UNIVERSITY OF BIRMINGHAM

IMMUNOTACE TRIAL

A Randomised phase II Clinical Trial of conditioning cyclophosphamide and Chemoembolisation with or without Vaccination with Dendritic Cells pulsed with HepG2 lysate in vivo in Patients with Hepatocellular Carcinoma

EUDRACT NUMBER: 2011-001690-62

Protocol VERSION: 1.0 1st August 2011

PROTOCOL NUMBER: HE2016

ISRCTN:

Sponsor Number Rg_10-148

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This Protocol has been approved by :

Professer David Adams **Signature :** **Date :**

Chief Investigator

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

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AMENDMENTS

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

Amendment No.	Date of Amendment	Version No.	Type of amendment? (e.g. substantial/non-substantial/administrative change)

PRINCIPAL INVESTIGATOR PROTOCOL SIGNATURE

I have thoroughly read and reviewed the study protocol:

A RANDOMISED PHASE II CLINICAL TRIAL OF CONDITIONING CYCLOPHOSPHAMIDE AND CHEMOEMBOLISATION WITH OR WITHOUT VACCINATION WITH DENDRITIC CELLS PULSED WITH HEPG2 LYSATE IN VIVO IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

VERSION 1.0
1ST AUGUIST 2011

I confirm I have read and understood the requirements and conditions of the aforementioned version of the trial protocol. I confirm my team and I will adhere to this version of the protocol following receipt of the required local approvals.

PRINCIPAL INVESTIGATOR'S NAME

……

SIGNATURE

……

DATE

……

The Principal Investigator should sign this page and return a copy to the Trials Office.
The signed protocol should be kept in the Investigator Site File.

TRIAL SYNOPSIS

Title

A randomised phase II trial of low dose cyclophosphamide and chemoembolisation with or without vaccination with dendritic cells (DC) pulsed *ex vivo* with HepG2 cell lysate in patients with hepatocellular carcinoma.

Background

A previous study by the Liver Research Team (Birmingham) using DC pulsed *ex vivo* with the lysate of the HepG2 cell line has shown clinical benefit with evidence of antigen-specific T-cell responses in some patients with advanced HCC. This project intends to investigate the efficacy of this vaccine in combination with chemoembolisation compared to chemoembolisation alone in patients with intermediate stage HCC. All patients will receive a conditioning regimen comprising low dose cyclophosphamide.

Study Design

Phase II randomised, open label, multi centre study

Objectives

To determine whether activity due to the addition of DC vaccine to chemoembolisation and preconditioning cyclophosphamide warrants further investigation in a large randomised phase III clinical trial.

Recruitment / Patient Population

Patients over the age of 18 years, with HCC with performance status 0 - 2, with adequate renal function and hepatic function and in Child-Pugh category A or B and considered suitable for chemoembolisation. There should be no active concurrent infections or other malignancies and the patients must not be taking immunosuppressive therapy. Patients must not be immunocompromised.

Planned Interventions

Group 1: TACE therapy + preconditioning Cyclophosphamide only

Group 2: TACE therapy + preconditioning Cyclophosphamide + dendritic cells infusions.

Proposed Outcome Measurements

Primary outcome measure:

1. Progression free survival

Secondary outcome measures:

1. Radiological response rate (RECIST criterion)
2. Rate of change in the tumour marker serum AFP
3. Assessment of toxicity using NCI-CTCAE version 4
4. Immune response rate
5. Overall survival

Proposed Sample Size

A total of 70 (35 in each group) patients with HCC will be recruited.

TRIAL SCHEDULE

	Screening	Leukopheresis Group 2 patients only	Cy infusions visits		TACE therapy visit	Post TACE FU visits	Repeat cyc infusions visits	Repeat DC infusions visits	Three monthly FU visits
Day	-14 to 0	-14 to -1	1	29	31	38,45,52	60,90,120	62,92,122	152,242,332,422
Informed Consent	X								
Clinical Assessment[1]	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X
Screening Blood Tests[2]	X								
Mandatory Microbiology screening[3]	X								
Tumour assessment[4]	X						X		X
Auto-antibody screening tests[5]	X					X	X		X
Standard Blood Tests[3]			X	X		X	X		X
Immune responses blood tests	X		X	X		X	X		X
Blood collection for generation of MCM[6] via Leukopheresis		X							
Dendritic Cell infusion[7]					X			X	
Adverse Events	X	X	X	X	X	X	X	X	X
Clinical Events	X	X	X	X	X	X	X	X	X

1. Clinical assessment: This will include medical history, confirmation of diagnosis, examination, documentation of ECOG performance status and assessment for potential difficulties in intra-hepatic artery cannulation (i.e. severe atherosclerotic disease) and venous assess for leukopheresis at screening. Subsequent visits will only involve focussed history and relevant examination.

2. FBC: full blood count including measurement of haemoglobin, white blood cell count, platelets. INR: international normalized ratio – standardized measurement of coagulation. U+Es: urea, sodium, potassium, creatinine. LFT: liver function tests including albumin protein, alkaline phosphatase, aspartate transaminase, bilirubin, amylase. AFP: alpha fetoprotein.

3. As part of the screening procedure all patients will require mandatory testing for blood borne infectious agents as per National Blood Service requirements for screening of blood products prior to processing and storage. These tests must be performed within 30 days of Leukopheresis and consist of serological testing for HBV, HCV, Human Immunodeficiency Virus (HIV), Human T-Lymphotropic Virus 1 and 2 (HTLV-1, HTLV-2) and Syphilis. Appropriate pre-test counselling will be available and in the event of an unexpected positive result, the investigator will provide initial counselling and referral to the appropriate specialist service.

4. Tumour assessments: A baseline CT or MRI must be carried out in the 28 days prior to randomisation. Repeat Scan will be carried on day 60 and every 3 months (90 days) thereafter until disease progression

5. Auto-antibody screen: Rheumatoid factor, anti-nuclear antibody, anti-mitochondrial antibody, anti-thyroid antibodies, smooth muscle antibodies and LKM-antibodies

6. Leukophersis: Only patients randomised to group 2 will undergo leukopheresis for isolation of monocytes.

7. DC infusions: Only patients in Group 2 will receive dendritic cell infusions. First infusion will be given intra-hepatic at the same time as TACE. Subsequent infusions will be given by intravenous route.

ABBREVIATIONS

AE	Adverse Event
AFP	Alpha-Fetoprotein
ALD	Alcoholic Liver Disease
APC	Antigen Presenting Cell
ATC	Anatomical Therapeutic Chemical Classification
CI	Chief Investigator
CRF	Case Report Form
CTCAE	Common Toxicity Criteria Adverse Event
Cy	Cyclophosphamide
CT	Computed tomography
CTL	Cytotoxic T cells
DC	Dendritic Cells
DoH	Department of Health
ECOG	Eastern Cooperative Oncology Group
GCP	Good Clinical Practice
Hb	Haemoglobin
HbeAg	Hepatitis B Core Antigen
HbsAg	Hepatitis B Surface Antigen
HIV	Human Immunodeficiency Virus
HCC	Hepatocellular Carcinoma
HLA	Human Leukocyte Antigen
ICH GCP	International Conference on Harmonisation of Good Clinical Practice
IHA	Intra-Hepatic Arterial
IL-4	Interleukin 4
KLH	Keyhole limpet hemocyanin
LFTs	Liver Function Tests
MCM	Macrophage Conditioned Medium
MHC	Major Histocompatability Complex
MPL	Monophosphoryl Lipid A
MRI	Magnetic resonance imaging
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
Plts	Platelets
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TIL	Tumour Infiltrating Lymphocyte
U&Es	Urea and Electrolytes
WBC	White Blood Cell
WHO	World Health Organisation

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1. BACKGROUND AND RATIONALE

1.1. Background

Hepatocellular carcinoma (HCC) is the most common primary hepatic tumour and is one of the commonest cancers worldwide. It is especially common in Asia and Sub-Saharan Africa. Risk factors for its development worldwide include Hepatitis B, Hepatitis C, alcohol abuse, aflatoxin exposure and metabolic liver disease.

Although HCC is relatively rare in the western hemisphere with a prevalence of 4 cases per 100,000 populations in the United States its incidence is rising both in the United States[1] and the United Kingdom[2]. This is likely to be a reflection of the increased prevalence of cirrhosis from three main causes: hepatitis C, fatty liver disease and alcoholic liver disease (ALD). It is therefore likely to become a major health burden in the UK in the coming years.

Current treatment for HCC is limited and 5-year survival for all stages combined is less than 5%. A retrospective study evaluated survival in North American patients with all stages of HCC and found median survival to be only 10 months.[3] At present surgery, either tumour resection or liver transplantation is the only potentially curative treatment for HCC. However resection is feasible in less than 10% of patients as they are required to have small tumours, limited stage disease and good hepatic function. Hence, the majority of patients present with advanced disease that is deemed unresectable. Survival is relatively poor even in those who undergo surgical resection with high recurrence rates and a 5-year survival of about 30-60%.[4]

Treatment for unresectable but localized HCC includes local ablative therapy such as percutaneous ethanol injection or percutaneous radiofrequency ablation.[5] These interventions have become the mainstay of treatment for patients with unresectable HCC and in carefully selected cases have shown good outcomes. Patients who are unsuitable for surgery or local ablation but with liver-confined disease may derive palliative benefit from transarterial chemoembolisation (TACE). Two randomised controlled trials have shown that TACE performed with doxorubicin or cisplatin improves survival in selected patients compared to best supportive care. A subsequent meta-analysis including 7 trials and 545 patients reported a 2-year overall survival rate in treated patients of 41% (range, 19%-63%) versus 27% (range, 11%-50%) in the control group (odds ratio, 0.53; 95% confidence patients with large interval, 0.32-0.89; P =0.017).[6,7,8] Such benefit has not been demonstrated for trans-arterial embolisation (TAE) alone. On this basis TACE became the recommended first line non-curative therapy for non-surgical/multifocal HCC by the 2006 American Association for the Study of Liver Diseases (AASLD) guidelines.[9] In spite of the proven efficacy of TACE, treatment is associated with toxicity and mortality. Post embolisation syndrome consisting of pain, nausea and fever occurs in 60-80% of patients but is self-limiting lasting for 3-4 days. Less common but more serious side effects include liver failure 7.5%, ascites 8.3%, gastrointestinal bleeding 3%, liver abscess 1.3%, renal failure 1.8% and bile duct injury, 2%.[10] Treatment related 30-day mortality has been reported in 0-9.5% of patients treated with a median rate of 2.4%. Appropriate patient selection reduces the risk of serious side effects as demonstrated in the two positive RCTs.[7,8] Even so, TACE remains palliative and disease progression is inevitable such that combination with novel therapies is attractive. Since TACE may liberate an abundance of tumour antigens it may lend itself to combination with immunotherapeutic strategies.

1.2. The immunotherapy of malignancy: Antigens

Because immune mediated mechanisms play an important role in controlling the growth of some types of cancer,[11] immunotherapy could in theory exploit such responses to generate therapeutic immune responses against tumour antigens. Many tumours have altered gene regulation resulting in expression of specific tumour antigens or over expression of other proteins. One such category is the cancer/testis antigens, a category of tumour antigens that are normally expressed in male germ cells but not in adult somatic tissues. In malignancy, this gene regulation is disrupted, resulting in cancer/testis antigen expression in a proportion of tumours of various types, for instance alpha fetoprotein (AFP) is a serum marker for HCC.

Proteins that are specifically or predominantly expressed by tumours are potential targets for immunotherapy. Standard vaccination strategies have had only limited success in stimulating anti-tumour immunity leading to the use of adoptive therapy with T cells or dendritic cells (DC) to stimulate more potent immune responses against the cancer which selectively kill malignant cells expressing specific antigens. Tumour antigens expressed in varying degrees in HCC and thus potential targets for immunotherapy include AFP[12,13], MAGE-1 and 3[14] and SSX-1 and 4[14], TSPY[15], NY-ESO-1[16] and Glypican-3.[17] Furthermore, we recently identified the polycomb proteins BMI-1 and EZH2 as HCC-associated antigens.[18] However no single antigen has been proven to be present in all cases of HCC and, with the exception of AFP the knowledge of T cell reactivity against HCC tumour antigens are limited.

1.3. The immunotherapy of malignancy: Dendritic cells

Dendritic cells (DC) are potent antigen presenting cells which exist in peripheral tissues where they take up and process antigens, short peptide fragments of which (epitopes) are presented on the cell surface in association with the major histocompatibilty complex (MHC). Signals released by infected or damaged tissues act through receptors on the DC to promote their activation and maturation. Maturation is associated with expression of co-stimulatory molecules that allow them to activate T cells and chemokine receptors that mediate DC migration from peripheral tissue into draining lymph nodes where they interact with and activate T cells that recognise the presented epitopes resulting in the generation of effector T cells that can mount antigen-specific anti-tumour immune responses.

Following the demonstration that activated DCs were potent inducers of immune responses when adoptively transferred into animals, the development of techniques to generate large numbers of DC from peripheral blood enabled DC based immunotherapy to be been tested in clinical trials.[19] Trials in several cancers including malignant melanoma, renal cell cancer and HCC demonstrate the safety of DC administration with variable efficacy. The majority of trials used autologous monocyte-derived DC matured and loaded with antigen ex vivo but some studies have re-infused naïve DC in the expectation that they will pick up endogenous antigen and become activated and mature in vivo.[20] The rationale and success to date of each technique is discussed below.

1.4. Dendritic cell therapy: Antigen loading *ex vivo*

In order to be used therapeutically DC need to express a target antigen. If an antigen can be identified it can be loaded into DC *in vitro* before reinfusion into the patient allowing the investigator to control the antigen used and the state of DC maturation. Various techniques have been employed to load DC including pulsing with recombinant proteins, peptides or tumour lysates, RNA transfection and transfection with plasmid vectors encoding tumour associated antigens.[21] Although approaches using transfection are theoretically attractive they are expensive to implement under GMP conditions and the evidence that they are more clinically effective than vaccines using peptides or tumour lysates is lacking. Published clinical trials have used DC matured and loaded with antigens *in vitro* to treat patients with melanoma[22] HCC,[23] prostate and renal cell carcinoma.[24] Several of these studies report that infusion of *in vitro* sensitised DC enhances CTL responses *in vivo* in some patients with variable evidence of clinical efficacy. A study of 31 patients with melanoma[25] vaccinated with autologous DC pulsed with tumour peptides reported successful vaccination in 91% of patients 13 of whom had objective clinical responses. The vaccine was safe and well tolerated with mild erythema and induration at the injection site the only reported toxicity. Nestle *et al* reported 16 patients with metastatic melanoma who received autologous DC pulsed with autologous tumour lysates weekly for twelve weeks. All vaccinations were well tolerated; 11 patients developed functional responses to vaccination and 13 had objective clinical responses.

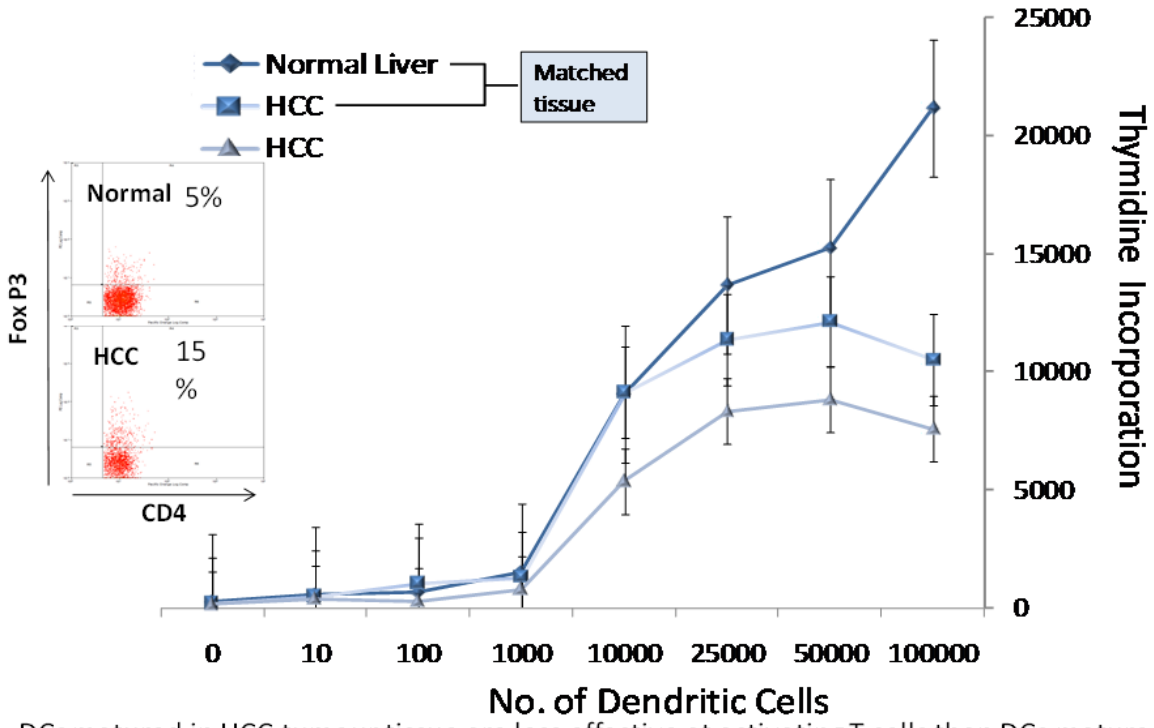
A previous study conducted at our centre demonstrated the safe intravenous administration of autologous DC pulsed with a cell lysate made from HepG2 cells (a hepatoblastoma tumour line) in patients with HCC.[21] 35 patients with advanced HCC not suitable for radical or loco-regional therapies received between 1 and 6 DC vaccinations each at 3-week intervals. In total, 134 DC infusions were administered with no significant toxicity. The treatment was well tolerated with only mild self-limiting toxicity; 8 patients experienced grade 1

myalgia occasionally associated with low-grade fever. No hepatic toxicity, autoantibody formation or dose limiting toxicity was reported. 25 patients who received at least 3 vaccine infusions were assessed clinically for response. The radiologically determined disease control rate (combined partial response and stable disease >3months) was 28%. In 17 patients the baseline serum AFP, a tumour marker which correlates with tumour load and response to therapy in HCC was >1,000 ng/mL; in 4 of these patients it fell to<30% of baseline following vaccination. In 1 patient there was a radiological partial response associated with a fall in AFP to <10% baseline. Immune responses were assessed using an ELIspot assay of interferon-γ (IFN-γ) release. In several cases T-cell responses to the vaccine and/or AFP were detected following vaccination. Lee *et al*[26] vaccinated 31 patients with advanced HCC with a course of intravenous infusions of autologous DC pulsed with tumour cell lysate. 12.9% achieved a partial response and 54.8% had stable disease. These results are encouraging and make it important to continue to investigate the role of DC therapy in HCC.

1.5. Dendritic cell therapy: Antigen loading *in vivo*

Studies have shown that DC loaded with antigens *ex vivo* and administered to tumour bearing hosts can promote T-cell mediated tumour destruction. However these studies necessitate maturation and loading of DC *in vitro*. This is a timely and costly procedure which has yet to show consistent and lasting anti-tumour responses.

Another therapeutic strategy involves inducing intra-tumoural DC to take up endogenous antigens within the tumour environment. Unfortunately DC found near or in most tumours are phenotypically and functionally immature and unable to stimulate T-cells to produce an anti-tumour response. Indeed they often promote a tolerogenic response which suppresses anti-tumour immunity.[27] Our own data (figure) show that DC within HCC express low levels of costimulatory molecules when compared with DC from surrounding non-malignant liver tissue and tend to induce the activation of regulatory T cells rather than CTL. This is a consequence of tumour-associated stromal cells which secrete factors including IL-6 that prevent full DC maturation. Thus if immature DC are injected into the tumour site they would be unlikely to fully mature to activate anti-tumour immunity. Song *et al*[28] evaluated the ability of DC to induce an anti-tumour response when injected directly into a B cell lymphoma. Mice with transplanted tumours were given intratumoural DC alone, systemic chemotherapy alone or intratumoural DC plus systemic chemotherapy. Intratumoural DC injection alone had no anti-tumour effect & systemic chemotherapy resulted in only transient tumour regression. However combined therapy led to complete, long term tumour regression in the majority of mice. This effect was systemic resulting in regression of tumour at other injected sites & was resistant to tumour rechallenge. The effect was the same whether immature or *in vitro* matured DCs were used. Thus intratumoural DC therapy has potential if DCs are given in combination with treatment that causes apoptotic or necrotic cell death allowing loading with tumour antigens & subsequent T-cell activation *in situ*.



DCs matured in HCC tumour tissue are less effective at activating T cells than DCs matured in normal liver tissue. Blood monocytes were matured to DCs in vitro in the presence of conditioned media from either non-malignant liver tissue or tumour tissue (HCC) and their ability to activate T cells in an MLR assessed. T cell proliferation was less in the presence of tumour tissue and more of the resulting T cells were regulatory cells CD4+CD25+Cd127low.

1.6. Evidence of an immune response in hepatocellular carcinoma

Cancers which evoke a lymphocytic infiltrate are generally more susceptible to immunotherapy.[20] These include malignant melanoma, renal cell carcinoma and hepatocellular carcinoma (HCC). HCC is heavily infiltrated by T-lymphocytes and the tumour shows strong expression of MHC Class 1 antigens and ICAM-1.[29] Furthermore, tumour-specific CTL can be expanded from HCC tissues which lyse autologous tumour cells more efficiently than non-specific targets. Thus HCC is potentially a good candidate tumour for immunotherapy. Further support for immunotherapy was provided by studies from the 1990s reporting clinical responses in HCC treated with local IL-2 and in vitro expanded adoptively transferred lymphocytes.[30] More recent studies, including our own phase I/II clinical trial, have reported clinical responses to adoptive immunotherapy with dendritic cells (see below). Thus a substantial body of evidence supports the development of immunotherapy to treat HCC.

1.7. Rationale for combining DC therapy with ablative therapy

Studies in experimental animals and more recently in humans show that ablative therapy of liver tumours can stimulate anti-tumour immune responses presumably by releasing tumour antigens and providing an environment of tissue damage and inflammation that activates local DC resulting in anti-tumour immune responses. These responses include the generation of CTLs against AFP in patients undergoing ablative therapy for HCC.[12,31-33] Thus it is logical to combine ablative therapy with DC vaccination in the present clinical trial because a) the tumour burden will be reduced b) DC vaccination will be taking place in a highly immunogenic environment that will enhance the probability of overcoming local tumour-mediated immune suppression c) both DC vaccination and ablative therapy have been shown to stimulate immune responses

against AFP d) it provides a route for administering at least one dose of DC directly into the tumour rather than peripherally.

1.8. Rationale for Cyclophosphamide conditioning

Some chemotherapeutic drugs including the anthracyclines, DNA-damaging compounds such as cyclophosphamide (Cy), are immunogenic and activate DC by molecularly defined pathways in the absence of additional stimuli. Cy has been used extensively in chemotherapy of solid tumours and lymphomas and as an immunosuppressive agent in some autoimmune conditions. Cy has a differential effect on lymphocyte compartments, rapidly depleting B and T cells followed by a recovery phase characterised by extensive proliferation and bone marrow mobilisation. Low doses of Cy selectively deplete immunosuppressive CD4+25+ T regulatory cells.[34,35]

Greten et al recently determined the optimal dose of Cy required to deplete Tregs in patients with HCC and showed that it unmasked CTL responses against AFP but had no direct effect on the tumour.[36,37] Previous studies have reported that Tregs suppress anti-tumour immunity in patients with HCC[37-39] and thus depleting Tregs should enhance the chance of DC vaccination inducing lasting anti-tumour immunity. Earlier phase II studies have indicated that Cy, even at higher doses, is not active against HCC and so we do not anticipate any direct cytotoxic effects from its use in this protocol.

1.9 Justification for patient population

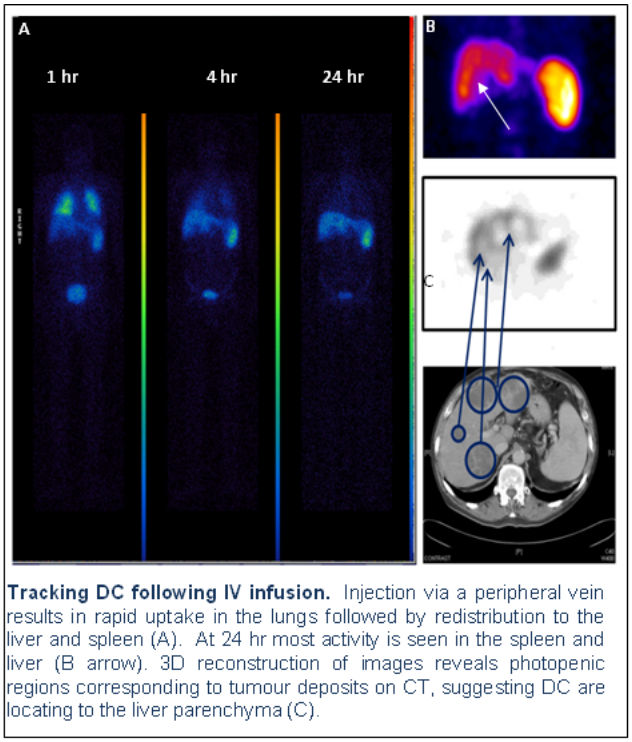
New approaches are required for the treatment of HCC as the worldwide incidence of this condition is rising rapidly. In the UK this is a direct consequence of the rising prevalence of chronic liver disease and cirrhosis. There are currently no satisfactory treatments for the majority of patients who present with locally advanced or metastatic disease and the overall survival rate is less that 5% at 5 years. DC vaccination therapy could potentially be used as an adjuvant treatment following resection or transplantation as well as in the treatment of more advanced disease making it potentially applicable to most patients with HCC. There is thus an unmet need for new therapies for HCC and the opportunity to have a major effect on an increasing and fatal disease.

1.10 Justification for trial design

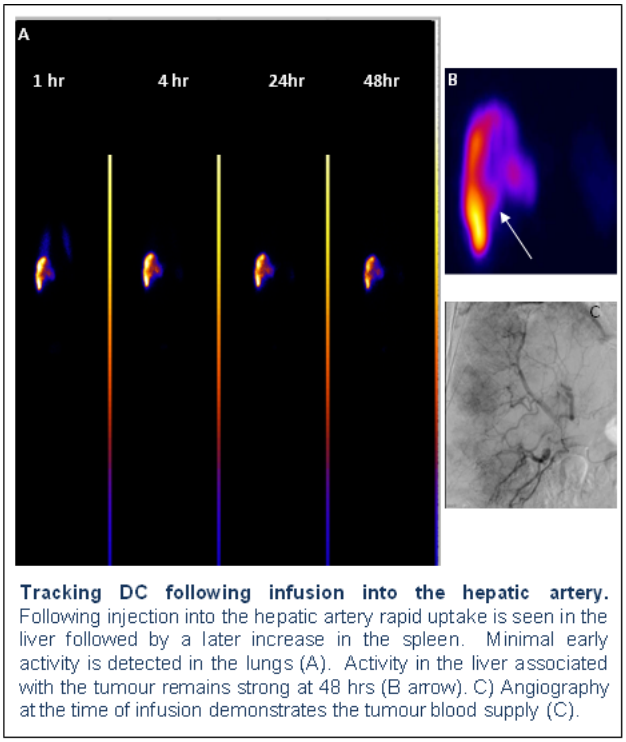
The studies discussed above, including our phase II clinical trial, show that some patients with HCC respond to DC therapy. The 28% disease control rate seen in our study was even more remarkable given that we studied patients with otherwise untreatable disease and large tumour loads which will hinder the efficacy of immunotherapy. There is thus a pressing need to build on these promising studies to develop a more effective way of using DC vaccination. We believe that combining DC vaccination with ablative therapy and regulatory T cell depletion mediated by low-dose Cy will greatly enhance the chances of success. The current randomised phase II clinical trial will determine the toxicity and efficacy of DC vaccination in combination with low-dose Cy conditioning and chemoembolisation compared with low-dose Cy and TACE. We have outlined the reasons for this combination of therapies above. We have not included an arm in which patients receive either low-Cy alone or TACE alone because there is good evidence from previous studies that low-dose Cy either alone or in combination with ablative therapy is ineffective. Thus if DC vaccination is effective we would expect to see significant differences between the two groups.

We decided to mature DC in autologous macrophage conditioned medium (MCM). Our own *in vitro* studies suggest that MCM is a highly effective maturation agent in the generation of DC from blood monocytes and an autologous maturation agent reduces the risk of toxicity. We have chosen to pulse the DC with HepG2 lysate because a) it was effective in our previous clinical trial b) it is readily available and we can standardise DC loading c) it contains several potential antigens including AFP, glypican-3 and polycomb antigens. DC

will also be loaded with Keyhole limpet hemocyanin (KLH), a model antigen available to GMP grade, which will allow us to monitor vaccine specific immune responses. The effectiveness of infused DC depends on their ability to activate effector T-cells that migrate to and destroy the tumour. This requires that infused DC migrate to hepatic and associated lymphoid tissue after transfer. The intravenous route of administration is the one that is clinically most straightforward and the one that has been used in many previous trials of DC therapy. We have studied the patterns of migration of DC in patients with liver tumours after both intravenous and intrahepatic delivery (see figure). Predictably intrahepatic administration results in accumulation of DC in the tumour and surrounding liver. Although intravenous delivery results in accumulation in the lungs within hours of infusion this is followed by redistribution of the DC to the liver and spleen at 12-24 hours suggesting that intravenous administration is an appropriate route of delivery. The present design allows us to deliver the first treatment directly into the tumour via the hepatic artery (IHA) under radiological guidance at the time of chemoembolisation, with further DC re-infused intravenously through a peripheral vein.



Tracking DC following IV infusion. Injection via a peripheral vein results in rapid uptake in the lungs followed by redistribution to the liver and spleen (A). At 24 hr most activity is seen in the spleen and liver (B arrow). 3D reconstruction of images reveals photopenic regions corresponding to tumour deposits on CT, suggesting DC are locating to the liver parenchyma (C).



Tracking DC following infusion into the hepatic artery. Following injection into the hepatic artery rapid uptake is seen in the liver followed by a later increase in the spleen. Minimal early activity is detected in the lungs (A). Activity in the liver associated with the tumour remains strong at 48 hrs (B arrow). C) Angiography at the time of infusion demonstrates the tumour blood supply (C).

Considerable previous information demonstrates the safety of dendritic cell infusion both within this organisation and in the published literature. Therefore the primary objective for this trial is to determine if the addition of DC vaccination is effective enough to warrant further investigation. Efficacy will be measured using progression free survival. A progression-based primary endpoint has been selected as the basis for statistical assumptions rather than radiological response rate, which is consistent with the AASLD guidelines for HCC clinical trial design (JNCI, 2008:698-711; www.aasld.org/practiceguidelines.aspx). It is recognised that conventional radiological response does not necessarily reflect anti-tumour efficacy in the context of HCC whereas progression is a reliable surrogate. Data from an audit of our own TACE experience (Palmer, personal communication) and from a recently presented TACE trial (J Clin Oncol 2010;28:15s; abstr 4025) indicate the PFS for TACE to be approximately 8 months and this has been used as the baseline for statistical calculations. All patients will be carefully monitored for unexpected adverse events. An important end-point for immunotherapy is to demonstrate appropriate immune activation and we are setting up a series of assays that will allow us to demonstrate whether the vaccine activates CD4 or CD8 T-cell responses and/or induces antibodies against cancer antigens. It is likely that host-dependent and tumour dependent factors will influence how well individuals respond to immunotherapy. We will attempt to define patients who are likely to respond using a proteomics approach to look for factors that might be associated with a clinically significant immune response.

3 AIMS, OBJECTIVES AND OUTCOME MEASURES

3.1 Aims and Objectives

To determine whether activity due to the addition of DC vaccine to chemoembolisation and preconditioning warrants further investigation in a large randomised phase III clinical trial.

3.2 Primary Outcome Measures

- Progression free survival

3.3 Secondary Outcome Measures

- Radiological response assessment (RECIST criterion)
- Rate of change in the tumour marker Serum AFP
- Assessment of toxicity using NCI-CTCAE (version 4)
- Immune Response Rate
- Overall survival

3.4 Demonstration of Immune Response

The demonstration of immune response to the vaccine is a crucial outcome measure and will be quantified in both groups because ablative therapy alone has been shown to induce anti-tumour immune responses. Patients will be monitored for immune responses on a weekly basis for the first month and then at monthly intervals following the first DC infusion. Multiple assays will be used to assess CD4 and CD8 T Cell responses during treatment. The numbers and percentages of T lymphocytes sub-populations in the peripheral blood of trial patients will be monitored by flow cytometry using serial blood samples before, during and after treatment. T lymphocytes responses will be measured by the detection of TAA lymphocytes against AFP, glypican-3, HepG2 lysate and responses to KLH. If cell numbers allow, the presence of antibodies against AFP and glypican-3 will be measured using quantitative ELISA techniques and T lymphocytes functions will be further characterised by intracellular flow cytometry.

It will be important in future studies to be able to predict which patients will respond to immunotherapy. It is likely that many factors will be involved and these may not be predictable. Thus we will collect blood serum samples to allow us to compare gene expression and protein profiles in responders versus non-responders using expression microarray analysis of expressed mRNA, multiplex analysis of secreted proteins and proteomic analysis. Prof Philip Johnson has previously used similar approaches to predict prognosis and outcome in patients with HCC.[40] These analyses will be done in the Functional Genomics and Proteomics Facility in the School of Bioscience with the input of Dr Wenbin Wei, Senior Bioinformatician, CRUK Centre for Cancer Sciences, University of Birmingham.

4 TREATMENT DETAILS

4.1 Medication preparation

All Groups

TACE Chemotherapy Type

Trade name: **Doxorubicin Hydrochloride** (*Generic product*)

Active Substance: **Doxorubicin Hydrochloride**

Pharmaceutical form: Powder for solution for injection

Route of administration: Intra-Hepatic

Investigational Medicinal Product Status: Non-IMP

ATC code: L01DB01

Doxorubicin hydrochloride is now a *generic product* and can be produced by multiple manufacturers. Doxorubicin Hydrochloride will be packaged and labelled in the standard manner according to the current marketing authorisation by the manufacturer. The drug will be purchased by the participating hospital via the normal NHS purchasing procedure. The hospital pharmacy at trial site will add the sponsor's details and trial specific labels to each individual drug package to meet the requirements of the EU's Good Manufacturing practice for Medicinal Products guidelines (Annex 13, Manufacture of investigational Medicinal Products, The Rules Governing medicinal products in The European Community, Volume IV). Doxorubicin hydrochloride will be stored and prepared in accordance with the specific manufacturer's Summary of Medicinal Product Characteristics (SmPC). A copy of the SmPC for the product used should be kept in the Pharmacy file.

The actual Doxorubicin chemotherapy regimen starting dose for use on this clinical trial will be dependent on the individual patient's Bilirubin (µmol/L) level (see section 10 for additional information in relation to dose modifications):

Initial Dose only

Bilirubin (µmol/L)	Actual Dose Level	Percentage equivalent of maximum dose
≤22	60mg/m^2	100%
23-50	30mg/m^2	50%

Conditioning Regimen

Trade Name: **Cyclophosphamide** (*Generic Product*)

Active Substance: **Cyclophosphamide monohydrate BP**

Pharmaceutical form: Powder for injection

Route of administration: Intravenous

Investigational Medicinal Product Status: IMP

ATC Code: L01AA01

Cyclophosphamide is now a *generic product* and can be produced by multiple manufacturers. Cyclophosphamide monohydrate will be packaged and labelled in the standard manner according to the current marketing authorisation by the manufacturer. The drug will be purchased by the participating hospital via the normal NHS purchasing procedure. The hospital pharmacy at each trial site will add the sponsor's details and trial specific labels to each drug to meet the requirements of the EU's Good Manufacturing practice for Medicinal Products guidelines (Annex 13, Manufacture of investigational Medicinal Products, The Rules Governing medicinal products in The European Community, Volume IV). Cyclophosphamide will then be stored in accordance with the SmPC. Cyclophosphamide will be stored in the original container and in at room temperature (below 25^{o}C).

Cyclophosphamide is provided as a powder for injection and will be stored in the original container and in at room temperature (below 25^{o}C). . The shelf life of Cyclophosphamide when stored as per SmPC and in original packaging is 36 months. After reconstitution for intravenous administration, the shelf-life recommended in the SmPC should be used and the final product should be protected from light and stored between 2-8^{o}C.

It is also permitted to purchase cyclophosphamide as a reconstituted solution ready for further dilution, if this is local practice by pharmacy at site. A copy of the SmPC for the product used should be kept in the Pharmacy file.

The conventional dose for Cyclophosphamide is 80-300mg/m^2 daily as a single i.v. dose. The actual conditioning regimen starting dose for use on this clinical trial will be (see section 10 for additional information in relation to dose modifications):

Day 1 +/- 3 (Individual 31 day cycle of therapy): 250 mg/m^2

Day 29 +/- 3 (Individual 31 day cycle of therapy): 250 mg/m^2

Subsequent infusion (Day 60, 90,121) +/-3 day: 250 mg/m^2

The initial starting dose is in accordance with the SmPC.

Group 2 patients only

Dendritic Cell pulsed with HepG2 lysate vaccine

Trade Name: Not applicable

Active Substance: Not applicable

Pharmaceutical form: Dendritic cells suspended in 10ml 0.9% sodium chloride solution and 0.5% serum albumin

Route of administration: Via intraheptic arterial (1st infusion only, given the same time as TACE therapy)
Intravenous (subsequent infusions)

Investigational Medicinal Product Status: IMP

ATC Code: Not Applicable

The dendritic cell vaccine product will be prepared as per the current Investigator Brochure

Peripheral blood mononuclear cells (PBMC) will be isolated from the leukapheresis product using the CliniMACS™ closed bag system provided by Miltenyi Biotec Ltd. CD14+ monocytes are enriched by immunomagnetic separation and all washing steps completed using the Cobe Spectra 2997 cell washing system. All reagents and hardware are GMP grade CE marked.

The CD14+ monocyte enriched fraction is cultured at $2x10^6$ cells/ml in cell culture bags (40 – 60ml per bag) at 37^oC / 5% CO_2 in a humidified incubator in DC medium containing Dentimax (GMP grade culture medium) GM-CSF 1000IU/ml and IL-4 1000IU/m. The medium is replenished by adding 10% of the start volume to the culture bag of DC medium on day 2 and 4. On day 5 the DC are washed, resuspended in freezing medium and separated into four equal portions (closed bag system as previously described) for cryopreservation until time near to each infusion. The stored DC will be thawed according to standard protocol and treated in the same manner as detailed below prior to each subsequent infusion.

Two days prior to each DC infusion, one bag of DC suspension will be thawed according to standard protocol. HepG2 cell lysate is added to the DC culture bag to give a final lysate concentration of 20μg/ml. KLH is added to give a final concentration of 10μg/ml. MPL is added to give a final concentration of 1μg/ml. The cells are then incubated for 24-48 hours at 37^oC / 5% CO_2 in a humidified incubator, ready for infusion. The resultant cells are harvested and washed twice in CliniMACS buffer containing 0.5% AB serum. After the second wash the cells are resuspended in 10ml 0.9% sodium chloride solution containing 0.5% AB serum and transferred to the WTCRF for infusion into the patient.

The release criteria are that there should be >50% of the cells are viable DC based on morphology and exclusion of trypan blue under light microscopy. The documentation for the cell preparation process is organized so that it can be verified that the appropriate reagents were used and that they were free of microbiological contamination and that all steps of the cell preparation procedure were carried out according to the work instructions.

No standardised dosage has been set out for the DC vaccine. However, data from the first 3 DC vaccine treated patients will be validated to provide information on the optimal dosage.

A master cell bank (MCB) of HepG2 cells is maintained by the Health Protection Agency Culture Collections (HPACC). The cells in the MCB are screened and determined to be free from infectious agents.

The procurement, processing, storage and distribution of the DC vaccine will be performed and licensed in accordance with the Quality and Safety Regulations of the Human Tissue Authority and Medicines and Healthcare products Regulatory Agency.

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5 Trial Design

5.1 Number of centres

This is a multicentre clinical trial. Patents will be recruited from up to three specially selected centres in the UK. The University Hospital Birmingham NHS Trust, Queen Elizabeth Hospital – Birmingham UK will be the lead research centre. .

5.2 Trial Design

This is an open label randomised phase II clinical trial

Patients will undergo a screening period (following written informed consent) of up to 14 days prior to entry into trial.

The trial will involve the preparation and infusion of HepG2 lysate loaded mature DC.

Patients who are eligible to enter into the trial will be randomised between the two treatment groups.

GROUP 1: TACE therapy + preconditioning Cyclophosphamide therapy only

GROUP 2: TACE therapy + preconditioning Cyclophosphamide + Dendritic cells infusions

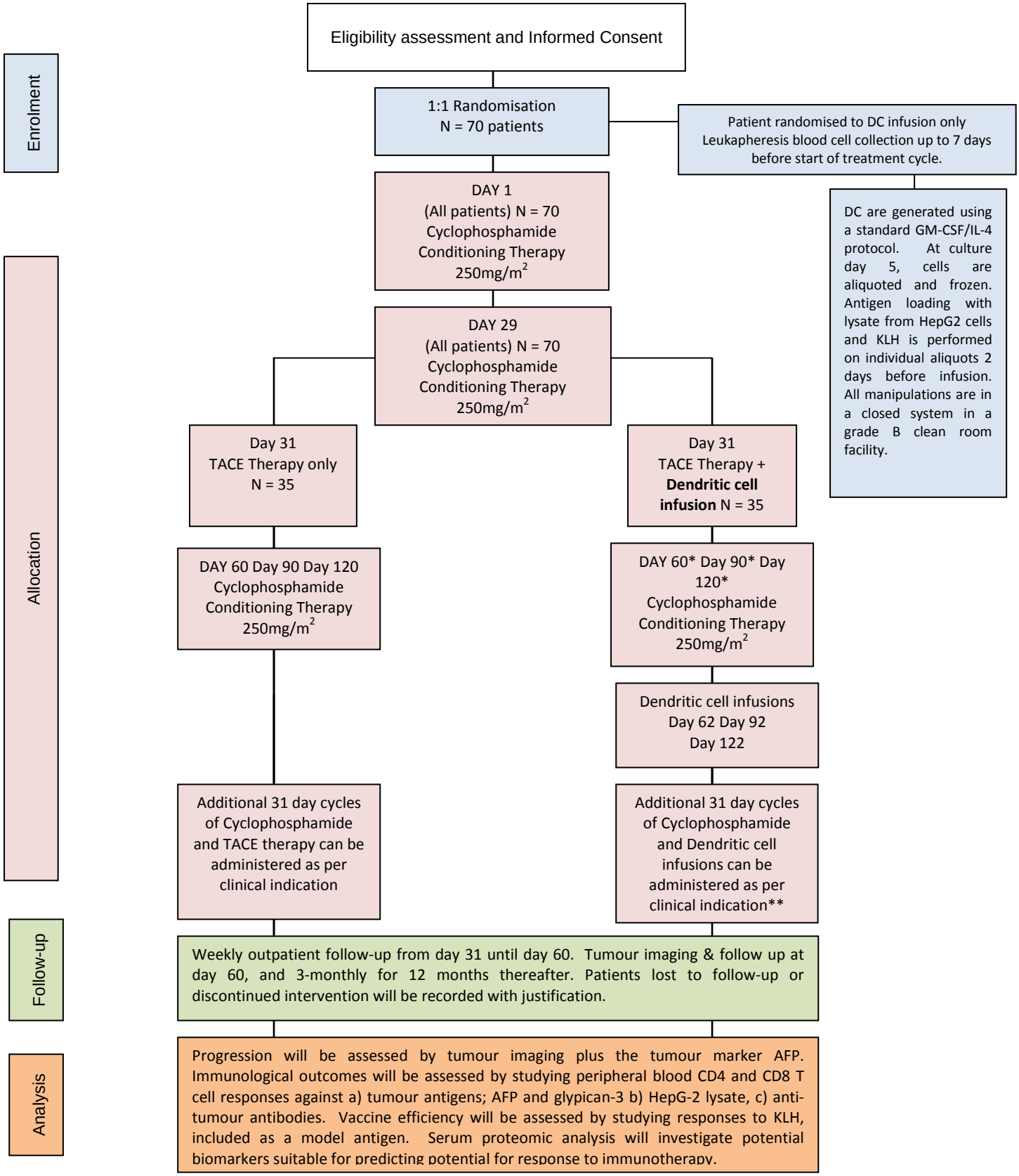
Group 1 patients will receive conditioning regimen with Cyclophosphamide at a dose of 250 mg/m^2 on day 1 and day 29 and chemoembolisation on day 31 (and subsequent repeats as clinically indicated) as the standard treatment. Standard TACE will require in- patient admission for 3 to 7 days. Patients in group 1 with no evidence of disease progression will receive additional Cyclophosphamide at a dose of 250mg/m2 on day 60, 90 and 120.

Patients in group 2 will additionally receive DC vaccination, the first of which will be given via the intrahepatic route at the time of chemoembolisation on day 31 (through cannulation of the femoral artery under radiological guidance). Otherwise TACE will be carried as per standard protocol. Three further vaccinations will be given intravenously at monthly intervals (day 62, 92 and 122) with a single dose of Cyclophosphamide at a dose of 250mg/m2 two days before each vaccination (day 60, 90 and 120). Patients in group 2 with no evidence of disease progression will receive further vaccinations at 3 monthly intervals**. They will be assessed for treatment-related toxicity in the weeks following the infusion. Subsequently patients will be followed up in the outpatient department and receive standard care. For this study there is a +/- 3 days visits window for all visits except for DC infusion visits which will always take place 2 days following each Cyclophosphamide infusion. If any of the visits are performed early or late, subsequent visits should adhere to the original schedule in relation to the start of the study.

Patients can withdraw at any time during the trial.

A total of 70 patients with HCC will be recruited in this randomised phase II trial

Figure 1: SUMMARY OF IMMUNOTACE TRIAL



*Timing schedule for repeated preconditioning (Cyclophosphamide therapy) and dendritic cell infusion therapy cycles +/- 3 Days. Preconditioning therapy must always be completed two days prior to dendritic cell infusion.

**Additional therapy cycles (conditioning Cyclophosphamide therapy and dendritic cell infusion) can be given at 3 monthly (93 days +/- 3 days) intervals post day 122 as long as the patient shows no signs of disease progression (see section 16.1). The amount of additional therapy cycles that any one individual may receive is dependent on the patient's disease status and the quantity and availability of dendritic cells available for that individual patient.

5.3 Study duration

The trial is expected to be open for randomisation of patients for 48 months.

6. ELIGIBILITY

6.1 Inclusion Criteria

1. Histological or cytological diagnosis or meet the AASLD criteria (Appendix 2) for diagnosis of HCC and at least one uni-dimensional lesion measurable according to the RECIST criteria by CT-scan or MRI (Appendix 3).
2. Suitable for TACE
3. Aged ≥ 18 years and estimated life expectancy ≥6 months
4. Not a candidate for surgical resection or transplantation
5. No previous chemotherapy, radiotherapy, immunotherapy or other experimental treatment for HCC prior to entry into the trial
6. ECOG performance status ≤ 2 (Appendix 4)
7. Adequate haematological function: Hb ≥9g/L, Absolute neutrophil count ≥1.5x10^9/L, platelet count ≥ 50x10^9/L
8. Bilirubin ≤ 50 µmol/L, AST or ALT ≤ 5 x ULN
9. Adequate renal function: Cockroft and Gault estimation ≥ 40ml/min (Appendix 5)
10. INR ≤ 1.5
11. Child-Pugh score ≤ 7 (Appendix 6)
12. Women of child-bearing potential should have a negative pregnancy test prior to trial entry
13. Women of child-bearing potential and men who have partners of child-bearing potential must be willing to practise effective contraception for the duration of the study and for three months after the completion of treatment.
14. Written informed consent

6.2 Exclusion Criteria

1. Extra-hepatic metastasis
2. Prior embolisation, systemic or radiation therapy for HCC
3. Investigational therapy or major surgery within 4 weeks of trial entry
4. Any ablative therapy (RFA or PEI) for HCC (this should not exclude patients if target lesion(s) have not been treated and occurred >6 weeks prior trial entry)
5. Child Pugh score >7(Appendix 6)
6. Hepatic encephalopathy
7. Ascites refractory to diuretic therapy
8. Documented invasion of the main portal vein
9. Hypersensitivity to intravenous contrast agents
10. Active clinically serious infection >grade 2 NCI-CTC version 4 (Appendix 7) within preceding 2 weeks
11. Pregnant or lactating women
12. History of second malignancy except those treated with curative intent more than three years previously without relapse and non-melanotic skin cancer or cervical carcinoma in situ
13. Evidence of severe or uncontrolled systemic diseases, congestive cardiac failure >NYHA class 2 (Appendix 8), MI within 6 months or laboratory finding that in the view of the investigator makes it undesirable for the patient to participate in the trial
14. Psychiatric or other disorder likely to impact on informed consent
15. Known history of HIV
16. Patient is unable and/or unwilling to comply with treatment and trial instructions
17. Patients with active auto-immune disorder

7. Screening and Consent

7.1 Informed Consent

The Investigator (or designated co-investigator as documented on the Site Signature and Delegation Log) must obtain written informed consent for each patient prior to performing any trial related procedure. A Patient Information Sheet will be provided to facilitate this process. The Investigator will ensure that they adequately explain the aim, trial treatment, anticipated benefits and potential hazards of taking part in the trial to the patient. The Investigator should also stress that the patient is completely free to refuse to take part or withdraw from the trial at any time.

The patient will be given ample time (greater than 24 hours) to read the Patient Information Sheet and to discuss their participation with others outside of the site research team. The patient will be given an opportunity to ask questions which should be answered to their satisfaction. The right of the patient to refuse to participate in the trial without giving a reason will be respected.

If the patient expresses an interest in participating in the trial they should be asked to sign and date the latest version of the Informed Consent Form. The Investigator (or designated representative) will then sign and date the form. A copy of the Informed Consent Form will be given to the patient, a copy should be filed in the hospital notes, and the original placed in the Investigator Site File (ISF). Once the patient is entered into the trial the patient's trial number will be entered on the Informed Consent Form maintained in the ISF. In addition after the patient has been entered into the clinical trial a copy of the consent form will be sent to the Trials Office with the patient's explicit consent.

Details of the informed consent discussions should be recorded in the patient's medical notes, this should include date of, and information regarding, the initial discussion, the date consent was given, with the name of the trial and the version number of the Patient Information Sheet and Informed Consent Form. Throughout the trial the patient should have the opportunity to ask questions about the trial and any new information that may be relevant to the patient's continued participation should be shared with them in a timely manner. On

occasion it may be necessary to re-consent the patient in which case the process above should be followed and the patient's right to withdraw from the trial respected.

Details of all patients approached about the trial should be recorded on the Patient Screening/Enrolment Log and with the patient's prior consent their General Practitioner (GP) should also be informed that they are taking part in the trial. A GP Letter is provided electronically for this purpose.

7.2 Screening

Potential participants will be identified by their usual direct healthcare team, namely their treating oncologist or hepatologist. The treating physician will either introduce the potential participant to the trial team or ask permission from the potential participant for the trial team to contact them. After informed consent has been obtained the following screening procedures will be performed within 14 days (unless otherwise stated) prior to randomisation.

- Written informed consent
- Vital signs
- Documentation of concomitant medications
- Medical history (including assessment of suitability for hepatic artery injection if appropriate and in particular a history of arterial disease)
- Physical examination including documentation of peripheral pulses
- Documentation of ECOG performance status (Appendix 3)
- FBC, U&Es, LFTs, serum AFP, coagulation
- Microbiology screen for HBV, HCV, HIV, Human T-Lymphotropic Virus 1 and 2 (HTLV-1, HTLV-2) and Syphilis.
- Serum pregnancy test if female of child-bearing potential
- HLA typing
- ECG
- Imaging of the chest by computed tomography (CT) and a scan of the abdomen by either CT or contrast enhanced MRI scan will be performed within 28 days of randomisation
- Estimation of GFR (by Cockcroft Gault Formula)

The screening procedures detailed above are required to take place before randomisation

8 TRIAL ENTRY

8.1 Confirmation of Eligibility

Once the results of the screening visit are available (usually the same day) the following must be checked:

Patient Informed Consent completed
Confirm all Inclusion Criteria
Review of Exclusion Criteria

8.2 Randomisation

Patients will be randomised to one of two treatment groups:

- **GROUP 1**: TACE therapy + preconditioning Cyclophosphamide therapy only
- **GROUP 2**: TACE therapy + preconditioning Cyclophosphamide + Dendritic cells infusions

Patients will be assigned to either treatment group on a 1:1 basis using a computer generated minimisation algorithm. This will ensure balance of important factors across treatment groups. The stratification factor to be used in this trial is viral hepatitis vs. non-viral aetiology. Patients will also be stratified by randomisation centre. At randomisation, patients will be allocated a unique patient trial number and scheduled for treatment and follow up visits as detailed in section 10.

Contact Details for Randomisation:

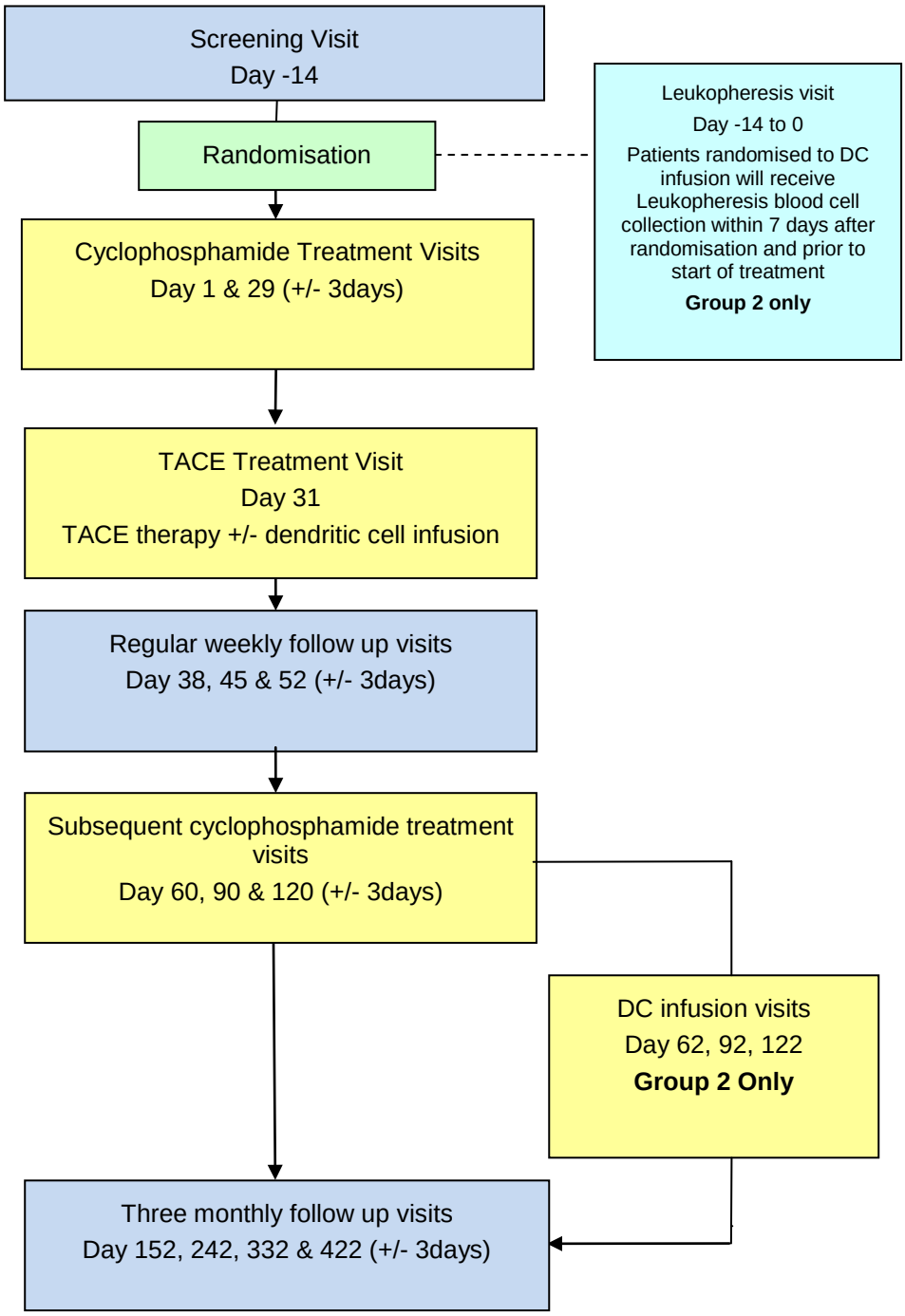
CRUK Clinical Trials Unit
School of Cancer Sciences
University of Birmingham
Edgbaston
Birmingham
B15 2TT

Phone: 0800 371 969 or 0800 731 7625
(Mon – Fri, 9am – 5pm)

Fax: 0121 414 3700

9. TREATMENT FOLLOW UP

Scheduling Timeline:



Screening Visit (Day -14)

- Obtain Bloods for FBC, INR, UE, LFT, AFP, autoantibodies, HLA typing, mandatory microbiology and immune response assessment (Total 100mls)
- Patient History data collection (Current adverse events)
- Evaluation of inclusion and exclusion criteria

Leukopheresis visit (Day -14 to 0) Group 2 only

- Clinical assessment to document clinical events, adverse events and concomitant medications
- Vital signs

Cyclophosphamide infusion visits (Day 1, 29)

- Clinical assessment to document clinical events, adverse events and concomitant medications
- Obtain Bloods for FBC, INR, UE, LFT AFP and immune response assessment. (60mls on each occasion, total 120mls)
- Vital signs

TACE visit +/- dendritic cells infusion (Day 31)

- Clinical assessment to document clinical events, adverse events and concomitant medications
- Vital signs

Regular weekly visits post TACE (Day 38, 45 & 52)

- Clinical assessment to document clinical events, adverse events and concomitant medications
- Obtain Bloods for FBC, INR, UE, LFT, AFP, autoantibodies and immune response assessment. (60mls on each occasion, total 120mls)
- Vital signs

Subsequent Cyclophosphamide infusions visits (Day 60, 90 & 120)

- Clinical assessment to document clinical events, adverse events and concomitant medications
- Obtain Bloods for FBC, INR, UE, LFT, AFP and immune response assessment. (60mls on each occasion, total 180mls)
- First tumour radiological assessment by CT or MRI of the abdomen will carried out on day 60.
- Vital signs

DC infusion visits *(Group 2 patients only)* (Day 62, 92 & 122)

- Clinical assessment to document clinical events and adverse events
- Vital signs

Three monthly visits (Day 152, 242, 332 & 422)

- Clinical assessment to document clinical events, adverse events and concomitant medications
- Obtain Bloods for FBC, INR, UE, LFT, AFP autoantibodies and immune response assessment. (100mls on each occasion, total 400mls)
- Tumour radiological assessment by CT or MRI of the abdomen (tumour assessment procedures can be performed at this visit or a maximum of +/-10 days prior to or after this visit).
- Vital signs

All patients will be followed for a minimum of 14-months following randomisation.

Visit Scheduling

All visits can be performed **(In sequence)** within a +/- 3 days scheduling window to allow for weeknds and Bank holidays. It is very important that the overall timing between visits is maintained ie: Cyclophosphamide infusions are always given 2 days prior to DC infusions.

9.1 Immune response assessment

Immune responses will be monitored for all patients at weekly intervals following TACE treatment until day 62 and then monthly intervals until the completion of the final Cy infusion on day 120. Thereafter, three monthly blood tests for immune response will be taken until the end of trial visit.

9.2 Assessment of efficacy

All patients will be assessed clinically and receive a formal radiological tumour assessment every 3 months from the date of randomisation. The tumour assessment will consist of a chest, abdomen and pelvic CT or MRI scan. The formal assessment of tumour response on the CT scans will be performed by independent radiologists. All scans will be assessed using RECIST criteria and the target lesions will be formally measured and any additional non-target lesion(s) assessed at every tumour assessment. The Consultant radiologist will be informed of the sequence of the scans for each individual patient and therefore will be able to assess the change in target lesions and depending on individual patient responses will determine the efficacy of the protocol defined therapy in relation to radiological response to therapy. (Appendix 2)

9.3 Unscheduled Visits

On enrolment participants will be provided with contact details (telephone, e-mail) for trial staff who can be contacted for advice.

An unscheduled visit for assessment will be arranged should the study patient have any clinical or adverse events.

9.3 Treatment compliance

Proportions of planned protocol dose of treatments received will be reported together with the number of treatment delays and reductions.

9.4 Treatment Discontinuation

In the event of discontinuation of trial treatment, full details of the reason(s) for discontinuation should be recorded on the appropriate pages on the CRF. All patients, including non-compliant subjects, should be followed up according to the protocol unless they withdraw consent (see section 11).

A patient should discontinue trial drug in the event of any of the following:

- Disease Progression (Radiological disease progression, Clinical Disease progression (see Section 16)
- Unacceptable toxicity
- Any other adverse event which, in the Investigator's opinion, requires termination of the trial medication
- Administration of radiotherapy, an investigational agent or any anti-tumour therapy other than TACE during the trial
- Pregnancy
- Any other reason given by the Investigator
- The patient uses illicit drugs or other substances that may, in the opinion of the Investigator, have a reasonable chance of contributing to toxicity or otherwise interfering with results
- The development of a second malignancy that requires treatment
- Request by the patient or a legal representative/relative to stop the treatment
- Death or End of Trial

10. DOSE MODIFICATIONS AND TOXICITY MANAGEMENT RECOMMENDATIONS

Trial medication will be continued until the criterion for stopping is reached. In response to toxicities or patient blood test values (Bilirubin, ANC or Platelets) described below, the dose will be reduced to predefined levels:

DOXORUBICIN ONLY

Initial Dose only[a]

Bilirubin (µmol/L)	Actual Dose Level	Percentage equivalent of maximum dose
≤22	60mg/m^2	100%
23-50	30mg/m^2	50%

[a]Patients that start therapy on the reduced dose value (50%) due to elevated Bilirubin levels will not be allowed to dose escalate (receive 60mg/m^2) irrespective of any improvement in Bilirubin levels (return to normal) after initiation of therapy.

During Treatment

Bilirubin (µmol/L)	Actual Dose Level	Percentage equivalent of maximum dose (Dose Level)
≤22	60mg/m^2	100% (Full dose)
23-50	30mg/m^2	50% (Level-2)
>50	DISCONTINUE	0% (NA)

Combined Doxorubicin and Cyclophosphamide regimen (31 day cycle)

Dose Level	Doxorubicin Hydrochloride [b] (Day 31)	Cyclophosphamide[c] (Day 1 & Day 29)
Full Dose (100%)	60 mg/m^2	250 mg/m^2
Level-1 (75%)	45 mg/m^2	250 mg/m^2
Level-2 (50%)	30 mg/m^2	250 mg/m^2

[b] The actual starting dose of Doxorubicin Hydrochloride that a patient receives will be determined by the individual patient's Bilirubin levels prior to the initiation of therapy.

[c] It is anticipated that no dose modifications will be required in relation to Cy infusions. If any dose reductions need to be applied to Cy this can be done as per the local investigator/study doctor discretion and should be clearly documented in the patient's medical notes.

Individual drug dosage levels can be modified independently of each other however no dose escalation of any trial drug will be allowed after a patient has received a reduced dose for toxicity or any other reason. Once a patient reaches dose Level-2 no further dose reduction will be allowed if further dose limiting toxicity is experienced by an individual patient; the patient will need to be withdrawn from trial therapy and continue to be followed up as per the trial patient follow-up schedule

Dendritic cell infusion

It is anticipated that no dose modifications will be required in relation to DC infusion. The immature DC will be split into four culture bag of equal volume prior to freezing. DC cells count will take place prior to the release of each bag of DC vaccine for infusion.

Haematological toxicity management: Dose changes and actions

Definition of Grade – see NCI Common Terminology Criteria for Adverse Events version 4 (NCI CTCAE v4)

ANC (x10^9/L)	NCI CTC Grade	Action (Dose level)
≥ 1.0 - < 2.0	N/A – Grade 2	100% (Full Dose)
0.5 - <1.0	Grade 3	Delay until ≥ 1.0 and then reduce to 75% (Level-1)
<0.5	Grade 4	Delay until ≥ 1.0 and then reduce to 50% (Level-2)

Platelets (x10^9/L)	NCI CTC Grade	Action (Dose level)
≥ 75- < 150	Grade 1	100% (Full Dose)
50 - <75	Grade 1 – Grade 2	Reduce dose to 50% (Level-1)
<50	Grade 3 or 4	Stop therapy

Other Haematological toxicity: not neutrophil or platelets (*for neutrophils and platelets see section above*)

Definition of Grade – see NCI Common Terminology Criteria for Adverse Events version 4.03 (NCI CTCAE v4.03)

NCI CTC Grade	Action (Dose level)
Grade 0 - 2	100% (Full Dose)
Grade 3	Reduce dose by one level and continue treatment [d]
Grade 4	Discontinue treatment until resolved to grade 0-2 and restart at reduced level [e]

[d] If no recovery to grade 0-2 within 30 days then reduce level again. If no recovery to grade 0-2 within a further 30 days, or dose already reduced, discontinue trial treatment.

[e] If no recovery after 30 days discontinue trial treatment.

Non-Haematological toxicity Management

Definition of Grade – see NCI Common Terminology Criteria for Adverse Events version 4 (NCI CTCAE v4)

NCI CTC Grade	Action (Dose level)
Grade 0 - 2	100% (Full Dose)
Grade 3	Reduce dose by one level and continue treatment [f]
Grade 4	Discontinue treatment until resolved to grade 0-2 and restart at reduced level [g]

[f] If no recovery to grade 0-2 within 30 days then reduce level again. If no recovery to grade 0-2 within a further 30 days, or dose already reduced, discontinue trial treatment.

[g] If no recovery after 30 days discontinue trial treatment.

11. POSSIBLE DISCOMFORTS OR RISKS

Inconveniences:

This trial will require fourteen visits (additional 4 visits for patients in group 2) including screening, treatment and follow-up.

Venepuncture:

Several blood samples are required as part of the trial protocol. These may be associated with localised discomfort and bruising.

Leukopheresis:

To obtain DCs, patients will undergo leukopheresis which will usually take four hours. This will require two cannulas to be placed into peripheral veins in the arms and these may result in some temporary discomfort during insertion and some bruising on the arm after removal. No significant risk is expected from the insertion or the removal of cannulas. The main side effect of leukopheresis is a sensation of buzzing or tingling around the mouth and lips during the procedure and rarely this may develop into muscle spasm or cramps in the hands, arms or legs. This is due to the anticoagulant required for the procedure causing a drop in calcium levels and can be prevented and treated by the consumption of calcium containing foods or calcium supplements. Very rarely if the calcium level drops too low it can result in arrhythmia and cardiac arrest. Hence, patients are closely monitored throughout the procedure according to standard protocols and treated accordingly. Hypotension can occur but is short lasting and usually be managed conservatively.

Dendritic cell infusion:

Previous studies have shown the toxicity from DC infusion to be mild and self limiting. Our own published study in HCC involved vaccination with mature autologous DC loaded with lysates of the hepatoblastoma cell line HepG2. 134 infusions were administered to 35 patients with the following toxicities: 11% of patients grade 1 fever only occasionally associated with rigors; 28% transient grade one myalgia post infusion; no dose-limiting toxicities, hepatic toxicity or development of autoimmunity. There is a small theoretical risk of an allergic reaction or a cytokine storm being induced when the cells are infused and thus a trained medical practitioner or research nurse will be present for the duration of the infusion and for a period of time post infusion and treated accordingly as per Resuscitation Guidelines 'Emergency Treatment of Anaphylactic Reactions' January 2008. This will be done in clinical research facility and the diagnostic radiology room where there are facilities for prompt and appropriate treatment in the unlikely event of a severe reaction. No such reactions have been reported in the trials discussed in this protocol. Administering autologous DC has the potential to induce autoimmunity and this has been reported in some of the melanoma studies, often associated with good anti-tumour responses. We did not see any evidence of autoimmunity in our previous study but the measurement of autoantibodies and immunoglobulins will be used to look for activation of autoimmunity and transaminases to exclude autoimmune hepatitis.

TACE & Doxorubicin:

Previous experience suggests there will be few adverse events related to the DC cell vaccination with the majority resulting from the TACE therapy and unlikely to be exacerbated by the addition of DC therapy or low dose cyclophosphamide. TACE is the current standard treatment for locally advanced liver cancer. Like any procedure it can be associated with side effects. However, this can be reduced by strict selection of suitable candidates (inclusion & exclusion criteria) and close monitoring after the procedure. Side effects of

embolisation include post embolisation syndrome, which consists of a low grade pyrexia, constipation, nausea, vomiting and abdominal pain. Injury to surrounding organs due to disruption of blood supply can occur, resulting in bile duct stricture, gall-bladder and bowel infraction, but these once again are rare. Very rarely, formation of liver abscess, renal failure, gastrointestinal bleeding and liver failure can also occur. The procedure will involve arterial access via the femoral artery which can result in discomfort, but can also result in vascular complications such as haematoma, prolong bleeding and false aneurysm.

TACE will involve the intra-hepatic arterial injection of doxorubicin under radiological guidance. Side effects of doxorubicin are well documented, which include: reversible alopecia, fatigue, mucositis, anorexia, diarrhoea, dehydration, changes in urine colour, nausea, vomiting and hepatic impairment. There is also a possibility of bone marrow suppression, but this is rare. This can result in an increased risk of infection, bleeding and anaemia. To ensure future safety, patients with anaemia (haemoglobin <10), leukopenia (white blood count <3.5), thrombocytopenia (platelet <50) or immunnosuppression as a result of medications or medical conditions at the time of recruitment are excluded. Cardiovascular complications such as cardiomyopathy, congestive heart failure and supraventricular tachycardia can occur. However, cardiovascular side effects usually occur with cumulative dose of doxorubicin (450 - 500 mg/m2). The planned dose is below those at which cardiovascular side effects are expected to be a problem.

Other rare side effects include urticarial rash, onycholysis, hyperpigmentation of nail beds, fever, chills, anaphylaxis, drowsiness, conjunctivitis, lacrimation and renal damage.

Doxorubicin proved to be highly teratogenic in rats, but its effect in human is uncertain. Pregnancy should therefore be avoided during doxoxrubicin therapy and for six months thereafter. It is for this reason that all patients taking part in the study will be required and prepared to use adequate contraceptive methods which must be continued for 6 months after the completion of treatment.

Secondary neoplasia is possible but unlikely to be a problem with the doses used and in the context of the patient population.

Cyclophosphamide:

Cyclophosphamide has been used for many years in patients with solid organ cancer and autoimmune disorder. Known side effects have been well documented in these patients treated with standard dosage. In this trial we are using a low dose regime and as a result it is likely to be well tolerated with minimal side effects.

Bone marrow suppression are dose limiting and nadir is reached about 10 to 14 days after IV dose with recovery by day 21. Platelets and haemoglobin are relatively spared. The planned doses are below those at which this is expected to take place. Nausea and vomiting are said to be frequent with large IV doses and symptoms begin several hours after treatment and are usually over by the next day. Other gasterintestinal symptoms including altered taste and anorexia can also occur. The planned doses are below those at which this is expected to be dose limiting. Reversible alopecia is common, usually starting after 2 to 3 weeks. Skin and nails may become darker. Mucositis is uncommon. Haemorrhagic or non haemorrhagic cystitis may occur in 5% to 10% of patients treated. It is usually reversible with discontinuation of the drug, but it may persist and lead to fibrosis or death. Frequency is diminished by ample fluid intake and morning administration of the drug. The planned doses are below those at which this is expected to be a problem. Amenorrhoea and azoospermia are recognised adverse events. The risks of this are low at the doses planned in this trial. However, young male participants will be advised to store sperm prior to treatment. Inhibition of antidiuretic hormone is of significance only with very large doses. Secondary neoplasia is possible but unlikely to be a problem with the doses used and in the context of the patient population. Acute and potentially fatal cardiotoxicity occurs with high dose therapy, abnormalities include pericardial effusion, congestive cardiac failure, decreased electrocardiographic voltage, and fibrin microthrombi in cardiac capillaries with endothelial injury and haemorrhagic necrosis. These are all very unlikely to be problems at the doses used. Lung fibrosis and hepatic toxicity can occur, but has rarely been reported.

Cyclophosphamide has been shown to be teratogenic. Pregnancy should therefore be avoided during cyclophosphamide therapy and for three months thereafter. It is for this reason that all patients taking part in the study will be required and prepared to use adequate contraceptive methods which must be continued for 3 months after the completion of treatment.

It has been reported that doxorubicin may enhance the severity of the toxicity of other anticancer therapies, such as cyclophosphamide induced haemorrhagic cystitis. However, this is unlikely to occur at the low dosage we are planning to use for this trial.

Imaging & radiation:

As part of standard TACE treatment patients will undergo CT or MRI scans to monitor their response to the treatment. Patient taking part in the trial may need an additional CT scan of the chest, abdomen and pelvis if not carried out 28 days prior to randomisation. This will result in additional radiation exposure as a result of taking part in the trial. The risk of development of further malignancy as a result of additional radiation for this patient group is negligible. Also the standard treatment of TACE will involve screening X-rays, but taking part in the study will not increase the radiation exposure compared to standard TACE treatment outside of the trial.

In very rare cases, contrast agents used in CT or MRI scan can result in allergic reactions presenting as itching or skin rash. This is usually mild and will be treated accordingly as required. Signs of a more serious anaphylactic allergic reaction can also occur, but will be monitored by trained staff and treated accordingly as per Resuscitation Guidelines 'Emergency Treatment of Anaphylactic Reactions' January 2008.

11.1 Concomitant Therapy

All medication that the participant is taking at the time of enrolment will be recorded. Any changes or new medications added during the study will be recorded.

The generic drug name, daily dose, route of administration, treatment start/stop date and indication will be recorded.

Participants will be asked to limit alcohol consumption and participants with Alcoholic Liver Disease advised to abstain completely.

Any drug, if considered necessary for the participant, is permitted at the discretion of the Investigator, with the following exceptions: Participation in another trial of an investigational product

12. PARTICIPANT WITHDRAWAL & TRIAL COMPLETION

12.1 Participant Withdrawal

Participants are free to withdraw from the study at any stage and may be withdrawn by the Investigator at any stage.

The following are justifiable reasons for the Investigator to withdraw a patient from study:

- Unacceptable toxicity
- Unforeseen events: any event which in the judgement of the Investigator makes further treatment inadvisable
- SAE requiring discontinuation of treatment
- Withdrawal of consent
- Serious violation of the study protocol (including persistent patient attendance failure and persistent non-compliance)
- Withdrawal by the Investigator for clinical reasons not related to the study drug treatment

Participant withdrawals will not be replaced. All participants will be included in the analysis unless they have withdrawn consent to remain in the study in which case participants will be included in the analysis up to the date they withdraw consent.

Withdrawal of Consent

Patients may withdraw consent at any time during a trial. The details of withdrawal should be clearly documented and communicated to the Trial Office.

The following should be clearly documented in the medical notes:

The date and reason the patient withdraws consent. If no reason is for withdrawal is specified by the patient concerned this will also need to be documented in the medical notes. The patient should not be pressured in any way to give a reason for withdrawal if he/she does not wish to supply this information.

12.2 Trial completion

A patient will be considered to have completed the trial following 12-months follow-upat the time of patient withdrawal, death or if lost to follow-up.

13. ADVERSE EVENT REPORTING

The collection and reporting of Adverse Events (AEs) will be in accordance with the Medicines for Human Use Clinical Trials Regulations 2004 and its subsequent amendments. Definitions of different types of AE are listed in Appendix 6. The Investigator should assess the seriousness and causality (relatedness) of all AEs experienced by the patient (this should be documented in the source data) with reference to the (compendium of) Summary of Product Characteristics. In the case of DC vaccination the latest version of the IMPD will be used.

13.1 Reporting Requirements

13.1.1 Adverse Events

All medical occurrences which meet the definition of an AE (see Appendix 9 for definition) should be reported. Please note this includes abnormal laboratory findings.

13.1.2 Serious Adverse Advents

Investigators should report AEs that meet the definition of an SAE (see Appendix 8 for definition).

13.1.3 Events that do not require reporting on a Serious Adverse Event Form

Hospitalisation for the purpose of the TACE treatment, and lasting for up to 7 days after that treatment, does not require reporting unless associated with other serious events. Hospitalisations lasting for > 7 days post TACE or re-admissions within 7 days (i.e. if the patient is discharged from hospital and then returns within 7 days), require reporting in the usual manner as outlined above.

Although not reported as a serious adverse event, details of length of stay in hospital will be captured on the relevant page of the CRF.

13.1.4 Monitoring pregnancies for potential Serious Adverse Events

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

In the event that a patient or their partner becomes pregnant during the SAE reporting period, a Pregnancy Notification Form (providing the patient's details) will be completed and returned to the Trial Office as soon as possible. If it is the patient who is pregnant information regarding outcome of the pregnancy will be provided on a follow-up Pregnancy Notification Form. Where the patient's partner is pregnant, consent must first be obtained and the patient should be given a pregnancy release of information form to give to their partner. If the partner is happy to provide information on the outcome of their pregnancy they should sign the form. Once consent has been obtained, details of the outcome of the pregnancy will be recorded on the follow-up Pregnancy Notification Form.

13.2 Reporting Period

The reporting period for AEs will commence from date of consent and will continue until 30 days post last trial drug infusion. For Group 1 patients this will be 30 days post last Cyclophosphamide infusion, for Group 2 patients this will be 30 days post last dendritic cell infusion. All AEs must be followed up until resolution of the event, irrespective of the time period elapsed.

The reporting period for serious adverse events (SAEs) is from the date of consent until 30 days after last trial drug administration infusion i.e.; for Group 1 patients this will be 30 days post last cyclophosphamide infusion, for Group 2 patients this will be 30 days post last dendritic cell infusion. The length of time of the SAE reporting period will be slightly different for each treatment group. The maximum reporting period for an individual patient will therefore be determined by both the treatment allocated (via the randomisation process) and the actual number of treatments (dendritic cell or cyclophosphamide infusions) that a patient receives. All SAEs that occur within 30 days of any cyclophosphamide or dendritic cell infusion received by trial participants will be reported to the Trials Office and will continue to be followed up until resolution of the serious adverse event.

13.3 Reporting Procedure

13.3.1 Site

13.3.1.1 Adverse events

AEs should be reported on an AE Form (and where applicable on an SAE Form). An AE Form should be completed at each visit and returned to the Trials Office.

AEs will be reviewed using the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0 (see Appendix 6). Any AEs experienced by the patient but not included in the CTCAE should be graded by an Investigator and recorded on the AE Form using a scale of (1) mild, (2) moderate or (3) severe. For each sign/symptom, the highest grade observed since the last visit should be recorded.

A pre-existing condition must not be reported as an AE unless the condition worsens by at least one CTC grade during the trial. The condition, however, must be reported in the CRF.

13.3.1.2 Serious adverse events

AEs defined as serious and which require reporting as an SAE (excluding events listed in Section 13.1.3 above) should be reported on an SAE Form. When completing the form, the Investigator will be asked to define the causality and the severity of the AE which should be documented using the CTCAE version 4.0.

On becoming aware that a patient has experienced an SAE, the Investigator (or delegate) must complete, date and sign an SAE Form. The form should be faxed together with a SAE Fax Cover Sheet to the Trials Office using one of the numbers listed below as soon as possible and no later than 24 hours after first becoming aware of the event:

To report an SAE, fax the SAE Form with an SAE Fax Cover Sheet to:

0121 414 8286 (Primary number)

Or

0121 414 2230 (Secondary number)

On receipt the Trial Office will allocate each SAE a unique reference number. This number will be transcribed onto the SAE Fax Cover Sheet which will then be faxed back to the site as proof of receipt. If confirmation of receipt is not received within 1 working day please contact the Trial Office. The SAE reference number should be quoted on all correspondence and follow-up reports regarding the SAE. The SAE Fax Cover Sheet completed by the Trial Office should be filed with the SAE Form in the ISF.

For SAE Forms completed by someone other than the Investigator the Investigator will be required to countersign the original SAE Form to confirm agreement with the causality and severity assessments. The form should then be returned to the Trial Office in the post and a copy kept in the ISF.

Investigators should also report SAEs to their own Trust in accordance with local practice.

13.3.1.3 Provision of follow-up information

Patients should be followed up until resolution or stabilisation of the event. Follow-up information should be provided on a new SAE Form.

13.3.2 Trials Office

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

13.4 Reporting to the Competent Authority and main Research Ethics Committee

13.4.1 Suspected Unexpected Serious Adverse Reactions

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

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

13.4.2 Serious Adverse Reactions

The Trials Office will report details of all SARs (including SUSARs) to the MHRA and main REC annually from the date of the Clinical Trial Authorisation, in the form of an Annual Safety Report.

13.4.3 Adverse Events

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

13.4.4 Other safety issues identified during the course of the trial

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

13.4.5 Investigators

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

13.4.6 Data Monitoring Committee

The independent Data Monitoring Committee (DMC) will review all SAEs.

13.5 Notification of deaths

All deaths must be reported to the Trial Office within 24 hours of the investigator site becoming aware of the event, irrespective of whether the death is related to disease progression, the Investigational Medicinal Product, or an unrelated event.

14 Data Handling and Record Keeping

14.1 Data Collection

The Case Report Form (CRF) will comprise the following forms:

Form	Summary of data recorded
Eligibility checklist	Check of Inclusion and Exclusion Criteria
Randomisation Form	Patient demographics, patient trial number and details of treatment group
On-Study Form	History and examination findings, vital signs, pregnancy test (in patients of childbearing potential, Baseline blood results, Medications at Start of Study
Treatment Form(s)	Dates, dosages and routes of administered treatments including length of stay in hospital for protocol defined therapy, Toxicity
Follow Up assessments	Focused history and examination, Vital signs, Blood results, Progression, Survival
Concomitant Medications	, Changes during Study
Clinical Events	Record of Events - Dates, Severity, Management and Outcomes
Adverse Effects	Record of Adverse Effects – Dates, Severity, Management and Outcomes

Ad hoc forms

Serious Adverse Event form

Pregnancy Notification Form

The CRF will be completed, signed/dated and returned to the Trials Office by the Investigator or an authorised member of the site research team (as delegated on the Site Signature and Delegation Log).

The exception is the SAE Form which must be co-signed by the Investigator. See Adverse Event reporting section 8 for further details.

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

Data reported on each form should be consistent with the source data or the discrepancies should be explained. If information is not known, this must be clearly indicated on the form. All missing and ambiguous data will be queried. All sections are to be completed before returning.

In all cases it remains the responsibility of the Investigator to ensure that the CRF has been completed correctly and that the data are accurate.

Completed CRFs submitted to the Trial Office will be reviewed by the Trial Co-ordinator who will enter the data into an electronic database. Any queries raised on the submitted data will be sent to the site, answered queries will be returned to the Trial Co-ordinator who will update the database.

Trial forms may be amended by the Trials Office, as appropriate, throughout the duration of the trial. Whilst this will not constitute a protocol amendment, new versions of the form must be implemented by participating sites immediately on receipt.

14.2 Archiving

The Investigator will ensure all essential trial documentation and source records (e.g. signed Informed Consent Forms, Investigator Site Files, Pharmacy Files, patients' hospital notes, copies of CRFs etc) at their site are securely retained for at least 5 years after the end of the trial. The Trial Office will retain the original CRFs.

15. Quality Management

All sites will be required to sign a clinical study site agreement prior to participation. In addition all participating Investigators will be asked to sign the necessary agreements and supply a current CV to the Trials Office. All members of the site research team will also be required to sign the site signature and delegation log, which should be returned to the Trial Office. Prior to commencing recruitment the site will undergo a process of initiation. Key members of the site research team will be required to attend a meeting covering aspects of the trial design, protocol procedures, Adverse Event reporting, collection and reporting of data and record keeping. The site will be provided with an Investigator Site File containing essential documentation, instructions, and other documentation required for the conduct of the trial. The Trial Office must be informed immediately of any change in the site research team.

15.1 On-site Monitoring

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

15.2 Central Monitoring

Trial staff will be in regular contact with the site research team to check on progress and address any queries that they may have. Trial staff will check incoming Case Report Forms for compliance with the protocol, data consistency, missing data and timing. The site will be sent Data Clarification Forms requesting missing data or clarification of inconsistencies or discrepancies.

The site may be suspended from further recruitment in the event of serious and persistent non-compliance with the protocol and/or GCP, and/or poor recruitment. Any major problems identified during monitoring may be reported to the trial management group and the relevant regulatory bodies. This includes reporting serious breaches of GCP and/or the trial protocol to the main Research Ethics Committee (REC) and the Medicines for Healthcare products Regulatory Agency (MHRA).

15.3 Audit and Inspection

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

The site is also requested to notify the Trial Office of any MHRA inspections.

15.4 Notification of Serious Breaches

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

- The conditions and principles of GCP in connection with that trial or;
- The protocol relating to that trial, within 7 days of becoming aware of that breach

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

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

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

16. End of Trial Definition

The trial will end when the final participant has completed their final follow up assessment.

17 STATISTICAL CONSIDERATIONS

17.1 Definition of Outcome Measures

Primary:

- Progression free survival is defined as the time from date of randomisation to the date of progression or death from any cause. Progression will be determined according to RECIST as compared against the baseline scan. In the absence of radiological disease, patients may be considered to have clinically progressed based on clinical evidence. Whether progression is classed as radiological or clinical will be documented. Alive patients without documented progression will be censored at the date of last follow-up.

Secondary:

- Radiological response will be assessed by 3-monthly scan compared against baseline scan and categorised according to RECIST criteria.
- Serum AFP will be collected at randomisation and then monthly to investigate changes over time.
- Toxicity: Adverse events and serious adverse events will be graded according NCI-CTC v4.02 during treatment. Feasibility is defined as the proportion of protocol treatment administered (dose intensity) as well as treatment delays recorded in days. .
- Immune response will be based on CD4 and CD8 T cell counts collected at monthly intervals from randomisation.
- Overall survival is defined as the time from date of randomisation to the date of death from any cause. Alive patients will be censored at the date of last follow-up.

17.2 Analysis of Outcome Measures

As an early phase, the objective is to determine if the TACE+cyclophosphamide+vaccine regimen is feasible and warrants further investigation in the phase III setting. As such, analyses will be based on descriptive statistics with no significance testing across groups.

Primary:

- Progression free survival estimates will be calculated using the method of Kaplan and Meier. Median and 12-month progression free survival rates with confidence intervals will be presented by treatment group.

Secondary:

- Radiological response over time will be reported. Patients will be categorised according to their 'best' response and proportions reported by treatment group.
- Mean change in serum AFP from randomisation, with confidence intervals, will be reported descriptively by treatment group. With complete data, mean area under the curve will be reported descriptively by treatment group.
- Toxicity: the proportions of patients with specific grade 3/4 toxicities will be reported descriptively across treatments. The proportion of protocol treatment administered (dose intensity) as well as treatment delays recorded in days will be reported descriptively by treatment group.
- Overall survival estimates will be calculated using the method of Kaplan and Meier. Median and 12-month overall survival rates with confidence intervals will be presented by treatment group
- Immune response will be based on CD4 and CD8 T cell analyses based on change in counts over time. Proteomic analysis will be carried out to investigate potential biomarkers predictive of response based on logistic regression analyses but using relaxed error levels due to the limited power.

17.3 Power Calculations

The Jung design[47] is an extension of Simon's two-stage design for single arm phase II trials to the randomised setting. A two-stage minimax design has been used. Local unpublished data from the Queen Elizabeth Hospital estimates progression free survival in this group of patients to be 30% at 12-months. This design is based on ensuring that the type I and type II error rates are less than or equal to 0.2, and assumes a progression free survival rate on the experimental control arm (TACE+cyclphosphamide) of 30% and a 20% expected absolute improvement in the experimental treatment arm (TACE+cyclphosphamide+vaccine) to 50%.

Stage 1: The trial will recruit 23 patients to each arm in the first stage and at this interim analysis, if the number of patients in the experimental treatment arm who are alive and progression free by 12-months is greater than or equal to the number on the experimental control arm (i.e. the number of patients in the experimental treatment arm with progression/ death is less than or equal to the number on the experimental control arm), then recruitment will continue. Whilst the first 23 patients are assessed for progression, recruitment will continue.

Stage 2: The trial will continue to recruit until there are 35 patients per arm. If the difference between treatment arms in terms of the number of patients who have progressed/ died by 12-months is greater than or equal to 4 (i.e. the number of patients in the experimental treatment arm with progression/death is at least 4 less than the number on the experimental control arm) then this provides evidence that the efficacy of the experimental treatment (TACE+cyclophosphamide+vaccine) warrants further investigation in the phase III setting.

In summary, the statistical design stated above requires 35 patients to be randomised to each treatment arm and as such the total recruitment target is 70 patients randomised in total, to provide evidence that the experimental treatment warrants further investigation in the phase III setting. The number of participants lost to follow-up, or who withdraw consent prior to initial treatment is expected to be minimal. The Data Monitoring Committee may advise recruitment of additional participants if numbers are higher than anticipated.

17.4 Interim and Final Analysis

The trial will have one interim analysis which will be presented to an independent data monitoring committee (DMC) who will assess safety of patients during the recruitment phase of the study. The first stage of the trial will recruit 23 patients into each treatment group. The results of stage 1 will be presented to the independent DMC by the trial statistician. Based on the results seen in stage 1, the trial may continue to recruit to 35 patients in each treatment arm.

Final analyses will be carried out when all participants in stage 1 and stage 2 have been followed for at least 12-months after randomisation.

All analyses will be based on an intention to treat basis including all randomised patients analysed according to their randomised treatment allocation. A secondary 'per protocol' sensitivity analysis may be undertaken if the numbers of ineligible patients or protocol violators is larger than expected and will be based on actual treatment received as opposed to randomised treatment group.

18 Trial Organisational Structure

18.1 Sponsor

IMMUNOTACE is an investigator led trial, co-ordinated by the Liver Research Group within the Cancer Research UK Clinical Trials Unit (CRCTU) in Birmingham. The University of Birmingham will act as a sponsor. CRCTU, Birmingham will coordinate the study on behalf of the Sponsors.

In terms of liability, NHS Hospitals have a duty of care to patients treated, whether or not the patient is taking part in a clinical trial. Compensation is only available in the event of clinical negligence being proven. There are no specific arrangements for compensation made in respect of any serious adverse events occurring through participation in the trial, whether from side effects listed, or others yet unforeseen

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

Sponsor address:

University of Birmingham
Edgbaston
Birmingham
B15 2TT
United Kingdom

18.2 Data Monitoring Committee

Data analyses will be supplied in confidence to an independent Data Monitoring Committee (DMC), whose primary role is patient safety. The DMC will give advice on whether the accumulated data from the trial, together with the results from other relevant research, justifies the continuing recruitment of further patients. The DMC will operate in accordance with a trial specific charter based upon the template created by the Damocles Group. During the recruitment phase of the trial the DMC is scheduled to meet one month prior to the due date of the Annual Safety Report and annually thereafter. Additional meetings may be called if recruitment is much faster than anticipated and the DMC may, at their discretion, request to meet more frequently. An emergency meeting may also be convened if a safety issue is identified. The DMC will report directly to the Trial Management Group who will convey the findings of the DMC to the sponsors as applicable. The DMC may consider recommending the discontinuation of the trial if the recruitment rate or data quality are unacceptable or if any issues are identified which may compromise patient safety.

18.3 Finance

The trial is funded by the National Institute of Health Research Efficacy and Mechanism Evaluation programme (NIHR EME).

18.4 Trial Management Group

Membership	Chief Investigator
	Co-investigators
	Research Physicians
	Senior Trial Coordinator
	Research Nurse
	Trial Statistician
Responsibilities	Design and Conduct of Trial
	Preparation of Protocol and Amendments
	Preparation of Patient Information Sheets and Consent Forms
	Preparation of Case Report Forms (CRF)
	Reviewing progress of Trial and if necessary agreeing changes to the protocol
	Providing Annual Report to MHRA and Ethics Committee
	SUSAR Reporting to MHRA
	Data Verification
	Data analysis
	Preparation of Trial Reports including DMC Reports
	Publication and Presentation of Results

18.5 Delegation

The Principal Investigator at each centre will be ultimately responsible for patient identification, recruitment, data collection, completion of CRFs, follow up of trial participants and adherence to study protocol.

These duties may be delegated to appropriate medical or nursing trial staff as detailed in the Site Signature and Delegation Log.

19 Ethical considerations

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

The trial will be conducted in accordance with the Research Governance Framework for Health and Social Care, the applicable UK Statutory Instruments, (which include the Medicines for Human Use Clinical Trials 2004 and subsequent amendments and the Data Protection Act 1998 and the Human Tissue Act 2008 and the International Conference on Harmonisation Guidelines for Good Clinical Practice (ICH GCP). This trial will be carried out under a Clinical Trial Authorisation in accordance with the Medicines for Human Use Clinical Trials regulations. The protocol will be submitted to and approved by the main Research Ethics Committee (REC) prior to circulation.

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

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

20 Confidentiality and data protection

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the Data Protection Act 1998. With the patient's consent, their initials, date of birth, hospital number and National Health Service number will be collected at trial entry.

Patients will be identified using only their unique trial number, initials, hospital number and date of birth on the Case Report Form and correspondence between the Trials Office and the participating site

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

The Trials Office will maintain the confidentiality of all patients' data and will not disclose information by which patients may be identified to any third party. Representatives of the trial team may be required to have access to patients' notes for quality assurance purposes but patients should be reassured that their confidentiality will be respected at all times.

21 Insurance and Indemnity

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

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

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

22 Publication Policy

Final results of this trial will be submitted for publication in a peer reviewed journal. The manuscript will be prepared by the Trial Management Group (TMG) and authorship will be determined by mutual agreement. Any publication of trials data, interim or otherwise, will be prepared and approved by the TMG.

23 Reference List

1. El-Serag HB et al. Rising incidence of Hepatocellular Carcinoma in the United States. N.Engl.J.Med. 1999; 340: 745-750.

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APPENDIX 1 - WMA DECLARATION OF HELSINKI

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Recommendations guiding physicians

in biomedical research involving human subjects

Adopted by the 18th World Medical Assembly

Helsinki, Finland, June 1964

and amended by the

29th World Medical Assembly, Tokyo, Japan, October 1975

35th World Medical Assembly, Venice, Italy, October 1983

41st World Medical Assembly, Hong Kong, September 1989

and the

48th General Assembly, Somerset West, Republic of South Africa, October 1996

INTRODUCTION

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

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

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

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

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

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

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

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

I. BASIC PRINCIPLES

Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation. Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

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

In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, reestablishing health or alleviating suffering.

The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

In any medical study, every patient - including those of a control group, if any - should be assured of the best proven diagnostic and therapeutic method. This does not exclude the use of inert placebo in studies where no proven diagnostic or therapeutic method exists.

The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).

The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

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

In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

The subject should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.

The investigator or the investigating team should discontinue the research if in his/her or their judgement it may, if continued, be harmful to the individual.

In research on man, the interest of science and society should never take precedence over considerations related to the wellbeing of the subject.

APPENDIX 2 – DIAGNOSES OF HCC : AASLD CRITERIA

Diagnosis of suspected HCC may be confirmed by biopsy or by application of the following non-invasive diagnostic criteria that have been proposed by the AASLD. Reference: Bruix, J and Sherman, M. Management of Hepatocellular carcinoma. *Hepatology* (2005). 42(5):1208-36.

1. Nodules between 1-2 cm found on ultrasound screening of a cirrhotic liver should be investigated further with two dynamic studies, either CT scan, contrast ultrasound or MRI with contrast. If the appearances are typical of HCC (i.e., hypervascular with washout in the portal/venous phase) in two techniques the lesion should be treated as HCC. If the findings are not characteristic or the vascular profile is not coincidental among techniques the lesion should be biopsied.

2. If the nodule is larger than 2 cm at initial diagnosis and has the typical features of HCC on a dynamic imaging technique, biopsy is not necessary for the diagnosis of HCC. Alternatively, if the AFP is > 200 ng/mL biopsy is also not required. However, if the vascular profile on imaging is not characteristic or if the nodule is detected in a non-cirrhotic liver, biopsy should be performed.

APPENDIX 3 - RESPONSE EVALUATION CRITERIA IN SOLID TUMOUR

The following contains excerpts from the RECIST criteria plus trial specific instructions.

For more information regarding RECIST and a full copy of criteria, go to http://www.eortc.be

Ref. E.A. Eisenhauera, P. Therasseb, J. Bogaertsc *et al*. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). 45, 228-247 (2009).

1. Measurability of Tumour Lesions at Baseline

1.1 Definitions

Only patients with measurable disease at baseline should be included. At baseline, tumour lesions will be categorised as follows:

Measurable (lesions that can be accurately measured in at least one dimension [longest diameter to be recorded] with a minimum size of 10mm by CT scan (CT scan slice thickness no greater than 5mm), 10mm caliper measurement by clinical exam and 20 mm by chest X-ray. For malignant lymph nodes to be considered pathologically enlarged and measureable, a lymph node must be ≥15 mm in short axis when assessed by a CT scan (at baseline and in follow-up, only the short axis will be measured and followed).

Nonmeasurable (all other lesions, including small lesions [longest diameter <10 mm or pathological lymph nodes with ≥10 to >15mm short axis] and truly nonmeasurable lesions).

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

All measurements should be recorded in metric notation by use of a ruler or callipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

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

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

1.2 Specifications by methods of measurements

The same method of assessment and the same technique should be used to characterise each identified and reported lesions at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the anti tumour effect of a treatment. The following examinations are allowed in the IMMUNOTACE study for determining response and progression free survival.

CT and MRI: Dynamic phase contrast enhanced CT or MRI should be used so that arterial enhancement and venous phase washout can be demonstrated. CT and MRI are the best currently available and reproducible methods for measuring target lesions selected for response assessment. Conventional CT and MRI should be performed with contiguous cuts of 10 mm or less in slice thickness. Spiral CT should be

performed using a 5 mm contiguous reconstruction algorithm; this specification applies to tumours of the chest, abdomen and pelvis, while head and neck tumours and those of extremities usually require specific protocols. More details concerning the use of this method of assessment can be found in Appendix II of RECIST criteria.

Chest X-ray. Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable. More details concerning the use of this method of assessment can be found in Appendix II of RECIST criteria

2 Tumour Response Evaluation

2.1 Baseline evaluation

2.1.1 Baseline documentation of "target" and "nontarget" lesions

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

Target lesions should be selected on the basis of their size (those with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).

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

All other lesions (or sites of disease) should be identified as “nontarget” lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

2.2 Response criteria

A. Evaluation of target lesions

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

B. Evaluation of non target lesions

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

[1]To achieve “unequivocal progression” on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumour burden has increase sufficiently to merit discontinuation of therapy (examples can be found in Appendix II of RECIST criteria).

C. Evaluation of best overall response

The best overall **response** is the best **response** recorded from the start of treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). In general, the patient's best **response** assignment will depend on the achievement of both measurement and confirmation **criteria.**

Table 1: Overall responses for all possible combinations of tumour responses in target and non-target lesions with or without the appearance of new lesions

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

CR = complete response; PR = partial response; SD = stable disease; and PD = progressive disease.

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

APPENDIX 4 – ECOG PERFORMANCE STATUS

These scales and criteria are used by doctors and researchers to assess how a patient's disease is progressing, assess how the disease affects the daily living abilities of the patient, and determine appropriate treatment and prognosis. They are included here for health care professionals to access.

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

* As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982

APPENDIX 5 - COCKROFT AND GAULT FORMULAE

The Cockcroft-Gault Formula is used to calculate Creatinine Clearance.

MEN : GFR = (140 - age) x Weight (kg) / (72 x serum creatinine(mg/dl)) X 1

WOMEN : GFR = (140 - age) x Weight (kg) / (72 x serum creatinine(mg/dl)) X 0.85

OR

MEN : GFR = (140 - age) x Weight (kg) / (72 x serum creatinine/88.6(µmol/L)) X 1

WOMEN : GFR = (140 - age) x Weight (kg) / (72 x serum creatinine/88.6(µmol/L)) X 0.85

APPENDIX 6 - CHILD-PUGH SCORE

Measure	1 point	2 points	3 points	units
Bilirubin (total)	<34 (<2)	34-50 (2-3)	>51 (>3)	µmol/l (mg/dL)
Serum albumin	>35	28-35	<28	mg/L
INR	<1.7	1.71-2.20	> 2.20	no unit
Ascites	None	Suppressed with medication	Refractory	no unit
Hepatic encephalopathy	None	Grade I-II (or suppressed with medication)	Grade III-IV (or refractory)	no unit

APPENDIX 7 – NCI COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS

Adverse Events will be recorded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4 (NCI CTCAE (v4)).

The full NCI CTCAE (v4) document is supplied in the Investigator Site Folder and can also be requested from the IMMUNOTACE Trials Office.

It is also available on the National Cancer Institute (NCI) website, at the following address:
http://ctep.cancer.gov/reporting/ctc.html.

APPENDIX 8 – STAGING : NEW YORK HEART ASSOCIATION (NYHA).

Class 1:	Subjects with no limitation of activities; they suffer no symptoms from ordinary activities.
Class 2:	Subjects with slight, mild limitation of activity; they are comfortable with rest or mild exertion.
Class 3:	Subjects with marked limitation of activity; they are comfortable only at rest.
Class 4:	Subjects who should be at complete rest, confined to a bed or chair; any physical activity brings on discomfort and symptoms occur at rest

APPENDIX 9 - DEFINITION OF ADVERSE EVENTS

Adverse Event

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

Comment:

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

Adverse Reaction

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

Comment:

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

Serious Adverse Event

Any untoward medical occurrence or effect that at any dose:

- Results in death
- Is life-threatening*
- Requires hospitalisation** or prolongation of existing inpatients' hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly/birth defect
- Or is otherwise considered medically significant by the Investigator***

Comments:

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

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

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

*** Medical judgment should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should be considered serious.

Serious Adverse Reaction

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

Suspected Unexpected Serious Adverse Reaction

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

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

Unexpected Adverse Reaction

An AR, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator Brochure for an unapproved IMP or (compendium of) Summary of Product Characteristics (SPC) for a licensed product).

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