ImmunoTACE TRIAL

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

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CHIEF INVESTIGATOR: **Professor David Adams**

University of Birmingham

CHIEF INVESTIGATOR SIGNATURE PAGE

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This Protocol has been approved by :

Professor David Adams **Signature :** **Date :** 24/11/16

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.

This protocol was written using CRCTU-PRT-QCD-001, version 1.0

SPONSOR University of Birmingham

TRIAL PERSONNEL

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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	Protocol Version No.	Type of Amendment (e.g. substantial/non-substantial/administrative change)	Summary of amendment?
1	**21_Nov_ 2013**	**n/a**	Substantial	Change of PI at site
2	**02_Mar_ 2015**	**5.0**	Substantial	- Change to study personnel, contact details and change of CI - Typographical errors corrected - Updated Abbreviated List
3	**05_Oct_ 2015**	**6.0**	Substantial	- Change to study personnel, and contact details - Typographical errors corrected -RECIST criteria updated to v1.1 throughout - Section 3 – Treatment Details (Autologous Dendritic Cells Pulsed with HepG2 Lysate matured with Monophosphoryl Lipid A)
N/A	**22_Mar_ 2016**	**7.0**	N/A	Protocol was updated and signed off internally, but not submitted to the Regulatory Authorities. This was subsequently further amended and any changes included in V8.0 (see details in row below)
4	**17_Nov_2016**	**8.0**	Substantial	- Schedule updated to match text (and vice versa) and footers clarified - Day of Leukapheresis moved to prior to Day 1 Cyclophosphamide - Randomisation timelines clarified - Addition of 3 month survival status follow up - **3.1** Group 2 patients updated information - **4.2** clarified windows for visits and progressive disease in first 12 months - **6.2** Screening – HLA typing removed - **6.3** contraception section added - **7.2** Randomisation – text updated - **8.0** Treatment and follow up – updated to

				match schedule - **9.0** Dose Modifications – clarified - **10** Leukapheresis procedure time updated - **11** Evaluable patient term clarified - **12.1.4** text updated - **12.2** Reporting periods clarified - **12.3** Reporting procedures clarified and SAE contact details updated - **13** Updated and clarified. - **16.3** Sample size calculation updated - **16.4** Analysis plan updated - Amendment table streamlined - Change to trial personnel and contact details - Terminology and typographical errors clarified or corrected

TRIAL SYNOPSIS

Title

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

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 activity 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

Randomised, open label, multicentre, phase II trial

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 1.1 criterion)
2. 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 (within 14 days of random-isation)	Leuka-pheresis Group 2 patients only	Day 1 (first Cy infusion; up to 14 days after random-isation) ALL patients	Day 29 (Cy infusion) ALL patients	TACE visit (Day 31) ALL patients	Post TACE Follow Up visits (Day 38, Day 45, Day 52) ALL patients	Repeat Cy infusion visits (Day 60, Day 90, Day 120) ALL patients	Repeat DC infusion visits (Day 62, Day 92, Day 122) Group 2 patients only	Follow Up visits[¥] (Three monthly; Day 152, Day 242, Day 332, Day 422) ALL patients	Survival Status Follow Up (Three monthly) ALL patients
Informed Consent	X									
Medical History	X									
Physical Examination	X[1]		X	X			X		X	
ECOG Performance Status	X	X	X	X	X	X	X	X	X	
Concomitant Medications	X	X	X	X	X	X	X	X	X	
Adverse Events	X	X	X	X	X	X	X	X	X	
Clinical Events	X	X	X	X	X	X	X	X	X	
Vital Signs	X	X	X	X	X	X	X	X	X	
Mandatory Microbiology screening[2]	X	X								
Tumour assessment[3]	X						X (DAY 60 ONLY)		X	
Auto-antibody tests[4]	X					X	X		X	
Standard Blood Tests[5]	X	X	X	X		X	X		X	
Immune response blood tests			X		X	X	X		X	
Blood collection for generation of DC vaccine[6] via Leukapheresis		X								
Pregnancy Test[7]	X									
Electrocardio-gram (ECG)	X									
Height and weight	X									
eGFR[8]	X									
Dendritic Cell infusion[9]					X			X		
Cyclo-phosphamide infusion			X	X			X			
Survival Status[10]										X

1. This will include assessment for potential difficulties in intra-hepatic artery cannulation (i.e. severe atherosclerotic disease) and venous access for leukapheresis at screening.

2. All patients will require mandatory testing for blood borne infectious agents of blood products prior to processing and storage. These tests must be performed during screening and on day of leukapheresis – Group 2 only. This consists of serological testing for HBV, HCV, HIV, Human T-Lymphotropic Virus 1 and 2 (HTLV-1, HTLV-2) and Syphilis**, and** NAT (PCR) testing for HBV, HCV and HIV. 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. N AT (PCR) testing **should only be performed on the first sample (i.e. not the sample on day of leukapheresis).**

3. Tumour assessments: A baseline chest CT and triple phase CT or contrast enhanced MRI scan of abdomen must be carried out in the 28 days prior to randomisation. If a standard of care scan has been performed within 28 days of randomisation, that scan can be used provided it can be reported according to RECIST 1.1. Repeat imaging will be performed on day 60 and every 3 months (90 days) until 12 months and thereafter every 3 months (90 days) until disease progression. Tumour assessment can be carried out within **10 days** of the scheduled visit.

4. Auto-antibody screen: Rheumatoid factor, anti-nuclear antibody, anti-mitochondrial antibody, anti-thyroid antibodies, smooth muscle antibodies and Liver Kidney Microsomal (LKM)-antibodies

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

6. Leukapheresis: Only patients randomised to Group 2 will undergo leukapheresis for isolation of monocytes.

7. Pregnancy test: a pregnancy test should be undertaken for all women of child bearing potential.

8. Estimation of glomerular filtration rate (GFR) by Cockcroft Gault Formula (Appendix 4)

9. 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.

10. Survival Status: This should be obtained every 12 weeks starting from the last Follow Up visit (Day 422) until the last patient on the trial reaches 2 years post the Day 422 Follow Up Visit, or until patient death. This can be from checking hospital records, contacting the GP or patient/ family.

¥ If a patient discontinues the trial treatment early then a Treatment Discontinuation Form should be completed, and the patient enter the Follow Up part of the trial, unless the patient has withdrawn their consent.

ABBREVIATIONS

AE	Adverse Event
AFP	Alpha-Fetoprotein
ALD	Alcoholic Liver Disease
APC	Antigen Presenting Cell
ATC	Anatomical Therapeutic Chemical Classification
ATMP	Advanced Therapy Medicinal Product
CI	Chief Investigator
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
Cy	Cyclophosphamide
CT	Computed tomography
CTL	Cytotoxic T lymphocytes
DC	Dendritic Cells
DoH	Department of Health
eRDC	Electronic Remote Data Capture
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
FBC	Full Blood Count
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GP	General Practitioner
Hb	Haemoglobin
HbeAg	Hepatitis B Core Antigen
HbsAg	Hepatitis B Surface Antigen
HIV	Human Immunodeficiency Virus
HCC	Hepatocellular Carcinoma
HTLV	Human T-lymphotrpohic Virus
IB	Investigator Brochure
IFN_γ	Interferon-gamma
IHA	Intra-Hepatic Arterial
IL-2	Interleukin 2
IL-4	Interleukin 4
IL-6	Interleukin 6
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
INR	International Ratio
ISF	Investigator Site File
IV	Intravenous

KLH	Keyhole Lympet Haemocyanin
LFTs	Liver Function Tests
LKM-antibodies	Liver Kidney Microsomal Antibodies
MAGE A1	Melanoma-associated Antigen-1
MCM	Macrophage Conditioned Medium
MHC	Major Histocompatability Complex
MHRA	Medicines and Healthcare products Regulatory Agency
MI	Myocardial Infarction
MLR	Mixed Lymphocyte Reaction
MPLA	Monophosphoryl Lipid A
MRI	Magnetic Resonance Imaging
NAT	Nucleic Acid Testing
NHS	National Health Service
NIHR WTCRF	National Institute for Health Research Wellcome Trust Clinical Research Facility
NY-ESO-1	New York-Esophageal Squamous Cell Carcinoma-1
NYHA	New York Heart Association
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
PEI	Percutaneous Ethanol Injection
PFS	Progression Free Survival
PHE	Public Health England
Plts	Platelets
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
RECIST	Response Evaluable Criteria in Solid Tumours
RFA	Radio Frequency Ablation
SAE	Serious Adverse Event
SmPC	Summary of Product Characteristics
SAP	Statistical Analysis Plan
SOP	Standard Operating Procedure
SSX-1	Synovial Sarcoma X Antigens
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAA	Tumour Associated Antigen
TACE	Trans-arterial Chemoembolisation
TAE	Transarterial Embolisation
TIL	Tumour Infiltrating Lymphocyte
TMG	Trial Management Group
TSPY	Testis-Specific Protein Y
U&Es	Urea and Electrolytes
ULN	Upper Limit of Normal
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 trial 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 Trial 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 randomised controlled trials (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

As 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 histocompatibility 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[19].

Following the demonstration that activated DC 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 tested in clinical trials[20]. 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*[21]. 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[23], HCC[24], prostate and renal cell carcinoma[25]. Several of these studies report that infusion of *in vitro* sensitised DC enhances cytotoxic T lymphocyte (CTL) responses *in vivo* in some patients with variable evidence of clinical efficacy. Rosenberg *et al* (1998) reported a trial of 31 patients with melanoma, 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[26]. Nestle *et al* (1998)[23] 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 trial 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[22]. Thirty-five 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* (2005)[24] 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 that suppresses anti-tumour immunity[27,28]. Our own data (Figure 1) 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 interleukin (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.* (2005)[29] 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 DC were used. Thus intratumoural DC therapy has potential if DC are given in combination with treatment that causes apoptotic or necrotic cell death allowing loading with tumour antigens & subsequent T-cell activation *in situ*.

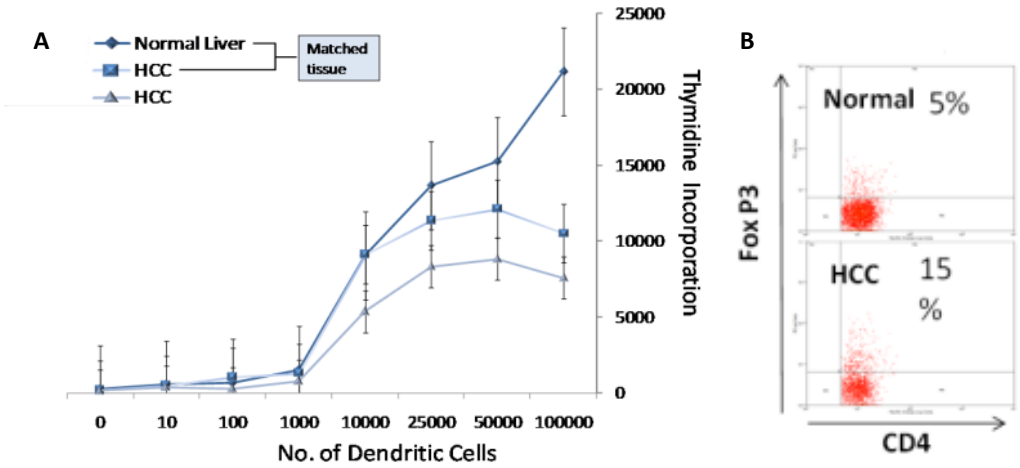


Figure 1. DC Matured in Conditioned Media From Liver Tumour Are Less Functional.

(A) DC matured in HCC tumour tissue are less effective at activating T-cells than DC matured in normal liver tissue. Blood monocytes were matured to DC 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. Data are expressed as median and interquartile range. (B) DC matured in HCC tumour tissue are able to induce a population of suppressive regulatory T-cells (CD4+Foxp3+)

1.6 Evidence Of An Immune Response In Hepatocellular Carcinoma

Cancers which evoke a lymphocytic infiltrate are generally more susceptible to immunotherapy[21]. These include malignant melanoma, renal cell carcinoma and HCC. HCC is heavily infiltrated by T-lymphocytes and the tumour shows strong expression of MHC Class 1 antigens and ICAM-1[30]. Furthermore, tumour-specific CTL can be expanded from HCC

tissues, which lyse autologous tumour cells more efficiently than non-specific targets[30]. 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[31]. 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[12,32-34]. 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[35,36].

Greten *et al* (2010)[38] 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[37,38]. Previous studies have reported that Tregs suppress anti-tumour immunity in patients with HCC[38-40] 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 therefore 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 than 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.

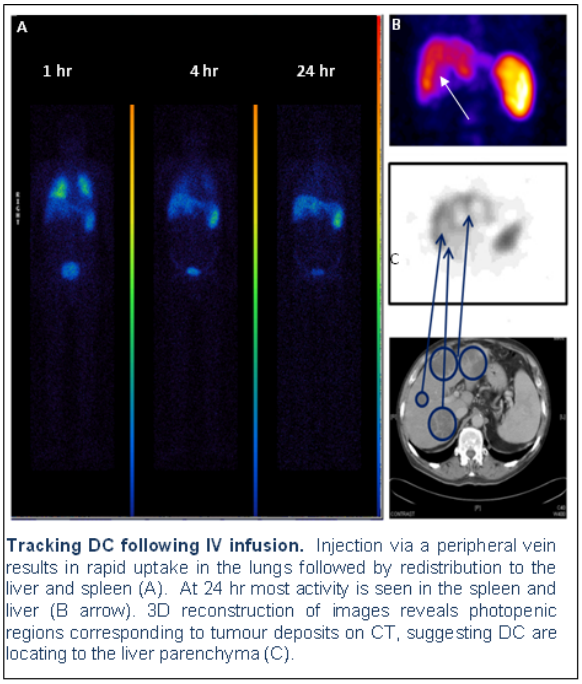
1.10 Justification For Trial Design

The studies discussed above, including our phase II clinical trial, show that some patients with HCC respond to DC therapy. The disease control rate of 28% seen in our trial was even more remarkable given that we studied patients with otherwise untreatable disease and large tumour loads which will hinder the efficacy of immunotherapy. Therefore there is 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.

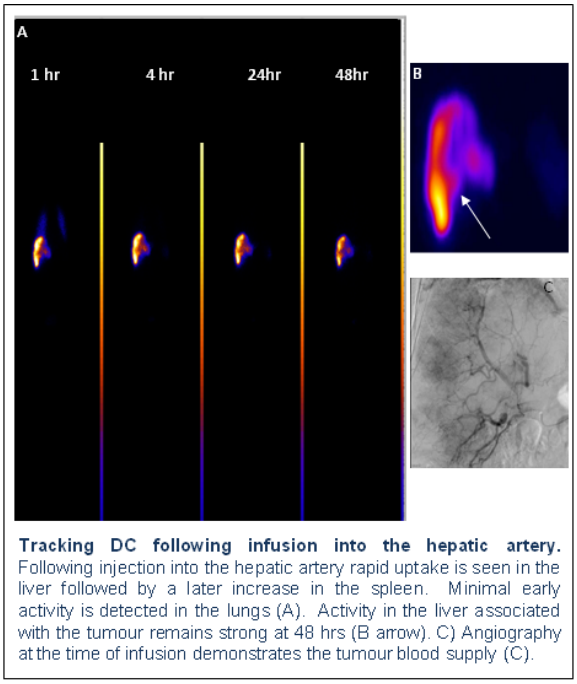
We will mature DC in monophosphoryl lipid A (MPLA). Our own *in vitro* studies suggest that MPLA is a highly effective maturation agent in the generation of DC from blood monocytes and an autologous maturation agent reduces the risk of toxicity. We have chosen to pulse the DC with HepG2 lysate because 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 haemocyanin (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 (Figures 2 and 2a). 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 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).

Figure 2. Tracking DC following intravenous infusion.



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).

Figure 2a. Tracking DC following intra-hepatic infusion

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[41,42]. 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[43] indicate the progression free survival (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.

2 AIMS, OBJECTIVES AND OUTCOME MEASURES

2.1 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.

2.2 Primary Outcome Measures

- Progression free survival

2.3 Secondary Outcome Measures

- Radiological response assessment (Response Evaluable Criteria in Solid Tumours [RECIST 1.1]))
- Change in the tumour marker Serum AFP
- Assessment of toxicity using Common Terminology Criteria for Adverse Events (CTCAE) (version 4)
- Immune Response Rate
- Overall survival

2.4 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[44]. These analyses will be undertaken in the Functional Genomics and Proteomics Facility in the School of Bioscience.

3 TREATMENT DETAILS

3.1 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
IMP 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. 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 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 9 for additional information in relation to dose modifications):

Initial Dose only

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

Conditioning Regimen

Trade Name:	**Cyclophosphamide** (*Generic Product*)
Active Substance:	**Cyclophosphamide monohydrate BP**
Pharmaceutical form:	Powder for injection
Route of administration:	Intravenous
IMP Status:	A copy of the SmPC can be found in the Pharmacy File.
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 is provided as a powder for injection and will be stored in the original container and 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 80-300 mg/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 9 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 days:	250 mg/m^2

The initial starting dose is in accordance with the SmPC.

Group 2 Patients Only

Autologous Dendritic Cells Pulsed with HepG2 Lysate matured with Monophosphoryl Lipid A

Trade Name:	Not applicable
Active Substance:	Not applicable
Pharmaceutical form:	Dendritic cells suspended in up to 16 ml CyroStor CS10 as a frozen product for thawing at the patient bedside, for intra-arterial infusion (first infusion only) or resuspended with up to 20 ml normal saline for intravenous infusion for all subsequent infusions.
Route of Administration:	The initial infusion is thawed at the patient bedside before being transferred to a sterile single use syringe via a sterile 40 μm blood filter. This is administered to the patient via the hepatic artery at the same time as TACE. Subsequent infusions are presented in a sterile infusion bag, which is then thawed in a water bath at the patients' bedside. A peripheral cannula is inserted into the patient's vein, and a standard infusion set is used to administer 250 ml 0.9% normal saline prior to the patient receiving the thawed cryopreserved cells intravenously. After the cells have been administered another 250 ml of 0.9% normal saline is given prior to the cannula being removed
IMP Status:	IMP (Advanced Therapy Medicinal Product [ATMP])
ATC Code:	Not Applicable

The dendritic cell vaccine product will be prepared as per the current Investigational Medicinal Product Dossier

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.

4 TRIAL DESIGN

4.1 Number of Centres

This is a multicentre clinical trial. Patients will be recruited from specially selected centres in the UK.

4.2 Trial Design

This is an open label randomised phase II clinical trial (see Figure 3 for schematic representation). 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 will receive additional Cyclophosphamide at a dose of 250 mg/m^2 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).

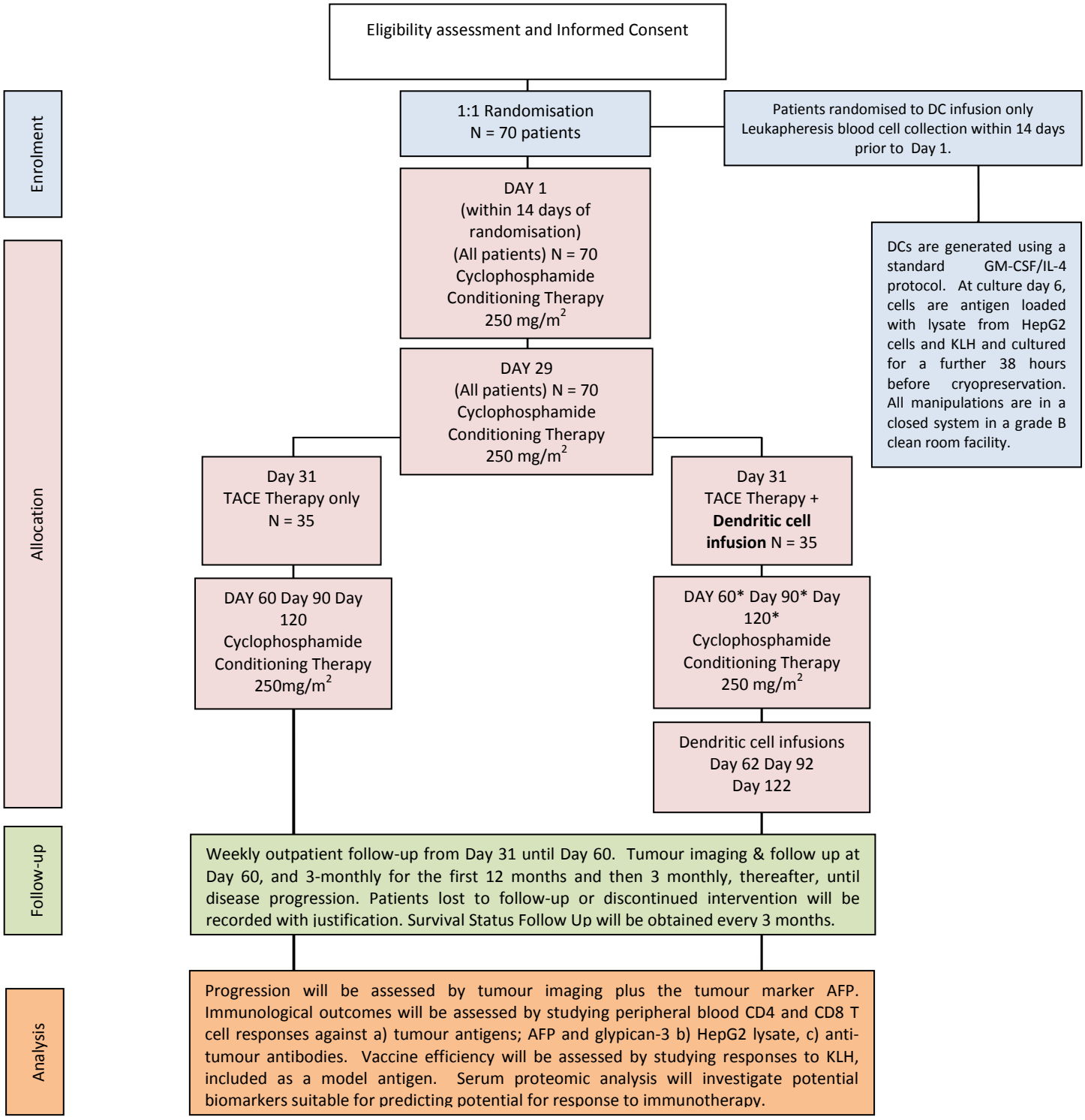


Figure 3: Summary of ImmunoTACE Trial

*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.

Otherwise TACE will be performed 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 250 mg/m^2 two days before each vaccination (Day 60, 90 and 120). Subsequently patients will be followed up in the outpatient department and receive standard care. Survival status information will be collected on a 3 monthly basis after the end of active trial

participation. This information can be obtained from the patient, their nominated representative of their GP. **Please see section 4.2.1 for patients who have evidence of disease progression in the first 12 months.**

N.B. For this trial there is a +/- 3 days visits window for all visits except for:

a) Day 1 cyclophosphamide infusion (within 14 days of randomisation)

b) DC infusion visits (Group 2 only), which will always take place 2 days following each cyclophosphamide infusion.

c) CT scans (after screening), can take place up to 10 days before or after the scheduled visit

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 trial.

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.

4.2.1 Progressive Disease Within First 12 Months

Patients who have progressive disease within the first 122 days should continue to receive the protocol scheduled cyclophosphamide infusions (Group 1 and Group 2 patients) and DC vaccinations (Group 2 patients only) if possible (see section 8.4). All patients who develop progressive disease within the first 12 months should continue to have imaging and follow up visits as per protocol, up to and including the scan at 12 months. Where possible, this imaging should performed even if the patient is receiving standard of care treatment i.e. Sorafenib. Patients who enter another interventional trial should discontinue from the ImmunoTACE trial completely.

4.3 Trial Duration

It is anticipated that recruitment will take approximately 3 years and the trial will continue until all patients have completed all the protocol defined visits. .

5 ELIGIBILITY

5.1 Inclusion Criteria

1. Written informed consent
2. Histological or cytological diagnosis or meet the AASLD criteria (Appendix 1) for diagnosis of HCC and at least one uni-dimensional lesion measurable according to the RECIST 1.1 by CT-scan or MRI (Appendix 2).
3. Suitable for TACE
4. Aged $\geq$ 18 years and estimated life expectancy $\geq$ 6 months
5. Not a candidate for surgical resection or transplantation
6. No previous chemotherapy, radiotherapy, immunotherapy or other experimental treatment for HCC prior to entry into the trial
7. Eastern Cooperative Oncology Group (ECOG) performance status $\leq$ 2 (Appendix 3)
8. Adequate haematological function: Hb $\geq$90g/L, Absolute neutrophil count $\geq 1.5 \times 10^9$/L, platelet count $\geq 50 \times 10^9$/L
9. Bilirubin ≤50 µmol/L, AST or ALT ≤5 x ULN
10. Adequate renal function: Cockcroft and Gault estimation $\geq$ 40ml/min (Appendix 4)
11. INR ≤1.5
12. Child-Pugh score $\leq$7 (Appendix 5)
13. Women of child-bearing potential should have a negative pregnancy test prior to trial entry
14. 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 trial and for six months after the completion of treatment.

5.2 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 (Radio Frequency Ablation [RFA] or Percutaneous Ethanol Injection [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 5)
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 CTCAE version 4 (Appendix 6) 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 > New York Heart Association (NYHA) class 2 (Appendix 7), Myocardial Infarction (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
18. Hypersensitivity to cyclophosphamide or to any of its metabolites
19. Current cystitis infection
20. Urinary outflow obstruction

6 SCREENING AND CONSENT

6.1 Informed Consent

The Investigator (or designated co-investigator as documented on the Site Signature and Delegation Log [SSDL]) 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.

6.2 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
- Medical history (including assessment of suitability for hepatic artery injection if appropriate and in particular a history of arterial disease)
- Documentation of concomitant medications
- Documentation of any clinical and Adverse Events that have occurred since Informed Consent
- Physical examination including documentation of peripheral pulses and assessment of venous access for leukapheresis
- Vital signs including height and weight
- Documentation of ECOG performance status (Appendix 3)
- Blood samples for FBC, U&Es, LFTs, serum AFP, coagulation and auto-antibody tests
- Estimation of glomerular filtration rate (GFR) (by Cockcroft Gault Formula; Appendix 4)
- Microbiology screen for HBV, HCV, HIV HTLV-1, HTLV-2, Syphilis and NAT (PCR) testing for HIV, HBV, HCV
- Pregnancy test if female of child-bearing potential
- Electrocardiogram (ECG)
- Imaging of the chest by computed tomography (CT) and a scan of the abdomen by either triple phase CT or contrast enhanced MRI scan will be performed within 28 days of randomisation

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

6.3 Contraception

Women of child bearing potential must agree to use a reliable form of contraception during the trial, e.g. oral contraceptive, intra-uterine device (IUD), diaphragm with spermicide, implant and/or condom. This should be continued for at least 3 months after the treatment has finished.

Men who have not had a vasectomy and who have a partner who could conceive must agree to use reliable forms of contraception during the trial. In addition to male sterilisation, acceptable contraceptive measures are the use of condoms.

7 TRIAL ENTRY

7.1 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

7.2 Randomisation

Randomisation can take place up to 14 days prior to the Day 1 Cyclophosphamide visit. 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 with an error component to prevent allocation predictions. 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, and treatment 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.

Once the Trial Office has received a copy of the SSDL, site staff who have been delegated the task of randomisation will be given access to the online database (by the Trial Coordinator) and will be able to randomise patients into the trial. If the site staff member(s) has/ have not had an account with CRCTU previously, a new account will be set up.

Contact Details for Randomisation (*If online randomisation process is unavailable)*:

CRUK Clinical Trials Unit
Institute of Cancer and Genomic Sciences University of Birmingham
Edgbaston
Birmingham
B15 2TT

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

Fax: 0121 414 3700 Email: immunotace@trials.bham.ac.uk

8. TREATMENT AND FOLLOW UP

8.1 Treatment and Follow Up Visits

NB. PATIENTS RANDOMISED TO GROUP 2 WILL HAVE 1 VISIT PRIOR TO DAY 1:

Leukapheresis Visit – GROUP 2 ONLY Blood collection for generation of DC vaccine via leukapheresis

- Mandatory microbiology screen (HBV, HCV, HIV, HTLV-1, HTLV-2, Syphilis)
- Document clinical events, adverse events and concomitant medications
- ECOG performance status
- Obtain bloods for FBC, INR, U&E, LFT and AFP
- Vital signs

Cyclophosphamide Infusion Visits (Day 1 within 14 days of randomisation & Day 29)

- Document clinical events, adverse events and concomitant medications
- Physical examination
- ECOG performance status
- Obtain bloods for FBC, INR, U&E, LFT AFP

- Vital signs
- Cyclophosphamide infusion

TACE Visit +/- Dendritic Cells Infusion (Day 31)

- Document clinical events, adverse events and concomitant medications
- ECOG performance status
- Obtain bloods for immune response assessment
- Vital signs
- Dendritic cell infusion

Regular Weekly Visits Post TACE (Day 38, 45 & 52)

- Document clinical events, adverse events and concomitant medications
- ECOG performance status
- Obtain bloods for FBC, INR, U&E, LFT, AFP, autoantibodies and immune response assessment
- Vital signs

Subsequent Cyclophosphamide Infusions Visits (Day 60, 90 & 120)

- Document clinical events, adverse events and concomitant medications
- Physical examination
- ECOG performance status
- Obtain bloods for FBC, INR, U&E, LFT, AFP, autoantibodies and immune response assessment
- Radiological assessment of response using same modality as baseline, will be carried out on Day 60.
- Vital signs
- Cyclophosphamide infusion
- Tumour assessment (**Day 60 only**)

DC Infusion Visits *(Group 2 patients only)* (Day 62, 92 & 122)

- Document clinical events, adverse events and concomitant medications
- ECOG performance status
- Vital signs
- Dendritic cell infusion

Follow Up Visits (3 monthly; Day 152, 242, 332 & 422)[¥]

- Document clinical events, adverse events and concomitant medications
- Physical examination
- ECOG performance status
- Obtain bloods for FBC, INR, U&E, LFT, AFP autoantibodies and immune response assessment.
- Radiological assessment of response using same modality as baseline (tumour assessment procedures can be performed at this visit or a maximum of +/-10 days prior to or after this visit).
- Vital signs

[¥] If a patient is withdrawn from treatment during the first 122 days, a Treatment Discontinuation Form should be completed and they should enter the Follow Up part of the trial, unless the patient has withdrawn their consent.

Survival Status Follow Up (3 monthly)

- Survival status

Survival status can be obtained from hospital records, the GP or by contacting the patient/ family as appropriate. This should take occur every 3 months until the last patient on the trial reaches 2 years post the Day 422 Follow Up Visit, or until patient death.

Visit Scheduling

Visits can be performed **(in sequence)** within a +/- 3 days scheduling window to allow for weekends and Bank Holidays. Radiological assessment of response using same modality as baseline (tumour assessment procedures can be performed at this visit or a maximum of +/-10 days prior to or after a scheduled visit).Visits can be performed **(in sequence)** within a +/- 3 days scheduling window to allow for weekends and Bank Holidays for all visits except for:

a) Day 1 cyclophosphamide infusion (within 14 days of randomisation)

b) DC infusion visits (Group 2 only), which will always take place 2 days following each cyclophosphamide infusion.

c) CT scans (after screening), can take place up to 10 days before or after the scheduled visit

8.2 Immune Response Assessment

Immune responses will be monitored for all patients at weekly intervals following TACE treatment until Day 60 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.

8.3 Assessment of Radiological Response

All patients will receive a formal radiological tumour assessment on Day 60 and every 3 months (90 days) thereafter until disease progression. The tumour assessment will consist of a chest CT and a triple phase CT or contrast enhanced MRI scan of the abdomen. Independent radiologists will perform the formal assessment of tumour response on the CT scans. All scans will be assessed using RECIST 1.1 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 activity of the protocol defined therapy in relation to radiological response to therapy (Appendix 2).

Accumulating clinical evidence indicates some subjects treated with immune system stimulating agents may develop disease progression by conventional response criteria before demonstrating clinical objective responses and/or stable disease.

Therefore, subjects will be allowed to continue trial therapy after an initial investigator-assessed RECIST 1.1 defined progression as long as they meet the following criteria:

- Investigator assessed clinical benefit
- Subject is tolerating trial treatment

The appearance of new malignant lesions denotes disease progression. The finding of a new lesion should be unequivocal: ie, not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumour (for example, some ‘new’ bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient’s baseline lesions show partial or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a ‘new’ cystic lesion, which it is not.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents new disease. If repeat scans confirm that there is a new lesion, then progression should be declared using the date of the initial scan.

8.4 Unscheduled Visits

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

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

8.5 Treatment Compliance

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

8.6 Treatment Discontinuation

In the event of discontinuation of trial treatment, full details of the reason(s) for discontinuation should be recorded in the patient's medical notes and on the appropriate pages on the Case Report Form (CRF) or eRDC. 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 after Day 122 (Radiological disease progression, Clinical disease progression [see Appendix 2]) **Please see section 4.2.1 for further details.**
- 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

9 DOSE MODIFICATIONS

9.1 Dendritic Cell Infusion

It is anticipated that no dose modifications will be required in relation to DC infusion.

9.2 Cyclophosphamide Infusion

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/trial doctor discretion and should be clearly documented in the patient's medical notes.

9.3 Doxorubicin

In response patient bilirubin values described below, the dose will be reduced to predefined levels.

Bilirubin (µmol/L)	Actual Dose Level	Percentage equivalent of maximum dose (Dose Level)
≤22	100 mg	100% (Full dose)
23-50	50 mg	50% (Level-1)

10 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.

Leukapheresis:

To obtain DC, patients will undergo leukapheresis which will typically take four-six 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 leukapheresis 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 can 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 trial 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 trial 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 immunosuppression 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 humans is uncertain. Pregnancy should therefore be avoided during therapy and for six months thereafter. It is for this reason that all patients taking part in the trial 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 gastrointestinal 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 trial will be required and prepared to use adequate contraceptive methods which must be continued for 6 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. Patients taking part in the trial may need an additional CT scan of the chest, and abdomen 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 trial 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.

10.1 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 trial 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.

11 PARTICIPANT WITHDRAWAL & TRIAL COMPLETION

11.1 Participant Withdrawal

Participants are free to withdraw from the trial 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 trial:

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

Participants in both groups will be considered evaluable once they have received TACE (Day 31). Patients who withdraw before this will be replaced. All participants who have received TACE (Day 31) will be included in the analysis unless they have withdrawn consent to remain in the trial 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 in the patient's hospital notes and communicated to the Trial Office on a Withdrawal Form.

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 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 8. 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 CySmPC. In the case of DC vaccination the latest version of the Investigator Brochure (IB) will be used.

12.1 Reporting Requirements

12.1.1 Adverse Events

All medical occurrences which meet the definition of an AE (see Appendix 8 for definition) should be reported. **Please note this includes abnormal laboratory findings.**

12.1.2 Serious Adverse Events

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

12.1.3 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.

12.1.4 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 trial or up to 60 days afterwards, please complete a Pregnancy Notification Form (providing the patient's details) and return to the Trials Office as soon as possible, If it is the patient who is pregnant provide outcome data on a follow-up Pregnancy Notification Form the patient must also discontinue from the trial immediately. 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 Pregnancy Release of Information Form. Once consent has been obtained, provide details of the outcome of the pregnancy on a follow-up Pregnancy Notification Form. If appropriate also complete an SAE Form as detailed below.

12.2 Reporting Period

The reporting period for AEs (including SAEs) 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 after the last cyclophosphamide infusion **OR** 30 days after the last dendritic cell infusion – whichever is the latter. All AEs must be followed up until resolution of the event, irrespective of the time period elapsed.

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 will be reported to the Trials Office and will continue to be followed up until resolution of the serious adverse event.

All SAEs that are at possibly related to the trial IMP / ATMP treatment must be reported to the Trials Office even after the reporting period.

12.3 Reporting Procedure

12.3.1 Site

12.3.1.1 Adverse Events

AEs should be reported on an AE Form (and where applicable on an SAE Form). An AE Form - this will either be on paper or on an electronic Remote Data Capture (eRDC) system, should be completed at each visit and sent to the Trials Office.

AEs will be reviewed using the 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, all grades 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.

12.3.1.2 Serious Adverse Events

For more detailed instructions on SAE reporting refer to the SAE Form Completion Guidelines contained in Section 5 of the Investigator Site File (ISF). AEs defined as serious and which require reporting as an SAE should be reported on an SAE Form. When completing the form, the Investigator will be asked to define the causality and the severity of the AE which should be documented using the CTCAE version 4.0.

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 371 8028 (Primary number)

Or

0121 414 3700 (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.

12.3.1.3 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.

12.3.2 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).

12.4 Reporting to the Competent Authority and Research Ethics Committee

12.4.1 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.

12.4.2 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.

12.4.3 Adverse Events

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

12.4.4 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.

12.4.5 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.

12.4.6 Data Monitoring Committee

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

12.5 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 Products, or an unrelated event.

13 DATA HANDLING AND RECORD KEEPING

13.1 Data Collection

Data will be collected during this clinical trial. Data will be captured on case report forms (CRF), initially this will be on paper forms. Once the electronic remote data capture (eRDC) systems is implemented sites will be informed and further details provided. The Case Report Form (CRF / eRDC system) will comprise the following forms:

Form	Summary of data recorded	Schedule for submission
Eligibility Checklist	Confirmation of eligibility and satisfactory staging investigations where necessary	Faxed at point of randomisation.
Dosing Forms		
Cyclophosphamide	Dose level, Visit, Date, infusion details	Within 2 weeks
Dendritic Cell	Date, Visit, infusion details	Within 2 weeks
Assessment Forms		
Adverse Event	Start and stop dates, grade according to CTCAE version 4.0, causality	Every 4 weeks and as requested
Concomitant Medication	List of concomitant medication	Every 4 weeks and as requested
Microbiology	Date, Visit, results,	Within 2 weeks
Immune Response	Date, Visit, time	Within 2 weeks
Auto Antibody	Date, Visit, time, results, clinical significance	Within 2 weeks
Clinical Chemistry	Date, Visit, results, clinical significance	Within 2 weeks
ECG	Date, Visit, results	Within 2 weeks
Haematology	Date, Visit, results, clinical significance	Within 2 weeks
Medical History	Prior conditions, diagnosis, grade	Within 2 weeks
TACE	Date, procedure details	Within 2 weeks
Physical Examination	Date, Visit, investigations	Within 2 weeks
Pregnancy Test	Date, Visit, result	Within 2 weeks
Vital Signs	Date, Visit, results	Within 2 weeks
Imaging Form	Date, Visit, method of assessment, target lesions, non-target lesions, evaluation	Within 2 weeks
Ad Hoc Forms		
Treatment Discontinuation	As required	Within 4 weeks of trial visit date.
Serious Adverse Event Form	Details of the SAE	Within 24 hrs of being made aware of the SAE
Pregnancy Notification Form	Patient details and details of pregnancy	Within 24 hrs of being made aware of the pregnancy
Death Form	Date and cause of death	Immediately upon notification of death
Deviation Form	Completed in the event of a deviation from the protocol	Immediately upon discovering deviation.
Withdrawal Form	Used to notify the Trials Office of patient withdrawal from the trial	Immediately upon patient withdrawal

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 SSDL) within the timeframes listed above.
The exception is the SAE Form, which must be co-signed by the Investigator. See Adverse Event reporting section 12 for further details.

Entries on the paper-based 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 paper-based 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.
Any completed paper originals should be sent to the ImmunoTACE Trials Office and a copy filed in the ISF.

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.

13.2 Archiving

The Principal 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. **As this trial involves an ATMP all accountability records must be retained for 30 years.** The Trial Office will retain the original CRFs. Do not destroy any documents without prior approval from the Cancer Research UK Clinical Trials Unit (CRCTU) Document Storage Manager.

14. QUALITY MANAGEMENT

All sites will be required to sign a clinical trial site agreement prior to participation. In addition all participating Investigators will be asked to sign the necessary agreements and 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.

The trial is being conducted under the auspices of the Cancer Research UK Clinical Trials Unit (CRCTU) according to the current guidelines for Good Clinical Practice (GCP) and their local procedures. Participating sites will be monitored by CRCTU staff to confirm compliance with the protocol and the protection of patients' rights as detailed in the Declaration of Helsinki: October 1996 (Appendix 9).

14.1 On-site Monitoring

Monitoring will be carried out as required following a risk assessment and as documented in the ImmunoTACE Quality Management Plan. Additional on-site monitoring visits may be triggered for example by poor CRF return/ eRDC completion, 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.

14.2 Central Monitoring

Where a patient has given explicit consent sites are requested to send in copies of signed Informed Consent Forms.
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 Research Ethics Committee (REC) and the Medicines for Healthcare products Regulatory Agency (MHRA).

14.3 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.

14.4 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.

15 END OF TRIAL DEFINITION

For the purposes of the MHRA the end of trial will be 12 months after all patients have completed all their protocol trial defined visits. This will allow sufficient time for the completion of protocol procedures, data collection and data input. For the purposes of REC approval, the trial end date is deemed to be 6 months after the last data capture following 3 years of long term follow up.

After closure of the trial with the MHRA the Sponsor is no longer required to notify the MHRA and REC of changes of Principal Investigator. However, sites should continue to notify the Trials Office of changes in Principal Investigator by completing and returning (where required) an Investigator Registration Form together with a current signed and dated CV.

The Trials Office will notify the MHRA and REC that the trial has ended at the appropriate time and will provide them with a summary of the clinical trial report within 12 months of the end of trial.

16 STATISTICAL CONSIDERATIONS

16.1 Definition of Outcome Measures

Primary:

- The number of patients in each treatment group that are progression-free at 12 months from Day 1 will be assessed. Progression will be determined according to RECIST 1.1. 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.

Secondary:

- Radiological response will be assessed by 3-monthly scan compared against baseline scan and categorised according to RECIST 1.1.
- Serum AFP will be collected at Day 1 and then monthly to investigate changes over time.
- Toxicity: Adverse events and serious adverse events will be graded according CTCAE (Appendix 6) 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 Day 1 .
- Overall survival is defined as the time from date of Day 1 to the date of death from any cause. Alive patients will be censored at the date of last follow-up.

16.2 Analysis of Outcome Measures

As an early phase trial, 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:

- Assessment of progression free survival is based on the frequency of events across treatment arms. Further, PFS estimates will be calculated using the method of Kaplan and Meier, and median and 12-month progression free survival rates with confidence intervals will be presented by treatment group. Alive patients without documented progression will be censored at the date of last follow-up.

Secondary:

- Radiological response over time will be reported. Patients will be categorised according to their 'best' response and proportions reported by treatment group.
- The change in serum AFP from Day 1 , will be reported descriptively by treatment group. With complete data, the 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 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 that are predictive of response.

16.3 Power Calculations

The primary outcome measure in this trial is progression free survival based on the frequency of events across treatment arms. The sample size was calculated using the Lachin and Foulkes[45] method as it provides a sample size in terms of the underlying risk difference and accounts for accrual duration, follow-up period and a hazard dropout rate for each group.

Local unpublished data from the Queen Elizabeth Hospital estimates progression free survival in patients to be 30% at 12 months. The aim of this trial is to increase progression free survival to 50% using the TACE + cyclophosphamide + vaccine regime. In order to detect this 20% increase with a one-sided significance level of 20%, power of 80%, and accounting for an accrual period of two years, minimum follow-up period of one year and a patient dropout rate of 20%, 70 patients will need to be randomised into the trial (35 patients per treatment arm).

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.

16.4 Final Analysis

This trial design does not include an interim analysis. The final analysis will be carried out when all 70 participants 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.

17 TRIAL ORGANISATIONAL STRUCTURE

17.1 Sponsor

ImmunoTACE is an investigator led trial, co-ordinated by the D^3B Team within the CRCTU, University of Birmingham. The University of Birmingham will act as a sponsor. CRCTU, Birmingham will coordinate the trial 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.

17.2 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 Development Safety Update 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 is unacceptable or if any issues are identified which may compromise patient safety.

17.3 Trial Steering Committee

An independent Trial Steering Committee (as per the NIHR EME programme requirements) will be set up by the Trial Management Group. This committee will meet annually from the start of trial recruitment (ideally after the DMC committee report has been received). This committee will review the trial progress and will review the Data Monitoring reports and well as advising the Trial Management Group. Independent members of the committee will consist of representatives from the funder, Trial sponsor and independent clinicians.

17.4 Finance

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

17.5 Trial Management Group

Membership	Chief Investigator
	Co-investigators
	Invited Principal Investigators
	Senior Trial Coordinator
	Trial Coordinator
	Research Nurse
	Trial Statistician
	D^3B Trial Management Team Leader
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)/ eRDC Forms
	Reviewing progress of Trial and if necessary agreeing changes to the protocol
	SUSAR Reporting to MHRA
	Data Verification
	Data analysis
	Preparation of Trial Reports including DMC Reports
	Publication and Presentation of Results

17.6 Delegation

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

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

18 ETHICAL CONSIDERATIONS

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects, adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964, amended at the 48th World Medical Association General Assembly, Somerset West, Republic of South Africa, October 1996 (website: http://www.wma.net/en/30publications/10policies/b3/index.html) (Appendix 9).

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 Good Clinical Practice (GCP). This trial will be carried out under a Clinical Trial Authorisation in accordance with the Medicines for Human Use Clinical Trials regulations. The protocol will be submitted to and approved by 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 the Trials Office receives written confirmation of R&D approval.

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.

19 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 CRF 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.

20 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.

21 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.

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APPENDIX 1 - DIAGNOSIS OF HCC: AASLD CRITERIA

Diagnosis of HCC may be confirmed by biopsy or by application of the following non-invasive diagnostic criteria that have been proposed by the AASLD[a]. The application of dynamic imaging criteria should be applied only to patients with cirrhosis of any aetiology and to patients with chronic hepatitis B who may not have fully developed cirrhosis or have regressed cirrhosis.

1. Nodules larger than 1 cm found on ultrasound screening of a cirrhotic liver should be investigated further with either 4-phase multi-detector CT scan or dynamic contrast enhanced MRI.
    - If the appearances are typical* of HCC, the lesion should be treated as HCC.
    - If the findings are not characteristic or the vascular profile is not typical, a second contrast enhanced trial with the other imaging modality should be performed, or the lesion should be biopsied.

2. Nodules found on ultrasound surveillance that are smaller than 1 cm should be followed with ultrasound at intervals from 3-6 months.

*Typical characteristics: hypervascularity in the arterial phase with washout in the portal venous or delayed phase.

[a]Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 53, 1020-1022 (2011).]

APPENDIX 2 - RESPONSE EVALUATION CRITERIA IN SOLID TUMOUR 1.1

The following contains excerpts from the RECIST version 1.1 plus trial specific instructions.
A free copy of the revised guidelines is available from http://www.eortc.be/recist/default.htm
(Eisenhauer *et al*., 2009)

Measurability of Tumour Lesions at Baseline

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

- Measurable
- Non-measurable

Measurable lesions are those that can be accurately measured in at least one dimension (longest diameter to be recorded) with a minimum size of 10 mm by CT scan (CT scan slice thickness no greater than 5 mm), 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with callipers should be recorded as non-measurable) and 20 mm by chest X-ray. For malignant lymph nodes to be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by a CT scan (at baseline and during treatment, only the short axis will be measured and followed).
Non-measurable lesions are all other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to > 15 mm short axis) and truly non-measurable lesions.
Lesions considered to be truly non-measurable include the following: bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, abdominal masses that are not confirmed and followed by imaging techniques, and cystic lesions.
Tumour lesions that are situated in a previously irradiated area are not considered measurable.
The term "evaluable" in reference to measurability is not recommended and will not be used because it does not provide additional meaning or accuracy.
All measurements should be recorded in metric notation using callipers (or a ruler) if clinically assessed. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of treatment.

Specifications by Methods of Measurements

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

Tumour Response Evaluation

Baseline Documentation of "Target" and "Non-target" Lesions
All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as “target” lesions and recorded and measured at baseline.
Target lesions should be selected on the basis of their size (those with the longest diameter) and their suitability for accurate, reproducible, repeated measurements.
A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as the reference by which to characterise the objective tumour response.

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

Response Criteria

A. Evaluation of Target Lesions

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

B. Evaluation of Non-target Lesions

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

Notes:

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

C. Evaluation of Best Overall Response

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

D. 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
Complete response (CR)	CR	No	CR
Complete response (CR)	Non-CR/non-PD	No	PR
Complete response (CR)	Not evaluated	No	PR
Partial response (PR)	Non-PD	No	PR
Stable disease (SD)	Non-PD	No	SD
Not all evaluated	Non-PD	No	Not evaluable (NE)
Progressive disease (PD)	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

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

Frequency of Tumour Re-evaluations

For the ImmunoTACE trial, clinical response rate, disease control rate (CR+PR+SD), PFS and duration of response will be evaluated by triple phase CT or contrast enhanced MRI scan of the abdomen at baseline. Repeat imaging will be performed on Day 60 and every 3 months (90 days) for the first 12 months (including for those who have progressed and may be receiving standard of care treatment) and thereafter until disease progression. trialtrial

APPENDIX 3 - ECOG PERFORMANCE STATUS

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 self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.:

Oken MM, Creech RH. Tormey DC, et al. Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5, 649-655 (1982).

APPENDIX 4 - COCKCROFT-GAULT EQUATION

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 5 - 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 6 - COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS

Adverse Events will be recorded according to the Common Terminology Criteria for Adverse Events version 4 (CTCAE).

The full 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 7 - 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 8 - 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 affect 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.

APPENDIX 9 - 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 fulfilment of this mission.

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

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

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

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

In the field of biomedical research a fundamental distinction must be 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 foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

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 trial 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 trial 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 trial 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, re-establishing 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 trial, 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 trial 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.