditions and the recipient pharmacy department at each site will be required to confirm by return of a completed delivery note as instructed that correct shipment conditions have been maintained. A copy of the QP release will be provided to each site to file in the Pharmacy Site File (PSF).

Full IMP accountability will be maintained at all participating site pharmacy departments.

Temperature records are to be maintained with daily minimum/maximum readings via a calibrated/certificated thermometer. The records will be available for monitoring visit purposes and either a copy of those records or a signed statement of compliance will be placed in the PSF following the end of IMP treatment phase.

The IMP – artesunate/placebo 100 mg tablets must be stored below 25°C.

8.2 Accountability procedures for the IMP(s)

Each participating site's pharmacy department will be responsible for maintaining and updating the IMP Accountability Log, filed in the pharmacy site file. All used/unused IMP(s) will be returned to the site pharmacy. If research pharmacy is not available at the study site, a qualitfied study coordinator that is not part of the investigator's team can perform this task.

IMP(s) destruction will be conducted, following verification by the Clinical Trials Monitor and agreement by the Sponsor, in accordance with local pharmacy practice. Destruction records will be maintained and filed in the site pharmacy site file.

8.3 Assessment of compliance

An assessment of medication compliance will be made by the principal investigator or delegated research team, member on Day 7 and at the end of the treatment course based on verbal answers given to direct questioning and a count of untaken medication on Day 15. These data will be entered into the Case Report Form.

Following completion of the patient's IMP treatment period any unused medication will be returned to the site Pharmacy department for the IMP accountability log to be updated. Patients who take the study drug for < 8 days in the 14 day treatment period will be considered 'non-compliant'. All patients will continue to be followed up according to the study protocol regardless of compliance.

8.4 Post-trial IMP arrangements

This is a neoadjuvant study. There will be no further IMP supplied to participants following colorectal surgery.

8.5 Name and description of each non-IMP (NIMP)

No NIMPs used.

8.6 Concomitant treatment

There are no reports of negative drug interactions with artesunate to date. Concomitant medication details will be collected at baseline and any changes recorded at subsequent study visits.

We will document adjuvant treatment following surgery such as chemotherapy and aspirin therapy. We will include this in our final data analysis in order to account for any confounding results that could arise as a result of adjuvant therapy. If patients develop Grade 3 neutropenia or more (according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0) Definition of neutropenia: A disorder characterized by an ANC <1000/mm3 and a single temperature of >38.3 degrees C (101 degrees F) or a sustained temperature of >=38 degrees C (100.4 degrees F) for more than one hour. Grade 3 neutropenia : ANC <1000/mm3 with a single temperature of >38.3 degree C (101 degrees F) or a sustained temperature of >=38 degrees C (100.4. degrees F) for more than one hour. They should be managed with granulocyte colony stimulating factor (GCSF) supplied via routine hospital pharmacy in accordance with local policy and prescribing practice.

9. Participant Selection criteria

There will be no exceptions (waivers) to eligibility criteria prior to participant inclusion into the study. Any questions raised about eligibility should be addressed prior to entering the participant.

The eligibility criteria have been carefully considered and are standard to ensure both the safety of the participants and that the trial results can be appropriately used to make future treatment decisions for other patients with similar disease or medical condition. It is therefore vital exceptions are not made to the following detailed selection criteria.

Deviations from the eligibility criteria are considered to be protocol violations and may be reported to the NPRA as a serious breach.

All participants that are screened for inclusion into the study must be entered onto the screening log and will be assigned a sequential number. Participants will be considered eligible for enrolment into this trial if they fulfil all of the inclusion criteria and none of the exclusion criteria as defined below.

Eligible participants will be entered onto the Subject ID log and assigned a Trial specific Identification number in a pre-agreed format in accordance with Site identifier and next sequential numerical value e.g. SG001.

Each site will be provided with a unique site identifier communicated via the Sponsor.

Eligibility Criteria

9.1 Inclusion criteria

1. Aged 18 or over
2. Histologically proven single primary site colorectal adenocarcinoma or high grade dysplasia plus unequivocal radiological evidence of invasive cancer
3. Stage II/III colorectal cancer planned for surgical resection and no clinical indication for neoadjuvant preoperative chemotherapy/chemoradiation therapy
4. WHO performance status 0,1 or 2
5. Adequate full blood count: White Cell Count (WCC) >3.0 x 10^9 /l; Platelets >100 x 10^9/l; Haemoglobin (Hb) >80g/L
6. Adequate renal function : Glomerular Filtration Rate >30ml/min by Cockcroft-Gault formula.
7. Adequate hepatobiliary function : Total bilirubin < 3 x Upper limit norm
8. Female participants of child bearing potential must have a negative pregnancy test <72 hours prior to initiating study intervention and agree to avoid pregnancy using adequate, medically approved contraceptive precautions for up to 6 weeks after the last dose of study treatment interventions.
9. Male participants with a partner of childbearing potential must agree to use adequate, medically approved contraceptive precautions during and for up to 6 weeks after the last dose of the study treatment intervention.
10. Patient able and willing to provide writen, informed consent for the study.

9.2 Exclusion criteria

1. Contraindication to use of artesunate due to hypersensitivity
2. Pregnancy or lactation
3. Male or female participants unwilling to use an effective method of birth control (either hormonal in the form of the contraceptive pill or barrier method of birth control accompanied by the use of a proprietary spermicidal foam/gel or film) ; or agreement of true abstinence from time consent is signed until 6 weeks after the last dose of study treatment intervention (i.e. withdrawal, calendar, ovulation, symptothermal and post ovulation are not acceptable methods)
4. History of hearing or balance problems
5. History of immunosuppression
6. Patient weight < 52 kg or > 110 kg
7. Other planned intervention, apart from standard of care
8. Any other malignant disease diagnosis within the preceding 2 years with the exception of non-melanomatous skin cancer and carcinoma in situ
9. Lactose intolerance

10. Subject/Patient Recruitment process

10.1 Participant Recruitment

Participant recruitment at any site will only commence once evidence of the following are in place in the Sponsor site file.

1. Relevant ethics committee approval, and NPRA
2. Delegation of Duties and Sponsorship Agreement signed by the CI
3. Final sponsorship (following evidence of Pharmacy green light) issued by the Sponsor representative.

All sites participating in the trial will also be asked to provide a copy of the signed Clinical Trial Site Agreement (CTSA) and Confirmation of Capacity and Capability.

Once the trial initiation procedure is completed the Sponsor will issue the 'Open to recruitment' letter. All participants who wish to enter the study will be fully screened and consented by the Principal Investigator, or one of the qualified clinicians involved in the study as Clinical Co-investigator.

NeoART aims to randomise 200 participants worldwide with 120 patients in Malaysia. We aim to recruit 8-12 participants to the study per month to meet our recruitment target over 24 months. Our Phase I pilot trial (Krishna *et al.,* 2015) was the first trial to investigate neoadjuvant artesunate for colorectal cancer and demonstrated the feasibility, practicability, and safety of this novel approach.

Each patient will receive a minimum of RM150 reimbursement for their transportation cost at each additional visit.

11. Study procedures

11.1 Informed consent

It is essential that all trial personnel/staff undertaking the informed consent process have signed the Delegation of Responsibilities Log to ensure that the person has been delegated the responsibility by the study CI/PI. All personnel taking informed consent must be GCP trained. Refer to sponsor's SOP 'TMF Maintainence'.

Informed consent from the participant must be obtained following explanation of the aims, methods, anticipated benefits and potential hazards of the trial and before any protocol-specific procedures are performed. If a potential participant is illiterate or visually impaired and does not have a legally acceptable representative, the investigator must provide an impartial witness to read the informed consent form to the participant and must allow for questions. Thereafter, both the participant and the witness must sign (or mark in the case of illiteracy) the informed consent form to attest that informed consent was freely given and understood. Where appropriate, written translation will be provided in addition to professional verbal translation.

The only procedures permitted in advance of written informed consent are those that would be performed on all participants in the same situation as routine clinical practice.

The Investigator or designee will explain that the participants are under no obligation to enter the trial and that they can withdraw at any time during the trial, without having to give a reason.

A copy of the signed Informed Consent Form (ICF) along with a copy of the most recent approved Patient Information Sheet (PIS) will be given to the study participant. An original signed and dated consent form will be retained in the ISF and a copy will be placed in the medical notes. If new safety information results in significant changes to the risk–benefit assessment, the consent form will be reviewed and updated if necessary. All participants, including those already treated, will be informed of the new information, given a copy of the revised patient information sheet and will be asked to re-consent if they choose to continue in the study. This process must also be recorded and documented as above.

11.2 Randomisation procedure

Participants recruited to the study will be randomised to receive either artesunate or placebo 1:1. Randomisation will be by a computer-generated code. An unblinded randomisation list will be provided to each participating site and held in Pharmacy or held by a study coordinator that is not directly involved in the study. Following completion of the study the participant treatment allocation list held in the site Pharmacy files will be anonymised prior to collection by the Clinical Trials Monitor. The anonymised lists will be handed over to the statistician to facilitate data analysis.

All patients will be given a study specific 24 hrs emergency contact card immediately after being randomised. The card includes details of the Trial: Study title, details of IMP, patient trial number, CI/PI's contact details along with out of hours contact details in case of emergency.

11.3 Prescribing & Dispensing of IMP

The Sponsor will construct a protocol specific Clinical Trial Procedure which will detail participant management in relation to IMP dosing and pharmacy dispensing procedures. The Sponsor will also provide a study specific prescription template to be used for the study. All IMP prescriptions presented to pharmacy must be on the Sponsor approved template and be signed and dated by an approved prescriber. The prescriber must have been signed off by the CI /PI on the Staff delegation of duties log for that task. A sample signature of all delegated prescribers must be provided for the Pharmacy Site File prior to receipt of the 1st trial prescription. Upon site initiation completed delegation logs will be copied by the Sponsor Clinical Trials Monitor and filed in the Pharmacy/Institute Site File. Prescriptions received by a non-approved prescriber will not be acceptable and will cause the participant unnecessary delays.

Upon receipt of a valid prescription the Research Pharmacy staff or study coordinator who is not part of the investigator's team will assign the next available IMP allocation either artesunate or placebo as indicated on the randomisation list. A single supply of IMP in a bottle of 28 tablets of 100 mg artesunate/placebo tablets will be made, sufficient to cover the duration of the trial. The label of the IMP will contain instructions to the trial participant of the date to commence artesunate/placebo dosing calculated as 15 days prior to the day of surgery. The surgery date will be annotated on the NeoART prescription by the prescriber. The peel-off/tear-off labels on the outside of the IMP bottle will be removed and affixed to the IMP accountability forms held in the pharmacy site file.

The treatment allocation should not be revealed to the investigator team nor the participant. The Patient Trial Number and Site ID should be completed on each label. The label must follow the Appendix E: Labelling Requirements of the Rules and guidance for Malaysian-Guideline-for-Application-of-CTIL-and-CTX-7.1-Edition and have the following details completed :- Patient initials, Patient Trial Number, date of dispensing and the start date – calculated as 15 days prior to day of surgery as indicated on the prescription to facilitate a 14 day of course of treatment.

A pharmacy dispensing label may also be affixed to the bottle in accordance with local hospital policy but must not obscure the approved bottle label. IMP accountability records must be completed for each participant indicating date of dispensation, Participant Trial ID, treatment start date – dispenser and checker. Tear off/ removable labels must be affixed to this accountability record. The IMP will be provided to the participant together with a 24 hour emergency contact card and instructions to request the return of the IMP medication to pharmacy.

The IMP accountability logs will be checked for the presence of the tear off/peel off label for each participant by the Clinical Monitor.

11.4 Emergency unblinding

Each study site will have the capabilities of revealing patient treatment allocation in case of emergency. In the case where there is a need for emergency unblinding, follow the local SOP. The investigators will remain blinded. An SOP on local policy for unblinding must be file in the local ISFand PSF. Unneccessary unblinding should be avoided.

All code break requests must be directed via the local PI who can contact their local assigned personel to reveal treatment allocation. A file note and NeoART code break form documenting clearly the reason for code break must be completed and sent to the Sponsor within 24 hours (or on the next working day if a weekend or bank holiday).

The delegated personel will;

Complete a NeoART-M code-break record form with the details supplied by the NeoART-M study team member.

Cross reference the NeoART-M dispensing record labels with the Participant Trial ID provided to determine artesunate or placebo treatment.

The treatment reveal should be communicated to the originator of the request only. Do not reveal treatment allocation to the NeoART-M Sponsor Emergency Out of hours cover.

Add details to the NeoART-M code break form –email completed NeoART-M code break form to the Sponsor within 24 hours.

File the original copy in the Pharmacy site file code break section.

Annotate against participants IMP accountability record – code broken- initial and date.

Site team will need to be informed the following working day to facilitate completion of a Serious Adverse Event Form and reporting timelines must be followed. Refer to protocol section 14.2

Certain serious adverse events such as a trial patient developing anaphylaxis, a serious heart arrhythmia or neutropenic sepsis may require emergency unblinding. The participant should be managed accordingly and full medical support provided as necessary.

11.5 Overdose of Trial medication

In the event of an overdose of trial medication the patient should attend the Accident and Emergency (A&E) and be urgently reviewed by a member of the research team during working hours or the oncall Medical Officer. A thorough medical history and events leading to the overdose should be documented as well as a complete physical examination including blood pressure monitoring. Blood tests should be performed including full blood count, urea and electrolytes and liver function tests. There is no antidote or documented role for gastric lavage. The overdose should be documented in the participants medical notes and CRF.The Adverse Event log and the SAE reporting form should be completed. The completed SAE form should be emailed to the Sponsor in accordance with protocol section 14.2.

11.6 Discontinuation/withdrawal of participants and stopping rules

11.6.1 Discontinuing/withdrawal of participants

In consenting to the trial, participants are consenting to trial treatments, trial follow up and data collection. However, an individual participant may stop treatment early or be stopped early for any one of the following reasons :

- Unacceptable treatment toxicity or an monitoring
- Investigator decides that it is in the best interests of the patient to terminate his/her participation in the study
- Poor patient compliance and/or major protocol deviation
- Intercurrent illness that prevents further protocol treatment

Withdrawal of consent from the participant

As participation in the trial is entirely voluntary, the participant may choose to discontinue treatment at any time without penalties or loss of benefits to which they may be entitled. Although not obliged to give a reason for discontinuing their trial treatment/study participation a reasonable effort should be made to establish this reason, whilst remaining fully respectful of the participant's rights. Participants who discontinue protocol treatment, for any of the above reasons, should remain in the trial for the purpose of follow up and data analysis.

Temporary discontinuations of study medication are not permitted. If a patient decides to discontinue treatment then the patient's decision should be documented on the Subject Tracking log, CRF and the IMP returned to the study site Pharmacy. Participants should continue to be followed up as closely as possible to the follow-up schedule defined in the protocol, providing they are willing. However if the participant confirms they do not wish to participate in the scheduled follow up data collection visits then data that has already been collected should be kept and analysed according to the ITT principle for all participants who stop follow up early. Patients withdrawn from the study will continue to be followed up according to the study protocol to the scheduled date of study completion, or to recovery or stabilisation of a followed-up AE, whichever comes last. Routine medical and surgical care of withdrawn participants will continue as planned. The investigator will make every effort to contact the participant in the case of non attendance to a planned review appointment. Participants who stop the trial follow up early will not be replaced.

11.6.2 List of criteria for premature discontinuation of the study

The study may be discontinued prematurely by the Sponsor, the DMC or the Chief Investigator if:

- As a result of the interim analysis or any other event during the study there is significant doubt based on statistical evaluation as to the risk/benefit ratio
- The aim of the study has become outdated or is no longer of interest
- There is a serious breach by the investigator of a fundamental obligation under this agreement, including but not limited to breach of the Study Protocol, breach of the applicable laws and regulations or breach of the ICH guidelines for Good Clinical Practice

In all cases the relevant ethics committee, NPRA and CRM will be informed in accordance with statutory guidance.

11.7 Participant transfers

If a participant moves from the area making continued follow up at their consenting centre inappropriate, every effort should be made for them to be followed up at another Sponsor approved trial centre. Written consent should be taken at the new centre and then a copy of the participant's CRF should be provided to the new centre. for the participant remains with the original consenting centre until the new consent process is complete.

11.8 Lost to Follow up

For all participants that qualify for randomisation, information in relation to their Next of Kin (NoK) and registered GP details will be confirmed as correct and current in the patient medical records to facilitate ease of contact throughout the study and data collection period. Following a missed scheduled visit, effort to contact the participant and/or Next of Kin on up to 3 occasions over a 7-10 day period should be made. Each occasion will be documented in the medical notes and on the Telephone log . If following 3 attempts contact has failed via the participant/ NoK then participant status will need to be confirmed via the GP.

Definition of the End of Trial

The end of the trial will be defined as the last participant to have completed follow up at 5 years (last patient last visit) or to have died 5 years following surgery. The relevant ethics committee and the NPRA will be notified of the end of the trial within 90 days of its planned completion or within 15 days if the study is terminated early in accordance with.

12. Study Assessments

Each study related visit and any interventions, tests or procedures will be documented in the participant medical notes and clearly marked as relative to their NeoART-M participation

12.1 Screening assessments

For potential participants who appear to meet the criteria for study participation, the Investigator will provide the patient information sheet and answer any questions to allow the patient to make an informed decision regarding study participation. Investigators will be expected to maintain the Screening Log of all potential participants. This Log will include limited information about the potential participant.

If informed consent is given, the Investigator will conduct a full screening evaluation to ensure that the patient satisfies all inclusion and exclusion criteria. Site research staff must record Patient screening activities on Screening Log. A patient who gives written informed consent and who satisfies all the inclusion and exclusion criteria may enrol in the study, be added to the Subject ID log and proceed to randomisation.

Note that assessments conducted as standard of care do not require informed consent and may be provided as screening data if conducted within the acceptable timeframe prior to registration. Assessments required at baseline are detailed in section 12.2.

12.2 Baseline assessments

All participants who consent to enter the study and fulfill all of the eligibility criteria will be entered onto the Subject ID log and be assigned a unique subject ID and undergo a review at the 1st study visit prior to randomisation and commencing the artesunate/placebo treatment course. A full medical and concomitant medication history will be taken. Staging investigations will be reviewed and vital signs and baseline blood samples will be performed. Baseline blood tests will include FBC, urea and electrolytes, liver function tests (LFT), and carcinoembryonic antigen (CEA). A pre-treatment blood sample will be taken for study molecular analyses. Patients will be asked to complete EORTC validated quality of life questionnaires (EuroQol EQ-5D, EORTC QLQ C-30, QLQ-CR29). Potential female participants of child bearing age must have a negative pregnancy test.

12.3 Treatment procedure

[Refer to Appendix 2 for a summary chart of study assessments]

Participants entered into the study will be randomised 1:1 to receive artesunate 200 mg or matched placebo once a day orally for 14 days to commence 15 days prior to day of scheduled surgery. The artesunate/placebo packs dispensed to the participant will provide clear instructions on when to start taking the IMP and when to stop.

Study medication will be started fifteen days before the scheduled surgery with the final dose taken on Day 14 (the day before surgery).
Day 7 of study medication (telephone assessment)
Medical History
Concomitant medication and patient self reported adverse events
QOL –questionnaires – EuroQol EQ-5D, EORTC QLQ C-30, QLQ-CR29

Day 14 of study medication/Pre-operative admission assessment
Medical history
Concomitant medication and patient self reported adverse events
QOL –questionnaires – EuroQol EQ-5D, EORTC QLQ C-30, QLQ-CR29

Day 15 Surgery
Blood tests – FBC, Kidney and liver function tests, CEA tumour marker
Post treatment serum sample for study molecular analyses, these blood tests will be done on either day 14 or 15.
Safety blood tests (FBC, U&E, LFT) must be available prior to patients having surgery to exclude risk of neutropenia and deranged biochemistry.
Post surgery/in-patient stay/or at a relevant time point in the case of cancellation of surgery
Patient self-reported adverse events.

Day 28
Blood test – FBC

Day 42 (Routine follow up 4 weeks post surgery)
Medical history
Blood tests – FBC, Kidney and liver function tests, CEA tumour marker

6 month follow up visit post surgery
Medical history
Blood tests – CEA tumour marker

12 month follow up visit post surgery
Medical history
Blood tests – CEA tumour marker
Colonoscopy
CT scan chest, abdomen and pelvis

18 month follow up visit post surgery
Medical history
Blood tests – CEA tumour marker

24 month follow up visit post surgery
Medical history
Blood tests – CEA tumour marker
CT scan chest, abdomen and pelvis

30 month follow up visit post surgery
Medical history
Blood tests – CEA tumour marker

36 month follow up visit post surgery
Medical history
Blood tests – CEA tumour marker

42 month follow up visit post surgery
Medical history
Blood tests – CEA tumour marker

48 month follow up visit post surgery
Medical history
Blood tests – CEA tumour marker

54 month follow up visit post surgery
Medical history
Blood tests – CEA tumour marker

60 month follow up visit post surgery
Medical history
Blood tests – CEA tumour marker
CT scan chest, abdomen and pelvis

At the end of 5 years follow up patients with no evidence of disease recurrence will be discharged back to the care of their GP. All patients with disease recurrence will have their management discussed at the Colorectal Multidisciplinary meeting (if available).

For pragmatic trial management, the following visit windows are permitted :
Day 1 and Day 7 -/+3 days of the study review date. Any baseline blood tests within 1 week of starting the study treatment are acceptable.

The Day 28 review may be performed within 7 days of the set date.

The Day 42 review may be performed within 2 weeks of the set date.

The 6 to 60 month follow up visits post surgery may be performed +/- 6 weeks of the set date.

If for any reason study follow up visits defer from the scheduled study time scales (for example the 12 month follow up visit is scheduled before the timeframe window due to a rising CEA or post adjuvant chemotherapy) this will be at the managing clinicians discretion. However a minor Deviation Reporting Form should be completed to document the reason for the deviation and reported according to SOP.

12.5 Methods

12.5.1 Samples

12.5.1.1 Obtaining, labelling, storing

Following informed consent, blood samples will be collected into vacutainer systems or similar tubes (usually 2 x 10 ml EDTA vacutainers) on D1 and D14/D15 of the study. The collected blood should be centrifuged, processed and stored at -80ºC according to the NeoART- trial: Blood sample collection, processing and storage SOP . NB. The Sponsor representative should be notified in instances where the storage freezer temperature deviation from -80 ºC by more than +/-10 º C

These samples will be used for genomic and proteomic analysis. Blood samples will be suitably stored in the pathology department of each site till required for batch analysis. When requested for batch analysis at various recruitment time points throughout the study, blood samples will be transferred for centralised study sample storage under the Human Tissue Authority License/ Permit to Import or Export of Human Tissues or Part Thereof Health Office/District Health Office (BLESS)

The Sponsor 'Record of retained body fluids log' will be maintained throughout the study at each site to track all participant blood and Tumour samples accountability and location. These completed logs should be made available upon request at study monitoring visits.

Tumour samples will be derived from tumour biopsies performed during colonoscopy and surgical samples following surgery. Any excess tumour tisue left over after the tissue sample has been processed for routine diagnostic purposes will be utilised. Formalin fixed, paraffin embedded blocks will be prepared and stored in the pathology department of each site till requested for batch analysis. All tissue samples will be transferred for centralised study sample storage under the Human Tissue Authority License/ Permit to Import or Export of Human Tissues or Part There of Health Office/District Health Office (BLESS).

Each sample will be coded with the unique participant study ID to maintain confidentiality. The date of collection will also be recorded on study sample log and the relevant CRF completed. The samples will be stored indefinitely, with patient consent. However patients have the right to have the samples destroyed by contacting the Chief Investigator who will then arrange for the samples to be confidentially destroyed. Tumour samples will be analysed to identify tumour markers predictive of response to artesunate and elucidate potential mechanisms of action. Samples will be sent for further molecular analyses to the labs of other collaborators involved in the NeoART project collaboration in accordance with the NeoART-M Material Transfer Agreement (MTA).

Patient treatment allocation blinding will be maintained throughout the trial including during the sub-study biomarker/mechanism of action analysis. An independent lab researcher will de-link the patient sample from the trial patient ID and allocate the sample a separate random study sample lab number. Once the serum and tissue samples have been processed, the exploratory molecular endpoint results

will be entered into a lab results excel spreadsheet. The sponsor will then be provided with the de-linked study sample lab number and they will notify the independent lab researcher of the study arm allocation for each de-linked study sample.

12.5.1.2 Transporting samples

An approved specialised courier will transfer the samples at the appropriate temperature (e.g. -80ºC for specimens stored at -80 ºC) with in-transit monitoring and transport labelling of specimens. Arrangements for confirmation of receipt will be clearly outlined and logged on. A copy of this will be kept within the Trial Master File.

12.5.1.3 Laboratory procedures

The CI or the CI delegate will ensure that any laboratory that is used for the trial is adequate for "the foreseen duration of the trial to conduct the trial properly and safely" (ICH GCP 4.2.3 and ICH GCP 5.184b). The CI or the CI delegate will ensure that the laboratory the study will use has been verified and is compliant with accreditation standards and that all necessary contracts for the contracted activities have been finalised. The CI or the CI delegate will ensure that any laboratory used for research purposes sets up and maintains a study specific Laboratory Site File that contains a current approved version of the protocol. Access to the laboratory files will be provided to the Sponsor trial monitor. ct s

Samples will be stored and shipped to any external labs involved in the NeoART project collaboration according to the agreed NeoART Material Transfer Agreement (MTA). Each sample will be coded with the participants Trial ID to maintain confidentiality. The date of shipping of the samples will be recorded on the Sample Collection Log.

12.6 Radiology or any other procedure(s)

Patients will have a routine baseline CT Chest/Abdomen/Pelvis as part of their staging investigations prior to entering the study. Following surgery they will have a routine restaging CT Chest/Abdo/Pelvis at 1 year, 2 year and 5 year follow up visits to exclude disease recurrence as per routine clinical follow up.

13. Translational research / Pharmacodynamic Studies

For instructions on processing and storage of study bloods, tissue samples please refer to the study lab manual.

14. Pharmacovigilance

14.1 Definitions

Adverse Event (AE) — Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

An adverse event (AE) can therefore be any unfavourable and unintended sign (e.g. an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. Pre-existing conditions should only be reported as an adverse event if the condition worsens by at least 1 CTCAE grade. As the IMP has a short half-life, we would expect drug clearance within 24 hours of stopping the IMP.

As part of pharmacovigilance, adverse event data will be collected from the day 1 of IMP dose until 4 weeks following the end of the IMP 14 day treatment schedule. This coincides with the D42 post surgical review.

Adverse Reaction (AR) — All noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions.

Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR) — any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability/incapacity or
- Is a congenital anomaly/birth defect.

Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered “serious”.

Suspected Unexpected Serious Adverse Reaction (SUSAR) — an Adverse Reaction which is classed in nature as both serious and unexpected

An ‘Unexpected Adverse Reaction’ is when both the nature and severity of the event is not consistent with the reference safety information available for the IMP in question.

14.2 Investigator responsibilities relating to safety reporting

All Adverse Events whether serious or not will be recorded in the hospital notes in the first instance. A record will also be kept in the participant’s CRF and the Sponsor’s AE Log. In the event of a mildly deranged blood test or clinical observation of no clinical significance to the patient (for example a mildly elevated liver function test or a mildly elevated blood pressure reading in a participant with known hypertension taking prescribed antihypertensive medication), the local PI will be informed and will sign and date the participants CRF. However it will not be necessary to complete an AE CRF or the Sponsor’s AE Log for these minor events.

SAEs and SARs must be notified to the sponsor as soon as the investigator becomes aware of the event (within 24 hours). Refer to sponsor’s SOP and ensure the completed SAE report form is sent to the sponsor via E-mail.

The sponsor will inform the National Pharmaceutical Regulatory Agency (NPRA) and relevant ethics committee of SAE and SUSARs as soon as possible, but no later than 7 calendar days after first knowledge by the sponsor that a case qualifies. Any additional information will be reported within 8 additional calendar days. This report must include an assessment of the importance and implication of the findings, including relevant previous experience with the same or similar medicinal products. Follow-up information should be actively sought and follow-up reports should be submitted to the NPRA when it becomes available.

Causality Assessment — All cases judged by either the reporting health care professional or the sponsor as having a reasonable suspected causal relationship to the medicinal product qualify as ARs.

Definitely — there is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.

Probably — there is evidence to suggest a causal relationship and the influence of other factors is unlikely.

Possibly — there is some evidence to suggest a causal relationship (e.g. the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (i.e. the patient's clinical condition, other concomitant events).

Unlikely — there is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, or other concomitant treatments).

Unrelated — there is no evidence of any causal relationship.

Not Assessable — Note if this description is used the sponsor will assume the event is related to the IMP until follow up information is received from the investigator to confirm a definitive causality assessment.

Any SUSAR assessed as related to the IMP will need to be reported to the Sponsor irrespective of how long after IMP administration the reaction has occurred.

Expectedness should be based solely on the available RSI for the IMP and will be described using following categories :

Expected — an AE that is classed in nature as serious and which is consistent with the information about the IMP listed in the RSI or clearly defined in this protocol.

Unexpected — an AE that is classed in nature as serious and which is not consistent with the information about the IMP listed in the RSI.

The completed AE Log will be sent to Sponsor upon request and/or every 2 months.

The Chief Investigator (CI) will respond to any SAE queries raised by the Sponsor as soon as possible. Follow up reports must continually be completed within acceptable timeframes and sent to the sponsor as detailed above until the reportable event is considered resolved.

Notification of deaths

All deaths will be reported to the Sponsor irrespective of whether the death is related to disease progression, the IMP or an unrelated event.

14.3 Development Safety Update Reports (DSURs)

The CI or the CI delegate will prepare the DSUR, using the Sponsor's template and in accordance with the Sponsor's DSUR SOP. It will be reviewed by the Sponsor and when necessary be referred to an independent committee (i.e. Research Governance Safety Sub Committee). The CI or the CI delegate will provide to the relevant ethics committee and the Sponsor will provide to NPRA the prepared DSUR annually within the defined reporting timelines.

14.4 Annual Progress/Safety Reports (APRs)

The CI or the CI delegate will prepare the APR in accordance with SOP. Following review by the Sponsor, the CI or the CI delegate will send to the relevant ethics committee to the reviewed report. The APR is due for submission annually within 30 days of the anniversary date on which the favourable opinion was given by the Ethics committee, until the trial is declared ended.

14.5 Pregnancy

The limited data available on exposure during pregnancy does not indicate any adverse effects of artesunate on the health of the foetus/newborn child. However, there is inadequate information for determining the foetal risk of artesunate treatment during pregnancy. Data from preclinical embryo foetal development studies in rats and rabbits showed a low incidence of cardiovascular malformation and syndrome of skeletal defects at doses close to embryolethal doses starting at around 6mg/kg/day. The dose used in our study of artesunate 200 mg will be below this threshold. In the event that pregnancy occurs whilst on the study drug, the pregnancy will be recorded in both the participant notes and CRF, the Sponsor and DMC will be notified. The female participant/pregnant partner of a male participant will be urgently reviewed by the research team and obstetric specialist and emergency un-blinding of the trial arm will be undertaken as per protocol section 11.4. If the participant is found to be receiving artesunate, the female participant or pregnant partner of a male participant will be followed up closely on a fortnightly basis together with an Obstetric Specialist and Foetal Medicine Specialist. The pregnancy will be monitored closely for any intrauterine abnormalities. Following successful delivery the newborn child will be monitored for up to 12 months together with a Paediatrician to ensure no adverse developmental effects have occurred.

14.6 Reporting Urgent Safety Measures

The Sponsor and Investigators may take appropriate urgent safety measures in order to protect the participants against any immediate hazard to their health or safety. The CI must telephone the NPRA to discuss the circumstances leading to the decision and the proposed measures to be taken. No later than 3 days following the conversation the CI must give written notice to the NPRA of that conversation and agreed actions. The Sponsor must also receive a copy of this communication to facilitate oversight of participating PI and participating site notification of imposed safety measures. A formal Substantial Amendment will be required for preparation and submission to the relevant

ethics committee/NMRR and NPRA at the earliest opportunity. Refer to sponsor SOP Management of Amendments for guidance.

14.7 Notification of Serious Breaches of GCP and/or the protocol

Any Study Deviations or Violations will be documented using Sponsor's Protocol Deviation form, and entered onto the Sponsor's log. Potential Serious Breaches and Urgent Safety Measures will be reported according to SOP.

A "serious breach" is a breach which is likely to effect to a significant degree :

(a) The safety or physical or mental integrity of the participants of the trial; or
(b) The scientific value of the trial

The CI or the CI delegate will notify the Sponsor immediately of any case where there exists a possible occurrence of a serious breach.

15. Data management and quality assurance

15.1 Confidentiality

All data will be handled in accordance with the Data Protection Act. The Case Report Forms (CRFs) will not bear the participant's name or other directly identifiable data. The participant's trial Identification Number (ID) only will be used for identification. The Sponsor Subject ID log can be used to cross reference participant's identifiable information.

15.2 Data collection tool

Case Report Forms will be designed by the CI and the CI delegate and the final version will be approved by the Sponsor.

It is the Investigator's responsibility to ensure the accuracy of all data entered and recorded in the CRFs. The Staff Delegation of Responsibilities Log will identify all trial personnel responsible for data collection, entry, handling and managing the database. Basic demographic and clinical information will be gathered at the time of the initial interview and by review of the medical notes if needed. The following information will be recorded for all subjects in the CRF :

- Demographic details: Date of enrolment, age, gender, ethnicity
- Clinical data: symptomatology, risk factors for development of CRC, weight, height, vital signs
- Findings at endoscopy
- Imaging: findings of ultrasound, CT and/or MRI (performed as clinically indicated)
- Disease staging: TNM staging prior to surgery and definitive histological staging (TNM, Duke's stage)
- Histology: routine histological grading, apoptosis count and specialist assays (see below)
- Concomitant medication: a full record of all non-study drugs taken will be kept
- Study-drug treatment: compliance with study medication, all adverse events, serious or otherwise
- Validated Quality of Life Tool (EORTC QLQ C-30, EuroQol EQ-5D, QLQ-CR29)

The following blood sample analysis will be performed:

- Baseline - FBC, urea and electrolytes, LFTs, CEA, pre-treatment serum sample for study molecular analyses
- Day 14/15 of treatment - FBC, urea and electrolytes, LFTs, CEA, post-treatment serum sample for study molecular analyses
- Day 28 - FBC
- Day 42 (4 weeks post-surgery) - FBC, urea and electrolytes, LFT, CEA
- 6 monthly visits for 5 years following surgery - CEA
- Serum plasma samples will be taken at baseline (Day 1) and end of treatment (Day 14), processed and stored at -80°C (+/- 10°C) for future analyses
- Any other blood or clinical sample at any time if deemed necessary by the investigators in case of a suspected adverse event

All laboratory analysis should be reviewed by the PI and where results reported are not within the normal range the PI must clearly annotate 'Not clinically Significant' or 'Clinically significant' and sign and date the results. The relevant results should be carefully transcribed to the Case Report Forms.

In order to ensure completeness of data collected, participants will be contacted by the research team (telephone call/email) in the event of missing data such as patient quality of life questionnaires.

15.3 Incidental findings

Incidental findings arising during the study will be discussed with the medical and research team involved in the patients care. The medical and research team will always act in the patient's best interest and take appropriate clinical action in accordance with good clinical practice.

15.4 Data handling and analysis

Data will be stored in anonymised form in a password-protected database. We will use a 44 Randomize44 study database storage programme. Study documentation will be stored for fifteen years after the completion of the study. Quality Control will be applied at each stage of data handling to ensure that all data are reliable and are processed correctly. Study data is destroyed after period of storage.

16. Archiving arrangements

The trial essential documents (e.g. TMF) along with the trial database will be archived in accordance with the sponsor SOP. The agreed archiving period for this trial will be defined within the Delegation of Duties Sponsorship Agreement.

17. Statistical design

17.1 Statistical input in trial design

Dr Irina Chis Ster – Senior Lecturer in Biostatistics, provided statistical expertise in clinical trial design and statistical planning. Professor Bertrand Lell will conduct the statistical analyses and data interpretation as data is now being combined from 2 studies implementing the same protocol

(NeoART-UK and NeoART-Vietnam). Professor Bertrand Lell is also providing the statistical expertise and support for NeoART-Vietnam.

17.2 Endpoints

17.2.1 Primary endpoints

The primary endpoint for the main comparison between the artesunate treatment and placebo arms will be persistent disease or disease recurrence, two years following surgery. All analyses will be made on an intention to treat basis, although per protocol analyses will also be reported. Statistical significance will be assumed at the 5% level unless otherwise indicated.

17.2.2 Secondary endpoints

Secondary endpoints will include:

- Overall survival at 2 and 5 years
- Recurrence free survival at 5 years
- Colon cancer specific death at 2 and 5 years
- Artesunate drug related toxicity
- Adverse events
- Pathological assessment of tumour regression (involvement of lymph nodes; serosa; resection margin)
- Quality of life (EuroQol EQ-5D, EORTC QLQ C-30, EORTC QLQ-CR29)
- Surgical morbidity/mortality
- Predictive value of tumour biomarkers in terms of predicting response to artesunate therapy
- Biomarker and molecular profiling studies

17.3 Sample size and recruitment

17.3.1 Sample size calculation

We performed our sample size calculation largely based on inferences drawn from our pilot Phase I study (Krishna *et al*., 2015). The input assumes that the analysis would be conducted using a univariate Cox proportional hazard survival model from the time of surgery to the first disease recurrence with one binary covariate representing treatment groups (artesunate or placebo). We calculated that a minimum sample size of 168 patients is necessary to test a hazard ratio (HR) of 0.56 for the intervention versus placebo. This value is within the confidence intervals estimated in our phase I study (Krishna *et al*., 2015) and somewhat between the point estimate in the worse case scenario inference in the paper and a value of 0.7 commonly circulated in oncology clinical trials. For this Phase II study, we set the power at 0.8 and accepted a type one error of 0.1 for a one-sided test.

1) The null nypothesis H0: HR=0.56 (equivalent ≤)

2) The alternative hypothesis H1: HR>0.56

These settings would result in a sample size of 168 patients. Inflating this sample size by 15-20% to allow for loss at follow-up would result in approximately 194-202 patients. We believe that 200 patients therefor represents a feasible and achievable target given our time and financial constraints.

The rationale and sensitivity analyses for these calculations are outlined below. The general setting is that of a survival analysis where the event is defined as the first disease recurrence after surgery.

A cohort of Stage II/III colorectal cancer patients undergoing surgery will be randomised as per recruitment to receive the artesunate intervention or placebo. Patients will be followed-up for 5 years following surgery. The minimum sample size to test a hazard ratio (HR) of 0.56 for the intervention vs. placebo group is based on:

Calculation framework:

1. The minimum sample size calculation has been calculated as for a univariate Cox proportional hazard survival model with one binary covariate representing treatment groups (artesunate or placebo).

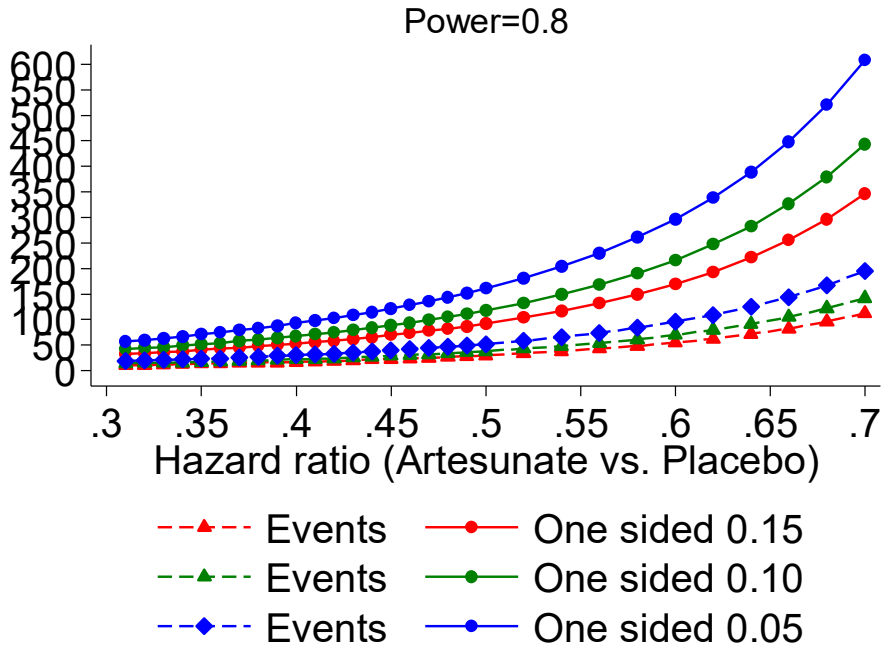
2. The necessary number of events are calculated as

$$\mathrm{D} = \left(\mathrm{Z}_{1-\alpha} + \mathrm{Z}_{1-\beta}\right)^2 \left[\mathrm{P} \times (1-\mathrm{P}) \times \left(\log(\mathrm{HR})\right)^2\right]^{-1}$$

Where P represents the proportion of the sample assigned to the treatment group (0.5 corresponding to a 1:1 treatment assignment in our setting) and the HR is the estimated hazard ratio of the event (disease recurrence) for the treatment group vs. placebo. The values of the inverse of the standard normal cumulative distribution function are
$Z_{1-\alpha} = 1.28$ for one sided α=0.1 and $Z_{1-\beta} = 0.842$ for β=0.20 (equivalent to 80% power).

3. Hence, the minimum total number needed for the trial is calculated as

$$\mathrm{N} = \mathrm{D} \times (100/32)$$



Graph 1: Study sample size- a sensitivity analysis to various settings for hazard ratios and type one error for one-sided test.

Total number participants	One sided level of significance	HR	Number of events
53	.15	.4	17
56	.15	.41	18
59	.15	.42	19
62	.15	.43	20
66	.15	.44	22
70	.15	.45	23
74	.15	.46	24
78	.15	.47	25
82	.15	.48	27
87	.15	.49	28
92	.15	.5	30
104	.15	.52	34
117	.15	.54	38
132	.15	.56	43
149	.15	.58	48
169	.15	.6	55
193	.15	.62	62
222	.15	.64	72
256	.15	.66	82
297	.15	.68	96
347	.15	.7	112
68	.1	.4	22
71	.1	.41	23
75	.1	.42	24
80	.1	.43	26
84	.1	.44	27
89	.1	.45	29
94	.1	.46	31
99	.1	.47	32
105	.1	.48	34
111	.1	.49	36
118	.1	.5	38
132	.1	.52	43
149	.1	.54	48
168	.1	.56	54
190	.1	.58	61
216	.1	.6	70
247	.1	.62	80
283	.1	.64	91
327	.1	.66	105
379	.1	.68	122
443	.1	.7	142
93	.05	.4	30
98	.05	.41	32
103	.05	.42	33
109	.05	.43	35
115	.05	.44	37
122	.05	.45	40
129	.05	.46	42
136	.05	.47	44
144	.05	.48	47
152	.05	.49	49
161	.05	.5	52
181	.05	.52	58
204	.05	.54	66
230	.05	.56	74
261	.05	.58	84
297	.05	.6	96
339	.05	.62	109
389	.05	.64	125
448	.05	.66	144
520	.05	.68	167

608	.05	.7	195

Table 2: Details sensitivity analyses to various settings for hazard ratio values. Power is set at 0.8.

The minimum sample size has been calculated within the same general circumstances and similar outcome (disease recurrence) as the pilot study. Namely, the calculations are initially based on the HR of treatment group versus placebo group values ranging from 0.15-0.31 as obtained in our pilot study (assuming a 1:1 randomization). The overall ratio of events (disease recurrence) was 0.32 (7/22). However, given the small sample size of the pilot study, we have extended our sensitivity analysis up to the value of 0.56 for the hazard ratio and the corresponding sample size to 200 patients (see graph 1 and table 2). May we add that, under the assumption that the intervention is not worse, the numbers would also be valid for a setting in which one-sided alpha level of significance is set at 0.15 and HR = 0.6 and for one sided alpha level of significance of 0.05 and HR = 0.5 – with power held at 0.8.

17.3.2 Planned recruitment rate

NeoART aims to randomize 200 participants in total. We have faced challenges meeting our recruitment target in. We have therefore discussed these issues with the DMC and TSC in the UK and a decision has been made to combine the data from the UK with a mirror study in Vietnam (NeoART-Vietnam) and Malaysia (NeoART-Malaysia). Although different study populations, these are mirror studies in terms of trial protocol, study procedures and Investigational Medicinal Product source. The target recruitment in the UK will now be 34, Vietnam will be 46 and Malaysia will be 120 patients and a target trial recruitment of 200 patients.

In Malaysia, 5 Ministry Of Health, Universiti Malaya are ready to open the trial. We aim to recruit 8-12 participants to the study per month in each sites in Malaysia over 24 months.

17.3.3 Statistical analysis plan

The event of interest (failure defined as cancer recurrence from a survival analysis perspective) is defined as the first recurrence of the disease after the patients' treatment allocation and surgery. Patients will be followed-up for 2 years; if the event of interest does not occur the patient will be considered (right) censored. The general assumption for time-to-event (survival analysis) is that the censoring is non-informative, i.e. it is independent of the event process. The Cox proportional hazard (PH) model would be applied to investigate the hazard ratio of disease recurrence for the artesunate group versus placebo group. The semi-parametric technique allows no assumption to be made on the baseline hazard; nevertheless proportionality of the hazards in the two arms should hold. The latter assumption can be tested on the basis of Shoenfeld residuals after fitting the model to the data.

We discussed an interim analysis in December 2021 with the DMC and TSC. At this time point 78 patients will have reached the primary end point (recurrence free survival 2 years after surgery). This analysis will be performed by an independent trial statistician familiar with both trials (Prof Bertrand Lell) and a report presented to the DMC and TSC members for review and recommendations thereafter.

17.3.3.1 Summary of baseline data and flow of patients

Details of the number of patients eligible for the trial, the number of patients consenting and the number randomised along with a breakdown for each group of the numbers of participants assigned, receiving the intended treatment, completing the study protocol, and analysed for the primary outcome will be clearly documented. This information will be displayed as a flow diagram (http://www.consort-statement.org/).

The analyses will be performed in STATA (StataCorp. 2014. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.) and/or (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/). GraphPad (Prism) or other comparable statistical package.

Descriptive statistics, preliminary analysis and graphics will be used to understand the data structure, the nature of the variables and the appropriateness of the subsequent tests and analyses. Data will be summarized as mean, standard deviation and range if normally distributed. If the data are skewed then additional statistics such as median and inter-quartile ranges will be used to correctly describe the selected sample in the population. Parametric or non-parametric tests will be used as appropriate to ensure/verify that the two groups do not differ statistically with respect to any aspects but the intervention.

Three safety interim analyses are planned as the trial data collection proceeds, approximately after 10%, 50% and 75% of patients have completed their day 28 assessments.

The appropriate statistical framework for the analysis of the primary outcome is that of a survival analysis. Patients will be followed-up up to 2 years; if the event of interest does not occur within this time frame, the patient is considered (right) censored. The general assumption is that the censoring is independent of the event process. The Cox proportional hazard (PH) model would be applied to investigate the hazard ratio of disease reccurrence for the artesunate group vs. placebo group. The semi-parametric technique allows no assumption to be made on the baseline hazard; nevertheless proportionality of the hazards in the two arms should hold. The latter assumption can be tested on the basis of Shoenfeld residuals after fitting the model to the data.

The inference is expressed in survival type metrics: i.e. proportion of individuals surviving recurrence beyond 1 year, 2 years and median time to recurrence in each group. Predictions (survival prognostics) will refer to the colon cancer population within our data range.

Missing data (attrition) will be assessed and sensitivity analysis will be employed upon the particularities exhibited by the data, i.e. missing data pattern and whether the missing information is associated with the main outcome. This will include data analysis under a 'missing at random assumption' and imputation methods tailored to interval censored data will also be applied.

Both a frequentist and Bayesian framework for statistical inference are envisaged. The uncertainty will be expressed in terms of 95% confidence intervals (CI) or 95% credible intervals (CrI) for their corresponding estimates. The frequentist statistical significance would be deemed at the 0.05 (5%) level.

17.3.3.2 Primary endpoint analysis

Additional exploratory analyses will be undertaken of the potential impact of tumour biomarkers on treatment efficacy and prognosis. The analyses of these outcomes will be decided upon depending on data structure and nature, i.e. whether to use one measurement or multiple measurements per individual. Appropriate multilevel approaches will be implemented in case of the later. Suitable variable transformations will be applied if required by modelling assumptions. Missing data patterns will also be evaluated and appropriate methodologies and sensitivity analyses will be used to assess the results sensitivity compared to the statistical assumptions.

17.3.3.3 Secondary endpoint analysis

Overall survival

Overall survival and cancer specific survival analyses at 2 and 5 years will be treated in the same manner as progression free survival analysis.

Pathological Response/assessment of tumour regression

Pathological tumour response will be assessed for all patients and comparisons examined using the chi-squared test with 95% confidence intervals expressed for response rates.

Toxicity assessments

The proportion of patients experiencing grade 3 or 4 toxicity will be compared between the two treatment arms using the chi-squared test. When cell frequencies are less than 5, Fisher's exact test will be used. The chi-squared test for trend will also be used to compare all toxicity scores.

Locoregional failure

Patterns of locoregional failure will be reported descriptively.

Quality of life

The functional and symptomatic scales of the EORTC quality of life questionnaires (EuroQol EQ-5D, EORTC QLQ C-30, EORTC QLQ-CR29) will be examined by calculating the differences in measurements from baseline. The standard EORTC recommended methods will be used to calculate function and symptomatic scores. The nature of the variable which quantifies this EORTC score will be investigated and summary statistics will be presented for values at baseline and post treatment. A multivariable regression type analysis will be conducted with post-treatment scores and pre- treatment (baseline) scores as an outcome and groups defined by the interventions as explanatory variables. Additional explanatory variables such as age, gender, disease stage may also be included. Missing data will be investigated in a similar fashion as previously described.

Surgical morbidity/mortality

The proportion of patients experiencing an adverse event in relation to surgical morbidity/mortality will be compared between the two treatment arms using the chi-squared test. When cell frequencies are less than 5, Fisher's exact test will be used. The chi-squared test for trend will also be used to compare rates of adverse events. Length of hospital stay will be reported as a mean.

Molecular biomarkers

Predictive value of tumour biomarkers in terms of predicting response to artesunate therapy and molecular profiling studies will be evaluated. Where appropriate, paired analyses will be performed to evaluate the effect of known prognostic factors or potential prognostic factors on outcomes observed.

17.4 Randomisation

Patients recruited to the study will be randomised to receive either artesunate or placebo in equal proportions. Randomisation lists will be generated for each site by computer-generated code, supplied by the study Sponsor's representative to the Clinical Trials Pharmacist or a study coordinator who is not part of the investigator's team only. The code will only be broken in exceptional

circumstances, when knowledge of the investigational drug is essential for medical treatment of the patient.

17.5 Interim analysis

An interim analysis reviewing feasibility and tolerability will be performed once 10%, 50% and 75% of patients have completed their day 42 assessments. Upon data collection availability, further refined analysis may be possible using advanced multistate modelling methodologies (Schmoor *et al* 2013).

We have discussed an interim analysis in December 2021 with the DMC and TSC. At this time point 78 patients will have reached the primary end point (recurrence free survival 2 years after surgery). This analysis will be performed by an independent trial statistician familiar with both trials (Prof Bertrand Lell) and a report presented to the DMC and TSC members for review and recommendations thereafter.

17.6 Other statistical considerations

It is possible that the general Cox proportional hazard model assumption, i.e. proportional hazard may not hold. Parametric survival analyses may then be required, such as accelerated time failure (AFT) models. Whereas a proportional hazards model assumes that the effect of a covariate is to multiply the hazard by some constant, an AFT model assumes that the effect of a covariate is to accelerate or decelerate the life course of a disease by some constant. However, this is especially appealing in a technical context where the 'disease' is a result of some mechanical process with a known sequence of intermediary stages and more appropriate in case of fast dynamic diseases. It may also be possible that it is more appropriate to assume that the failure rate is proportional to a power of time – in which case a Weibull type distribution may be a more accurate description of the process. However, all these statements are speculative as they depend on the patterns in the data which are impossible to accurately predict at this stage.

18. Committees involved in the trial

Appropriate oversight mechanisms will be put in place for the trial.

1. Trial Management Group (TMG) – will include those individuals responsible for the day-to-day management of the trial, such as the CI, statistician, trial manager, research nurse, data manager, Clinical Trial Monitor/Sponsor representative. The role of the group is to monitor all aspects of the conduct and progress of the trial, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the trial itself. Decisions about continuation or termination of the trial or substantial amendments to the protocol will be the responsibility of the Trial Management Group as informed by the TSC and DMC.

2. Trial Steering Committee (TSC) - provides overall supervision of the trial and ensures that it is being conducted in accordance with the principles of GCP and the relevant regulations. The Trial Steering Committee should agree the trial protocol and any protocol amendments and provide advice to the Investigators on all aspects of the trial. A Trial Steering Committee may have members who are independent of the Investigators, in particular an independent chairperson. The TSC will discharge its saftey assessment of the study to the DMC. The TSC will advise the Sponsor directly of any decision regarding any change to study status in accordance with the NeoART TSC Charter.

3. Data Monitoring Committee (DMC) - will meet to review the interim analysis results and to assess whether there are any safety issues that should be brought to the attention of the TSC. The Data Monitoring Committee should be independent of both the Investigators and the funder/Sponsor and should be the only body that has access to unblinded data. The DMC will operate in accordance with the NeoART DMC Charter.

All formally constituted trial groups/committees will be provided with a charter that sets out group composition, quorum requirements, responsibilities, structure of meetings, processes for decision making and reaching consensus.

19. Direct access to source data

The Investigator(s)/institution(s) will permit trial-related monitoring, audits, relevant ethics committee review, and regulatory inspection(s), providing direct access to source data/documents. Trial participants are informed of this during the informed consent discussion. Participants will consent to provide access to their medical notes.

20. Site approval and ongoing regulatory compliance

Before any site can enroll patients into the trial, the Sponsor will be in possession of ethics approval. The site will have a fully executed Clinical Site Agreement and have received the Open to Recruitment letter issued by the Sponsor. The site must conduct the trial in compliance with the protocol as agreed by the Sponsor and, by the regulatory authorities as appropriate and which was given a favourable opinion by the relevant ethics committee and NMRR.

The Chief Investigator will be provided (via the Sponsor) with file indexes i.e. TMF index and ISF index for use with SOP of 'Preparation and Maintenance of the TMF'. The CI will be responsible for the maintenance of the TMF and will delegate the responsibility of ISF file maintenance to the PI at each participating site.

It is the responsibility of the PI at each site to ensure that all subsequent amendments gain the necessary approvals. Refer to SOP of 'Management of Amendments'. This does not affect the individual clinician's responsibility to take immediate action if thought necessary to protect the health and interest of individual patients (see section 14.6 for details of reporting procedures/requirements).

Within 90 days after the end of the trial, the CI and Sponsor will ensure that the relevant ethics committee and the National Pharmaceutical Regulatory Authority are notified that the trial has finished. If the trial is terminated prematurely, those reports will be made within 15 days after the end of the trial. Refer to SOP 'End of study declaration'.

The CI will supply an End of Study report of the clinical trial to the NPRA and relevant ethics committee within one year after the end of the trial. The Sponsor can provide the End of study Report template.

21. Monitoring plan for the trial

The CI will be requested to complete the Risk Assessment Questionnaire and forward to the Sponsor to facilitate appropriate costing and Sponsorship in Principle to be issued prior to NMRR application. The trial will be monitored according to the risk based monitoring plan agreed by the Sponsor and CI. The governance team will determine the initial project risk assessment and justify change as the study

progresses. The PI at each collaborating site in addition to site monitoring visits will required to complete self-monitoring form(s) and must return the forms to the Sponsor for review and action. Failure for any PI to comply with requests for on behalf of the Sponsor may be escalated in accordance with SOP Escalation Procedure ; the site may also be selected for a GCP audit.

It is the Sponsor's Clinical Research Associate' s (CRA) responsibility to ensure that any findings identified in any monitoring report are actioned appropriately and in a timely manner and that any violations of GCP or the protocol will be reported to the CI and Sponsor. Any serious breach will be handled according to SOP Serious Breach Reporting.

Any urgent safety measures at either the CI or a PI site must be reported by that site, as per NPRA Regulations. The CI and/or the CI delegate will be provided with a copy of the study monitoring plan during the Trial Initiation monitoring visit.

22. Finance

Funded by the Malaysian Federal Government, Selangor State Government, charitable and philanthropic donations provided financial support for the study.

23. Insurance and Indemnity

Metanoic Health Limited as the study Sponsor holds insurance to cover participants for injury caused by their participation in the clinical trial. Participants may be able to claim compensation if they can prove that sponsor has been negligent. This includes negligence in the writing of the protocol, or selection of trial resources.

Where the trial is conducted in a hospital, the hospital has a duty of care to participants. Metanoic Health Limited will not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. Hospitals selected to participate in this clinical trial shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to Metanoic Health Limited ,upon request.

Participants may be able to claim compensation for injury caused by participation in this Trial without the need to prove negligence on the part of sponsor or another party.

If a participant indicates that they wish to make a claim for compensation, this needs to be brought to the attention of Metanoic Health Limited immediately. Failure to alert Metanoic Health Limited without delay and to comply with requests for information by the sponsor or any designated Agents may lead to a lack of insurance cover for the incident.

24. Intellectual Properties and development policy

Unless otherwise specified in agreements, the following guidelines shall apply : All Intellectual Property Rights and Know How (IP) related to the Protocol and the Trial are and shall remain the property of the Sponsor excluding :

1) pre-existing IP related to clinical procedures of any Hospital.
2) pre-existing IP related to analytical procedures of any external laboratory.

All contributors shall assign their rights in relation to all Intellectual Property Rights and in all Know How, not excluded above, to the Sponsor and at the request and expense of the Sponsor, shall execute all such documents and do all such other acts as the Sponsor may reasonably require in order to vest fully and effectively all such Intellectual Property Rights and Know How in the Sponsor or its nominee.

All contributors shall promptly disclose to the Sponsor any Know How generated pursuant to this Protocol and not excluded above and undertake treat such Know How as confidential information jointly owned it and the Sponsor.

Nothing in this section shall be construed so as to prevent or hinder the medical professional team from using Know How gained during the performance of the Trial in the furtherance of its normal business activities, to the extent such use does not result in the disclosure or misuse of Confidential Information or the infringement of any Intellectual Property Right of the Sponsor.

Transfer of ownership of Intellectual Property and Know How from Sponsor will be arranged through the principal funding and coordinating agency in Malaysia (based on funding for the study coming from the Malaysian Federal Government and Selangor State Government).

25. Publication Policy

Publication: "Any activity that discloses, outside of the circle of trial investigators, any final or interim data or results of the Trial, or any details of the Trial methodology that have not been made public by the Sponsor including, for example, presentations at symposia, national or regional professional meetings, publications in journals, theses or dissertations."

All scientific contributors to the Trial have a responsibility to ensure that results of scientific interest arising from Trial are appropriately published and disseminated. The Sponsor has a firm commitment to publish the results of the Trial in a transparent and unbiased manner without consideration for commercial objectives.

To maximise the impact and scientific validity of the Trial, data shall be consolidated over the duration of the trial, reviewed internally among all investigators and not be submitted for publication prematurely. Lead in any publications arising from the Trial shall lie with the Chief Investigator in the first instance, in discussion with Sponsor.

25.1 Before the official completion of the Trial

All publications associated with this trial will be discussed with the Sponsor but duties regarding publication will be delegated to the Chief Investigator. Any disputes regarding authorship will be discussed and settled by the Trial Steering Committee. Exempt from this requirement are student theses that can be submitted for confidential evaluation but are subject to embargo for a period not shorter than the anticipated remaining duration of the trial.

25.2 Up to 180 days after the official completion of the Trial

- During this period the Chief Investigator shall liaise with all investigators and strive to consolidate data and results and submit a manuscript for peer-review with a view to publication in a reputable academic journal or similar outlet as the Main Publication.

- The Chief Investigator and Surgical Oncology Trial Adviser shall be senior and corresponding/co-corresponding authors of the Main Publication.

- Insofar as compatible with the policies of the publication outlet and good academic practice, the other Investigators shall be listed according to contributions and/or under a Trial title such as Neo-ART.

- Providers of analytical or technical services shall be acknowledged, but will only be listed as co-authors if their services were provided in a non-routine manner as part of a scientific collaboration.

- Members of the Steering Group will be acknowledged as co-authors.

- Members of the DMC will be acknowledged in publications . If there are disagreements about the substance, content, style, conclusions, or author list of the Main Publication, the Chief Investigator and Surgical Oncology Trial Adviser shall arbitrate and ask the Steering Group to assist.

25.3 Beyond 180 days after the official completion of the Trial

After the Main Publication or after 180 days from Trial end date any Investigator or group of investigators may prepare further publications. In order to ensure that the Sponsor will be able to make comments and suggestions where pertinent, material for public dissemination will be submitted to the Sponsor for review at least thirty (30) days prior to submission for publication, public dissemination, or review by a publication committee. In exceptional circumstances this should be expedited. Sponsor's reasonable comments shall be reflected. All publications related to the Trial shall credit the Chief and Surgical Oncology Trial Adviser as co-authors where this would be in accordance with normal academic practice and shall acknowledge the Sponsor and the Funders.

26. Statement of compliance

The trial will be conducted in compliance with the protocol, Sponsor's Standard Operating Procedures (SOPs), GCP and the applicable regulatory requirement(s).

The study conduct shall comply with all relevant laws of Malaysia in which the study site is located including but not limited to, the Human Rights Act 1998, Personal Data Protection Act 2010 (PDPA) of Act 709., the Human Medicines Regulations 2012, the Medicines for Human Use (Clinical Trial) Regulations 2004, and with all relevant guidance relating to medicines and clinical studies from time to time in force including, but not limited to, the ICH GCP, the World Medical Association Declaration of Helsinki entitled 'Ethical Principles for Medical Research Involving Human Subjects' (2008 Version). This study will be conducted in compliance with the protocol approved by the relevant ethical committee and according to GCP standards and NPRA. No deviation from the protocol will be implemented without the prior review and approval of the Sponsor except where it may be necessary to eliminate an immediate hazard to a research participant. In such case, the deviation will be reported to the Sponsor and relevant ethical committee as soon as possible.

27. List of Protocol appendices

Appendix 1 Protocol Amendment/Revision History

Appendix 2	Summary chart of study assessments
Appendix 3	Quality of Life Questionnaires EuroQol EQ-5D, EORTC QLQ C-30, EORTC QLQ CR29
Appendix 4	Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0

28. References

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Appendix 1. Protocol amendment /Revision History

Protocol Version and Date	Amended text Section details and change
Version 2.0 6 January 2023	Title: Changed the short name of the study from NeoART Trial to NeoART-M trial
Version 2.0 6 January 2023	Front page: Added protocol ID: 2023-MHL-001 Changed protocol version and date Changed Chief Investigator's email address to skrishna@cadt.org.uk Changed of sponsor to Metanoic Health Limited Changed of sponsor representative to Dr Isaac John
Version 2.0 6 January 2023	Signature page and statement - Changed sponsor representative
Version 2.0 6 January 2023	2. Roles and Responsibilities - Added Co-Principal Investigator - Added Lead Research Pharmacists
Version 2.0 6 January 2023	3. Study Synopsis -Anticipated primary recruitment completion date: 31stJuly 2025 Sponsor: Metanoic Health Limited
Version 2.0 6 January 2023	8. IMP Dosage regimen and rationale - 'The study product does not contain porcine, bovine or animal components.' - Methods of contraception that are acceptable for the trial include the following: The implant, the coil and male or female sterilisation will be acceptable for the trial. The injection and most forms of hormonal contraception will also be deemed acceptable for the trial if used in combination with condoms or other barrier methods. However, condoms alone won't be sufficient during the study.
Version 2.0 6 January 2023	11.3 Prescribing & Dispensing of IMP 'The label must follow the Appendix E: Labelling Requirements of the Rules and guidance for Malaysian-Guideline-for-Application-of-CTIL-and-CTX-7.1-Edition and
Version 2.0 6 January 2023	11.4 Emergency unblinding - Each study site will have the capabilities of revealing patient treatment allocation in case of emergency. In the case where there
Version 2.0 6 January 2023	12.5.1.1 Obtaining, labelling, storing Permit to Import or Export of Human Tissues or Part Thereof Health Office/District Health Office (BLESS) to
Version 2.0 6 January 2023	13. Translational research / Pharmacodynamic Studies

	For instructions on processing and storage of study bloods, tissue samples please refer to the study lab manual.
Version 2.0 6 January 2023	17.3.2 Planned recruitment rate .The target recruitment in the UK will now be 40 to 50, Vietnam will be 40 to 50 and Malaysia will be 120 patients and a target trial recruitment of 200 patients in total by 31/07/2025. We aim to recruit 8-12 participants to the study per month in each site in Malaysia to meet our recruitment target by 31st July 2025.
Version 2.0 6 January 2023	22. Finance The study will be funded through Centre for Affordable Diagnostics and Therapeutics
Version 2.0 6 January 2023	23. Insurance and Indemnity Added Metanoic Health Limited holds insurance to cover participants
Version 2.0 6 January 2023	Added Intellectual Properties
Version 3.0 11 July 2023	12.5.1.1 Obtaining, labelling, storing A new ethics approval will be obtained from University Malaya Medical Centre Medical Research Committee (UMMC-MREC) should we need to use the transferred samples for any further exploratory analysis.
Version 4.0 10 May 2024	Changed email to sponsor: clinicaltrials@metanoichealth.com
Version 4.0 10 May 2024	Removed Co-PI Associate Professor Khong Tak Loon and Dr Norfadhlina Binti Abdul Satar and replaced with Dr David Lee Dai Wee Pusat Perubatan Universiti Malaya Lembah Pantai,59100 Kuala Lumpur Email: daiwee@ummc.edu.my Dr Ruben Gregory Xavier Pusat Perubatan Universiti Malaya Lembah Pantai,59100 Kuala Lumpur Email: rubengregory@ummc.edu.my

	Removed Dr Wong Pak Kai as Co-PI from USM.
Version 4.0 10 May 2024	Study Synopsis Changed funding through Centre for Affordable Diagnostics and Therapeutics to Funded by the Malaysian government, Selangor State Government, charitable and philanthropic donations provided finaleencial support for the study. Changed Start date from 'August 2023' to 'Open to recruitment in Malaysia: Q22024' Changed Anticipated primary recruitment completion date from '31st July 2025' to 'Q4 2025' Changed email clinicaltrials@metanoichealth.com to contact details
Version 4.0 10 May 2024	8. IMP Dosage regimen and rationale Packaging and storage conditions Changed One patient box will provide 28 tablets – sufficient for the duration of the study to One patient bottle will provide 28 tablets – sufficient for the duration of the study.
Version 4.0 10 May 2024	10. Subject/Patient Recruitment process 'NeoART aims to randomise 200 participants worldwide with 120 patients in Malaysia. We aim to recruit 8-12 participants to the study per month to meet our recruitment target over 24 months. Our Phase I pilot trial (Krishna et al., 2015) was the first trial to investigate neoadjuvant artesunate for colorectal cancer and demonstrated the feasibility, practicability, and safety of this novel approach.'

Version 4.0 6 January 2024	<u>17.3.2 Planned recruitment rate</u> Changed 'NeoART aims to randomize 200 participants in total. Due to the COVID19 pandemic, the clinical trial has been on hold, and we have faced challenges meeting our recruitment target. We have therefore discussed these issues with the DMC and TSC and a decision has been made to combine the data from the UK with a mirror study in Vietnam (NeoART-Vietnam) and Malaysia (NeoART-Malaysia). Although different study populations, these are mirror studies in terms of trial protocol, study procedures and Investigational Medicinal Product source. The target recruitment in the UK will now be 40 to 50, Vietnam will be 40 to 50 and Malaysia will be 120 patients and a target trial recruitment of 200 patients in total by 31/07/2025.Principal Investigators at the 5 National Health Service sites throughout the UK are ready to re-open the trial and committed to recruiting to the study. In Vietnam, 2 large teaching hospitals will run the study (108 Military Central Hospital and the National Cancer Hospital which is under Ministry of Health). In Malaysia, 5 Ministry Of Health, University Malaya and University Science Malaysia are ready to open the trial. We aim to recruit 8-12 participants to the study per month in each sites in Malaysia to meet our recruitment target by 31st July 2025' to 'NeoART aims to randomize 200 participants in total. Due to the COVID-19 pandemic, the clinical trial has been on hold, and we have faced challenges meeting our recruitment target. We have therefore discussed these issues with the DMC and TSC and a decision has been made to combine the data from the UK with a mirror study in Vietnam (NeoART-Vietnam) and Malaysia (NeoART-Malaysia). Although different study populations, these are mirror studies in terms of trial protocol, study procedures and Investigational Medicinal Product source. The target recruitment in the UK will now be 34, Vietnam will be 46 and Malaysia will be 120 patients and a target trial recruitment of 200 patients. In Malaysia, 5 Ministry Of Health, University Malaya are ready to open the trial. We aim to recruit 8-12 participants to the study per month in each sites in Malaysia over 24 months.'
Version 4.0 10 May 2024	22. Finance Funded by the Malaysian Federal Government, Selangor State Government, charitable and philanthropic donations provided financial support for the study.
Version 4.0 10 May 2024	24. Intellectual Properties and development policy Added 'Transfer of ownership of Intellectual Property and Know How from Sponsor will be arranged through the principal funding and coordinating agency in Malaysia (based on funding for the study coming from the Malaysian Federal Government and Selangor State Government).'
Version 5.0 31 July 2024	8. IMP Dosage regimen and rationale We have removed bovine or animal components fromt the statement . The study product does not contain porcine, bovine or animal components.

Version 5.0 31 July 2024	Added 6.12 Assessment & management of potential risk Participants who develop CTCAE v5.0, Grade 3 or 4 serious adverse effects related to artesunate (or placebo) will be withdrawn from the study treatment but will continue in the study to enable an intention to treat analysis. The main possible risks associated with artesunate in this phase II trial are neutropenia and haemolytic anaemia. Patients should be monitored closely for these potential side effects and sites should take appropriate clinical action if such adverse events occur. Definition of anaemia is a disorder characterized by a reduction in the amount of hemoglobin in 100 ml of blood. Signs and symptoms of anaemia may include pallor of the skin and mucous membranes, shortness of breath, palpitations of the heart, soft systolic murmurs, lethargy, and fatigability. Patients developing neutropenia as defines by CTCAE 5.0 • Grade 3 anaemia: Hgb <8.0 g/dL; <4.9 mmol/L;<80 g/L; transfusion indicated • Grade 4 anaemia: Life-threatening consequences; urgent intervention indicated The treatment for anaemia is blood transfusion. Definition of neutropenia: A disorder characterized by an ANC <1000/mm3 and a single temperature of >38.3 degrees C (101 degrees F) or a sustained temperature of >=38 degrees C (100.4 degrees F) for more than one hour. Patients developing neutropenia as defines by CTCAE 5.0 • Grade 3 neutropenia : ANC <1000/mm3 with a single temperature of >38.3 degree C (101 degrees F) or a sustained temperature of >=38 degrees C (100.4. degrees F) for more than one hour • Grade 4 neutropenia: Life-threatening consequences; urgent intervention indicated Treatment for : GCSF for neutropenia
Version 5.0 31 July 2024	**8.6 Concomitant treatment** Added Definition of neutropenia: A disorder characterized by an ANC <1000/mm3 and a single temperature of >38.3 degrees C (101 degrees F) or a sustained temperature of >=38 degrees C (100.4 degrees F) for more than one hour. Grade 3 neutropenia : ANC <1000/mm3 with a single temperature of >38.3 degree C (101 degrees F) or a sustained temperature of >=38 degrees C (100.4. degrees F) for more than one hour.
Version 5.0 31 July 2024	**12.2 Baseline assessments** Removed **At sites participating in the biomarker sub-study, a pre-treatment blood sample will be taken for study molecular analyses.** Day 15 Surgery Blood tests – FBC, Kidney and liver function tests, CEA tumour marker Post treatment serum sample for study molecular analyses (at sites participating in biomarker substudy) – these blood tests will be done on either day 14 or 15.

Version 5.0 31 July 2024	17.3.2 Planned recruitment rate Changed ''NeoART aims to randomize 200 participants in total. Due to the COVID19 pandemic, the clinical trial has been on hold, and we have faced challenges meeting our recruitment target. We have therefore discussed these issues with the DMC and TSC and a decision has been made to combine the data from the UK with a mirror study in Vietnam (NeoART-Vietnam) and Malaysia (NeoART-Malaysia). Although different study populations, these are mirror studies in terms of trial protocol, study procedures and Investigational Medicinal Product source. The target recruitment in the UK will now be 40 to 50, Vietnam will be 40 to 50 and Malaysia will be 120 patients and a target trial recruitment of 200 patients in total by 31/07/2025.Principal Investigators at the 5 National Health Service sites throughout the UK are ready to re-open the trial and committed to recruiting to the study. In Vietnam, 2 large teaching hospitals will run the study (108 Military Central Hospital and the National Cancer Hospital which is under Ministry of Health). In Malaysia, 5 Ministry Of Health, University Malaya and University Science Malaysia are ready to open the trial. We aim to recruit 8-12 participants to the study per month in each sites in Malaysia to meet our recruitment target by 31st July 2025' to NeoART aims to randomize 200 participants in total. We have faced challenges meeting our recruitment target in. We have therefore discussed these issues with the DMC and TSC in the UK and a decision has been made to combine the data from the UK with a mirror study in Vietnam (NeoART-Vietnam) and Malaysia (NeoART-Malaysia). Although different study populations, these are mirror studies in terms of trial protocol, study procedures and Investigational Medicinal Product source. The target recruitment in the UK will now be 34, Vietnam will be 46 and Malaysia will be 120 patients and a target trial recruitment of 200 patients.
Version 5.0 31 July 2024	15.2 Data collection tool. Removed (*if participating in biomarker sub study)* •Baseline - FBC, urea and electrolytes, LFTs, CEA, pre-treatment serum sample for study molecular analyses (*if participating in biomarker sub study)* • Day 14/15 of treatment - FBC, urea and electrolytes, LFTs, CEA, post-treatment serum sample for study molecular analyses (*if participating in biomarker sub study)*
Version 5.0 31 July 2024	17.3.3 Statistical analysis plan Change Due to the COVID-19 pandemic, we have discussed an interim analysis in December 2021 with the DMC and TSC. At this time point 78 patients will have reached the primary end point (recurrence free survival 2 years after surgery). This analysis will be performed by an independent trial statistician familiar with both trials (Prof Bertrand Lell) and a report presented to the DMC and TSC members for review and recommendations thereafter to We have discussed an interim analysis in December 2021 with the DMC and TSC. At this time point 78 patients will have reached the primary end point (recurrence free survival 2 years after surgery). This analysis will be performed by an independent trial statistician familiar with both trials (Prof Bertrand Lell) and a report presented to the DMC and TSC members for review and recommendations thereafter.
Version 5.0 31 July 2024	17.5 Interim analysis

	Changed 'Due to the COVID-19 pandemic, we have discussed an interim analysis in December 2021 with the DMC and TSC. At this time point 78 patients will have reached the primary end point (recurrence free survival 2 years after surgery). This analysis will be performed by an independent trial statistician familiar with both trials (Prof Bertrand Lell) and a report presented to the DMC and TSC members for review and recommendations thereafter. to We have discussed an interim analysis in December 2021 with the DMC and TSC. At this time point 78 patients will have reached the primary end point (recurrence free survival 2 years after surgery). This analysis will be performed by an independent trial statistician familiar with both trials (Prof Bertrand Lell) and a report presented to the DMC and TSC members for review and recommendations thereafter.
Version 6.0 4 October 2024	Added The study products do not contain porcine but contains produced from bovine that is safe for human consumption.

Appendix 2. Summary chart of study assessments

Study Schedule	Screening	Day 1	Day 7	Day 14/Day 15	Day 15	Day 28	Day 42	6 months	12 months	18 months	24 months	30 months	36 months	42 months	48 months	54 months	60 months
		(±3 days)	(±3 days)			(±7 days)	(±2 weeks)	(±6 weeks)	(±6 weeks)	(±6 weeks)	(±6 weeks)	(±6 weeks)	(±6 weeks)	(±6 weeks)	(±6 weeks)	(±6 weeks)	(±6 weeks)
Study Procedures	Prior to consent & study entry	Baseline Clinical Review	Telephone review	Review Prior to Surgery	Surgery	Blood test check	Routine follow up 4 weeks post surgery	Follow up	Follow up	Follow up	Follow up	Follow up	Follow up	Follow up	Follow up	Follow up	Follow up
Drug Taken		X	X	X													
Medical History	X	X	X				X	X	X	X	X	X	X	X	X	X	X
Clinical Exam	X																
Concomitant Medication		X	X	X													
Vital signs	X																
Pregnancy Test																	
Blood Test (FBC/renal/ liver profile)		X		X		X	X										
Blood test-CEA tumour marker		X		X			X	X	X	X	X	X	X	X	X	X	X
Blood test- EDTA sample x 2 for molecular analyses (at relevant participating sites)		X		X													
Colonoscopy	X								X								
Surgery					X												
CT scan	X										X						X
QOL Tools: - EuroQol EQ-5D - EORTC QLQ C-30 - QLQ-CR29		X	X	X													

Appendix 3. QOL Tools (EuroQol EQ-5D, EORTC QLQ C-30 and EORTC QLQ CR29)

Appendix 4. Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0