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METILDA

A Randomised Phase II Study in Metastatic Melanoma to Evaluate the Efficacy of Adoptive Cellular Therapy with Tumour Infiltrating Lymphocytes (TIL) and Interleukin-2 Dose Assessment

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MAHSC Trials Coordination Unit Managed Trial

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SIGNATURES/PROTOCOL APPROVAL

A Randomised Phase II Study in Metastatic Melanoma to Evaluate the Efficacy of Adoptive Cellular Therapy with Tumour Infiltrating Lymphocytes (TIL) and Interleukin-2 Dose Assessment (METILDA)

Protocol Version: 1.0 23 August 2013
Sponsor reference number: 11_DOG14_12

This document describes the METILDA trial and provides information about procedures for entering patients into it.

The protocol should not be used as a guide for the treatment of patients outside the trial.

Every care was taken in drafting this protocol, but corrections or amendments may be necessary. These will be circulated to Investigators in the trial, but centres entering patients for the first time are advised to contact the Trial Manager at the MAHSC-CTU to confirm they have the most recent and approved version.

This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) regulations 2004 and ICH Good Clinical Practice guidelines. The trial will be conducted in compliance with the protocol, the Data Protection Act (DPA Z6364106), the Declaration of Helsinki, Human Tissue Act (2004), the Research Governance Framework (2005) and other regulatory requirements as appropriate.

Protocol Authorised by:

Name & Role	Site	Signature	Date
Professor Robert Hawkins (Co-Chief Investigator)	The Christie NHS Foundation Trust /University of Manchester		
Dr Paul Lorigan (Co-Chief Investigator)	The Christie NHS Foundation Trust		

TABLE OF CONTENTS

1 LIST OF ABBREVIATIONS

ACT	Adoptive Cell Therapy
AE	Adverse Event
ALK PHOS	Alkaline Phosphatase
ALT	Alanine amino-transferase
APTT	Partial thromboplastin time
AR	Adverse Reaction
ASR	Annual Safety Report
AST	Aspartate amino-transferase
bd	Twice a day
BP	Blood pressure
CA	Competent Authority
CI	Chief Investigator
CMV	Cytomegalovirus
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Computerised Tomography
CTA	Clinical Trial Authorisation
CTPM	Clinical Trial Project Manager
CTU	Cellular Therapeutics Unit
CTIMP	Clinical Trial of Investigational Medicinal Product
CXR	Chest X-ray
DMC	Data Monitoring Committee
DSMB	Drug Safety Monitoring Board
DTIC	Dacarbazine
EBV	Epstein Barr Virus
EC	European Commission
ECG	Electro-cardiogram
EDTA	Ethylenediamine tetraacetic acid
EMEA	European Medicines Agency
EU	European Union
EUCTD	European Clinical Trials Directive
EudraCT	European Clinical Trials Database
EudraVIGILANCE	European database for Pharmaco-vigilance
FBC	Full Blood Count
GAfREC	Governance Arrangements for NHS Research Ethics
GCP	Good Clinical Practice
GCSF	Granulocyte colony-stimulating factor
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
Hb	Haemoglobin
HCG	Human chorionic gonadotrophin
HD IL2	High dose interleukin-2
HIV	Human Immunodeficiency Virus
HSC	Haemopoetic Stem Cell
HTLV	Human T cell lymphotropic Virus
IB	Investigator Brochure
ICF	Informed Consent Form
IFNγ	Gamma Interferon
IL2	Interleukin-2
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
ISF	Investigator Site File

ISRCTN	International Standard Randomised Controlled Trial Number
LD-IL2	Low dose interleukin-2
LFT	Liver Function Test
LVEF	Left Ventricular Ejection Fraction
µl	Microlitre
MA	Marketing Authorisation
Main REC	Main Research Ethics Committee
MAHSC-CTU	Manchester Academic Health Science Centre Clinical Trials Unit
MHC	Major Histocompatibility Complex
MHRA	Medicines and Healthcare products Regulatory Agency
MRC	Medical Research Council
MRI	Magnetic Resonance Imaging
MS	Member State
MUGA	Multi Gated Acquisition Scan
NCI	National Cancer Institute
NHS R&D	National Health Service Research & Development
od	Once a day
PBMC	Peripheral blood mononuclear cells
PI	Principal Investigator
PICC	Peripherally Inserted Central Catheter
PICR	Paterson Institute for Cancer Research
PIS	Participant Information Sheet
Plts	Platelets
PK	Pharmacokinetics
PT	Prothrombin time
QA	Quality Assurance
QC	Quality Control
qds	Four times a day
QP	Qualified Person for release of trial drug
RCT	Randomised Control Trial
REC	Research Ethics Committee
REP	Rapid Expansion Protocol
RECIST	Response Evaluation Criteria in Solid Tumours
SAR	Serious Adverse Reaction
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedure
SmPC	Summary of Product Characteristics
SPL	Summary of product information
SSA	Site Specific Assessment
SSAR	Suspected Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBI	Total Body Irradiation
tds	Three times per day
TIL	Tumour infiltrating lymphocyte
TMG	Trial Management Group
TSC	Trial Steering Committee
U+E	Urea and electrolytes
ULN	Upper limit of normal
WBC	White blood cells
WHO	World Health Organisation

2 TRIAL SUMMARY

Title: **A Randomised Phase II Study in Metastatic Melanoma to Evaluate the Efficacy of Adoptive Cellular Therapy with Tumour Infiltrating Lymphocytes (TIL) and Interleukin-2 Dose Assessment**

Short title: TIL Therapy in Metastatic Melanoma and IL2 Dose Assessment (METILDA)

Design: This is a single site Phase II open labelled two arm randomised trial of tumour infiltrating lymphocytes (TIL) in metastatic melanoma using high versus low dose IL2.

Objectives:

Primary Objectives

1. To evaluate the anti-tumour activities of TIL therapy with HD-IL2 versus LD-IL2.
2. Evaluation of feasibility and tolerability of TIL therapy with HD-IL2 versus LD-IL2

Secondary Objectives

1. Evaluation of progression free survival.
2. Evaluation of the duration of response.
3. Assessment of overall survival.

Exploratory Objectives

1. Examination of potential tumour related or immunological biomarkers of response to facilitate better patient selection in the future.
2. Evaluation of response rate by immune related Response Criteria (irRC).
3. Evaluation of TIL harvest process.
4. Assessment of the cost of treatment.

Endpoints:

Primary Endpoint

- To evaluate the anti-tumour activities and response rates by RECIST v1.1 of TIL therapy with HD-IL2 versus LD-IL2.
- Evaluation of feasibility and tolerability of TIL therapy with standard HD-IL2 and LD-IL2. Feasibility will be assessed in terms of proportion of patients who undergo full therapy in both arms. Tolerability will be assessed according to CTCAE v4.0 grading.

Secondary Endpoints

1. Evaluation of the progression free survival.
2. Evaluation of the duration of response.
3. Assessment of overall survival.

Exploratory Endpoints

- Examination of potential tumour related or immunological biomarkers of response to facilitate better patient selection in the future.
- Evaluation of response rate by immune related Response Criteria (irRC).
- Evaluation of TIL harvest process at different stages.
- Assessment of the average per-patient cost of treatment.

Cohorts: This is a two-arm trial. Patients will be randomised into groups receiving HD-IL2 versus LD-IL2

ARM A: High Dose Interleukin-2
ARM B: Low Dose Interleukin-2

Eligibility: **There are TWO parts to TIL Therapy –TIL Harvest and TIL Therapy.**

To undergo TIL HARVEST patient must be potentially eligible for future TIL Therapy (either now or after intervening therapy/observation):

Inclusion Criteria for TIL Harvest

- Patients must have histologically confirmed malignant melanoma of cutaneous origin.
- They must have resectable metastatic lesion(s) of at least 2cm in total diameter.
- They must be likely to fulfil the full criteria for TIL therapy at a future date.
- The must give full written informed consent to the surgical procedure and the TIL harvest/storage.

Exclusions to TIL Harvest are:

- Patients known or found to be serologically positive for Hepatitis B, C, HIV or HTLV.
- Previous allogeneic transplant.
- Patient with ocular/mucosal (or any non-cutaneous primary site) melanoma.
- Patients who are high medical risks because of non-malignant systemic disease, including those with, uncontrolled cardiac or respiratory disease, or other serious medical or psychiatric disorders which in the lead clinicians opinion would not make the patient a good candidate for this therapy.
- Prior history of malignancies at other sites, with the exception of adequately treated cone-biopsied *in situ* carcinoma of the cervix uteri and basal or squamous cell carcinoma of the skin.
- Patients known or found to be serologically positive for Hepatitis B, C, HIV or HTLV.
- History of systemic autoimmune disease which could be life-threatening if reactivation occurred (for example hypothyroidism would be permissible, prior rheumatoid arthritis or SLE would not).
- Patients who are likely to require long-term systemic steroids or other
- Radiotherapy to >25% skeleton.

To be eligible for TIL THERAPY patients must have successfully completed TIL Harvest and fulfil the full eligibility criteria below:

Inclusion Criteria for TIL Therapy

- Patients must have histologically confirmed malignant melanoma of cutaneous origin with confirmed evidence of progressive metastatic disease and to have failed / refused standard therapies.
- Patients may enter if they have previously had TIL (successfully) harvested and stored at the Manchester Cell Therapy Unit.
- There must be measurable / evaluable disease.
- Patients may have had any previous systemic therapies including anti-CTLA4 (Ipilimumab) agent provided they are otherwise fit for treatment.
- Age equal to or greater than 18 years.
- World Health Organisation (WHO) performance status of 0 or 1.
- Life expectancy >3months.
- LVEF > 50% as measured by ECHO/MUGA and satisfactory stress ECHO (if over 60 or had previous cardiotoxic therapy).
- Haematological and biochemical indices:

Haemoglobin (Hb)	≥ 9.0 g/L
Neutrophils	≥ 1.0 x 10^9/L
Platelets (Plts)	≥ 100 x 10^9/L
Any of the following abnormal baseline liver function tests:	
serum bilirubin	≤ 1.5 x ULN
alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) and/or alkaline phosphatase (ALP)	≤ 5 x ULN
Serum creatinine	≤ 0.15 mmol/L

These measurements must be performed prior to tumour collection and repeated when TIL are growing and prior to commencing pre-conditioning chemotherapy. The chemotherapy to be used in this trial is non-myeloablative, but where patients have had previous high dose chemotherapy an autologous haemopoietic stem backup harvest, for stem cell rescue, will be obtained prior to commencing therapy in this trial. Similarly, where there is concern about a patient's bone marrow reserves, for example due to multiple previous lines of myelosuppressive chemotherapy a backup stem cell harvest will also be obtained.

- Female patients of child-bearing potential must have a negative serum or urine pregnancy test prior treatment and agree to use appropriate medically approved contraceptive precautions for four weeks prior to entering the trial, during the trial and for six months afterwards.
- Male patients must agree to use barrier method contraception during the TIL treatment and for six months afterwards.
- Full written informed consent

Exclusion criteria for TIL Therapy

- Those receiving radiotherapy, targeted therapy, immunotherapy, systemic steroids, or chemotherapy during the previous four weeks (six weeks for nitrosoureas and Mitomycin-C) prior to treatment or during the course of the treatment.
- All toxic manifestations of previous treatment must have resolved. Exceptions to this are alopecia or certain Grade 1 toxicities, which an investigator considers should not exclude the patient. For patients who had severe colitis (grade 3 / 4) on Ipilimumab (or similar therapy), this must be confirmed as resolved by colonoscopy.
- Previous radiotherapy treatment to the resectable metastatic site(s) within 1 year and no other suitable metastatic sites.
- Participation in any other clinical trial within the previous 30 days or during the course of this treatment.
- Previous allogeneic transplant.
- Patient with ocular/mucosal (or any non-cutaneous primary site) melanoma.
- Clinically significant cardiac disease. Examples would include unstable coronary artery disease, myocardial infarction within 6 months or Class III or IV AHA criteria for heart disease (see Appendix 6)
- Patients who are high medical risks because of non-malignant systemic disease, including those with, uncontrolled cardiac or respiratory disease, or other serious medical or psychiatric disorders which in the lead clinicians opinion would not make the patient a good candidate for this therapy.
- Evidence of active infection
- Prior history of malignancies at other sites, with the exception of adequately treated cone-biopsied *in situ* carcinoma of the cervix uteri and basal or squamous cell carcinoma of the skin.
- Patients known or found to be serologically positive for Hepatitis B, C, HIV or HTLV.
- History of systemic autoimmune disease which could be life-threatening if reactivation occurred (for example hypothyroidism would be permissible, prior rheumatoid arthritis or SLE would not).
- Patients with currently more than 3 brain metastases.
- Patients with symptomatic brain metastasis measuring more than 10mm in diameter or evidence of significant surrounding oedema on MRI will not be eligible until after treatment demonstrating no clinical or radiologic CNS progression for at least 2 months. Patient must be able to wean off any steroid use 3 weeks before treatment commencement.
- Patients who are likely to require long-term systemic steroids or other immunosuppressive therapy.
- Pregnant and lactating women.
- Radiotherapy to >25% skeleton.

TRIAL SUMMARY	
Trial treatment and methods	This is a two arm, open-labelled, phase II, randomised trial of Tumour Infiltrating Lymphocytes (TIL) in metastatic melanoma patients given with preconditioning chemotherapy and IL2. Potentially eligible patients will undergo surgical tumour excision from which TIL will be harvested and stored. Patients who have successful TIL Harvest and are fully eligible for TIL Therapy with have their TIL further expanded. Patients will then receive preconditioning chemotherapy with cyclophosphamide (60mg/kg) day -7 and day -6, followed by fludarabine (25mg/m^2) day -5 to day -1. The autologous TILs will be re-infused on day 0 and the patients will receive up to 12 doses of intravenous HD or LD-IL2 depending on the randomised arm. The primary objective of response rate according to RECIST 1.1 will be assessed and compared by CT scans carried out at week 6, week 12 and at 12 weekly intervals thereafter.
Trial duration per participant	Each participant will receive one cycle of treatment.
Estimated total trial duration	It is anticipated the trial will commence recruitment September 2013. Recruitment will occur over a 44 month period following opening. Trial analysis will be completed within 60 months of the trial opening to recruitment.
Total number of participants planned	Up to 90
Additional trials/Sub-trials	Not applicable

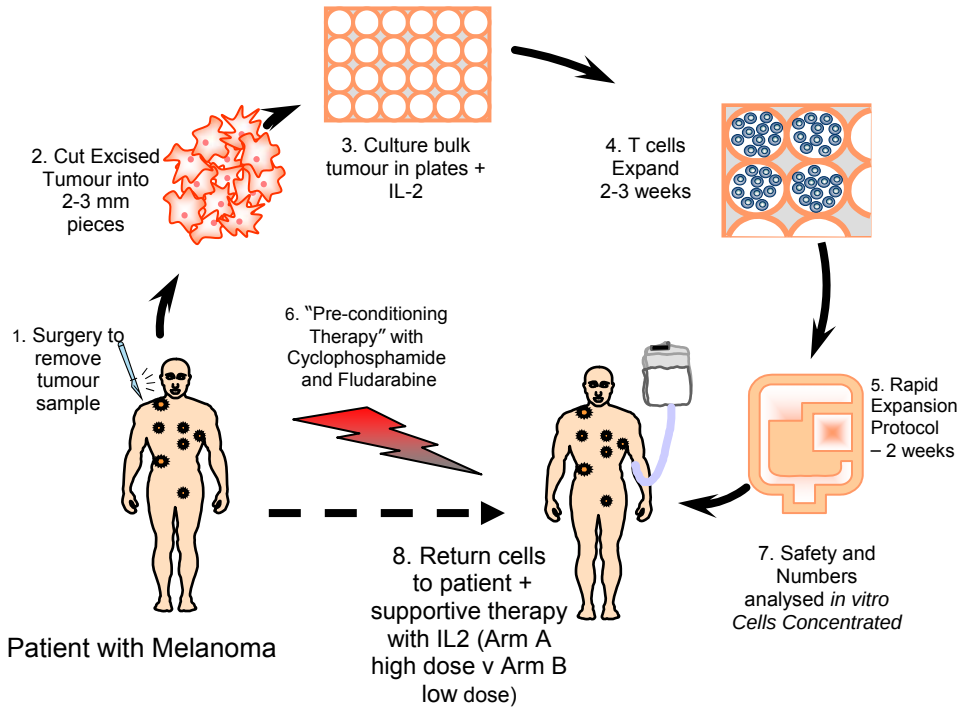


Figure 1 Summary Diagram of Treatment Process and Related Procedure

3 INTRODUCTION

3.1 Background

The incidence of malignant melanoma is increasing globally and although only representing 10% of all skin cancers, melanoma is the leading cause of skin cancer mortality with 66,000 deaths worldwide annually. In the United Kingdom, mortality from malignant melanoma has risen by approximately 18% between 2008-2010 (Cancer Research UK). Although there has been encouraging advancement in therapeutic options in recent years, the survival rate for patients with advanced or metastatic melanoma remains low with only 10-20% of patients surviving after 2-years.

Treatment for advanced unresectable or metastatic disease (stage IV) is difficult and remains primarily palliative. Treatment response is generally low and short-lived while complete response is rarely achieved. Single agent chemotherapy such as dacarbazine is well tolerated but with low response rate of 10% and no overall survival benefit. Combination regimes using platinum, vinca alkaloids or nitroureas can result in higher response rates but at the cost of significant toxicities with no durable remission or overall survival benefit over monotherapy. High dose Interleukin-2 (IL2) has been shown to benefit some patients with a possible complete response rate of about 6%(Atkins *et al*.,1999).However, the high toxicity rates of high dose IL2 requiring delivery of repeated cycles limits its use to only a very small subset of patients.

There have been discoveries of newer treatment options in Melanoma in recent years. Ipilimumab, an anti-CTLA4 (Cytotoxic T-Lymphocyte Associated Antigen 4) monoclonal antibody, has shown promising survival benefit in metastatic melanoma patients, as single agent or in combination with chemotherapy[14, 21]. A 2- year survival of more than 20% could be expected and some of these patients achieved long-term remission. Vemurafinib, a BRAF kinase inhibitor targeting the MAPK(Mitogen-Activated Protein Kinase) pathway has demonstrated improved overall and progression-free survival among patients with previously untreated, metastatic melanoma with BRAF V600E mutation in a randomised phase III trial (Chapman *et al*.,2011). This represents an attractive treatment option but limited only for patients with this mutation (40-60%). Furthermore, median duration response was reported to be 6.7 months (Sosman *et al*., 2012) with rapid clinical deterioration frequently observed in some patients following drug discontinuation. There is therefore a clear need to search for better and more durable therapeutic options for patients with metastatic melanoma of all sub-groups.

Adoptive Cell therapy (ACT) is a promising approach to the treatment of cancer. Much of the current clinical work in adoptive T cell therapy has been undertaken in melanoma by Rosenberg and colleagues at the NCI. The reasons to target melanoma include: the relatively frequent finding of tumour specific T cells in melanoma patients; the molecular characterization of their T cell receptors, the large number of tumour specific antigens discovered in melanoma; and the fact that metastatic melanoma has proven resistant to other forms of treatment, with standard chemotherapy having little clinical effect (recently reviewed by (Kirkwood *et al.*, 2008). A limited efficacy for interleukin-2 in the treatment of metastatic melanoma trials in conjunction with adoptive T cell transfer was first reported in 1988 (Rosenberg *et al*., 1988) with further updates in 1994 (Rosenberg *et al.*, 1994). Cells grown from melanoma tumours (“Tumour infiltrating lymphocytes”) were cultured and expanded ex vivo in interleukin-2 and returned to the patients either with or without cyclophosphamide. All patients received high-dose interleukin-2 after cell infusion. Overall, the response rate was good at 34% but the duration was often short (median 4 months for partial responses (Rosenberg *et al.*, 1994)). A key correlate of response was however, short time in culture/short-doubling time and in vitro anti-tumour reactivity also seemed beneficial (Schwartzentruber *et al.*, 1994) and these are markers of “Young cells”.

In a series of major trials following these initial observations a variety of improvements were explored. The outcome of these trials may be summarised as:

- Cloning cells to select individual reactive clones resulted in decreased in vivo T cell survival and a reduced clinical response rate (Dudley *et al*., 2001).
- Using cells that were only partially selected for specificity but introducing more intensive preconditioning resulted in better T cell survival, (indeed in many cases permitted significant in vivo expansion) enhanced both response rate and duration of response (Dudley *et al*., 2002). Key correlates of response were however, long telomere lengths and high surface expression of the CD27 molecule, both markers of 'young' cells (Zhou *et al*., 2005; Huang *et al*., 2006).
- Using intense preconditioning resulted in very high response rates (over 70%) with proven durability in some cases (Dudley *et al*., 2008).

These trials along with supporting pre-clinical data provide the current paradigm for the use of adoptive cell transfer in which infusion of cells is combined with pre-conditioning chemotherapy (**Figure 1**). In these trials, the importance of T cell characteristics was confirmed and the benefit of "young" rapidly growing cells held up from the initial trials. This has been further confirmed in trials using "Young TILs" whereby cells are not selected for specificity at all. Remarkably this produces excellent response rates – around 50% (Besser *et al*., 2009) or with CD8 selected TIL (Dudley *et al*., 2010). This approach is very practical, uses minimally manipulated cells and can potentially be applied widely in melanoma and perhaps other tumours.

Importantly the other established treatments for melanoma have very modest benefit and TIL appear to offer both high response rates and more durable benefits. Even though there are other interesting new therapies Young TIL appear to offer the unique advantage of a high response rate (~50%) with significant numbers of complete and durable responses **(Table 1).**

3.2 Risks and Benefits

The acute toxicities of treatment are largely those related to the chemotherapy and the interleukin-2. The chemotherapy is associated with nausea and vomiting that is generally well controlled with medication.

Table 1: Summary of clinical results obtained with current licensed agents for the treatment of melanoma. The results for TIL illustrate the high rate of response and the durable benefit obtained.

Treatment	Response Rate	Complete Response Rate	2 year survival	Reference
Dacarbazine/Temozolomide	12%	3%	10%	Middleton *et al*., 2000
Interleukin-2	18%	6%	20%	Atkins *et al*., 1999
Ipilimumab	11%	1%	20%	Hodi *et al*., 2010
Vemurafenib	81%	6%	Not known	Chapman *et al*., 2011
Ipilimumab + DTIC	15%	1.6%	28%	Robert *et al*., 2011
TIL	55%	21%	40%	Dudley *et al*., 2008 – updated 2010

Table 2: Acute Toxicities with Young TIL (Besser *et al*., 2010)

	Responders N = 10	Non-Responders N = 10
Days in hospital (average, including NMC)	19.0 ± 1.2	20.7 ± 3.5
Chemotherapy related toxicity		
Units RBC transfusion (median)	5	5
Units PLT transfusion (median)	18	24
Febrile neutropenia	9	10
Opportunistic infection (Herpes Zoster)	0	1
IL-2 related toxicity		
Pulmonary congestion	4	3
Renal failure	4	1
Prolonged hypotension	2	1
Hyperbilirubinemia	2	2
Diarrhea	2	2
Confusional state	0	1
Autoimmunity (vitiligo)	1	0

The interleukin-2 has major toxicities of flu-like symptoms with fever/rigors and symptoms due to vascular leak such as hypotension, oedema, pulmonary congestion, deterioration of renal function. Again these symptoms are generally easily managed and we have considerable experience of using the HD-IL2 regimen (>150 patients treated). The LD-IL2 regimen has the same toxicity but significantly less severe due to the reduced dose. The major toxicity arises from the combination of neutropenia / thrombocytopenia (as a result of the chemotherapy) and the interleukin-2. There is a significant risk of neutropenia infection and its management is complicated by the HD-IL2 as the symptoms can be similar. There are standard prophylactic measures that we will employ (G-CSF support, antibiotic prophylaxis) and again we are experienced in managing such adoptive cell therapy protocols with > 20 patients treated (5 with TIL therapy). Patients are expected to require blood and platelet transfusions. The toxicities actually observed at the Sheba Medical Center are shown in **Table 2**. The toxicities seen at the NCI are similar although there have been 2 treatment related deaths due to neutropenic sepsis (out of 56 patients) (Dudley *et al*., 2010). The toxicities of treatment generally resolve fairly rapidly. The IL2 related toxicities resolve within 24 hours of stopping. The toxicities related to neutropenia generally rapidly resolve after haematological recovery. With Young TIL, to date there have been no reports of long-term toxicities other than vitiligo. Nevertheless long term toxicity may occur and with other TIL protocols - uveitis requiring steroid eye drops was reported (Dudley *et al*., 2002). Other long-term toxicities have been reported with long-term follow up (Rosenberg *et al*., 2011): "The toxicities of treatment have been previously reported and were largely due to the lymphodepleting preparative regimen or IL-2. Most patients tolerated the treatment well and returned to baseline. There was one treatment-related death in the 93 patients; a 49 year old male who received 2 Gy TBI and died of sepsis four days after cell infusion from an undetected diverticular abscess present prior to treatment. One patient with a partial response who received 2Gy TBI developed prolonged pulmonary hypertension. Five patients who received 12 Gy TBI, all of whom experienced a complete regression developed a microangiopathic nephropathy with creatinine elevations in the range of 1.5 to 2.5 mg/dL. Renal function has not worsened over time and these patients are living normally."

As can be seen from this quote the longer term toxicity related largely to the protocols containing TBI and we are not using TBI within this protocol. However other rare toxicities can occur and certainly in a cell therapy protocol using Fludarabine chemotherapy we have seen fatal neurological toxicity (Thistlethwaite *et al*., 2008) – this is well recognised and occurs in around 1:200 patients treated with Fludarabine. Overall, with rare exceptions, patients return rapidly to their baseline state and leave hospital after about 21 days. Autoimmune toxicity is mild with TIL therapy and easily controlled without long-term consequences. The toxicity of chemotherapy / HD-IL2 is severe (it will be reduced in LD-IL2) but for most patients recovery is rapid. As with all systemic cancer treatment there is a risk of death. The available evidence suggests this would be 1-2% with the planned regimens. Overall, the toxicities and risks are not out of proportion to other cancer therapies and the potential benefits (particularly the ~20% durable remission rate) appear to justify these risks.

3.3 Rationale for testing Low-Dose Interleukin-2

Current TIL therapy, in both the US and Israel, uses HD-IL2 to support cells after return to the patient. Whilst there is considerable experience of delivering this treatment safely in Manchester (Shablak *et al*., 2011), it is rarely used elsewhere in the UK or Europe and is a toxic and complex treatment. There are a number of clinical and scientific reasons for believing that LD-IL2 is appropriate to test:

(i) Low Dose IL-2 therapy has reduced toxicity compared to that typically associated with High Dose IL-2 treatment. HD IL-2 therapy is able to drive objective clinical responses in a proportion of renal and melanoma patients. However, the high levels of toxicity associated with this therapy have been known for a long period of time (e.g. Atkins *et al*., 1999) with the result that few clinical centres currently offer HD-IL2 as a therapeutic option (2 in the UK). Conversely, low dose IL-2 is not associated with such toxicity and has been used as a component of a prolonged treatment schedule with IFNγ with no major evidence of toxicity (Vaglio, 2009). Thus, proving that LD-IL2 is equally effective in the adoptive transfer setting would encourage wider clinical participation in ACT. There is also a significant cost saving from using low dose interleukin-2.

(ii) High levels of IL-2 may hinder the ability of adoptively transferred effector T cells to respond to the homeostatic cytokines IL-7 and IL-15 and may also drive the selective expansion of regulatory T cells
The rationale for using HD-IL-2 has been the fact that the T cells are cultured in high dose IL2 prior to adoptive transfer. However, once transferred to the patient, it is now clear that IL-7/IL-15 play a major role in driving the homeostatic expansion of adoptively transferred T cells responding to the non-myeloablative regimes used to pre-condition patients prior to adoptive transfer. The IL-2, IL-7 and IL-15 receptors all utilise the common gamma (γc) chain and, consequently, it is possible that the presence of high levels of IL-2 will mean that the ligated IL-2 receptor will sequester all of the available γc chain and thereby reduce the ability of the effector T cell to respond to IL-7/IL-15. Moreover, recent studies suggest that a subset of host regulatory T cells that survive pre-conditioning can preferentially respond to IL-2 while effector T cells proliferate in response to IL-7 (Bayer, 2009). Therefore, high dose IL-2 may drive Treg expansion and may hinder effector T cell proliferation *in vivo*. It is expected that low dose IL-2 will cause a reduced level of sequestration of γc chain receptor.

Recent clinical activity using NY-ESO-1 specific TCR engrafted T cells employed HD-IL2 support (Robbins, 2011). However, a case study has been reported using a $CD4^+$ NY-ESO-1 reactive T cell clone that generated an objective clinical response in the absence of pre-conditioning or IL-2 support (Hunder, 2008). Furthermore, recent trials of adoptive transfer of chimeric antigen receptor T cells specific for the CD19 antigen have generated objective responses and long persistence of the T cells in

pre-conditioned patients but in the absence of IL-2 cytokine support (Kalos, 2011). Overall, these clinical studies highlight that antigen-specific T cell adoptive transfer can generate effective clinical responses but the role of IL-2 has not been fully elucidated. HD-IL2 may enhance Treg expansion and interfere with effector T cell function yet it may also prove to be critical to maintain effector cell activity for the period immediately post-adoptive transfer. Hence, this trial seeks to answer this question and determine whether a protocol employing less toxic LD-IL2 can be used to replace the HD-IL2 regimen.

(iii) Animal models indicate longer duration of IL2 may be beneficial

The toxicity of IL2 is much less in mice and therefore the issue of dose is difficult to directly compare. However, a recent realistic study (i.e. treating large established tumours) (Klebanoff *et al*., 2011) has shown that IL2 administration is important and that longer duration of administration is beneficial over shorter duration. A consequence of giving lower dose IL2 is that we expect to be able to give more doses. This is borne out by our own experience with two trials of engineered T-cells. One (targeting CEA) used HD-IL2 and was associated with no responses and toxicity leading to the trial stopping. The other targeting CD19 used LD-IL2 and treatment was well tolerated with little toxicity and 2/3 patients responded (unpublished). In the latter trial the patients on average received twice the number of IL2 doses. Obviously these are different trials in different diseases but again the combined clinical/animal data suggest that LD-IL2 may be at least as good, and conceivably better, than HD-IL2.

Overall, if LD-IL2 is of similar efficacy, it will facilitate the adoption of this therapy at more sites and will reduce toxicity and costs. This would represent a significant advantage.

3.4 Rationale of Preconditioning chemotherapy

Cyclophosphamide 60mg/kg/day (Day -7 and -6) and Fludarabine 25mg/m^2/day (Day -5 to -1) will be used in this study. This non-myeloablative regime was used in the pilot adoptive T cell therapy study (Rosenberg *et al*., 2011) and a well-established pre-conditioning chemotherapy regime for adoptive cell therapy studies in recent times. There is considerable justification for the use of pre-conditioning chemotherapy:

- To allow homeostatic mechanisms to facilitate expansion of lymphocytes (Baccala, *et al* 2005)
- To remove regulatory T cells, present in increased numbers in cancer patients (Wolf, *et al* 2003)
- To facilitate trafficking of the engineered T cells (Pinthus, *et al* 2004)

Under conditions of lymphopenia, adoptively transferred T cells can undergo marked proliferation, known as homeostatic proliferation (Baccala, *et al* 2005). In a series of experiments Dummer and colleagues showed that adoptive transfer of lymph node derived T cells into lymphopenic mice can confer anti-tumour immunity through homeostatic expansion (Dummer, *et al* 2002). Of note optimal inhibition of tumour growth was only obtained if the host mouse was made lymphopenic (in their case they used sub-lethal irradiation to induce lymphopenia).

Important evidence in the clinical setting showing that lymphodepletion can enhance survival and efficacy of transferred T cells comes from a series of clinical trials by Rosenberg's group using selected tumour-reactive TILs in metastatic melanoma patients (Rosenberg and Dudley 2004). They have used conditioning chemotherapy consisting of fludarabine and cyclophosphamide that is lymphodepleting, but importantly is non-myeloablative. It was administered prior to infusion of selected tumor-reactive TILs and the patients subsequently received high dose intravenous IL-2. An impressive 18 out of 35 patients had objective responses to this treatment and importantly there was a significant correlation between persistence of transferred TILs and tumour response (Rosenberg and Dudley 2004).

In a pre-clinical paper there is also strong evidence for the use of pre-conditioning chemotherapy in CAR T cell therapy (Pinthus, *et al* 2004). They administered erbB2-specific CAR expressing human T cells to a SCID mouse model with human prostate cancer bone marrow lesions. They used pre-conditioning treatment of either low dose irradiation or cyclophosphamide and combined it with IL-2 administration. The therapy resulted in decreased tumour growth and prostate-specific antigen secretion, prolongation of survival and even cure. They found that cyclophosphamide was the more effective of the two pre-treatments investigated and concluded that their results and the Rosenberg data 'strongly justify' the use of pre-conditioning of cancer patients prior to the adoptive transfer of tumour-specific T cells (Pinthus, *et al* 2004).

4 TRIAL OBJECTIVES AND ENDPOINTS

4.1 Trial Objectives

Primary Objectives

1. To evaluate the anti-tumour activities of TIL therapy with HD-IL2 versus LD-IL2.
2. Evaluation of feasibility and tolerability of TIL therapy with HD-IL2 versus LD-IL2

Secondary Objectives

1. Evaluation of progression free survival.
2. Evaluation of the duration of response.
3. Assessment of overall survival.

Exploratory Objectives

1. Examination of potential tumour related or immunological biomarkers of response to facilitate better patient selection in the future.
2. Evaluation of response rate by immune related Response Criteria (irRC).
3. Evaluation of TIL harvest process.
4. Assessment of the cost of treatment.

4.2 Trial Endpoints

Primary Endpoint

1. To evaluate the anti-tumour activities and the response rates by RECIST v1.1 of TIL therapy with HD-IL2 versus LD-IL2.
2. Evaluation of feasibility and tolerability of TIL therapy with standard HD-IL2 and LD-IL2 Feasibility will be assessed in terms of proportion of patients who undergo full therapy in both arms. Tolerability will be assessed according to CTCAE v4.0 grading.

Secondary Endpoints

1. Evaluation of the progression free survival.
2. Evaluation of the duration of response.
3. Assessment of overall survival.

Exploratory Endpoints

1. Examination of potential tumour related or immunological biomarkers of response to facilitate better patient selection in the future.
2. Evaluation of response rate by immune related Response Criteria (irRC).
3. Evaluation of the efficacy of TIL harvest process at different points in patient pathway.
4. Assessment of the average per-patient cost of treatment.

5 TRIAL DESIGN

5.1 Overall Design

The trial is a non-commercial randomised phase II design testing the standard TIL regimen given with high-dose IL2 versus low-dose IL2. It is not clear that the inclusion of high-dose interleukin-2 is necessary and as indicated above if low dose interleukin-2 were of similar efficacy there would be significant advantages.

The patient population will be patients with metastatic melanoma who have undergone standard treatment for metastatic melanoma and who are considered fit and potentially able to benefit from this TIL therapy. After careful planning, eligible patients will undergo surgical excision of tumour tissues to isolate in-tumour TILs. These will be cultured and expanded in the Cellular Therapeutics Unit at UMIC. Once satisfactory cell expansion is confirmed, patients will undergo preconditioning chemotherapy with cyclophosphamide (60mg/kg) day -7 and day -6, followed by fludarabine (25mg/m^2) day -5 to day -1. TILs will be re-infused on day 0 and depending on the trial arm drawn, patients will receive up to 12 doses of intravenous IL2 either at high-dose 600,000 (Arm A) or at 100,000 U/kg (Arm B) from day 0 to day 4(Figure 2).

Each participant will receive one cycle of treatment only.

The primary objective of response rate according to RECIST v1.1 will be assessed by CT scans carried out at week 6, week 12 and at 12 weekly intervals thereafter.

It is estimated that the trial will open in December 2013 and recruit patients for approximately 4 years. Up to 90 patients will be recruited and treated in a 2 stage process (Figure 3).

Figure 2 : Overview of Laboratory and Clinical Procedures

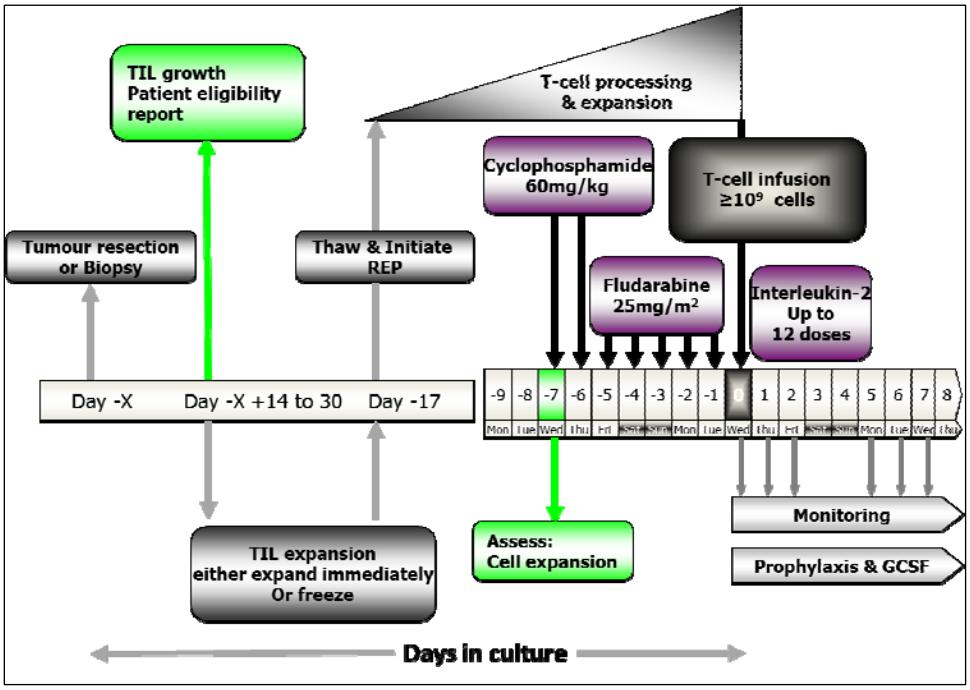
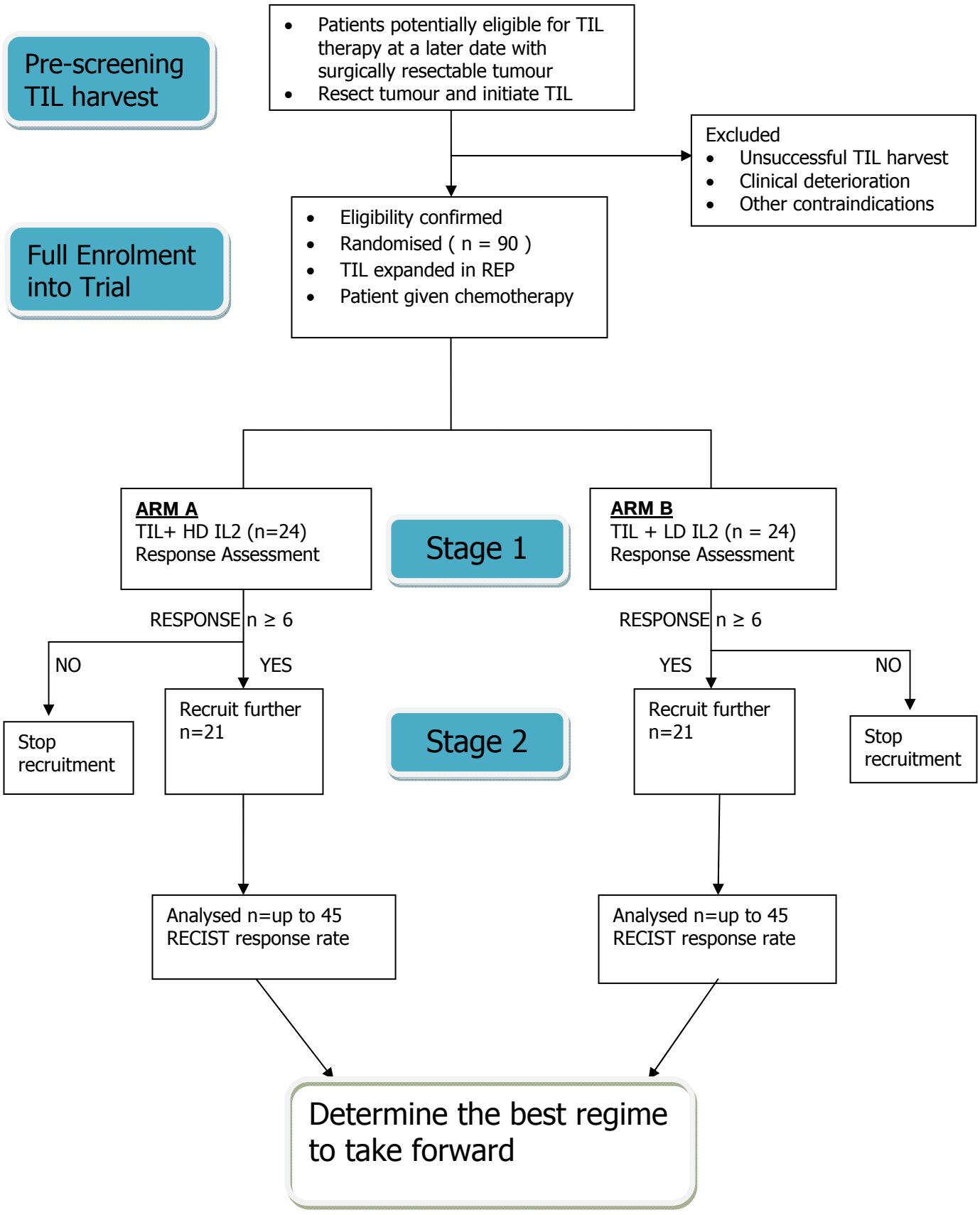


Figure 3. Overview Flow Chart of METILDA Trial (2 stage recruitment)



6 SELECTION OF TRIAL PARTICIPANTS

There are two parts to TIL Therapy –TIL Harvest and TIL Therapy. To be eligible for TIL Therapy patients must have successfully completed TIL Harvest and fulfil the full eligibility criteria below.

6.1 To undergo TIL Harvest patient must be potentially eligible for future TIL therapy (either now or after intervening therapy/observation):

6.1.1 Inclusion Criteria for TIL Harvest

- Patients must have histologically confirmed malignant melanoma of cutaneous origin.
- They must have resectable metastatic lesion(s) of at least 2cm in total diameter.
- They must be likely to fulfil the full criteria for TIL therapy at a future date.
- The must give full written informed consent to the surgical procedure and the TIL harvest/storage.

6.1.2 Exclusions to TIL Harvest are:

- Patients known or found to be serologically positive for Hepatitis B, C, HIV or HTLV.
- Previous allogeneic transplant.
- Patient with ocular/mucosal (or any non-cutaneous primary site) melanoma.
- Patients who are high medical risks because of non-malignant systemic disease, including those with, uncontrolled cardiac or respiratory disease, or other serious medical or psychiatric disorders which in the lead clinicians opinion would not make the patient a good candidate for this therapy.
- Prior history of malignancies at other sites, with the exception of adequately treated cone-biopsied *in situ* carcinoma of the cervix uteri and basal or squamous cell carcinoma of the skin.
- Patients known or found to be serologically positive for Hepatitis B, C, HIV or HTLV.
- History of systemic autoimmune disease which could be life-threatening if reactivation occurred (for example hypothyroidism would be permissible, prior rheumatoid arthritis or SLE would not).
- Patients who are likely to require long-term systemic steroids or other
- Radiotherapy to >25% skeleton.

6.2 TIL therapy

6.2.1 *Inclusion criteria for TIL Therapy*

- Patients must have histologically confirmed malignant melanoma of cutaneous origin with confirmed evidence of progressive metastatic disease and to have failed / refused standard therapies.
- Patients may enter if they have previously had TIL (successfully) harvested and stored at the Manchester Cell Therapy Unit.
- There must be measurable / evaluable disease.
- Patients may have had any previous systemic therapies including anti-CTLA4 (Ipilimumab) agent provided they are otherwise fit for treatment.
- Age equal to or greater than 18 years.
- World Health Organisation (WHO) performance status of 0 or 1.
- Life expectancy >3 months.

- LVEF > 50% as measured by ECHO/MUGA and satisfactory stress ECHO (if over 60 or had previous cardiotoxic therapy).
- Haematological and biochemical indices:

Haemoglobin (Hb)	≥ 80 g/L
Neutrophils	≥ 1.0 x 10^9/L
Platelets (Plts)	≥ 100 x 10^9/L
Any of the following abnormal baseline liver function tests:	
serum bilirubin	≤ 1.5 x upper limit of normal (ULN)
alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) and/or alkaline phosphatase (ALP)	≤ 5 x ULN
Serum creatinine	≤ 0.15 mmol/L

These measurements must be performed prior to tumour collection and repeated when TIL are growing and prior to commencing pre-conditioning chemotherapy. The chemotherapy to be used in this trial is non-myeloablative, but where patients have had previous high dose chemotherapy an autologous haemopoietic stem backup harvest, for stem cell rescue, will be obtained prior to commencing therapy in this trial. Similarly, where there is concern about a patient's bone marrow reserves, for example due to multiple previous lines of myelosuppressive chemotherapy a backup stem cell harvest will also be obtained.

- Female patients of child-bearing potential must have a negative serum or urine pregnancy test prior treatment and agree to use appropriate medically approved contraceptive precautions for four weeks prior to entering the trial, during the trial and for six months afterwards.
- Male patients must agree to use barrier method contraception during the TIL treatment and for six months afterwards.
- Full written informed consent

6.2.2 *Exclusion Criteria for TIL Therapy*

- Those receiving radiotherapy, targeted therapy, immunotherapy, systemic steroids, or chemotherapy during the previous four weeks (six weeks for nitrosoureas and Mitomycin-C) prior to treatment or during the course of the treatment.
- All toxic manifestations of previous treatment must have resolved. Exceptions to this are alopecia or certain Grade 1 toxicities, which an investigator considers should not exclude the patient. For patients who had severe colitis (grade 3 / 4) on Ipilimumab (or similar therapy), this must be confirmed as resolved by colonoscopy.
- Previous radiotherapy treatment to the resectable metastatic site(s) within 1 year and no other suitable sites available.
- Participation in any other clinical trial within the previous 30 days or during the course of this treatment.
- Previous allogeneic transplant.
- Patient with ocular/mucosal (or any non-cutaneous primary site) melanoma.
- Clinically significant cardiac disease. Examples would include unstable coronary artery disease, myocardial infarction within 6 months or Class III or IV AHA criteria for heart disease (see Appendix 6)

- Patients who are high medical risks because of non-malignant systemic disease, including those with, uncontrolled cardiac or respiratory disease, or other serious medical or psychiatric disorders which in the lead clinicians opinion would not make the patient a good candidate for this therapy.
- Evidence of active infection.
- Prior history of malignancies at other sites, with the exception of adequately treated cone-biopsied *in situ* carcinoma of the cervix uteri and basal or squamous cell carcinoma of the skin.
- Patients known or found to be serologically positive for Hepatitis B, C, HIV or HTLV.
- History of systemic autoimmune disease which could be life-threatening if reactivation occurred (for example hypothyroidism would be permissible, prior rheumatoid arthritis or SLE would not).
- Patients with currently more than 3 brain metastases.
- Patients with symptomatic brain metastasis measuring more than 10mm in diameter or evidence of significant surrounding oedema on MRI will not be eligible until after treatment demonstrating no clinical or radiologic CNS progression for at least 2 months. Patient must be able to wean off any steroid use 3 weeks before treatment commencement.
- Patients who are likely to require long-term systemic steroids or other immunosuppressive therapy.
- Pregnant and lactating women.
- Radiotherapy to >25% skeleton.

7 RECRUITMENT OF TRIAL PARTICIPANTS

Recruitment of patients to this trial will only commence once the MAHSC - CTU on the behalf of the Sponsor has confirmed that all necessary procedures are in place and the site has been notified by the MAHSC- CTU of the site being activated for recruitment.

7.1 Identifying Participants

Patients will be identified and referred by delegated clinicians through local and regional healthcare providers via standard referral process. Suitable patients will be discussed in the weekly immunotherapy meeting for subsequent steps. As there is no guarantee of successful TIL harvest every time (which will preclude subsequent proceedings of the trial), the recruitment and consenting process of this trial is divided in to 2 phases, namely **consenting to TIL Harvest** and **full consent to the TIL Therapy Trial** phase. See Figure 4. This will prevent patients with unsuccessful TIL harvest being exposed to unnecessary screening investigations specific to the trial. In addition, patient who may not yet be eligible for this trial at the time of consideration but undergoing planned tumour resection (curative or palliative) could have TILs harvested, cultured and frozen for future use, thus negating need of surgery when become eligible in the future. Please note informed consents need to be obtained separately at both phases.

7.2 Pre-Screening & TIL Harvest

Two categories of patients can be considered for pre-screening:

a) **Patient who potentially meets trial inclusion criteria at the time of consideration.**

b) **Patient who does not immediately meet trial inclusion criteria at the time of consideration but may become eligible in the future.** This applies to patients with metastatic or recurrent disease who were routinely being arranged to have surgical resection of metastatic lesion(s) with curative or palliative intent where their risk of further disease relapse or progression is considered to be high. This approach allows TILs to be harvested (and cryopreserved) from the resected tumour(s) during the planned surgery for future use within this trial (or indeed out of trial or in other trials) when the subject becomes eligible through disease relapse or progression.

7.2.1 Pre-screening - TIL Harvest Consent

Consent for pre-screening can be obtained following full discussion and review of PIS with suitable patients on the same day. Patients will need to have virology screen (HIV, HepB/C, HTLV1) undertaken and will only proceed to TIL Harvest when these are confirmed as negative.

All patients who underwent TIL Harvest process must be recorded in a separate log with details of outcomes at all stages.

7.2.2 TIL Harvest - Surgical Tumour Excision and TIL Isolation

As described in section 7.2.1, this stage involves pre-screening patients of category a) or b) undergoing surgery (standard planned surgery or potentially, trial-directed) to excise tumour specimen for the purpose of TIL harvesting. In both categories and for this purpose, pre-screening consent must be obtained prior to the surgery.

A patient with unsuccessful TIL harvest can choose to have re-attempt surgeries at suitable timing. The decision to re-attempt should be made jointly between the research team (including surgeons) and the patient. The risk of re-attempt surgery must be carefully considered every time and only to be carried out with patient agreement. Re-consent to multiple pre-screening attempts would not be necessary provided the patient remains well informed and eligible. The number of attempts is unlimited provided there is suitably sized resectable tumour nodule(s). Patient will be excluded if any of these conditions are not met or deemed unlikely to succeed further attempts by the research team. See Figure 4.

7.3 Main Trial Consent – TIL Therapy

Once eligible subject has successfully undergone the pre-screening process, i.e. successful TIL harvest, informed consent will be taken by the Principal Investigator or sub-investigators to enter the trial. The person taking consent must have up-to-date GCP training, be suitably qualified and have been delegated this duty by the CI/PI on the delegation log. In the case of patients in category b) as described in section 7.2, consent on to the main trial will only take place when become eligible in the future while harvested TILs are frozen and stored.

Informed consent must be obtained before any baseline screening or trial-related procedures.

The participant must have sufficient time to consider whether or not they wish to participate in the trial. For entry into the full study, there must be a minimum of 24hrs between the patient being given the participant information leaflet and informed consent being taken.

When informed consent is taken, three copies of the consent forms will be completed. One to be retained by the patient, one to be placed in the patient's notes and one to be placed in the patient trial file. The person taking consent must have up-to-date GCP training, be suitably qualified and have been delegated this duty by the CI/PI on the delegation log.

If new safety information results in significant changes in the risk/benefit assessment, the consent form will be reviewed and updated if necessary. All subjects, including those already being treated, will be informed of the new information, given a copy of the revised form and will be asked to sign the consent form if they wish to continue in the trial.

7.4 Registration and Randomisation Procedures

Patients deemed eligible for entry into the study (after successful pre-screening, TIL Harvest confirmation, review of diagnosis, treatment history and key eligibility criteria), will be provided with a verbal and written explanation of the study. After adequate time has been given (a minimum of 24 hours), all queries have been addressed and the clinical team is confident that the patient understands the study and all requirements, patients will be consented onto the study.
Consenting patients will be given a sequential 3 digit screening number, and be recorded on the trial screening log which will be maintained by the site research team. The screening log will contain patient initials, month and year of birth and date of consent. A copy of this screening log will be held in the MAHSC-CTU.

7.4.1 Cohort Randomisation

The research team will be issued with a randomisation password once the site is activated to start recruitment.

Once the patient has fulfilled all eligibility criteria and the Cellular Therapeutics Unit has confirmed that the TILs are successfully expanding, a member of the research team will complete the eligibility

checklist form and fax to the MAHSC-CTU. Once this has been acknowledged by the MAHSC-CTU, the research team member will randomise the patient as follows:

- Contact the MAHSC Trials Coordination Unit to randomise the patient on the telephone number below:

REGISTRATIONS AND RANDOMISATION LINE

Mon – Fri 9am to 5pm (UK time)

Tel: +44 (0)161 446 3311
Fax: +44 (0)161 446 8148

- The research team member will be asked for the trial randomisation password.
- The research team member will provide the patient initials, month and year of birth, and planned TIL dosing date.

The patient will be randomised to one of the two arms:

- ARM A: High Dose Interleukin-2
- ARM B: Low Dose Interleukin-2

A unique trial number will be allocated and verbally confirmed over the telephone. A confirmation email will be sent to specified members of the trial team and the TIL manufacturing contact.

Upon randomisation, participants will be given a trial specific participant card, which will have the trial title, IMP details, participant name and trial number, the contact details of the Principal Investigator and out of hours contact details in cases of emergency.

Screen failures and successes must be reported to The MAHSC-CTU and recorded as appropriate.

7.5 Ineligible and Non-Recruited Participants

Patients who consent to the main trial, but who are not subsequently registered, will be logged on the screen-fail log. They will be offered standard of care management or will be counselled as to whether there are any alternative trials available which they may be eligible to participate in.

7.6 Discontinuation/ withdrawal of participants

The Investigator must make every reasonable effort to keep each patient on study for the whole duration of the trial. However, if the Investigator removes a patient from the study, or if the patient declines further participation, prior to any therapeutic intervention, final off-study assessments should be performed, if possible on the day the decision is made to take the patient off-study or as soon as possible. All the results of the evaluations and observations, together with a description of the reasons for study withdrawal, must be recorded in the medical records and CRF.

Patients who come off treatment due to AEs (clinical or laboratory) will be followed up as per protocol. All pertinent information concerning the outcome of such treatment must be recorded in the CRF.

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

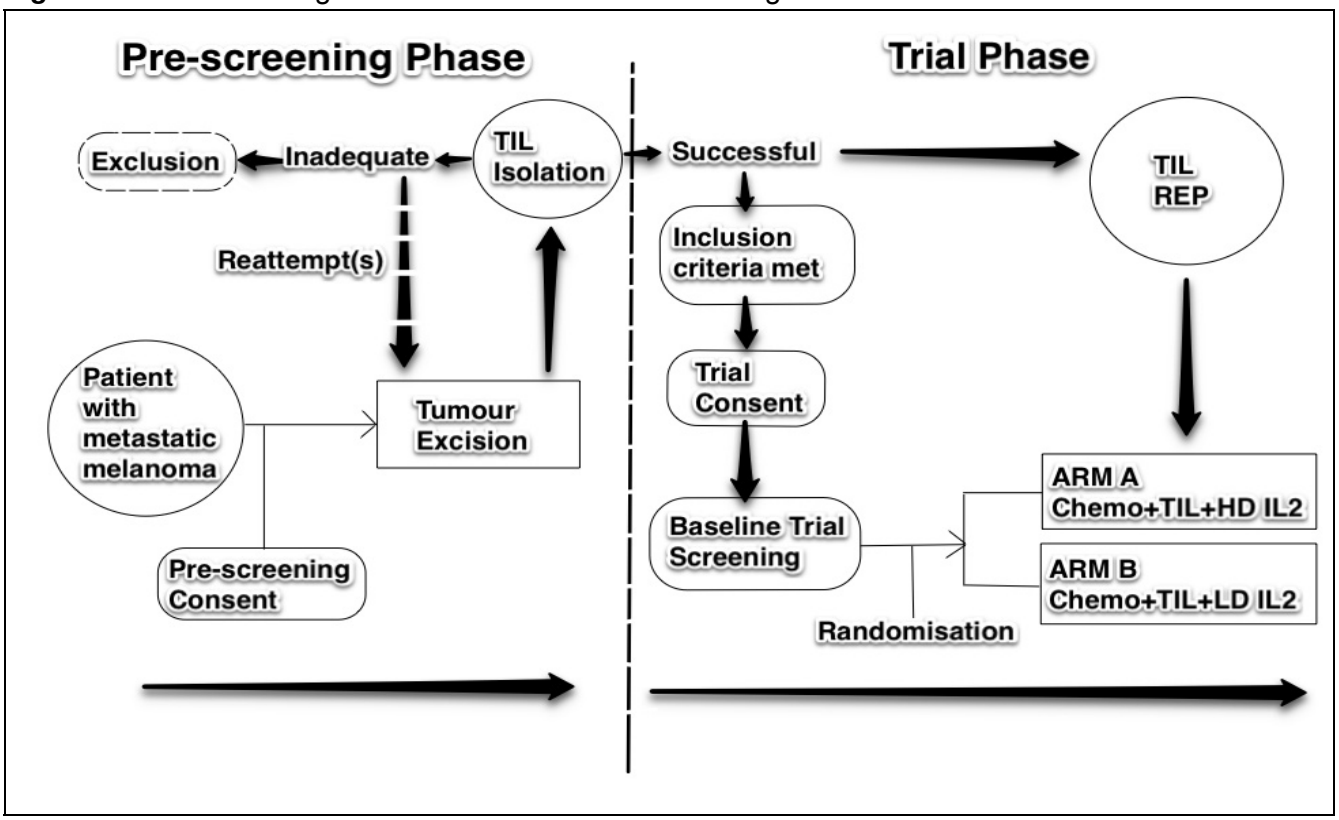
- Withdrawal of consent. (Note that where a patient is not evaluable, additional patients will be recruited to replace them).

- Did not receive any TIL therefore deemed non-evaluable for the purpose of this study. (Note that where a patient is not evaluable, additional patients will be recruited to replace them).

Any patient who has received some or all of the trial medication will continue to be followed up where possible according to the study calendar.

If a patient dies whilst on study, efforts will be made, with the permission of the next-of-kin, to undertake a full post-mortem examination and to obtain tissues for scientific studies including assays to look for the presence of TIL.

Figure 4 Schematic Diagram of Processes in Pre-screening and Trial Phase



8 TREATMENT DETAILS

8.1 Treatment summary

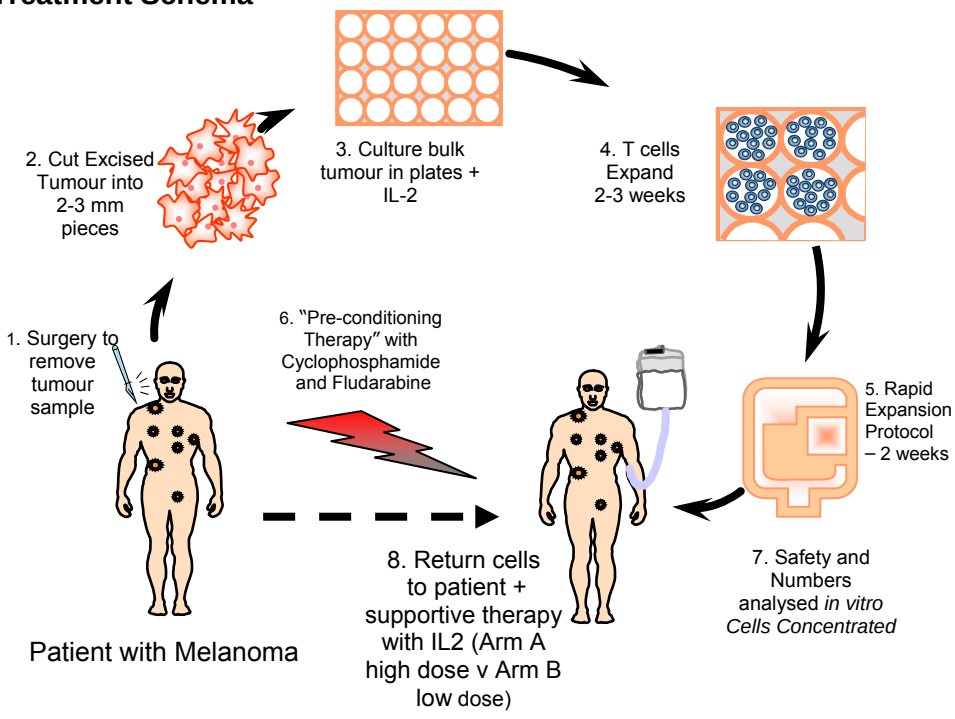
This is a trial of TIL therapy in patients with metastatic melanoma. Eligible patients will be randomised to Arm A (HD-IL2) or Arm B (LD-IL2) of the trial. They will undergo surgical tumour excision (primary or, more generally, metastatic tumour whichever is more appropriate) to isolate tumour TILs. Isolated TILs will be cultured and expanded and once successfully completed, the patient will commence preconditioning chemotherapy with cyclophosphamide (60mg/kg) day -7 and day -6, followed by fludarabine (25mg/m^2) day -5 to day -1. TILs will be re-infused on day 0 and the patients will receive up to 12 doses of intravenous IL2 from day 0 to day 4 at dose according to the arm drawn.

Each participant will receive one cycle of treatment only.

8.2 Details of trial treatment

Patients will be reassessed on the day they start treatment (chemotherapy) to confirm that they are still fit for therapy and that it is appropriate. If this is confirmed, they will receive pre-conditioning chemotherapy, TILs and intravenous IL2, as summarised in Section 5. **Chemotherapy will only be commenced following confirmation that the TILs have achieved satisfactory expansion and ready for use as defined in the IMPD, and an Investigator confirms that it is appropriate to commence chemotherapy having reviewed all of the patients results.**. All treatment will be administered to the patient as an inpatient at The Christie. Monitoring for adverse side effects and other parameters will be carried out both during and following treatment. Only one cycle of chemotherapy, TIL and IL2 of corresponding dose will be given to each patient

Treatment Schema



8.3 Route of administration, dosage, dosage regimen and treatment period(s)

8.3.1 Pre-Conditioning Chemotherapy (see appendix 5 for manufacturer details)

Patients will receive:

- Day -7 and -6 Cyclophosphamide 60mg/kg/day (see Table 1)
- Day -5 to -1 Fludarabine 25mg/m^2/day (see Table 2)

Table 3: Cyclophosphamide Treatment Regime

Order given	Day	Drugs	Dose	Volume	Route and duration
1	-8	Sodium Chloride	0.9%	1000ml	IV 12h (+/- 1h)
2	-7	Sodium Chloride	0.9%	500ml	To run concurrently IV over 1 hour (+/- 15mins)
		Cyclophosphamide	60mg/kg		
		Sodium Chloride	0.9%	500ml	
		Mesna	60mg/kg		
3	-7	Sodium Chloride	0.9%	1000ml	IV 4h (+/- 1h)
		Mesna	20mg/kg		
4	-7	Sodium Chloride	0.9%	1000ml	IV 4h (+/- 1h)
		Mesna	20mg/kg		
5	-7	Sodium Chloride	0.9%	1000ml	IV 8h (+/- 1h)
		Mesna	20mg/kg		
6	-6	Sodium Chloride	0.9%	500ml	To run concurrently IV over 1 hour (+/- 15mins)
		Cyclophosphamide	60mg/kg		
		Sodium Chloride	0.9%	500ml	
		Mesna	60mg/kg		
7	-6	Sodium Chloride	0.9%	1000ml	IV 4h (+/- 1h)
		Mesna	20mg/kg		
8	-6	Sodium Chloride	0.9%	1000ml	IV 4h (+/- 1h)
		Mesna	20mg/kg		
9	-6	Sodium Chloride	0.9%	1000ml	IV 8h (+/- 1h)
		Mesna	20mg/kg		

Cyclophosphamide will be given with additional hydration and Mesna to reduce the risk of haemorrhagic cystitis. Urine output will be maintained at above 150ml/h throughout Cyclophosphamide and Mesna infusions – where necessary intravenous boluses of Frusemide (20 mg iv) will be given.

Table 4: Fludarabine Treatment Regime

Order given	Day	Drugs	Dose	Volume	Route and duration
1	-5	Sodium Chloride	0.9%	10ml	IV 30mins (+/- 10mins)
		Fludarabine	25mg/m^2		
2	-4	Sodium Chloride	0.9%	10ml	IV 30mins (+/-10mins)
		Fludarabine	25mg/m^2		
3	-3	Sodium Chloride	0.9%	10ml	IV 30mins (+/- 10mins)
		Fludarabine	25mg/m^2		
4	-2	Sodium Chloride	0.9%	10ml	IV 30mins (+/- 10mins)
		Fludarabine	25mg/m^2		
5	-1	Sodium Chloride	0.9%	10ml	IV 30mins (+/- 10mins)

8.4 Infusion of Tumour Infiltrating Lymphocytes

Table 5: TIL treatment regime – supportive medication is given as an example and local equivalent anti-emetics/anti-histamines can be used. Steroids should be avoided.

Order given	Day	Drugs	Dose	Volume	Route & duration
1	0	Sodium Saline 0.9%		500 ml	IV 4 hours
2	0	Ondansetron	8mg		PO/IV bolus - 15 min prior to infusion
3	0	Chlorpheniramine	10mg		IV bolus - 15 min prior to infusion
4	0	Paracetamol	1000mg		PO/IV bolus - 15 min prior to infusion
5	0	TIL cells	$5x10^9$ to $2x10^{11}$	100-500mls	IV stat
6	0	Sodium Saline 0.9%		1000ml	IV 6 hours

- On day 0**, at least 24 hours after last dose of chemotherapy**, provided the TIL preparation fulfils the release criteria (as described in the IMPD) they will be washed and resuspended in buffered isotonic medium. TIL cells will be delivered fresh (i.e. non-cryopreserved).
- The number and condition of the cells will be confirmed by the CTU. An investigator will sign off the approval for the cells to be given prior to infusion. TIL infusion may be delayed for up to 7 days for clinical reasons or for issues regarding the cell specification. This decision must be made before final preparation for infusion. Once the cells are prepared for infusion they must be given within 4 hours or discarded.
- The patient will receive 1g PO/IV paracetamol and 10mg IV chlorpheniramine premedication 15 minutes prior to infusion of TIL which will be administered IV via the patient's central venous catheter over approximately 30-60 minutes depending on the final number / volume.

- The individual administering the TIL must wear sterile gloves and an apron. When the TIL infusion has begun, vital signs will be monitored every 15 mins for 2h, then every 30min for 2h, then every 1-4h for the duration that the patient is receiving IL2.
- Mild hypoxia is common and supportive oxygen should be given as necessary to keep oxygen-saturations at normal levels.
- Grade 1-2 hypersensitivity reaction should be dealt with as medically indicated without necessarily interrupting TIL infusion. If interruption is required and condition improves satisfactorily, TIL infusion can be resumed provided this is completed within 4 hours from cell preparation time..
- Please also refer The Christie NHS Foundation Trust Standard Operating Procedure (SOP) for detailed guideline on **Infusion of Non-cyropreserved Cells. Administrating staff to fill in TIL checklist(APPENDIX 9)**
- If a patient experiences a Grade 3-4 hypersensitivity reaction (i.e. bronchospasm, hypotension and angio-oedema), the infusion must be interrupted and the reaction treated as medically indicated. If the infusion is discontinued due to toxicity, remaining cells should be sent for microbiology analysis and the production centre informed. Please refer to section 10.7 for further information on management of infusion related toxicities.

8.5 Interleukin 2

- At least 90mins (and within 24 hours) after the TIL have been infused (and after subsidence of any infusion related toxicity), patients will commence intravenous interleukin-2
  Patients in Arm A will receive IL2 at a dose of 600,000 units per Kg. The IL2 will be administered over 15mins every eight hours for up to 12 doses according to the standard Christie Guidelines for HD IL2.
  Patients in Arm B will receive at a dose of 100,000 units per Kg. The IL2 will be administered over 15mins every eight hours for up to 12 doses according to the standard Christie Guidelines for HD IL2. The same management regimen will be followed but the toxicity is expected to be greatly reduced in this arm.
- Patients will also receive prophylactic and supportive medication as documented in section 8.7 (Table 6).

8.6 Stem Cell Harvest Backup

- For **very rare** patients stem cell backup may be prudent and can be done at any time prior to chemotherapy.
- The chemotherapy to be used in this trial is non-myeloablative, but where patients have had previous high dose chemotherapy an autologous haemopoetic stem backup harvest, for stem cell rescue, will be obtained prior to commencing therapy in this trial. Similarly, where there is concern about a patient's bone marrow reserves, for example due to multiple previous lines of myelosuppressive chemotherapy, a backup stem cell harvest should also be obtained according to local protocol.
- The patient with such a history will only proceed to chemotherapy if sufficient $CD34^+$ cells for stem cell rescue (in accordance with local guidelines) have been obtained or will be excluded.

- The stem cell backup (if available) will be administered on day 21 if there is failure of marrow recovery by this stage or sooner if required as determined by the clinical investigator.

8.7 Concomitant Medication

- Concomitant medication may be given as medically indicated. Details of the concomitant medication given must be recorded in the Case Report Form (CRF). The patients should also keep a record of any over the counter medicines used and these should be noted in the CRF.
- The use of concurrent systemic steroids (oral and intravenous) is likely to influence the efficacy of TIL and IL2. Steroid replacement is allowed (for example patients with adrenal or pituitary insufficiency). **All other patients must have stopped systemic steroids at least 3 weeks prior to trial entry if this can be done safely (this does not apply to steroids given as an anti-emetic during cyclophosphamide chemotherapy).**
- If a medical condition requires the prescription of systemic steroids (other than replacement) between consent and commencing chemotherapy, then the patient should be withdrawn from the trial.
- If a patient requires steroids after they have received their chemotherapy, TIL and IL2, this should be given at the lowest possible dose and shortest duration as required for the medical condition. This then must be documented in the CRF.
- The patient must not receive other anti-cancer therapy or investigational drugs while on study.
- The patients on the study will require certain supportive and prophylactic medication during and for some-time after they receive the chemotherapy, TIL and IL2 as documented below in Table 6. As with other concomitant medication this must be recorded in the CRF along with any changes to the regime documented below.

Table 6: Examples of Suitable Prophylactic and Supportive Medications

Drug	Days required (inclusive)	Dose regime
Ondansetron	D0 to D4	8mg PO bd
GCSF	d0 until neutrophil count >1.0 x 10^9/L	263mcg s/c od Long acting GCSF (e.g. Neulasta 6 mg once only is also acceptable).
Chlorphenamine	d0 15 mins prior to T cell infusion	10mg IV bolus once
Ibuprofen	d0 to d4	200-400mg PO 6 hourly prn
Paracetamol	d0 to d4	1g PO / IV 6 hourly prn
Ranitidine	d0 to d4	150mg PO bd
Levofloxacin	d0 until neutrophil count >1.0 x 10^9/L	500mg PO od
Co-trimoxazole	d0 for 3 months or until lymphocyte count >1.0 x 10^9/L	480 mg PO bd three times a week on non-consecutive days
Aciclovir	d0 for 3 months or until lymphocyte count >1.0 x 10^9/L	400mg PO bd
Fluconazole	d0 for 3 months or until lymphocyte count >1.0 x 10^9/L	100mg PO od

Patients may, if required, be prescribed dexamethasone 4mg PO/IV bd during and for 2 days after the cyclophosphamide as an antiemetic – **this must be discontinued no later than day -3**.

NOTE IF PATIENTS REQUIRE BLOOD PRODUCTS AFTER INITIATION OF THE CHEMOTHERAPY THESE MUST BE IRRADIATED
(The only exception is TIL and stem cells)

8.8 Accountability Procedures

Accurate records of all cell shipments, cell storage, cell usage and disposal (if applicable) must be maintained. This inventory record must be available for inspection by the Trial Sponsor at any time. TILs are to be used only in accordance with this protocol and under the supervision of the Investigator.

The Investigator must undertake not to destroy any unused cells unless directed to by the Trial Sponsor. Any discarded cells must be destroyed according to hospital procedures and properly accounted for using Hospital Drug Destruction Forms. At the conclusion of the study, the overall amount of the cells shipped to the centre, and the amount destroyed or returned will be provided and an account given of any discrepancy.

8.9 Participant Compliance

Due to the nature of this trial (intravenous IMP which is administered as an inpatient) this is not thought likely to be applicable.

8.10 Overdose

Due to the nature of this trial (IMP manufactured on a patient by patient basis) this is not thought likely to be applicable.

8.11 Dose Changes and Modification of Trial Treatment

Each patient will receive one cycle only of treatment. To be fully evaluable patients must receive all specified chemotherapy, TILs and IL2 at the correct dose.

9 TRIAL METHODOLOGY

9.1 Laboratory Procedure for Tumour Collection and Handling

The hospital recruiting the patient will identify a suitable surgeon & surgical site with the relevant experience for the type or surgical procedure required. The tumour sample will be surgically removed under aseptic conditions within an operating theatre at the surgical site. At least a 2cm tumour (in total in its longest diameter) will be resected and the material will be placed in a sterile container along with sterile saline or growth media (which may or may not also contain antibiotics to inhibit microbial growth). The bottles should be placed in a secondary container to reduce the chance of contamination. The life span of the tumour derived TIL outside of the patient has not been determined however, to increase the chance of a successful recovery using established methods, the tumour must be transferred to the Cellular Therapeutics Unit as soon as possible and certainly within 24 hours of collection. The cells should be transported between 15 to 30°C.

The hospital where the patient is undergoing treatment will request a manufacturing slot with the CTU and the manufacturing centre will arrange collection of the tumour from the site of surgical procedure. The treatment centre must provide the following details:

- Hospital Number
- DoB
- Sex
- Consent for Testing, Storage and Discard of Tissue or Cells and Storage of Personal Information for medical purposes only
- Name, date and Signature indicating of the patient demonstrating their consent
- Name, date and Signature of the individual performing the consent indicating that they have explained the procedures involved and the associated benefits and risks of the treatment

9.2 CTU Procedures for Receiving Tumour Material prior to growing and isolating TIL from the tumour

- Confirm the details on the tumour material match the request form
- Confirm that the patient has consented for the procedure
- Confirm receipt of the microbiology screen results for adventitious agents (i.e HIV, Hepatitis B, Hepatitis C, Syphilis and HTLV serology) and that the patient was negative within a window of 30 days prior to the tumour excision.
- Confirm patient has been approved by sponsor for trial recruitment

9.3 Description of Manufacturing Process and Process Controls

Isolation and expansion of the TIL will be produced under EU Good Manufacturing Practice (GMP) conditions. The processing route of TIL is schematically indicated in Appendix 7. The raw material (TIL isolated from a tumour excision) can be generated either by ex vivo enzymatic digestion or mechanical fragmentation of an excised metastatic melanoma tumour. TIL are subsequently expanded to at least

$3x10^7$ cells (this will take approximately 2 to 4 weeks). At this point TIL are either frozen for logistic reasons (e.g. when the patient/hospital can be scheduled for treatment), or directly expanded for 14 days using feeder PBMCs pre-irradiated using a dose between 2500-5000 cGy (PBMCs are either derived from: autologous apheresis or allogeneic donor buffy coats), anti-CD3 antibody and IL2. The expanded autologous TIL population will be harvested and washed prior to formulation in an infusion bag containing the excipients: isotonic saline and 1% HSA.

9.4 Pharmaceutical Information for Treatment Centre

9.4.1 Supply of TIL Cells

Autologous TIL cells will be supplied by the CTU and must be manufactured to the EU GMP following Standard Operating Procedures (SOPs) generated at the CTU according to Annex 1, 2 & 13 of EudraLex Vol 4 with the products being classified under (EC) 1394/2007 as ATMPs. The CTU is licensed, regulated and inspected by the MHRA to manufacture TIL within an Investigational Medicinal Products license. A complete certificate of analysis will be provided with each batch of TIL cells and must be retained in the Investigator Trial File (ITF).

For further information regarding the supply of TIL cells contact:

Name: Dr Ryan Guest
Address: Cellular Therapeutics Unit
University of Manchester
Manchester
48 Grafton Street
M13 9XX
United Kingdom

Tel: 0161 606 7278
Fax: 0161 606 7284
Email: ryan.guest@manchester,ac,uk

The CTU must send a copy of the drug shipment form to along with the TIL cells for the attention of the treating CI/PI identified in the request to manufacture form.

TIL cells will be shipped at 15 to 30°C

The CI/PI and the trial site are responsible for investigational product accountability. To this end, it is assumed that all clinical trial supplies will be the responsibility of a suitably qualified and authorised person at the treatment centre such as an appropriate research nurse, who will document drug disposition and accountability for the duration of the trial.

9.4.2 Acceptance Criteria for TIL cells

Assay	Details	Acceptance criteria
QC assay 1	Microbiology testing of starting tumour	No growth by day 6 to 10
QC assay 2	T cell dose	≥ $5x10^9$ to $2x10^{11}$CD3+ve, 7-AAD-ve/PI-ve of all CD3+
	Viability	≥ 70% CD3+ve & 7-AAD-ve/PI-ve of all CD3+ Or ≥ 70% of all cells are negative for Trypan blue
	Purity	≥ 80% CD3+ve of all CD45+ve
QC assay 3	Mycoplasma testing TIL (start of REP)	Negative
QC assay 4	Microbiology testing of TIL (final product)	No growth by day 6 to 10 (data available after infusion)

9.4.3 TIL cell Formulation

The TIL cells will be presented in an aseptically prepared bag containing the TIL cell dose (> $5x10^9$ but ≤ $2x10^{11}$), re-suspended in 100 to 500ml of buffered isotonic saline containing 1% Human Serum Albumin for direct infusion. The cells are to be administered in the form that they are supplied and must be administered without delay (within 4 hours).

9.4.4 Labeling and Transport of TIL Cells

All supplies must be stored in a secure, limited access storage area.

Packaging and labelling will be in accordance with the requirements of Directive Labelling and should comply with the requirements of Directive 2003/94/EC.

The primary bag of cells will be placed in a secondary bag and sealed to contain any potential spillage and to prevent contamination during storage, and transport. They will then be placed in an insulated Transport Box will be labelled with:

- Name and Telephone Number of the Receiving Clinician, the Department and Address.
- Name and Telephone Number of the CTU manager
- Description of the contents, including the number of containers and the volumes of each.

9.4.5 Receipt and Re-Infusion of TIL Cells

The transport container will be delivered from the CTU to the responsibility of a suitably qualified and authorised person at the treatment centre, such as an appropriate research nurse, who will document drug disposition and accountability for the duration of the trial.

Upon receipt, and prior to administration the bags must be checked for integrity. All problems associated with the product must be reported to the CTU using an Adverse Incident Report Form (supplied by the CTU).

Accompanying the bags of cells will be:

- Packages insert outlining the procedures to be used for re-infusion, this includes checking the product labels and documentation to ensure the correct product has been received
- A product certificate
- QP certification

Investigators should note that the clinical trial supplies:

- May only be used for the clinical trial for which they are indicated
- Are for the named patient only
- Unused bags of cells must be destroyed.
- At each visit, the CRA will review the drug-dispensing log and reconcile it with the documentation.

Tissue Storage for Translational Research
Blood samples for trial assays will be taken at the defined time points and stored as outlined in the laboratory manual.

Radiology and Other Procedures
Radiological assessment will be done using standard procedures and based on RECIST v1.1 Refer Appendix 2.

10 TRIAL ASSESSMENTS

10.1 Pre-Screening (Surgical Tumour Excision and TIL Harvest)

As described in section 7.2.1, this stage involves pre-screening patients of category a) or b) undergoing surgery (trial-directed or standard planned surgery, respectively) to excise tumour specimen for the purpose of TIL harvesting. In both categories, prior informed consent must be obtained and can be done on the same day following full discussion and provision of PIS. **Virology screening** is mandatory. Other pre-surgical clinical and blood work are as per standard practice.

The total excised tumour should be ≥ 2 cm in total diameter. The tumour nodule(s) will be trimmed to remove normal tissue, placed in a sterile container along with sterile saline and then transferred to the Cellular Therapeutics Unit at UMIC for immediate processing - see IMPD. Provided ≥30x10^7 TILs are obtained within 30 days of excision these may be directly expanded for therapy or frozen for future expansion and therapy.

Once successful TIL Harvest has been confirmed the treating team should inform the patient and either confirm continued storage of the TIL or agree a production date with the CPU. When the patient is potentially ready for treatment consent to TIL Therapy will be undertaken, full baseline screening will be undertaken (see 10.2) and the TIL rapid expansion protocol (REP) will commence. On day -7, once CPU confirms that the cells are expanding appropriately, pre-conditioning chemotherapy can be initiated.

At completion of the production process and after certification by the QP the cells will be transferred to the treating centre for use.

10.2 Baseline Screening Assessment (within 28 days of start of treatment)

- Informed consent for the main trial
- Demographic details including diagnosis and previous treatment for their cancer
- Complete medical history and baseline clinical conditions
- Listing concomitant illnesses and treatment
- Complete physical examination, including height, weight, WHO performance status, seated systolic and diastolic blood pressure (BP), pulse rate and neurological examination
- ECG
- CXR
- Baseline CT scan including brain CT (note: recent scan with clear evidence of measurable disease is acceptable for the purpose of screening. Repeat scan only if confirmation of measurable disease is required at this phase. Otherwise, first trial CT to be done as close to treatment as possible preferably week-2)
- Baseline MUGA scan or ECHO cardiogram (if appropriate)
- Laboratory tests to confirm eligibility:

  Haematology: Hb, white blood cells (WBC) with five point differential count, and Plts

  Clinical Chemistry: sodium, potassium, calcium, phosphate, Magnesium, urea, creatinine, total protein, albumin, bilirubin, Alk Phos, ALT/AST, gamma-GT, uric acid

  Coagulation screen: prothrombin time and partial thromboplastin time

CMV and EBV serology

HIV, Hepatitis B, Hepatitis C, Syphilis and HTLV serology

Explicit consent for these tests must be obtained including an explanation that it is a requirement that all patients undergoing surgery for tumour resection for TIL isolation must have negative HIV, HTLV, Hepatitis B, Hepatitis C and Syphilis serology. Counselling must be offered to patients prior to the tests

*Note: *CMV serology status does not affect trial entry eligibility. Routine leukodepleted blood product should be used during period of neutropenia. In both CMV seropositive and seronegative patient monitoring is not required but CMV PCR should be done upon clinical suspicion during and after trial treatment to rule out reactivation and new infection respectively.*

***EBV serology status does not affect trial entry eligibility. Patients may have positive EBV serology due to previous infection. This serves as baseline information for the purpose of long term monitoring as there has been report of association between EBV seropositivity and lymphoproliferative disorder.*

- Baseline trial assays (see section 11.3.1)
- Registration with the Trial Sponsor
- Women of childbearing age must have negative serum or urine HCG test **within 2 weeks prior to commencing treatment**; results must be obtained and reviewed before the first injection is administered.

10.3 Prior to Commencing Chemotherapy

Chemotherapy should commence as soon as practical on Day -7, but must not commence until the CTU confirm that the TIL expansion has proceeded satisfactorily. Prior to commencing chemotherapy the patient will be admitted and a central venous access line inserted. On the day the patient is due to commence chemotherapy the following must be (re)carried out:

- Physical examination, including weight, WHO performance status, seated systolic and diastolic blood pressure (BP), pulse rate, temperature and neurological examination.
- Confirmation of a satisfactory fresh baseline CT scan before commencing chemotherapy, preferably during week -2 or just before chemotherapy. (see Table 5b).
- Laboratory tests
  - **Haematology**: Hb, white blood cells (WBC) with five point differential count, and Plts
  - **Clinical Chemistry**: sodium, potassium, calcium, phosphate, magnesium, urea, creatinine, total protein, albumin, bilirubin, Alk Phos, ALT/AST, gamma-GT, uric acid, glucose
  - **Coagulation screen**: prothrombin time and partial thromboplastin time
  - **Trial assays:** see section 11.3.1 for detail.
- Listing concomitant illnesses and treatment
- An investigator should also confirm that the patient is fit to proceed to TIL Therapy and that it is appropriate for them to do so.

10.4 Evaluations During Treatment (Chemotherapy, TILs and IL2)

- The patient will be reviewed clinically at least once per day by an investigator. At each review, any adverse event experienced will be noted and graded according to NCI CTCAE v4.
- Laboratory tests to be taken as indicated in Table 5 Schedule of Events, with extra tests being done as clinically indicated:
  Haematology: Hb, white blood cells (WBC) with five point differential count, and Plts

Clinical Chemistry: sodium, potassium, calcium, phosphate, magnesium, urea, creatinine, total protein, albumin, bilirubin, Alk Phos, ALT/AST, gamma-GT, uric acid.
Coagulation screen: prothrombin time and partial thromboplastin time as indicated.
Trial assays: see section 11.3.1 for detail. Also see Table 7a and 7b.

- The patient will be discharged from hospital when clinically well and all toxicity has resolved to grade 2 toxicity or less. The total hospital stay is expected to be around 3 weeks.
- Patient should be commenced on pneumocystis, anti-viral and anti-fungal prophylaxis from Day 0 for total of 12 weeks (see Table 6).

10.5 Follow Up Evaluations Following Treatment

Patients will be followed up at regular intervals until they come off study (guidelines for patient withdrawal from the study are discussed in section 5.7). Details of timing of visits/investigations are given in Table 5 Schedule of Events. Subjects will be reviewed ***as a minimum*** at week 4, week 6 and week 12 following cell infusion, and then every three months for 1 year and subsequently at six monthly intervals.

At follow-up the following evaluations will be carried out:

- Physical examination, including weight, WHO performance status, seated systolic and diastolic blood pressure (BP), pulse rate, temperature and neurological examination.
- Listing concomitant illnesses and treatment
- Laboratory tests:
  Haematology: Hb, white blood cells (WBC) with five point differential count, and Plts
  Clinical Chemistry: sodium, potassium, calcium, phosphate, magnesium, urea, creatinine, total protein, albumin, bilirubin, Alk Phos, ALT, AST, gamma-GT, glucose, uric acid
  CMV-PCR: as clinically indicated
  Trial assays: as discussed in section 11.3.1
- CT scans week 6, week 12 and 12 weekly thereafter until irRC-confirmed disease progression.
- Biopsy at week 6. Patients may still remain in the main study even if they do not have the biopsy. The biopsy may be taken from any easily accessible tumour, and transported to the research centre laboratory as outlined in the laboratory manual.

10.6 Long-Term Follow Up After End of Trial and Study Withdrawal

All study patients will be followed up until death except patients who were withdrawn from study (see sections 7.7). Patients will be monitored for unexpected late side effects and late onset of adverse events linked to the therapy, e.g. oncogenesis or immunogenicity.

All surviving study patients will be followed up at 6-12 monthly intervals. Only adverse events potentially linked to the therapy will be recorded. Toxicities and adverse events due to subsequent therapy or their melanoma will not be recorded beyond progression.

In cases where patients are not able to attend the treating trial center for long-term follow up for example because of logistic reasons or fitness issue due to progressive disease, this information will be pursued by the research team from the health care establishment where the long term care of the patient is being held (for example general practitioner). Enquiries using standard questionnaires via phone calls will be made from research team annually regarding survival status or development of any late toxicity.

Patients who have progressive disease will continue to be followed up for survival and second malignancies. Toxicities and adverse events due to subsequent therapy or their underlying malignancy will not be recorded beyond progression. Only date of death and adverse events potentially linked to the therapy.

If a possibly related late onset toxicity was detected, a Chief Investigator will be consulted and patient will be recalled for attendance if possible for further assessment and investigation (for example blood and or tumour analysis)

If a patient dies whilst on study, efforts will be made, with the permission of the next-of-kin, to undertake a full post-mortem examination and to obtain tissues for scientific studies and assays.

Expected / Possible Trial Related Toxicity and Its Management

- Chemotherapy toxicity will be managed according to local guidelines
- Management of acute toxicity during infusion is described in section 8.4
- Management of neutropenic sepsis and other complications of chemotherapy – as per local guidelines.
- Management of anaemia and thrombocytopenia are generally as per local transfusion guidelines – transfusions should be given with the aim of maintaining the platelet count above $30x10^9$ / L and the haemoglobin above 9.0 g/dL during the IL2 therapy.
- In the event a stem cell transplant is needed / anticipated the involvement of the appropriate clinical support should be initiated.
- Management of IL2 related toxicities – as per local Christie guidelines. Specific toxicities include (but are not limited to): thyroiditis, immune thrombocytopenia and myocarditis
- Longer term immune-related toxicity related to TILs are rare and generally mild but severe toxicity has been reported for other T-cell trials. Investigators should be aware of potential toxicities and their treatment. In general management of toxicity will be supportive for grade 1-3 toxicity but may involve steroids or other immunosuppressive therapy for suspected grade 4 immune related toxicity.

  Skin

  - Vitiligo has been reported following TIL therapy – generally does not require treatment
  - Blistering is rare but may require topical /systemic steroids if severe. Generally self-limiting

  Eye

  - Uveitis has been reported following TIL therapy and in the event of clinical suspicion expert assessment should be sought and the patient treated with topical steroids

  Ear

  - Decreasing hearing has not been reported with TIL but has been reported with T cells targeting melanoma and is therefore possible. In the event of clinical suspicion expert assessment should be requested and treatment instituted with local steroids if appropriate.

  Colitis

  - This has been reported in T-cell trials targeting CEA. It can also occur after TIL therapy and appears more common if patients have had prior colitis with Ipilimumab therapy. Mild colitis should initially be treated with supportive therapy. Moderate colitis can be

treated with non-absorbable steroids. Severe colitis should be with intravenous prednisolone (1 - 2 mg / Kg) and if that fails anti-TNF antibodies.

Other autoimmune phenomena are possible and should be investigated and treated as appropriate to the condition.

- **Cytokine Release Syndrome (CRS)** – CRS is a clinical manifestation related to excessive release of pro-inflammatory cytokines such as those in acute sepsis but also a recognised reaction related to some cancer treatment especially with monoclonal antibodies (Winkler *et al*., 1999). There have been some cases reported in enhanced affinity genetically modified T cell therapy (for example Stephan *et al*., 2012) although no specific report has been linked to TIL therapy to date. Investigators should nevertheless be vigilant of such possibility and have a high index of suspicion during this treatment. Diagnosis of CRS is predominantly clinical with features mimic that of sepsis i.e. fever, chills, tachycardia and hypotension. Nausea and vomiting could also be a presenting feature. Non-cardiogenic respiratory distress and pulmonary infiltrates can also be expected. Cytokine Storm Syndrome (CSS) is the severe form of CRS with rapid onset and progression with potentially life-threatening sequelae such as multi-organ failure and disseminated intravascular coagulopathy (DIC). Lymphopenia, thrombocytopenia, and elevated liver enzymes have also been observed (Suntharalingam *et al*., 2006).

  Diagnosis and monitoring: Investigation in both suspected CRS and CSS should include careful septic screen to exclude infection. Other test includes chest x-ray, FBC, renal/ liver/coagulation profile and serum TNF and IL6 (in EDTA tubes at onset, at 60-90 minutes then 6 hourly after that). Close monitoring of clinical observations (temperature, pulse, blood pressure, oxygen saturation, respiratory rate, and urine output) should be carried out and rapid escalation of care should be considered if no improvement or clinical deterioration. Early intensive care team involvement should be incorporated in such event at the earliest possible opportunity.

  Treatment: In all cases with high index of suspicion, initial management should include steroids (intravenous hydrocortisone 100-200mg), anti-histamine (Chlorphenamine 10mg iv) and paracetamol 1g iv. Patient condition should be optimised with on-going supportive measures such as intravenous fluid, oxygen and anti-microbial. Where infection has been ruled out, patient with grade 3-4 CRS (see Appendix 8) or CSS should be considered for high dose steroids such as intravenous methylprednisolone and immunosuppressive agents such as anti-IL6 (e.g. Tocilizumab) or anti -TNF (e.g. Adalimumab).

- **Graft versus host disease (GVHD)** is not known with this therapy.

- **GVHD–like engraftment syndrome (ES) may be possible.** This is generally observed to be related to overproduction of pro-inflammatory cytokines associated with neutrophil recovery following cytotoxic chemotherapy or peri-neutrophil engraftment post haematopoietic stem cell transplantation. Clinical features can be difficult to differentiate from that of sepsis or cytokines release syndrome. Diagnosis of ES is mainly clinical supported by acute rise in CRP (C - reactive protein) in the absence of an infection. If ES is suspected, the following diagnostic criteria are proposed (Spitzer *et al*., 2001): *3 Major criteria or two major criteria and one or more minor criteria need to be met.*

Major Criteria	Minor Criteria
• Temperature ≥ 38.5˚C with no identifiable infectious etiology	• Hepatic dysfunction with either bilirubin ≥30 µm/l or transaminase ≥ 2x normal

• Erythrodermatous rash involving more than 25% of surface area and not attributable to a medication • Non-cardiogenic pulmonary oedema, manifested by diffuse pulmonary infiltrates , and hypoxia	• Renal impairment ≥ 2x baseline • Weight gain ≥ 2.5% of baseline body weight • Transient encephalopathy unexplainable by other causes

Treatment

Mild ES usually requires supportive care only. Full resolution without treatment can be expected upon haematological recovery and discontinuation of GCSF.

Progressive and more symptomatic ES (Grade 3 or more) particularly with pulmonary involvement requires further therapy. Steroid therapy may be necessary (intravenous methylprednisolone at 1mg/kg per day for 3 days then progressive reducing doses). Optimisation by other important supportive measures such as careful fluid balance, prophylactic antibiotic and diuretics should be considered at all times in such patients. If sepsis is ruled out and patients fail to respond to optimal supportive measures and intravenous steroids within five days, consideration will be given to other immunosuppressive agents such as anti-TNF antibodies or anti-IL6 antibodies.

Follow up will continue indefinitely in the absence of progression. Patients progressing after treatment should have other options discussed with them.

10.7 Blood Sampling

All blood samples routine laboratory tests (i.e. Haematology tests, Clinical Chemistry and coagulation screens) 2.5-10ml of blood should be placed in the standard hospital EDTA and lithium heparin tubes. The tubes should be labelled as per standard institution procedures and sent to the appropriate hospital laboratory for analysis.

All samples taken for trial assays must be labelled with the study number and DOB and date sampled and be delivered to the designated clinical research laboratories for processing and freezing as described in the laboratory manual.

Blood sample for laboratory research will be obtained at time points indicated below (also see table 7 Schedule of Events):

Cell and serum based research assays (50mls blood in EDTA tubes): Baseline, Day -7, Day 0, Week 1, 2, 4, 6, 12 and then 12 weekly until the subject comes off trial.

Cytokines Measurement (~2 mls blood in EDTA tubes): Day –7 (before administration of chemotherapy as baseline sample)**,** Day 0 and daily until day of discharge.

Table 7a: SCHEDULE OF EVENTS DURING TREATMENT AND FOLLOW UP ASSESSMENTS

Week / Parameters	*Pre-screening*	*Baseline Screening*	*Pre –chemo -2*	-1	0	1	2	4	6	12	***12 weekly***
Consent	X	X							X[l]		
Echo or MUGA		X									
ECG		X									
CXR		X									
CT Brain		X							X		X[n]
CT (TAP)[d]		X[d1]	X[d2]						X	X	X
Clinical Assessment[j]	X	X	X	X	X	X	X	X	X	X	X
Virology[k] screen	X	X									
CMV/EBV serology		X									
Pregnancy test[m]		X									
Prophylaxis					X	X	X	X	X	X	
Clinical Chemistry[b]	X	X	X	X	X	X	X	X	X	X	X
Haematology[a]	X	X	X	X	X	X	X	X	X	X	X
Coagulation Screen[f]	X	X	X	X	X	X					
Research/Cell Assays[c]		X		X	X	X	X	X	X	X	X
Cytokines Assays[g]				X	X						
Tumour Excision	X								X[l]		
Central line			X								

7b Treatment phase schedule of event and monitoring calendar Days -8 to +10

Parameters	Day of Treatment																			
	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11
In patient	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	According to clinical need →				
Toxicity Assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	According to clinical need →				
Chemotherapy [e]		C	C	F	F	F	F	F												
TILs infusion									X[h]											
IL2 (both arms)									X	X	X	X	X							
Haematology [a]	X	X		X		X		X	X	X	X	X	X	According to clinical need →						
Coagulation screen [f]	X								X		According to clinical need →									
Central line	X																			
Clinical Chemistry	X	X		X		X		X	X	X	X	X	X	According to clinical need →						
Cytokine Assays [g]		X*							X	X[i] Until discharge →										
Research/ cell Assays[c]		X							X							X	See Table 5a for more timepoints			

[a]FBC [b]U+E, LFT (bilirubin, Alk Phos, ALT/AST, gamma-GT), Calcium profile, Magnesium, urea, creatinine, total protein, albumin, uric acid [c]Research blood for cells assays. Take ~30 ml in EDTA bottles to be frozen and stored [d]CT scan –. Scan at Baseline [d1] is only required if evidence of measurable disease is uncertain on previous scans. Otherwise, first trial CT scan[d2] should be done as close to chemotherapy as possible. Scan older than 4 weeks before T cell infusion (Day 0) will need repeating. Further CT scans at approximately 6, 12 weeks, 24 weeks and 12 weekly thereafter until irRC confirmed PD [e]C=Cyclophosphamide, F=Fludarabine [f]Prothrombin time (PT), partial thromboplastin time (APTT) [g]Research blood for cytokine measurements. Take ~2mls in EDTA bottle. Day -7 sample should be taken before administration of chemotherapy [h]TIL infusion - may be deferred clinically indicated – see protocol. In this case subsequent steps will be delayed [i]Daily until discharge [j]Clinical assessment – Demographics at Baseline, history and examination, WHO performance status, blood pressure, concomitant medications [k]Virology - HIV, Hepatitis B, Hepatitis C, Syphilis and HTLV serology [l]consent(just for procedure itself) –only for patients have readily biopsiable tumour [m]Pregnancy test- Urine or serum HCG level no longer than 2 weeks from start of treatment. [n] follow up brain CT and/or MRI only if clinically indicated or brain metastases at baseline.

11 TRIAL EFFICACY / MEASURING

Response-based endpoints in this trial (namely response rate, duration of response and progression free survival) will be captured using two distinct but related criteria:

- RECIST v1.1
- Immune-related Response Criteria (irRC)

RECIST v1.1 will be used to define primary and secondary endpoints. The irRC will be used to define exploratory endpoints.

Due to the mechanism of action of T cell therapy and the dynamics of subsequent immune response, there could be potential initial tumour flare due to T cell infiltration. Also, there is good evidence of delayed tumour regression after initial mixed response or progression. Therefore, in this study, subjects with tumour volume increase detected prior to Week 12 but without rapid clinical deterioration will continue to be observed clinically along with scheduled imaging to allow detection of a subsequent tumour response until progression is confirmed at the subsequent time points.

11.1 Primary Endpoint

11.1.1 Response Rate

Response rate will be assessed using standard imaging procedure (cross-sectional CT or MRI scan as appropriate) at 6 weeks, then at 3 monthly intervals. Scans will be measured according to RECISTv1.1. Evaluable patients, defined as those who had received all planned treatments, will be assigned one of the following categories: 1) Complete response 2) partial response 3) stable disease 4) progressive disease 5) early death from malignant disease 6) early death from toxicity 7) early death of other cause 8) unknown (not assessable, insufficient data).

11.1.2 Best Overall Response (BOR)

This is defined by the best confirmed response designation over the study as a whole, recorded between the dates of treatment until the last tumour assessment prior to disease progression. The assessment of response at 12 weeks has particular emphasis due to the mechanism of action of immunotherapy inducing immune responses as basis for clinical responses. For assessment of BOR, all available assessments per subject are considered. CR or PR determinations included in the BOR assessment must be confirmed by a second confirmatory evaluation meeting the criteria for response and performed no less than 4 weeks after the criteria for response are first met.

The following table summarizes the criteria used to define BOR based on responses at different time points throughout the study.

11.1.3 Feasibility and Tolerability

Patient in both arms will be regularly assessed during all treatment phases using routine evaluation listed in section 10.4 and 10.5. Feasibility will be assessed in terms of proportion of patients who undergo pre-conditioning chemotherapy, TIL infusion and the allocated dose of IL2. Tolerability will be assessed in terms of CTCAE grading version 4.0.

Table 8: Criteria to Define BOR as per RECISTv1.1

Overall Response At Time Point Before Week 12	Overall Response At Week12	Overall response At Time Points After Week 12	Best Overall Response for All Time Points
CR* or PR*	Any	Any	CR or PR (at time point before Week12)
PD,SD or no data	CR* or PR*	Any	CR or PR (at 12 weeks)
PD,SD,CR**,PR** or no data	SD,CR**,PR**	CR* or PR*	CR or PR(at time point after Week12)
PD,SD,PR**,CR** or no data	SD,PR**,CR**	PD,SD,PR**,CR** or no data	SD
SD,PR**,CR** or no data	PD	Any	PD (at Week 12)
PD	PD	Any	PD (at time point before week12)

* Initial overall response of PR or CR confirmed by a second (confirmatory) evaluation meeting the criteria for response and performed no less than 4 weeks after the criteria for response are first met.
** Overall response of PR or CR not confirmed by a second (confirmatory) evaluation meeting the criteria for response and performed no less than 4 weeks after the criteria for response are first met.

11.2 Secondary Endpoints

11.2.1 Assessment of Progression Free Survival

This is defined as the time interval between the treatment starting date and the documented date of disease progression or death, whichever occurs first. For an alive and progression free patient, PFS is censored at the last follow-up date when patient is documented to be progression free.

11.2.2 Evaluation of the Duration of Response

Duration of Overall Response
Duration of overall response is measured from the time that measurement criteria are met for complete response or partial response (whichever status is recorded first) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The duration of overall complete response is measured from the time measurement criteria are first met for complete response until the first date that recurrent disease is objectively documented.

Duration of Stable Disease
It is recognised that durable stable disease in immunotherapy may represent anti-tumour activity (Wolchok *et al*., 2009), therefore duration of stable disease will be evaluated in this study. This is measured from the start of the treatment until the criteria for disease progression is met in patients

with achieved confirmed SD (taking as reference the smallest measurements recorded since the treatment started).

11.2.3 Assessment of Overall Survival

This is defined as the time interval between the treatment starting date and the documented date of death. For a surviving patient, OS is censored at the last follow-up date when the patient is documented to be alive.

11.3 Exploratory Endpoints

11.3.1 Analysis of TIL Tumour and Other Immunological Assessments Responses post-TIL infusion

Samples from all patients within the trial will be subjected to two types of laboratory based analysis:

- Detailed analysis of the tumour from which the TILs are isolated will be undertaken in an attempt to identify potential biomarkers of response.

- Following infusion of the TILs a hierarchy of research assays will be performed with the aim of monitoring the immunological response of the patient and determining correlates with clinical outcome.
  These will be performed on fractionated PBMC and plasma obtained from 50ml EDTA blood samples routinely collected according to the clinical protocol schedule. All samples will be processed and frozen following trial specific SOPs and analysed on-site or transported to an appropriate central monitoring facility.

Molecular characterization of Tumour excised for TIL preparation:

At the time of tumour resection a portion of the tumour will be collected into a RNA-stabilisation solution, clearly labelled and stored according to an appropriate SOP. The RNA from each of these samples will then be isolated and high-capacity profiling techniques be used to determine expression profiles from these samples with the aim of identifying potential prognostic markers. DNA will also be prepared. DNA and RNA will be retained for potential further analyses including sequence analysis.

Primary Laboratory Research Assays:

The primary research assays for the trial involve monitoring the immunological response of the patients following infusion of the TILs and will involve quantification of key cytokines in the plasma of the patients and analysis of immune-cell populations by flow cytometry (FCM).

i) Quantitation of key cytokines will be performed on plasma using a multiplexed array ran on a BioRad BioPlex platform. Cytokines analysed could include IL-2, IL-4, IL-6, IFNγ and TNF.

ii) Analysis of immune cell subsets by FCM from frozen PBMC. The number and activation status of key immune cells will be determined using a panel of antibodies including CD3, CD4, CD8 and CD69.

Secondary Laboratory Research Assays:

i) In addition the PBMC and plasma from each patient will be processed and stored according to GCP compliant SOPs and will be available for a range of secondary scientific assays will be performed to further elucidate the phenotype and activity of the infused cells as appropriate. The assays performed will depend upon availability of sample and clinical / scientific significance.

ii) During hospitalisation, all patients will also have daily 2ml EDTA blood samples taken from day 0 onwards until discharge for the collection of serum plasma. These samples will be collected according to standard clinical SOPs and stored frozen prior to analysis of relevant immune associated cytokines (e.g. IFNγ, IL-6) as described previously..

Where appropriate, biopsies at 6 weeks will be collected from tumour sites for downstream analysis. Prior to collection the processing should be discussed with the study team. If there is adequate tissue a portion should be collected fresh to grow TIL and potentially tumour cells. The remainder (or if there is inadequate tissue for culture tumour) will be snap frozen in liquid nitrogen and transported to research laboratories where the samples will be processed and potentially analysed for as for the tumour excised for TIL preparation (see above):

Analysis of general immune and T cell-specific parameters in tumour and pre-infused T cells:

i) Tumour: immunohistochemistry (IHC) of tumour biopsies after treatment
ii) Tumour: DNA and RNA extraction from tumour tissue after treatment (gene expression and mutational analysis in search for molecular correlatives to response). Potentially for genetic sequencing.
iii) PBMC and TIL: assay for recognition of tumour cells or tumour antigens.

11.3.2 Evaluation of Response Rate by Immune Response Criteria (irRC)

irRC will be used to capture and document overall response along with RECISTv1.1. See Appendix 3 for detail of definition criteria

11.3.3 Evaluation of TIL Harvest Process

All patients who undergone the pre-screening phase will be analysed in terms of success and failure rates at every stages. Outcome of source TIL between pre-screened patient category a) and category b) will be analysed and compared to further inform the merit of either approach for future development.

11.3.4 Assessment of the Cost of Treatment

Activity reports will be obtained from the treating centre with records of inpatient stays, tests undertaken (to include management of any toxicities) – these will be used to derive estimated costs of treatment as this will be potentially important for future development

12 TRIAL CLOSURE

The entire trial will be stopped when:

- An unexpected investigational product related adverse event (=CTC grade 4) is observed in more than 1 patient, the trial should be stopped and the data reviewed, before any further patients are treated.

- The stated number of patients to be recruited is reached.

- The interim analysis showed futility of therapy as defined by statistical design of this study.

Regardless of the reason for termination, all data available for the patient at the time of discontinuation of follow-up must be recorded in the CRF. All reasons for discontinuation of treatment must be documented.

In terminating the study, the Trial Sponsor and the Investigators will ensure that adequate consideration is given to the protection of the patient's interest.

13 STATISTICS AND DATA ANALYSIS

13.1 Trial Design and Sample Size Calculation

The study employs two parallel Simon 2-stage designs (Logan 2005), one for LD-IL2 and one for HD-IL2. Patients will be randomised to either of the arms and the best overall response to therapy will be the primary outcome. The characteristics of each design will be the same and a minimax design has been chosen. For each arm there will be 24 patients in the first stage and conditionally on the stage I response rates 21 further patients in the second stage i.e. there will be a minimum of 2x24=48 patients and a maximum of 2x45=90 patients. The study design regards a response rate of ≤ 20% to be not worthy of further consideration. A type I error of 5% with a power of 90% if the true response rate (π) is in fact 40% has been used to derive the design. The rates of 20% and 40% have been chosen as it is considered that if the response rate of either arm is less than 20% that arm would not be considered to have useful activity given the complexity of the therapy. A response of 40-50% is that reported in other series and hence a response rate of 40% should be achieved.

The main statistical analysis is based on the Binomial distribution. The purpose of the analysis is to ascertain whether each arm appears worthy of further consideration for example in a phase III trial. If at the interim analysis there are ≤ 5 responses out of 24 for either arm then that arm will be regarded as not worthy of further consideration (accept H0 $\pi \leq 0.20$) and no more patients will be recruited to that arm (If H0 is really true then the probability of early termination of that arm is ≥ 0.66). If there are at least 6 responses then 21 more patients will be recruited to that arm (if both arms pass this criterion then the additional patients will be randomised). After the second stage if there are more than 13 responses out of the 45 cases in an arm then that arm will be considered worthy of further consideration (reject H0 $\pi \leq 0.20$). If both arms meet this criterion then their respective response rate estimates and durability of response will be weighed against toxicity to decide on the 'best candidate' for further study. A significance test of equal true response rates will be applied but it is recognised that this will have low power (e.g. approximately 50% power if the true response rates were 0.30 and 0.50 respectively).

13.2 Interim Analysis

The trial will have one interim analysis as indicated above. The data will be presented to an independent data monitoring committee (IDMC) who will assess the safety and outcomes of this phase of the study. If appropriate the study will proceed to the second phase.

13.3 Primary and Secondary Analysis

Response-based endpoints in this trial (namely response rate, duration of remission and progression free survival) will be captured using two distinct but related criteria:

- RECIST v1.1
- Immune-related Response Criteria (irRC)

RECIST v1.1 will be used to define primary and secondary endpoints. The irRC will be used to define exploratory endpoints.

13.4 Primary Endpoints

13.4.1 Tumour Response

Objective tumour response will be evaluated by standard radiological assessment using RECIST v1.1 beginning at 6 weeks from Day 0, i.e. since TIL therapy. Further response should be done at 12 weeks and then 12 weekly until progression.

Proportion of CR, PR, and SD will be determined by patient's best overall response. This is defined by the best overall response recorded from the start of the treatment until disease progression. If response assessments taken after withdrawal from study treatment and/or alternative therapy are to be included in the main endpoint determination then this should be recorded clearly in the CRF.

Overall response rate will be estimated by the number of patients with complete and partial responses divided by the total number of evaluable patients. Ninety-five percent confidence intervals for the true success proportion will be calculated. Comparison will be made between the two treatment arms.

13.4.2 Feasibility and Tolerability

The primary feasibility outcome was number of subjects enrolled versus those completing the study at the main stages of this study, i.e. chemotherapy, T cells and IL2 and recovery phases. Tolerability will be measured utilising CTCAE v4.0.

13.5 Secondary Endpoints

Comparison will be made between the two treatment arms in terms of:

13.5.1 Progression Free Survival

This is defined as the time interval between the treatment starting date and the documented date of disease progression or death, whichever occurs first. For an alive and progression free patient, PFS is censored at the last follow-up date when patient is documented to be progression free.

13.5.2 Duration of Overall Response

Duration of overall response is measured from the time that measurement criteria are met for complete response or partial response (whichever status is recorded first) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The duration of overall complete response is measured from the time measurement criteria are first met for complete response until the first date that recurrent disease is objectively documented.

13.5.3 Duration of Stable Disease

This is measured from the start of the treatment until the criteria for disease progression is met in patients with achieved confirmed SD (taking as reference the smallest measurements recorded since the treatment started).

13.5.4 Overall Survival

This is defined as the time interval between the treatment starting date and the documented date of death. For a surviving patient, OS is censored at the last follow-up date when the patient is documented to be alive. This will be estimated and plotted using the Kaplan-Meier product-limit method with its estimates of the median and corresponding 95% confidence intervals.

13.6 Exploratory Endpoints

Laboratory analysis of TIL survival and other immunological biomarkers assessments will be hypothesis generating and there is no formal statistical plan at the point.

13.6.1 Evaluation of Response Rate by Immune Response Criteria (irRC)

As per response rate evaluation but using irRC criteria. Also see Appendix 3.

13.6.2 Evaluation of TIL Harvest Process

All patients who have undergone the pre-screening process (i.e. surgical tumour excision for TIL harvest and subsequent culture) will be analysed in terms of success and failure rates of TIL growth. Outcomes depending on the TIL harvest category (e.g. by category (A) or (B) or site of TIL harvest) will be examined to inform future development.

13.6.3 Assessment of the Treatment Cost

Average per-patient cost during the active treatment phase in both arms.

13.7 Randomisation

Once the TILs are available and the patient's eligibility confirmed the patient will be randomised centrally by The MAHSC Trials Co-ordination Unit. Randomisation will be 1:1 and to further protect against chance imbalance the allocation method will be minimisation (with a random element) using the following prognostic features for response: B-Raf (mutant, wild type), LDH (≤ULN, >ULN), M1 (A/B, M1c) and Anti-CTLA-4 (no, yes)

The researcher will telephone The MAHSC-CTU Trials Line to randomise a patient at the following details:

Tel: **0161 446 3311** (Monday – Friday 9am - 5pm)

After correctly providing the verbal password callers will be asked to provide the patient initials and month/year of birth in addition to the prognostic factors listed above. The trial number and treatment allocation will be returned verbally and a transported encrypted email will be sent to a pre-defined distribution list.

14 PHARMACOVIGILANCE

14.1 Definitions

14.1.1 Adverse Event (AE)

An adverse event is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product or trial-specific treatment and which does not necessarily have a causal relationship with this treatment.

Note: An AE may therefore be any unfavourable and unintended sign (including abnormal findings), symptom or disease concurrently associated with the use of the trial treatment, regardless of any relation to the treatment.

14.1.2 Adverse Reaction (AR)

An adverse reaction is any untoward and unintended response in a subject to a trial treatment which is related to any dose administered to that subject.

Note: Any adverse event judged by either the reporting investigator or the sponsor as having reasonable causal relationship to the trial treatment qualifies as an AR if there is evidence or argument to suggest a causal relationship.

14.1.3 Serious Adverse Event / Reaction (SAE / SAR)

A serious adverse event or reaction in a trial subject that:

- results in death,
- is life-threatening,
- requires in-patient hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability / incapacity
- is a congenital anomaly / birth defect, or
- other important medical condition (medical judgement should be exercised in deciding whether an adverse event / reaction should be classified as serious in other situations. Important adverse events / reactions 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 also be considered serious).

Note: For the purpose of this definition, life-threatening refers to an event in which the trial subject was at risk at the time of an event; not an event that might have caused death if it were more severe.

14.1.4 Suspected Unexpected Serious Adverse Reaction (SUSAR)

Any adverse reaction that is serious and unexpected (is not consistent with the reference safety information Investigator's Brochure [IB]. This may include serious adverse reactions which are expected but the severity or intensity of which exceeds that expected from the IB.

14.2 Detecting AEs and SAEs

For the purpose of this trial, Adverse Events (AEs) and Serious Adverse Events (SAEs) will be recorded from the point of surgical tumour excision until the end of trial/trial discontinuation. All events that occur prior to surgical tumour excision will be recorded in the subjects medical history, including those that occur following consent to the main study.

Trial subjects will be reviewed life long for unexpected late side effects and late onset of adverse events linked to the therapy, e.g. oncogenesis, autoimmunity or immunogenicity. Where possible, this will be until subject death.

14.3 Recording AEs and SAEs

All AEs, whether observed by the Investigator or reported by the patient during the study period outlined in section 14.2, and whether or not they are considered related to the drug, must be recorded in the CRF including those which are reported up to one month after the study drug administration. After this period, only those events causally related to the IMP will be recorded. The NCI CTCAE v4.0 must be used to grade the AE. The worse grade in a cycle for that particular event must be documented. If the AE resolves completely or resolves to baseline and then worsens again in the same cycle, this must be recorded as a separate AE event

.

To avoid excessive documentation of non-clinically significant AEs, the following exclusions will apply:

- Haematological Toxicities: Anything worse than the haematological ranges listed in the Inclusion Criteria will be reported.
- Liver Function: All liver abnormalities should be recorded according to CTCAE version 4.
- Creatinine: Anything worse than the range listed in the Inclusion criteria will be recorded.

14.4 Evaluation of AEs and SAEs

14.4.1 Severity Evaluation

The following definitions will be used for adverse events that are not included in CTCAE v4.0:

Mild: does not interfere with the conduct of the study, resolves spontaneously, does not need medications or any other therapy.

Moderate: requires treatment, interferes temporally with the conduct of the study.

Severe: forces withdrawal from the study.

14.4.2 Causality to the Investigational Product

Almost certainly:

- starts within a reasonable time after the study drug administration,
- stops/ improves when the study drug has been stopped,
- can reasonably be explained by known characteristics of the study drug

Probably:

- starts within a reasonable time after the study drug administration,
- stops/ improves when the study drug has been stopped,
- cannot be reasonably explained by known characteristics of the patient's clinical state

Possibly:

- starts within a reasonable time after the study drug administration but
- could have been produced by the subject's clinical state or other modes of therapy administered to the patient

Unlikely:

- the time association or the patient's clinical state is such that the study drug is not likely to have had an association with the observed effect

Unrelated:

- The AE is definitely not associated with the study drug administered

14.5 Reporting Procedures

14.5.1 Adverse Events (AEs)

All adverse events that occur between surgical tumour excision and end of trial treatment must be recorded in the patient notes and in the appropriate section of the trial CRF, as per CRF completion guidelines. Details on what information is required will be detailed in the CRF and CRF completion guidelines.

AEs meeting the definition of a Serious Adverse Event (SAE) must also be reported to the MAHSC-CTU using the trial specific SAE Report Form **within 24 hours of observing or learning about the event.**

Pre-existing conditions do not qualify as adverse events unless they worsen.

14.5.2 Serious Adverse Events (SAEs)

All SAEs that occur between surgical tumour excision and while the patient is still on study must be submitted to the MAHSC-CTU by fax within **24 hours** of observing or learning of the event, using the trial specific SAE Report Form. As many sections of the SAE form should be completed as possible (with further information being provided as it becomes available) and sent to the MAHSC-CTU. If the Principal Investigator is available the form should be signed and dated by the PI, but if the PI is unavailable the form can be signed by a designated representative and sent to the MAHSC-CTU, and then signed and dated by the PI as soon as possible..

All SAEs must be followed-up until resolution and site investigators must provide follow-up SAE Reports if the SAE had not resolved at the time the initial report was submitted.

All SAEs must be reported by faxing a completed SAE Report Form within 24 hours of becoming aware of the event to the Trial Manager at the MAHSC-CTU
Fax: 0161 446 8148

14.5.3 SAE Processing and Reporting at the MAHSC-CTU

On receipt of the SAE Report Form, the MAHSC Trials Coordination Unit will send an acknowledgement of the SAE to the relevant members of the trial team at the participating site. This acknowledgement will include an SAE reference number which should be included on all future correspondence regarding the SAE. The MAHSC Trials Coordination Unit MAHSC Trials Coordination Unit then passes the SAE form to the CI. The CI reviews the site PI's assessments of seriousness and causality (and expectedness where completed) and assigns their own judgment, including an assessment of expectedness in relation to the reference safety information. If an SAE is suspected to be a SUSAR, regulatory time constraints apply for expedited reporting. The MAHSC Trials Coordination Unit will then liaise with the CI to evaluate the event for seriousness, causality and expectedness to determine whether or not the case qualifies for expedited reporting.

Expectedness of the event to the trial treatment must be determined as follows:

- **Expected**
  The event is listed as an expected adverse event in the IB.

- **Unexpected**
  The event is not listed as an expected adverse event in the IB, or, the severity of the event is greater than that listed in the IB for example:

  - the event is life threatening or fatal.
  - the patient presents with an event which is considered to be moderate or severe, but only mild is listed as expected in the protocol appendix.

Note: If a difference of opinion between the PI and CI gives rise to an event being classified as an SAE by one clinician and a SUSAR by another, the "worst-case" assessment is assumed i.e. it is classified as a SUSAR. Similarly, the causality assessment made by the PI at site cannot be downgraded by the CI as the PI is more familiar with the patient's history, clinical signs and symptoms, lab findings and other investigations. The CI may, however, upgrade the PI's assessment of causality.

Centres should respond as soon as possible to requests from the CI or designated representative (via the MAHSC-CTU) for further information that may be required for final assessment of the SAE.

The CTPM will advise the CI and CTL by email of all SAEs within 24 hours of the unit becoming aware of the SAE. If required, the MAHSC-CTU will send copies of all SAE forms to the CI and CTL. The CTPM will also keep the CI and CTL informed of the status of SAEs with regular update emails and also will provide full details of how the SAE has resolved.

SAEs assessed as having a causal relationship to study drug and as being unexpected (SUSARs) will undergo expedited reporting to the relevant authorities by the MAHSC-CTU.

14.5.4 SUSAR Processing and Reporting at the MAHSC-CTU

If the event is evaluated as a SUSAR, the MAHSC Trials Coordination Unit will submit a report to the MHRA, REC, sponsor and within the following timeframes:

IMP trials

- Fatal or life-threatening SUSARs are reported to the MHRA and REC within 7 days. Additional information / follow-up reports are sent to the MHRA and REC as appropriate within an **additional 8 days**.
- Non-fatal or life-threatening SUSARs are reported to the MHRA and REC **within 15 days.**

SUSARs occurring in IMP trials are reported to the MHRA via the eSUSAR online reporting system by the CTPM, where agreed. Direct expedited reporting of SUSARs to the MHRA by investigators is not permitted. SUSARs are reported to the relevant REC by the CTPM using the National Research Ethics Service (NRES) process.

Where doubt exists whether an SAE is a SUSAR, it is only reported to the MHRA when it has been confirmed that it has fulfilled one of the criteria meeting the definition of a SUSAR. If the SUSAR represents a significant safety concern, all investigators participating in that trial will be informed as soon as possible by the CTPM. SUSARs will be notified to sponsors, investigators and third parties in accordance with the trial agreements.

A flow-chart overview of the entire notification and reporting process is given in Appendix 4.

14.6 Additional Safety Monitoring

Surviving patients will be followed indefinitely for any secondary malignancy and other late side effects. See section 10.6 for procedures of longer term follow up.

14.7 Pregnancy Reporting

If a patient or their partner becomes pregnant, the pregnancy must be reported to the MAHSC-CTU using the Pregnancy Notification and Outcome Form within **24 hours** of the participating site becoming aware of the pregnancy. The PI must obtain consent to follow the pregnancy and liaises with the obstetrician until delivery/outcome and for the postpartum period for 6 months. Parental and neonatal outcomes must be recorded (by updating the Pregnancy Notification and Outcome form) even if they are completely normal and without AEs. Abnormal outcomes for the foetus or child observed at birth or during the follow-up period (e.g. spontaneous or therapeutic abortion, stillbirth, neonatal death, congenital abnormality) are considered SAEs and are reported to the MAHSC-CTU on the standard SAE form.

14.8 Urgent Safety Measures

Where an urgent safety measure (to prevent immediate hazard to trial subject's health and safety) is necessary, prior authorisations from the MHRA and ethics are not required.

Where the PI takes urgent action that is not consistent with the protocol to prevent harm to a subject on a trial, the PI must immediately inform the CI / CTPM and give full details of the measures taken and the decision making process surrounding the action(s) taken. The CTPM will

inform the CI, sponsor, ethics and the MHRA of these measures immediately, but **no later than 3 days** of the actions being taken.

An amendment is formally submitted as soon as possible by the CI and CTPM to the relevant bodies in conjunction with the sponsor.

Examples of issues requiring urgent safety measures may be:

- A single report of a SAR with an unexpected outcome (e.g. death)
- An increase in rate of occurrence of SAR which is judged to be clinically important
- A post-trial SUSAR occurring after the subject has left the trial
- A new event relating to the use or development of an IMP likely to affect the safety of subjects e.g.:

    - an SAE that could be associated with the procedures and which could lead to modification of the trial conduct
    - lack of efficacy of an IMP used for a life-threatening disease
    - a major safety finding from a newly completed animal study.

14.9 Safety Reports

Annual Progress Reports (APR) and Development Safety Update Reports (DSUR) will be submitted annually to the MHRA and REC.

14.10 List of Expected Adverse Drug Reactions (ADR)

Any ADR listed below (Table 9) will <u>not</u> be reported to the REC and MHRA by MAHSC-CTU unless there is an increase in grade, severity or frequency of the ADR and the ADR also qualifies as 'serious'.

Note all toxicities relating to TILs will be considered 'unexpected'. Toxicities relating to IL2 are expected to resolve rapidly (within 72hr).

Table 9: Expected Toxicities from previous TIL Trials

General AE term	CTCAE term	Expected grade (s)/ severity	Reason for expecting the ADR
Chemotherapy related			
Reduction in white blood cells	Neutropenia	0-4	Previous clinical trial
Reduction in white blood cells	Lymphopenia	0-4	Previous clinical trial
Reduction in red blood cells	Anaemia	0-3	Previous clinical trial
Reduction in platelets	Thrombocytopenia	0-4	Previous clinical trial
Nausea	Nausea	0-2	Previous clinical experience
Vomiting	Vomiting	0-2	Previous clinical experience
Loss of appetite	Anorexia	0-2	Previous clinical experience
Sore mouth	Mucositis	0-2	Previous clinical experience
Tiredness	Fatigue	0-3	Previous clinical experience
Hair loss	Alopecia	0-2	Previous clinical experience
Cystitis	Cystitis	0-2	Previous clinical experience
Skin discolouration	Hyperpigmentation	0-1	Previous clinical experience
IL2 related (excluding any listed above)			
Rapid heart rate	Sinus tachycardia	0-2	Previous clinical experience
Diarrhoea	Diarrhoea	0-2	Previous clinical experience
Low blood pressure	Hypotension	0-2	Previous clinical experience
Infusion reaction	Infusion reaction	0-3	Previous clinical experience
Fluid retention	Oedema	0-2	Previous clinical experience
Rigors/chills	Rigors/chills	0-2	Previous clinical experience
Fever	Fever	0-3	Previous clinical experience
Confusion	Confusion	0-1	Previous clinical experience
Agitation	Agitation	0-1	Previous clinical experience
Shortness of breath	Dyspnoea	0-3	Previous clinical experience
Rash	Dermatitis	0-2	Previous clinical experience
Increase in creatinine	Raised creatinine	0-2	Previous clinical experience
Increase in liver function tests	Abnormal LFTs	0-1	Previous clinical experience
Low albumin	Hypoalbuminaemia	0-2	Previous clinical experience
Low calcium	Hypocalcaemia	0-2	Previous clinical experience
Low potassium	Hypokalaemia	0-3	Previous clinical experience

15 TRIAL MANAGEMENT AND OVERSIGHT ARRANGEMENTS

A delegation log will be prepared for each site detailing the responsibilities of each member of staff working on the trial

15.1 Oversight Committees

15.1.1 Independent Data Monitoring Committee (IDMC)

An IDMC will be instigated to review accruing trial data and to assess whether there are any safety issues that should be brought to the patient's attention, whether any safety amendments should be made or if there are any reasons for the trial should not continue. The IDMC will be independent of the investigators, funder and sponsor and will comprise of a statistician and at least 2 other experts in the disease area under investigation. The CI and TMG with the support of the MAHSC-CTU will be responsible for nominating IDMC members. The Committee's terms of reference, roles and responsibilities will be defined in a charter issued by Manchester CTU. This charter will outline any stopping rules and the frequency of analysis and IDMC meetings during the trial. The IDMC will meet in confidence at regular intervals.

Reports to the IDMC will be prepared and presented by the trial statistician and CTPM prior to the IDMC meeting. The IDMC Chairman will then report their recommendations to the Chairman of the TSC or TMG and may request additional reports or information if required. This report will be submitted to the TMG and TSC, and if required, the REC and the MHRA and the CI and CTPM will ensure that all actions and recommendations are followed up. There will not be a named medical monitor for safety related queries. The chief investigator will assume responsibility for this.

15.1.2 Trial Steering Committee (TSC)

A Trial Steering Committee (TSC) will be established and will include an independent Chairman (not involved directly in the trial other than as a member of the TSC), not less than two other independent members, a representative from a consumer group, plus the CI, one or two Principal Investigators, the Trial Manager and statistician.

The role of the TSC is to take responsibility for the scientific integrity of the trial, the scientific validity of the trial protocol, assessment of the trial quality and conduct (to ensure that the trial is being conducted in accordance with the principles of GCP and the relevant regulations) as well as for the scientific quality of the final study report. Decisions about the continuation or termination of the trial or substantial amendments to the protocol are usually the responsibility of the TSC.

The TSC will meet once ethics approval has been given and before the trial begins recruitment. Once the trial has started the TSC should meet at least annually to monitor the progress of the trial although there may be periods when more frequent meetings are necessary. Meetings should be organised by the CI via the CTPM. Minutes will be taken at TSC meetings and copies of the minutes will be filed in the Trial Master File. The trial manager and CI will ensure that all relevant issues and actions discussed during the meeting are followed up and resolved and details of significant issues will be made available to participating sites. Minutes of all TSC meetings are available on request.

The Committee's terms of reference, roles and responsibilities will be defined in a charter issued by the MAHSC-CTU.

15.2 MAHSC-CTU

The role and responsibilities of the MAHSC-CTU are outlined in the Delegation of Responsibilities agreement signed between the CI, Sponsor and MAHSC-CTU.

16 TRIAL MONITORING

16.1 Data Collection

The paper CRF will be used to collect the data. A study specific Data Entry Instructions document is provided by the MAHSC-CTU, and these instructions must be followed by all site personnel entering or correcting data. The Investigator is responsible for ensuring the accuracy, completeness, legibility and timelines of the data reported in the CRFs.

After all the queries have been resolved at the end of the study, the Investigator will confirm this by signing off the CRFs. The original CRFs will subsequently be submitted to Trial Sponsor for archiving. The Investigator will receive copies of the CRFs and Data Clarification Forms (DCFs).

16.2 Data Handling at MAHSC-CTU

Completed CRFs submitted to the Trial Sponsor will be reviewed by the designated MAHSC-CTU Data Manager who will enter the data into an electronic database. Data provided to the MAHSC-CTU will be checked for errors, inconsistencies and omissions. If missing or questionable data are identified, the MAHSC-CTU will request that the data be clarified. All aspects of data collection and handling throughout the life cycle of the trial will be described in trial specific documents.

16.3 Central Monitoring of received data

Central monitoring of the study data and documentation will be coordinated by the MAHSC-CTU by the CTPM and Data Manager.

The data will be checked by a series of manual and automated consistency checks and validations. Any queries raised on the submitted data will be sent to the site. The answered queries will be returned to the Data Manger who will then make the necessary database updates. This cycle may be repeated.

Appropriate members of the site trial team must be advised of any concerns regarding site or protocol compliance, and any issues that may impact planned reporting or analyses.

16.4 Direct Access to Data

By participating in the METILDA trial, the Principal Investigator is confirming agreement with his/her local NHS Trust to ensure that:

- Sufficient data is recorded for all participating patients to enable accurate linkage between hospital records and CRFs;
- Source data and all trial related documentation are accurate, complete, maintained and accessible for monitoring and audit visits;
- Trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s) are permitted and direct access to source data/documents is provided as required

16.5 Site Monitoring

On-site monitoring will be based on a risk-based strategy and a thorough risk assessment will be completed by the MAHSC-CTU as part of the site set-up process to ascertain the frequency and intensity of monitoring visits required (although additional monitoring may be conducted if necessary). This risk assessment and associated monitoring plan will be stored at the MAHSC-CTU.

The purpose of these visits is:

- To verify that the rights and well-being of patients/participants are protected
- To verify accuracy, completion and validity of reported trial data from the source documents
- To evaluate the conduct of the trial within the institution with regard to compliance with the currently approved protocol, GCP and with the applicable regulatory requirements

17 CONFIDENTIALITY AND DATA PROTECTION

17.1 Confidentiality

The CRFs for this trial will be anonymised; patients will only be identified by a numeric patient ID or unique trial number. All CRF pages will be kept in a secure storage area with limited access. The trial database will not contain any patient identifiable or personal data.

Patients will be made aware that data is collected as part of the informed consent process. Clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the sponsor, its designee, regulatory authorities or the Research Ethics Committee.

The investigator and trial staff involved with this trial may not disclose or use for any purpose other than performance of the trial, any data, record or other unpublished, confidential information disclosed to those individuals for the purpose of the trial.

Prior written agreement from the sponsor or its designee must be obtained for the disclosure of any confidential information to other parties.

The patient's consent to participate in the trial should be obtained after a full explanation has been given of the treatment options, including the conventional and generally accepted methods of treatment. Patients should be given sufficient time after being given the trial patient information sheet to consider and discuss participation in the trial with friends and family. A contact number should be given to the patient should they wish to discuss any aspect of the trial. Following this,

the recruiting investigator should determine that the patient is fully informed of the trial in accordance with ICH GCP guidelines.

The right of the patient to refuse to participate in the trial without giving reasons must be respected. After the patient has entered the trial, the clinician must remain free to give alternative treatment to that specified in the protocol, at any stage, if he/she feels it to be in the best interest of the patient. However, the reason for doing so should be recorded and the patient will remain within the trial for the purpose of follow-up and data analysis. Similarly, the patient must remain free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing his/her further treatment.

17.2 Data Protection

All investigators and trial staff involved with the trial must comply with the requirements of Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data and on the free movement of such data, and will uphold the directives core principles. According to the Directive, 'processing of personal data' ('processing') means any operation or set of operations which is performed upon personal data, whether or not by automatic means, such as collection, recording, organization, storage, adaptation or alteration, retrieval, consultation, use, disclosure by transmission, dissemination or otherwise making available, alignment or combination, blocking, erasure or destruction.

Access to collated participant data will be restricted to those clinicians treating the patients. Computers used to collate the data should have limited access measures via user names and passwords.

Published results should not contain any personal data that could allow identification of individual participants.

17.3 Publication Policy

Data will be analysed and published as soon as possible. All publications will include a list of investigators, and if there are named authors, these should include the Chief Investigator, clinical trial co-ordinator(s), and statistician(s) involved in the trial.

18 TRIAL CONDUCT

18.1 Protocol Amendments

Any changes in research activity will be reviewed and approved by the Chief Investigator and submitted in writing to the appropriate REC, Regulatory Authority and local R&D for approval prior to enrolment into an amended protocol.

18.2 Protocol Deviations

Protocol deviations should not be implemented without agreements from the appropriate REC, Regulatory Authority and assessment body approval except where necessary to eliminate an immediate hazard to trial participants.

Any deviation details (i.e. the nature of and reasons for the deviation) should be reported to the MAHSC Trials Co-ordination Unit, who will then process this information (e.g. if the deviation necessitates a subsequent protocol amendment, this will be submitted to the REC, Regulatory Authority and local assessment body for review and approval).

18.3 Serious Breaches

For Clinical Trials of Investigational Medicinal Products (CTIMPs), there is a legal requirement to report serious breaches of GCP or the trial protocol to the MHRA and REC within a defined timeframe.

The MAHSC-CTU will, in conjunction with the sponsor, assess all deviations reported to the unit and report serious breaches where necessary to the Regulatory Authorities.

19 ETHICAL AND REGULATORY REQUIREMENTS

The trial will be conducted in accordance with the principles of the good clinical practice (GCP).

The sponsor will ensure that the trial protocol, participant information sheet, consent form, GP letter and submitted supporting documents have been approved by the appropriate regulatory body (MHRA in the UK) and the appropriate research ethics committee (GTAC in the UK), prior to any participant recruitment. The protocol and all agreed substantial protocol amendments, will be documented and submitted for ethical and regulatory approval prior to implementation.

Due to the use of radiation in excess of that associated with standard of care the appropriate licence will be obtained (Administration of Radioactive Substances Advisory Committee (ARSAC) licence in the UK).

Each participating site must obtain local Research & Development (R&D) approval. It is the responsibility of the Principal Investigator at each site to ensure that all subsequent amendments gain the necessary approval.

The CI and sponsor will ensure that all associated Ethics Committees and competent authorities are notified that the trial has finished (either as expected or prematurely) within required timeframes with summary reports to be provided as required

Each participating site will be required to sign a trial agreement with the study sponsor, in order to confirm that they agree to follow the obligations, terms and conditions of the sponsor when carrying out the study. This agreement will be based on the National Institute of Health Research (NIHR) model non-commercial agreement template.

If any third party is required to carry out protocol specific procedures by one of the participating sites, other than those covered under the trial agreement, a service level agreement will need to be signed by the site requesting the service, and by the service provider

20 END OF TRIAL

The end of the trial is defined as the date of death of the last recruited patient or the date of transfer of the last patient to the potential epidemiological extension study if this is required. The sponsor will notify all trial investigators and institutions along with the appropriate regulatory authorities within the required timeframe.

The Chief Investigator and/or the trial steering committee have the right at any time to terminate the trial for clinical or administrative reasons. The end of the trial will be reported to the REC and Regulatory Authority within the required timeframe if the trial is terminated prematurely.

Investigators will inform participants of any premature termination of the trial and ensure that the appropriate follow up is arranged for all involved.

A summary report of the trial will be provided to the REC and Regulatory Authority within the required timeframe.

21 CONTINUATION OF DRUG FOLLOWING THE END OF TRIAL

TIL cells will be administered within this trial as a single infusion there is no intention to retreat within this trial.

22 PEER REVIEW

This trial was extensively peer reviewed by the NIHR Efficacy and Mechanism Evaluation Funding panel.

23 TRIAL RECORD RETENTION AND ARCHIVING

The sponsor will retain all sponsor-specific essential documents in conformance with the applicable regulatory requirements of the country(ies) where the sponsor intends to apply for product approval(s).

If the sponsor discontinues the clinical development of an investigational product (i.e., for any or all indications, routes of administration, or dosage forms), the sponsor should maintain all sponsor-specific essential documents for at least 30 years after formal discontinuation in conformance with Commission Directive 2005/28/EC 8 April 2005 and Directive 2001/20/EC 4 April 2001 of the European Parliament.

All essential documents will be retained until at least 30 years after the date of infusion of the IMP. These documents should be retained for a longer period however if required by the applicable regulatory requirement(s).

The MAHSC-CTU will inform the investigator(s)/institution(s) in writing of the need for record retention and should notify the investigator(s)/institution(s) in writing when the trial-related records are no longer needed.

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APPENDICES

APPENDIX 1 - WHO PERFORMANCE SCALE

Activity Performance Description	Score
Fully active, able to carry out all normal activity without restriction.	0
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.	1
Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	2
Capable of only limited self-care. Confined to bed or chair more than 50% of waking hours.	3
Completely disabled. Cannot carry out any self-care. Totally confined to bed or chair.	4

APPENDIX 2 – MEASUREMENT OF DISEASE

Response **E**valuation **C**riteria **i**n **S**olid **T**umours (RECIST v1.1)

Introduction

This appendix details the implementation of RECIST 1.1 Guidelines for the METILDA study with regards to Investigator assessment of tumour burden including protocol-specific requirements for this study.

Definition of Measureable, Non-Measureable, Target and Non-Target Lesions

Only patients with measurable disease at baseline should be included in the study. Measurable disease is defined by the presence of at least one measurable lesion which has not been previously irradiated.

Patients with at least one lesion (measurable and/or non-measurable) that can be accurately assessed by CT or MRI at baseline and follow up visits should be included in this study.

Measureable:

A lesion, not previously irradiated, that can be accurately measured at baseline as ≥ 10 mm in the longest diameter (except lymph nodes which must have short axis ≥ 15 mm) with computed tomography (CT) or magnetic resonance imaging (MRI) and which is suitable for accurate repeated measurements.

Non-Measureable:

- All other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15mm short axis at baseline*).
- Truly non-measurable lesions include the following: bone lesions, leptomeningeal disease, ascites, pleural / pericardial effusion, and inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses /abdominal organomegaly identified by physical examination that is not measurable by CT or MRI.
- Previously irradiated lesions**
- Skin Metastases

* Nodes with <10mm short axis are considered non-pathological and should not be recorded or followed as NTL.

**Localised post-radiation changes which affect lesion sizes may occur. Therefore, lesions that have been previously irradiated will not be considered measurable and must be selected as Non-Target Lesions (NTL) at baseline and followed up as part of the NTL assessment.

Special Cases

Lytic bone lesions or mixed lytic–blastic lesions, with identifiable soft tissue components, can be considered measurable if the soft tissue component meets the definition of measurability. Blastic lesions are considered non-measurable.

Cystic metastases can be considered measurable lesions if they meet the criteria for measurability from a radiological point of view, but if non-cystic lesions are present in the same patient, these should be selected as target lesions.

Target Lesions:
A maximum of 5 measurable lesions (with a maximum of 2 lesions per organ), representative of all lesions involved suitable for accurate repeated measurement, should be identified as target lesions (TL) at baseline.

Non-Target Lesions:
All other lesions (or sites of disease) not recorded as TL should be identified as NTL at baseline.

Methods of Assessment

THE SAME METHOD OF ASSESSMENT AND THE SAME TECHNIQUE SHOULD BE USED TO CHARACTERIZE EACH IDENTIFIED AND RECORDED LESION AT BASELINE AND DURING FOLLOW-UP VISITS.

A summary of the methods to be used for RECIST assessment is provided below and those excluded from tumour assessments for this study are highlighted, with the rationale provided.

Table 8: Summary of Methods of Assessment

TARGET LESIONS	NON-TARGET LESIONS	NEW LESIONS
CT (preferred) MRI	CT (preferred) MRI Clinical examination X-ray, Chest x-ray	CT (preferred) MRI Clinical examination X-ray, Chest x-ray Ultrasound Bone Scan FDG-PET

CT and MRI
CT and MRI are generally considered to be the best currently available and reproducible methods to measure TL selected for response assessment and to assess NTL and identification of any new lesions.

In the METILDA study it is recommended that CT examinations of the *chest abdomen and pelvis* will be used to assess tumour burden at baseline and follow-up visits. CT examination with intravenous (IV) contrast media administration is the preferred method. MRI should be used where CT is not feasible or it is medically contra-indicated.

Clinical Examination
In the METILDA study, clinical examination will not be used for assessment of TL. Clinically detected lesions can be selected as target lesions if they are assessed by CT or MRI scans. Clinical examination can be used to assess NTL and to identify the presence of new lesions.

Chest X-Ray
In the METILDA study, chest x-ray assessment will not be used for assessment of TL as they will be assessed by CT examination or MRI examination. Chest X-ray can, however, be used to assess NTL and to identify the presence of new lesions.

Plain X-Ray

In the METILDA study plain x-ray may be used as a method of assessment for bone NTL and to identify the presence of new bone lesions.

Ultrasound

In the METILDA study, ultrasound examination will not be used for assessment of TL and NTL as it is not a reproducible method, does not provide an accurate assessment of tumour size and it is subjective and operator dependent. Ultrasound examination can, however, be used to identify the presence of new lesions. If new clinical symptoms occur and an ultrasound is performed then new lesions should be confirmed by CT or MRI examination.

Endoscopy and Laparoscopy

In the METILDA study, endoscopy and laparoscopy will not be used for tumour assessments as they are not validated in the context of tumour assessment.

Tumour Markers

In the METILDA study tumour markers will not be used for tumour response assessments as per RECIST 1.1.

Cytology and Histology

In the METILDA study histology will not be used as part of the tumour response assessment as per RECIST 1.1.

Cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment is required when the measurable tumour has met criteria for response or stable disease. In such circumstances, the cytology is necessary to differentiate between response / stable disease (an effusion may be a side effect of the treatment) and progressive disease (if the neoplastic origin of the fluid is confirmed). Where cytology findings are not available, any effusion that significantly worsens (from trace to large) or appearance of clinically significant effusion (requiring change in drug therapy) during the study treatment will be considered to be progression of NTL, or disease progression due to new lesions.

Isopopic Bone Scan

In the METILDA study isotopic bone scans may be used as a method of assessment to identify the presence of new bone lesions at follow-up visits. New lesions will be recorded where a positive hot-spot that was not present on the baseline bone scan assessment is identified on a bone scan performed at any time during the study. The Investigator should consider the positive hot-spot to be a significant new site of malignant disease and represent true disease progression in order to record the new lesion. Confirmation by CT, MRI and x-ray is recommended where bone scan findings are equivocal.

FDG-PET Scan

In the METILDA study FDG-PET scans may be used as a method for identifying new lesions, according with the following algorithm: New lesions will be recorded where there is positive FDG uptake* not present on baseline FDG-PET scan or in a location corresponding to a new lesion on CT/MRI at the same follow-up visit. If there is no baseline FDG-PET scan available, and no evidence of new lesions on CT/MRI scans then follow-up CT/MRI assessments should be continued, scheduled as per protocol or clinical indicated, in order to confirm new lesions.

* A positive FDG-PET scan lesion should be reported only when an uptake greater than twice that of the surrounding tissue is observed.

Tumour Response Evaluation

Schedule of Evaluation

Baseline assessments should encompass all areas of known predilection for metastases in the disease under evaluation and should additionally investigate areas that may be involved based on signs and symptoms of individual patients and should be performed no more than 21 days before the start of study treatment. Follow-up assessments will be performed every 8 weeks after randomisation, and continue until objective disease progression as defined by RECIST 1.1. Any other sites at which new disease is suspected should also be adequately imaged at follow-up.

If an unscheduled assessment was performed and the patient has not progressed, every attempt should be made to perform the subsequent assessments at their scheduled visits. This schedule is to be followed in order to minimise any unintentional bias caused by some patients being assessed at a different frequency than other patients.

Target Lesions (TL)

Documentation of Target Lesions

A maximum of 5 measurable lesions, with a maximum of 2 lesions per organ (including lymph nodes), representative of all lesions involved should be identified as TL at baseline. Target lesions should be selected on the basis of their size (longest diameter for non-nodal lesions or short axis for nodal lesions), but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion, which can be measured reproducibly, should be selected.

The site and location of each TL should be documented as well as the longest diameter for non-nodal lesions (or short axis for lymph nodes). All measurements should be recorded in millimeters. At baseline the sum of the diameters for all TL will be calculated and reported as the baseline sum of diameters. At follow-up visits the sum of diameters for all TL will be calculated and reported as the follow-up sum of diameters.

Special Cases

For TL measurable in 2 or 3 dimensions, always report the longest diameter. For pathological lymph nodes measurable in 2 or 3 dimensions, always report the short axis.

- If the CT/MRI slice thickness used is > 5mm, the minimum size of measurable disease at baseline should be twice the slice thickness of the baseline scan.
- If a lesion has completely disappeared, the longest diameter should be recorded as 0 mm.
- If a TL splits into two or more parts, then record the sum of the diameters of those parts.
- If two or more TL merge then the sum of the diameters of the combined lesion should be recorded for one of the lesions and 0 mm recorded for the other lesion(s).
- If a TL is believed to be present and is faintly seen but too small to measure, a default value of 5mm should be assigned. If an accurate measure can be given, this should be recorded, even if it is below 5mm.
- If a TL cannot be measured accurately due to it being too large, provide an estimate of the size of the lesion.
- When a TL has had any intervention e.g. radiotherapy, embolisation, surgery etc., during the study, the size of the TL should still be provided where possible.

Evaluation of Target Lesions

This section provides the definitions of the criteria used to determine objective tumour visit response for TL.

Table 9: Evaluation of Target Lesions

Complete Response (CR)	Disappearance of all target lesions since baseline. Any pathological lymph nodes selected as target lesions must have a reduction in short axis to < 10 mm.
Partial Response (PR)	At least a 30% decrease in the sum of the diameters of TL, taking as reference the baseline sum of diameters
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD
Progressive Disease (PD)	At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5mm.*(Please see text below concerning new lesions)*
Not Evaluable (NE)	Only relevant if any of the target lesions were not assessed or not evaluable or had a lesion intervention at this visit. Note: If the sum of diameters meets the progressive disease criteria, progressive disease overrides not evaluable as a target lesion response

Non-Target Lesions

Evaluation of Non-Target Lesions

All other lesions (or sites of disease) not recorded as TL should be identified as NTL at baseline. Measurements are not required for these lesions, but their status should be followed at subsequent visits. At each visit an overall assessment of the NTL response should be recorded by the Investigator. This section provides the definitions of the criteria used to determine and record overall response for NTL at the investigational site at each visit.

Table 10: Evaluation of Non-Target Lesions

Complete Response (CR)	Disappearance of all non-target lesions since baseline. All lymph nodes must be non-pathological in size (< 10 mm short axis).
Non CR/Non PD	Persistence of one or more NTL
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD
Progressive Disease (PD)	Unequivocal progression of existing non-target lesions. Unequivocal progression may be due to an important progression in one lesion only or in several lesions. In all cases the progression MUST be clinically significant for the physician to consider changing (or stopping) therapy. *(please see text below concerning new lesions)*
Not Evaluable (NE)	Only relevant when one or some of the non-target lesions were not assessed and, in the Investigator's opinion, they are not able to provide an evaluable overall non-target lesion assessment at this visit. Note: For patients without target lesions at baseline, this is relevant if any of the non-target lesions were not assessed at this visit and the progression criteria have not been met.

To achieve 'unequivocal progression' on the basis of non-target lesions, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target lesions, the overall tumour burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status.

New Lesions

Details of any new lesions will also be recorded with the date of assessment. The presence of one or more new lesions is assessed as progression.

A lesion identified at a follow up assessment in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression.

The finding of a new lesion should be unequivocal: i.e. not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumour.

If a new lesion is equivocal, for example because of its small size, the treatment and tumour assessments should be continued until the new lesion has been confirmed. If repeat scans confirm there is a new lesion, then the progression date should be declared using the date of the initial scan.

Symptomatic Deterioration

Symptomatic deterioration is not a descriptor of an objective response: it is a reason for stopping study therapy.

Patients with 'symptomatic deterioration' requiring discontinuation of treatment without objective evidence of disease progression at that time should continue to undergo tumour assessments where possible until objective disease progression is observed.

Evaluation of Overall Response

The overall visit response will be derived using the algorithm shown in Table 11.

Table 11: Overall Visit Response

TARGET LESIONS	NON-TARGET LESIONS	NEW LESIONS	OVERALL RESPONSE
CR	CR	No	**CR**
CR	NA	No	**CR**
NA	CR	No	**CR**
CR	Non CR/Non PD	No	**PR**
CR	NE	No	**PR**
PR	Non PD or NE	No	**PR**
SD	Non PD or NE	No	**SD**

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, NE = not evaluable, NA = not applicable (only relevant if there were no <<*TL/NTLs - delete as appropriate*>> at baseline).

Specifications for Radiological Imaging

These notes are recommendations for use in clinical studies. The use of standardized protocols for CT and MRI allows comparability both within and between different studies, irrespective of where the examination has been undertaken.

CT Scan

CT scans of the chest, abdomen, and pelvis should be contiguous throughout all the anatomic region of interest.

The most critical CT image acquisition parameters for optimal tumour evaluation using RECIST 1.1 are *anatomic coverage, contrast administration, slice thickness, and reconstruction interval.*

A. Anatomic Coverage: Optimal anatomic coverage for most solid tumours is the chest, abdomen and pelvis. Coverage should encompass all areas of known predilection for metastases in the disease under evaluation and should additionally investigate areas that may be involved based on signs and symptoms of individual patients. Because a lesion later identified in a body part not scanned at baseline would be considered as a new lesion representing disease progression, careful consideration should be given to the extent of imaging coverage at baseline and at subsequent follow-up time points. This will enable better consistency not only of tumour measurements but also identification of new disease.

B. IV Contrast Administration: Optimal visualisation and measurement of metastases in solid tumours requires consistent administration (dose and rate) of IV contrast as well as timing of scanning. Typically, most abdominal imaging is performed during the portal venous phase and (optimally) about the same time frame after injection on each examination. An adequate volume of a suitable contrast agent should be given so that the metastases are demonstrated to best effect and a consistent method is used on subsequent examinations for any given patient. It is very important that the same technique be used at baseline and on follow- up examinations for a given patient. For patients who develop contraindications to contrast after baseline contrast CT is done, the decision as to whether non-contrast CT or MRI (enhanced or non-enhanced) should be performed should also be based on the tumour type, anatomic location of the disease and should be optimised to allow for comparison to the prior studies if possible. Each case should be discussed with the radiologist to determine if substitution of these other approaches is possible and, if not, the patient should be considered not evaluable from that point forward. Care must be taken in measurement of target lesions on a different modality and interpretation of non-target disease or new lesions, since the same lesion may appear to have a different size using a new modality. Oral contrast is recommended to help visualise and differentiate structures in the abdomen.

If iodine contrast media is medically contraindicated at baseline or at any time during the course of the study then the recommended methods are: CT thoracic examination without contrast and abdominal and pelvis MRI with contrast. If MRI cannot be performed then CT without IV contrast is an option for the thorax, abdomen and pelvis examination. For brain lesions assessment, MRI is the preferred method.

C. Slice Thickness and Reconstruction Interval: It is recommended that CT scans be performed at 5mm contiguous slice thickness and this guideline presumes a minimum 5 mm thickness in recommendations for measurable lesion definition. Exceptionally, particular institutions may perform medically acceptable scans at slice thicknesses greater than 5 mm. If this occurs, the minimum size of measurable lesions at baseline should be twice the slice thickness of the baseline scans.

All window settings should be included in the assessment, particularly in the thorax where lung and soft tissue windows should be considered. When measuring lesions, the TL should be measured on the same window setting for repeated examinations throughout the study. All images from each examination should be included in the assessment and not “selected” images of the apparent lesion.

For these reasons, CT is the imaging modality of choice:

MRI Scan

MRI has excellent contrast, spatial and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity and measurement. Furthermore, the availability of MRI is variable globally. The modality used at follow-up should be the same as was used at baseline and the lesions should be measured / assessed on the same pulse sequence. Generally, axial imaging of the abdomen and pelvis with T1 and T2 weighted imaging along with gadolinium-enhanced imaging should be performed. The field of view, matrix, number of excitations, phase encode steps, use of fat suppression and fast sequences should be optimised for the specific body part being imaged as well as the scanner utilised. It is beyond the scope appendix to prescribe specific MRI pulse sequence parameters for all scanners, body parts and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques if possible.

FDG-PET Scans

FDG-PET has gained acceptance as a valuable tool for detecting, staging and restaging several malignancies. If FDG-PET scans are included in a protocol, an FDG uptake period of 60 min prior to imaging has been decided as the most appropriate for imaging of patients with malignancy. Whole-body acquisition is important since this allows for sampling of all areas of interest and can assess if new lesions have appeared thus determining the possibility of interval progression of disease. Images from the base of the skull to the level of the mid-thigh should be obtained 60 min post injection. PET camera specifications are variable and manufacturer specific, so every attempt should be made to use the same scanner, or the same model scanner, for serial scans on the same patient. Whole-body acquisitions can be performed in either 2- or 3-dimensional mode with attenuation correction, but the method chosen should be consistent across all patients and serial scans in the clinical trial.

PET/CT Scans

At present, low dose or attenuation correction CT portions of a combined PET–CT are of limited use in anatomically based efficacy assessments and it is therefore suggested that they should not be substituted for dedicated diagnostic contrast enhanced CT scans for tumour measurements by RECIST 1.1. In exceptional situations, if a site can document that the CT performed as part of a PET–CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast) then the CT portion of the PET–CT can be used for RECIST measurements. However, this is not recommended

because the PET portion of the CT introduces additional data that may bias an investigator if it is not routinely or serially performed.

Reference

Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). European Journal of Cancer. 45 (2009) 228-247

APPENDIX 3 – Immune Related Response Criteria (irRC)

Definition of Tumour Response Using irRC

The immune-related sum of products of diameters (irSPD) incorporates measurable new lesions that may have developed on-study, providing an assessment that includes both index and new lesions. The tumour assessment performed during screening will serve as baseline for determination of tumour response using irRC.

irSPD at Baseline: The sum of product of the diameters for all index lesions identified prior to randomisation.

irSPD Nadir: For tumours that are assessed more than one time after baseline, the lowest value of the irSPD is used as irSPD Nadir.

At baseline the irSPD is measured and recorded.

At each subsequent assessment timepoint, a separate assessment of overall response will be obtained for that timepoint. The sum of products of perpendicular diameters calculated and recorded at each post-baseline timepoint for immune-related response purposes (irSPD) include measurements of index lesions and also include measurable new lesions which are not too small to measure. A value of 25mm2(5mmx5mm) is imputed for each index and previously measurable new lesion which is present but too small to measure.

Response assessment using irRC

The overall assessment of immune-related response reported will be based on the following criteria:

Immune-related Complete Respone (irCR): Complete disappearance of all tumour lesions (index and non-index together with no new measurable/unmeasurable lesions)

Immune-related Partial Response (irPR): A decrease, relative to baseline of the irSPD of 50% or greater.

Immune-related Stable Diseae (irSD): is defined as an evaluable response that fails to meet criteria for immune-related complete response or immune-related partial response, in the absence of immune-related progressive disease.

Immune-related Progressive Disease (irPD): At least a 25% increase in the irSPD (based on irSPD of all index lesions and any measurable new lesions, as defined above) over the nadir irSPD, or the occurrence of any new measureable lesions is the SPD nadir is “0”.

Immune-related Unknown Response (irUN): Tumour assessments which cannot be evaluated (e.g., due to image quality, inability to assess all relevant lesions, etc) will be reported as irUN.

Immune-related Response Criteria Definitions are summarised in table 12 below:

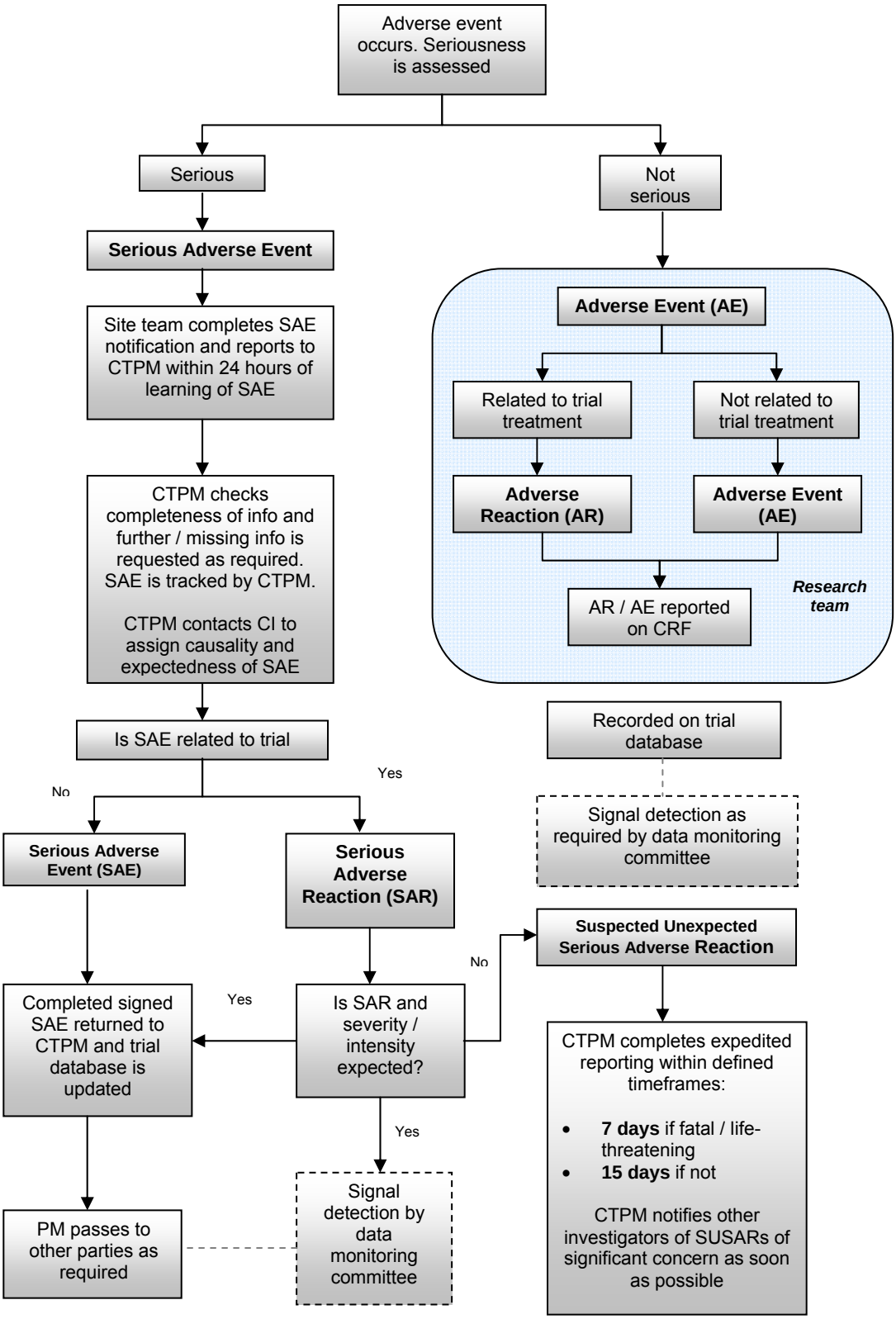
Table 12: Immune-related Response Criteria Definitions

Index Lesions	Non-index Lesions	New Lesions	% Change in Tumour Burden (including new lesions)	Overall irRC Response
CR	CR	No	-100%	IrCR
CR	Any	Any	>-100% to ≤-50%	IrPR
			>-50% to <+25%	irSD
			≥+25%	irPD
PR	Any	Any	>-100% to ≤-50%	irPR
			>-50% to <+25%	irSD
			≥+25%	irPD
SD	Any	Any	>-50% to <+25%	irSD
			≥+25%	irPD
PD	Any	Any	≥+25%	irPD
U	Any	Any	U	irUN

Reference

Wolchok *et al*. Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumours: Immune-Related Criteria. Clin Cancer Res 2009:15(23)

APPENDIX 4 – Safety Reporting



APPENDIX 5 - Details of Drugs to be used at the Christie

Equivalent supportive medication can be used at other centres or the same drugs made by other manufacturers.

Cyclophosphamide pharmaceutical information
Brand name: Endoxana
Manufacturer: Baxter Healthcare Ltd
PL number: 200mg 0116/0386
500mg 0116/0387
1g 0116/0388
For summary of product characteristics please see the Investigator Brochure.

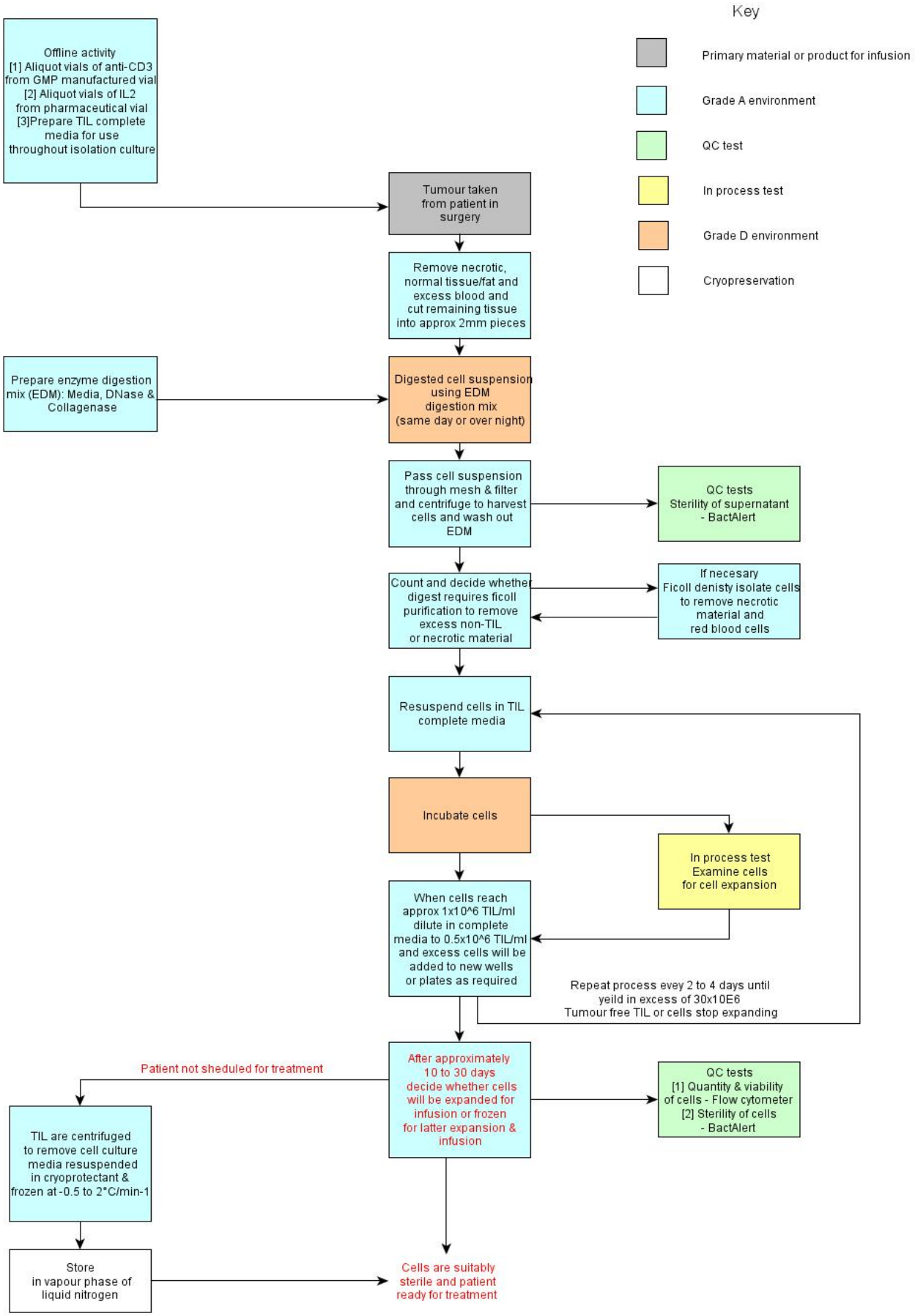
Fludarabine pharmaceutical information
Brand name: Fludara
Manufacturer: Schering Health care Ltd
PL number: 0053/0239
For summary of product characteristics please see the Investigator Brochure.

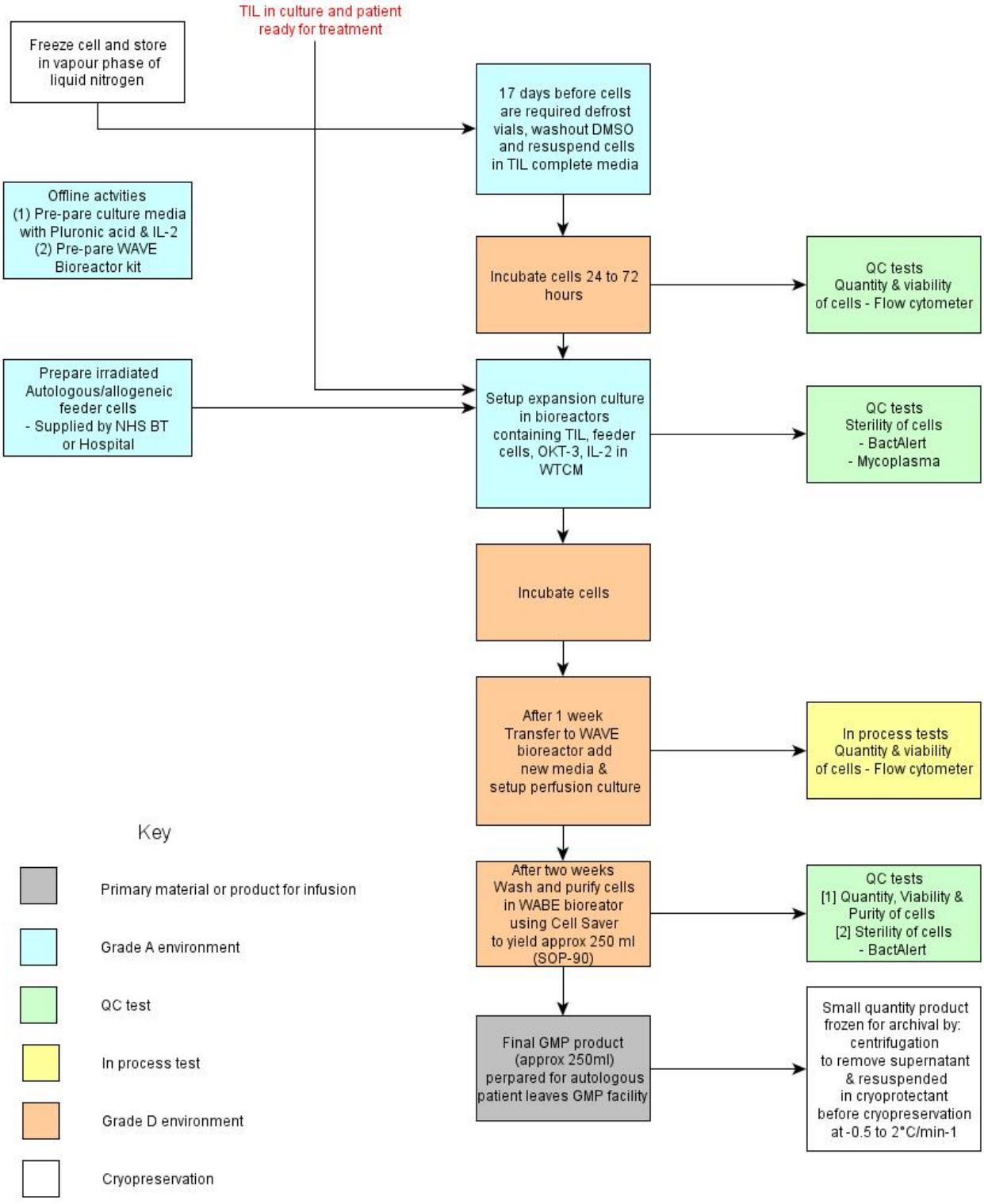
Interleukin 2 pharmaceutical information
Brand name: Proleukin
Manufacturer: Novartis Pharmaceuticals
PL number: 08800/0019A
For summary of product characteristics please see the Investigator Brochure.

APPENDIX 6 - American Heart Association Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels

Functional Capacity	Objective Assessment
Class I. Patients with cardiac disease but without resulting limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnoea, or anginal pain	**A** No objective evidence of cardiovascular disease.
Class II. Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnoea or anginal pain.	**B.** Objective evidence of minimal cardiovascular disease.
Class III. Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnoea, or anginal pain	**C.** Objective evidence of moderately severe cardiovascular disease.
Class IV. Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	**D.** Objective evidence of severe cardiovascular disease.

APPENDIX 7 – FLOW DIAGRAM OF THE TIL MANUFACTURING PROCESS




TIL in culture and patient ready for treatment
Freeze cell and store in vapour phase of liquid nitrogen
17 days before cells are required defrost vials, washout DMSO and resuspend cells in TIL complete media
Offline actvities
(1) Pre-pare culture media with Pluronic acid & IL-2
(2) Pre-pare WAVE Bioreactor kit
Incubate cells 24 to 72 hours
QC tests
Quantity & viability of cells - Flow cytometer
Prepare irradiated Autologous/allogeneic feeder cells
- Supplied by NHS BT or Hospital
Setup expansion culture in bioreactors containing TIL, feeder cells, OKT-3, IL-2 in WTCM
QC tests
Sterility of cells
- BactAlert
- Mycoplasma
Incubate cells
After 1 week
Transfer to WAVE bioreactor add new media & setup perfusion culture
In process tests
Quantity & viability of cells - Flow cytometer
After two weeks
Wash and purify cells in WABE bioreator using Cell Saver to yield approx 250 ml (SOP-90)
QC tests
[1] Quantity, Viability & Purity of cells
[2] Sterility of cells
- BactAlert
Final GMP product (approx 250ml) perpared for autologous patient leaves GMP facility
Small quantity product frozen for archival by: centrifugation to remove supernatant & resuspended in cryoprotectant before cryopreservation at -0.5 to 2°C/min-1
Key
Primary material or product for infusion
Grade A environment
QC test
In process test
Grade D environment
Cryopreservation

APPENDIX 8 -NCI Grading Definition of Cytokine Release Infusion Reactions

Grade	Definition
1	Mild reaction; no infusion interruption or intervention necessary
2	Therapy or infusion interruption but responsive to symptomatic treatment
3	Prolonged reaction, not rapidly responsive to symptomatic treatment, with possible recurrence of symptoms following initial improvement Hospitalization indicated for other clinical sequelae
4	Life-threatening; pressor or ventilatory support necessary

Based on information from National Cancer Institute (NCI), 2006.

APPENDIX 9 -CHECKLIST FOR ADMINISTERING OF TILs

Study Title: METILDA

Please complete and sign at the bottom of the sheet, keep 1 copy in patient notes and 1 copy for trial record

Date: _____

Subject Study Number: _____Subject Date of Birth: _____

	Bag Batch No:
Patient's details checked	
Doses checked against report	
Bag and seals checked and intact	
Patient wristband checked	
Time infusion commenced	
Time infusion completed	
Adverse reactions and/or other problems/ comments? **Yes/ No** If yes, please detail any action taken. Also make sure separate adverse reaction documentation is done according to trial protocol.	

ADMINISTERING STAFF

Name: _____ Signature: _____

CHECKED BY

Name: _____ Signature: _____