MSCTRAIL Treatment of Thoracic Cancer

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I, Alice Davies confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I conform that this has been indicated in the thesis

Signature:

Date: 12.5.20

Abstract

Lung cancer is the leading cause of cancer death worldwide with 5 year survival in the UK estimated at just 16%[1-3]. For patients with advanced non-small cell lung cancer treatment can offer some survival benefit but is often associated with poorly tolerated side effects.

Mesenchymal stromal cells (MSCs) possess innate characteristics which make them suited for use as a therapy, including the ability to home to and incorporate into sites of cancer [4].

TNF related apoptosis inducing ligand (TRAIL) has been shown to cause selective cancer cell death, via the extrinsic death pathway [5].

It has been demonstrated that MSCs can be transduced with a lentiviral vector to express TRAIL and that MSCTRAIL will home to and induce apoptosis of tumour cells *in vitro* and reduce tumour growth in multiple *in vivo* models[6] however it has not been trialled as a clinical therapy before.

In order to move from 'bench to bedside' a phase I/II trial was set up to establish the safety and efficacy of MSCTRAIL in combination with first line standard of care therapies for advanced adenocarcinoma of the lung- The TACTICAL trial.

TACTICAL has recruited and treated four patients and successfully delivered nine doses of MSCTRAIL to those patients.

At efficacy evaluation one patient had unconfirmed progressive disease, one has stable disease and two had partial response (by iRECIST criteria), no patients experienced dose limiting toxicities. The first three patients were incidentally found to have asymptomatic pulmonary embolisms. This led to a serious adverse event review, temporary pause to the trial and alterations to the protocol before it could re-open.

Lung cancer is a devastating disease with high mortality rates and poor treatment options. Presented here is the initial clinical work carried out to investigate if MSCTRAIL is a safe and effective treatment for metastatic adenocarcinoma of the lung.

Impact Statement

Lung cancer is the leading cause of cancer death worldwide. For advanced disease current chemo, immune or targeted therapies extend life by a number of months but can carry a heavy burden of side effects without any guarantee of benefit. This highlights the real need for novel targeted therapies. Work in this thesis proposes to test one such therapy.

Mesenchymal stromal cells have been studied extensively for a variety of diseases and treatments, however never in the treatment of lung cancer. TNF related apoptosis inducing ligand (TRAIL) has been trialled before in patients with lung cancer but a short half-life hindered its therapeutic potential [5].

It has been shown that MSCs can be transduced to express TRAIL and MSCTRAIL can kill multiple cancer types in *in vitro* and *in vivo* models [6] however it has never been tested in the clinical setting before.

This work will trial the use of a novel therapy in humans for the first time. It proposes to translate the pre-clinical work into the clinic and test a novel therapy for this devastating and life-limiting condition.

The implications of the results of this early phase work could lead to larger scale trials, commercialisation and ultimately a new, targeted treatment. Furthermore, it may open the door for the use of this therapy in other cancer groups such as malignant mesothelioma- a rare, incurable cancer. It will also pave the way and form the basis for wider translational work looking at the journey and fate of third-party allogeneic cells after intravenous administration. This will not only impact MSCTRAIL therapy but the future of all cellular therapies, broadening our understanding and allowing for optimisation of the use and delivery of cellular therapies.

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Dedication

I dedicate this thesis to my Dad, for his love and belief in me and for everything he should have been part of- *Er Cof*.

List of Abbreviations and Definitions

AE	Adverse event
ALK	Anaplastic lymphoma kinase
ARDS	Acute respiratory distress syndrome
ATIMP	Advanced Therapy Investigational Medicinal Product
BM-MSCs	Bone marrow derived mesenchymal stromal cells
CCGTT	Centre for Cell, Gene and Tissue Therapeutics
CK18	Cytokeratin 18
CRF	Clinical Research Facility
CMV	Cytomegalovirus
COPD	Chronic obstructive pulmonary disease
CT	Computer Tomography Scan
CTCAE	Common Terminology Criteria for Adverse Events
DISC	Death-inducing signalling complex
DLT	Dose Limiting Toxicity
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPBS	Dulbecco's phosphate buffered saline
EBUS	Endobronchial ultrasound
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal growth factor receptor
ESMO	European Society of Medical Oncology
FADD	FAS-activated death domain
FACS	Fluorescence-activated cell sorting
FBC	Full blood count
GCP	Good Clinical Practise
GDPR	General Data Protection Regulations

GMP	Good manufacturing practice
GvHD	Graft vs host disease
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HRA	Health Research Authority
IDMC	Independent Data Monitoring Group
IHC	Immunohistochemistry
IMPD	Investigational Medicinal Product
iRECIST	Response Evaluation Criteria in Solid Tumours for Immunotherapy
IVIS	In vivo imaging system
Luc	Luciferase
MHC	Major histocompatibility complex
MHRA	Medicines Healthcare Regulatory Authority
MOI	Multiplicity of infection
MPM	Malignant pleural mesothelioma
MRC	Medical Research Council
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal stromal cells
MSCTRAIL	MSCs modified to produce TRAIL
NICE	National Institute for Health Care and Excellence
NSCLC	Non-small cell lung cancer
OS	Overall survival
PET	Positron Emission Tomography
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate-buffered saline
PD	Progressive Disease
PD-L	Programmed cell death ligand

PFS	Progression Free Survival
PI	Principal Investigator
PR	Partial Response
PSS	Primary Seed Stock
QP	Qualified person
REC	Research Ethics Committee
RECIST	Response Evaluation Criteria in Solid Tumours
RFH	Royal Free Hospital
RNA	Ribonucleic acid
RP2D	Recommended phase 2 dose
rhTRAIL	Recombinant TRAIL
SAE	Serious adverse event
SCLC	Small cell lung cancer
SD	Stable Disease
SOC	Standard of Care
SIN	Self-inactivating (vectors)
TKI	Tyrosine kinase inhibitors
TL	Target Lesions
TMG	Trial management group
TNF	Tumour necrosis factor
TRAIL	TNF-related apoptosis inducing ligand (also known as APO2 ligand)
TTF	Transcription Termination Factor 1
UCL	University College London
UCL CTC	CR UK and UCL Cancer Trials Centre
UCT	Umbilical Cord Tissue
UCT-MSCs	Umbilical Cord derived mesenchymal stromal cells
WCS	Working cell stock

WOCBP	Woman of childbearing potential

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1 Introduction

Background

Lung cancer is the leading cause of cancer death both in UK and worldwide. The World Health Organization (WHO) estimates that it is the cause of 1.8 million deaths globally per year [2] with smoking responsible for over 80% [7]. In the UK alone 97 people die every day due to lung cancer and it carries a 10 year survival from diagnosis of just 9%[3].

The current treatments for lung cancer rely on a combination of surgery, radiotherapy, chemotherapy and newer targeted and immunotherapies. Despite recent advances these treatments can carry a heavy burden of toxic side effects with often only limited prolongation of life [8].

The global burden of cancer and cancer treatment is considerable and ever expanding; lung cancer remains one of the most accountable and because of this we must address the need for a novel effective treatment. A potential therapeutic option is MSCTRAIL, a cell and gene therapy that combines the tumour tropism of mesenchymal stromal cells (MSCs) with the selective anti-cancer properties of TNF related apoptosis inducing ligand (TRAIL). It has been shown that MSCs can be transduced with lentiviral vector to express TRAIL and that this product can home to and induce apoptosis *in vitro* and *in vivo* in lung metastasis in a murine model[6].

This thesis describes the set-up, challenges, implementation and early data from the TACTICAL trial, a first-in-human study using MSCTRAIL in patients with metastatic lung cancer. It aims to examine the safety and efficacy of a novel agent in clinical practise. The thesis will then go on to expand on some of the future work this project may lead to.

Lung Cancer

1.1.1 Classification

Lung cancer can be broadly divided histologically into small cell (SCLC) and non-small cell (NSCLC) with NSCLC accounting for 80%–90% of diagnosed lung cancers [9]. NSCLC can then be subdivided further based on histological findings into squamous and the more common adenocarcinoma [10]. The work in this thesis will focus primarily on adenocarcinoma.

Histological diagnosis, molecular characterisation and staging are vital for guiding treatment decisions as well as providing an indication to the likely natural history and behaviour of the tumour.

Diagnosis is a multi-step process and should be based on the WHO classification [11]. It begins with determining the histology by morphological diagnosis, this is then refined by immunohistochemistry (IHC) and the appropriate molecular characterisation.

IHC markers such as p63, p40 and cytokeratin CK 5/6 are associated with squamous cell carcinomas, while TTF1, Napsin A and CK7, as well as mucin stains, are associated with adenocarcinomas [9]. The sample can also be screened for activating driver mutations; epidermal growth factor receptor gene (EGFR), EML4-ALK and ROS1 fusion rearrangements as well as tumour cell expression of programmed death receptor 1 (PD-L1), which help guide final treatment decisions.

1.1.1.1 Adenocarcinoma of the lung

Adenocarcinoma is the most prevalent type of lung cancer, currently accounting for around 50% of all those diagnosed histologically[12]. It is derived from epithelial cells that have differentiated into glandular or mucin-producing tissue, and is typically TTF1 positive.While it is recognised it can be driven by smoking, the influence of tobacco smoke on the risk of adenocarcinoma is not considered as great as that of the other two major histological sub-types [13]. This may explain why its prevalence

has come to the forefront in recent years, as the proportion of ‘never smokers’ diagnosed with lung cancer increases. Radiologically, adenocarcinoma is more commonly seen in the periphery of the lungs, whereas squamous cell lung cancers are characterised by lesions arising from the airways..

Lung adenocarcinoma has five histologic patterns (acinar, papillary, micropapillary, lepidic, solid); with mucinous and non-mucinous subtypes. Through this it can represent a wide spectrum of disease ranging from preinvasive to metastatic invasive adenocarcinoma and identifying those in which the lesion is likely to remain early, indolent and slow growing, compared to those which rapidly spread and metastasise remains a key to curative treatment and improving survival rates.

Adenocarcinoma is thought to have a higher mutational status than other lung cancers due to considerable molecular heterogeneity, as demonstrated by genome-wide sequencing studies [14]. It often displays multiple somatic mutations in crucial signalling pathways that can be correlated to clinical characteristics of the patients [15]. A number of mutations are thought to be clinically relevant but there is still a considerable body of work to be done to identify not only further mutations, but those that can be targeted with effective therapies.

1.1.2 Staging

NSCLC is staged using the International Association for the Study of Lung Cancer’s (IASLC) ‘TNM’ system. ‘T’ represents the tumour size and ranges from T0 to T4, ‘N’ represents nodal spread of disease N0 to N3 and ‘M’ metastatic spread M0 to M1c (*table 1.1*). From these the final stage of the disease can be given (*table 1.2*).

TNM 8TH – Staging characteristics
T1 **≤3cm surrounded by lung/visceral pleural, not invading main bronchus** T1a ≤ 1cm T1b > 1cm to ≤ 2cm T1c >2 cm to ≤ 3cm
T2 **>3cm to ≤ 5cm** or involving main bronchus without carina or invasion or visceral pleural or atelectasis or post obstructive pneumonitis extending to hilum T2a >3cm to ≤ 4cm T2b >4m to ≤ 5cm
T3 **>5cm to ≤ 7cm in greatest diameter** or tumour of any size that involves chest wall, pericardium, phrenic nerve or satellite nodules in the same lobe
T4 **>7cm in greatest dimension** or invasion of mediastinum, diaphragm, heart, great vessels, recurrent laryngeal nerve, carina, trachea, oesophagus, spine or separate tumour in ipsilateral lung.
N N1 Ipsilateral peribronchial and/or hilar nodes and intrapulmonary nodes N2 Ipsilateral mediastinal and/or subcarinal nodes N3 Contralateral mediastinal or hilar; ipsilateral/contralateral/ supraclavicular
M1 distal metastasis M1a Tumour in contralateral lung and/or pleural/pericardial, nodule/effusion M1b Single extra thoracic metastasis, including single regional lymph node M1c Multiple extra thoracic metastases in one or more organs.

Table 1.1: IASCL TMN 8 Staging for Tumour Size

	N0	N1	N2	N3
T1	IA	IIB	IIIA	IIIB
T2A	IB	IIB	IIIA	IIIB
T2B	IIA	IIB	IIIA	IIIB
T3	IIB	IIIA	IIIB	IIIC
T4	IIIA	IIIA	IIIB	IIIC
M1A	IVA	IVA	IVA	IVA
M1B	IVA	IVB	IVA	IVA
M1C	IVB	IVB	IVB	IVB

Table 1.2: Stage grouping for 8th edition TNM staging

Stages shaded in yellow represent advanced disease and those eligible for the TACTICAL trial

1.1.3 Treatment of NSCLC

Treatment decisions should be based on detailed locoregional staging according to the 8th TNM staging system, the pathological status of the tumour as well as the cardiopulmonary fitness of the patient and, crucially, their wishes and treatment expectations.

Informed decision making is vital in tailoring treatment options for patients. Appropriate time, in conjunction with a nurse specialist, should be allowed to ensure comprehension for both the patient and their chosen family or carers before treatment paths are chosen.

For any stage or type of lung cancer, smoking cessation through recognised programmes should be encouraged whilst avoiding the patient feeling stigmatised or at fault. Benefit and improved outcomes have been seen in cessation even in advanced disease [16, 17].

Final Treatment decisions should be discussed within a multidisciplinary meeting. Appropriate treatment options should be recommended after evaluation of the results of investigations carried out in conjunction with the known performance status (PS) of the patient.

The Eastern Co-operative Oncology Group (ECOG) performance score is a measure of the patient's functional status and hence their ability to tolerate therapies and is used to further guide final treatment decisions (*table 1.3*).

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

Table 1.3: Eastern Co-operative Oncology Group (ECOG) Performance Status

Universal staging and diagnostic criteria ensure homogeneity of care through clinicians and trusts. For early stage lung cancer, i.e. stage I-II, surgery is the preferred treatment option. However if a patient is not fit enough, declines surgery, or if the tumour is thought to be inoperable, radiotherapy may be an option, either with stereotactic radiotherapy (SABR) or hypo-fractionated high dose radiotherapy [18]. For locally advanced stage III disease, a combination of surgery and chemotherapy (including targeted therapies) or radiotherapy with curative intent should be offered. More recently, following the PACIFIC trial [19] chemoradiotherapy with or without the immunotherapeutic Durvalumab has become licenced for stage III disease.

For advanced stage, metastatic NSCLC (stage IIIB-IV), the European Society of Medical Oncology (ESMO) and the National Institute for Health Care and Excellence (NICE) recommend that systemic treatment should be offered to anyone with an ECOG performance score 0-2 [20].

Chemotherapy has been the mainstay of treatment for many years until the development of therapies targeted towards driver oncogene mutations and, more recently, the introduction of immune therapy, specifically immune checkpoint inhibitors. Yet these therapies still often only extend life expectancy without offering cure, highlighting the need for novel effective therapies [8].

Below details the treatment landscape for advanced NSCLC at the start of this work. Just prior to opening the trial ESMO and NICE licensed the use of the immunotherapy, Pembrolizumab, in the first lining both as monotherapy and in combination with chemotherapy. Figure 3.2 describes that updated current treatment algorithm for advance non squamous NSCLC without actionable driver mutations as edited from NICE guidelines.

This change, the rationale and implications to both patients and the trial are discussed in *Chapter 3: Changing Paradigm of NSCLC Treatment.*

1.1.3.1 Chemotherapy

ESMO guidelines recommend dual agent chemotherapy with a platinum-based regime e.g. Cisplatin with Pemetrexed [21] as first line therapy for non-squamous NSCLC. These therapies carry a heavy burden of side effects and standard response rates (complete or partial) in patients with advanced NSCLC who receive these regimes are only in the region of about 25% [22, 23]. Meta-analysis showed the benefit over best supportive care, with a 23% reduction of risk of death, a 1 year survival gain of 9% and a 1.5 month absolute increase in median survival [24]. Two agents were found to be superior over 1 or 3 [25]. 4 treatment cycles are recommended followed by a less toxic maintenance monotherapy with 6 treatment cycles shown to be more toxic without survival gains.

Chemotherapy induces cell death via the intrinsic apoptotic pathway. This leads to DNA damage in cells which is in turn recognised by proteins such as P53. P53, through the signally gene BAX/BAK, makes the mitochondrial membrane permeable, enabling it to release cytochrome c (cyt-c) which activates caspase-9 through apoptotic protease-activating factor 1 (APAF-1). Caspase-9 in turn activates the effector caspases to carry out the function of apoptosis.

Chemotherapy does not distinguish rapidly dividing healthy cells from cancer cells effectively. This often leads to significant toxic side effects, which limits the delivery of higher doses as well as increases the associated patient morbidity and a reduction in quality of life.

1.1.3.2 Targeted Therapies

Research over the past decade has identified driver oncogene mutations within lung cancer cells. These acquired mutations can be responsible for the initiation and/or maintenance of the cancer [26]. Their presence confers growth advantage and promotes rapid tumor progression by activating tyrosine kinase receptors, disrupting the normal process of cell division [27]. Blocking these oncogene pathways, with targeted therapies such as tyrosine kinase inhibitors, halts tumour cell progression resulting in cell apoptosis. It is currently recommended that NSCLCs are screened for epidermal growth factor receptor tyrosine kinase (EGFR-TK) mutations,

echinoderm microtubule-associated protein-like 4 anaplastic lymphoma kinase gene re arrangement (EML40-ALK) and ROS1 mutations. Serine/threonine-protein kinase b-raf (BRAF) has also recently been identified as a target for therapy and while routinely screened for in the US and much of Europe is yet to be introduced in the UK. There is an ever-expanding cohort of identified oncogenic driver alterations in NSCLC, ranging in both incidence, targetability and relevance between adenocarcinoma and squamous cell cancer. Currently mutations for which therapies are being investigated for include, but are not restricted to, *RET* rearrangements, neurotrophic tyrosine kinase (NTRK) fusions loss of neurofibromin 1 (NF1), hepatocyte growth factor receptor (MET) exon 14 mutations, human epidermal growth factor receptor 2 (HER2) mutations, KRAS mutations and neurofibromin 1 (NF1) loss [28]

These mutations are only seen in a fraction of NSCLC; EGFR mutations occur in around 10-15% of a Caucasian population with lung adenocarcinoma but are much higher in Asian populations [9] and several therapies targeting mutations in exons 18-21 of EGFR are now licensed [29]. Fusion genes involving ALK, for which the most common is EML4, are seen in 2%–5% of lung adenocarcinoma and ROS1 fusion gene mutations, also with differing gene partners, are seen in 1%–4% [30].

A number of pharmaceutical tyrosine kinase inhibitors are available and used in everyday practice. These therapies, in the presence of relevant driver mutations, have high response rates and confer survival benefit [21], however all cancers eventually develop resistance to these agents. This resistance can be intrinsic, adaptive or acquired [31]. Intrinsic resistance relates to a failure to respond due to insensitivity to the chosen treatment, such as in *EGFR* exon 20 with an impaired apoptotic response to EGFR TKI therapy [32]. Adaptive changes are seen when the tumour undergoes early mutation or change which allow survival. Acquired resistance is where the tumour cell develops new alterations as a result of the selective pressure of therapy [28]. These changes may either be at the target site which the primary drug is acting upon, or ‘off-target’ at collateral or downstream sites. Work is ongoing to identify mechanisms for overcoming these resistances, and treatment strategies are being developed to ensure more prolonged progression-free survival.

Figure 1.1 below demonstrates some of the known driver mutations in adenocarcinoma and squamous cell lung cancer. While many driver mutations have been identified, there is still a large number of these mutations for which we do not screen, either because their relevance is not yet known, or because targeted therapies are not available.

The difference in the number of recognised mutations between adenocarcinoma and squamous cell should also be noted. This may be due to adenocarcinomas' 'higher mutational status' as discussed in S*ection 1.2.1.1: Adenocarcinoma of the lung.*

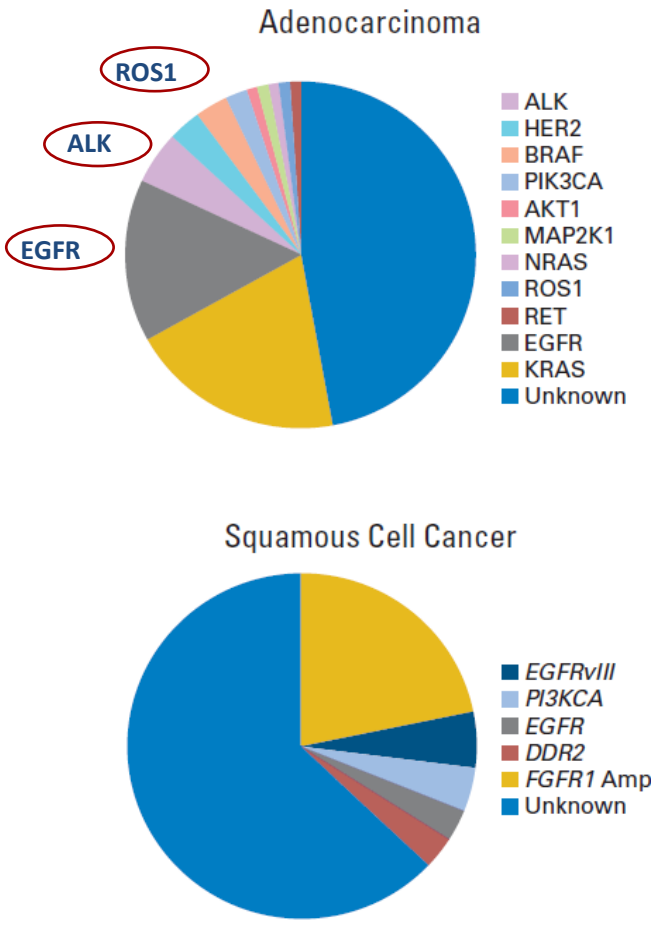


Figure 1.1: : Molecular base 'driver mutations' in NSCLC. [33]

1.1.3.3 Immune check point inhibitors

Immune therapy utilises the patient's own immune system to recognise and destroy cancer cells. Immune checkpoint inhibitors target the pathways that are exploited by tumours to evade recognition and hence destruction [34]. They achieve this via T cell modulation [35], disrupting the physiological balance between receptors that activate and inhibit the immune system. The development of these therapies has heralded a milestone in the treatment of lung cancers, which were previously thought to be poorly immunogenic.

Since starting this work the immune check point inhibitor Pembrolizumab has been licensed by NICE and is recommended by ESMO[36] in the first line setting for non-squamous NSCLC patient in combination with chemotherapy if the tumour PD-L1 expression is >1% or as a monotherapy if <1%.

Further details on immune check point therapy, the changing first line therapy and implications on the trial can be found in *Chapter 3: Changing Paradigm of NSCLC Treatment.*

Mesenchymal Stromal Cells

Mesenchymal stromal cells (MSCs) were first described by Friedenstein et al in the 1970s [37] and since then there has been a rapid advancement in knowledge about them and their subsequent therapeutic utility.

MSCs are undifferentiated cells that possess the ability of unlimited self-renewal as well as the capacity to differentiate into other more specialised cells. This multipotent characteristic is unique and not seen in any other mature cells [38]. The International Society for Cellular Therapy (ISCT) set out standards to define MSCs as being; 'adherent to tissue culture plastic under standard culture conditions, expressing the cell surface markers CD105, CD73 and CD90 and lacking expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules. In addition, they must be capable of differentiating into adipocytes, osteoblasts and chondroblasts under standard *in vivo* differentiating conditions' [39].

MSCs were originally identified in bone marrow but have since been isolated from multiple sources including amniotic fluid, umbilical cord, adipose, and muscle tissue, and dental pulp [40-43].

MSCs possess five key characteristics which make them ideal for use in therapeutics [4]:

- The potential to be readily and easily harvested from patients
- A high capacity for proliferation: MSCs can be cultured and expanded for numerous passages while still retaining their characteristics [44]
- Ease of manipulation, allowing modification using viral vectors
- An ability to home to and integrate into host target tissues or cancer
- Immune privilege: MSCs express the major histocompatibility complex class I (MHC class I) but lack MHC class II and the co-stimulatory molecules CD80, CD40 and CD86 [45] allowing injection into immunocompetent patients without donor matching [46]. This allows for MSCs to be given as an 'off the shelf' allogeneic product. A large biobank of cell-based therapy can be made, stored and given to any patient, reducing patient morbidity and therapy costs.

These characteristics combined with the inherent properties of unlimited self-renewal and differentiation potential make them ideal vehicles for targeted therapies.

1.1.4 Umbilical cord derived MSCs

As discussed above MSCs can be harvested from multiple sites, one such site is the umbilical cord after birth. Umbilical cord derived MSCs (UCT-MSCs) are phenotypically comparable to bone marrow derived MSCs (BM-MSCs) and meet all International Society for Cellular Therapy (ISCT) criteria for MSC characterisation[47]. They are harvested from the perivascular and intervascular region of the umbilical cord tissues within Wharton's jelly[48]. As the umbilical cord is discarded postnatally the collection of cells can be easily carried out and without ethical conflict[49], it also does not require invasive procedure which gives them an advantage over BM derived MSCs. They are foetal derived and as a result some consider them to possess multipotent properties between embryonic stem cells and adult stem cells including a relatively higher rate of proliferation and self-renewal[50]. Further details on the work up and characterisation of UCT-MSCs can be seen in section 2.1.16.

1.1.5 MSC homing

MSCs possess the unique ability to home to as well as incorporate into tumours, termed tumour tropism or to sites of cellular injury [51].

Tumour tropism was first demonstrated by Nakamizo et al who, in 2005, showed SP-DiI–labelled human MSCs (hMSC) when injected into the left carotid homed to and engrafted into a right hemisphere glioma (*figure 1.2*) [52]

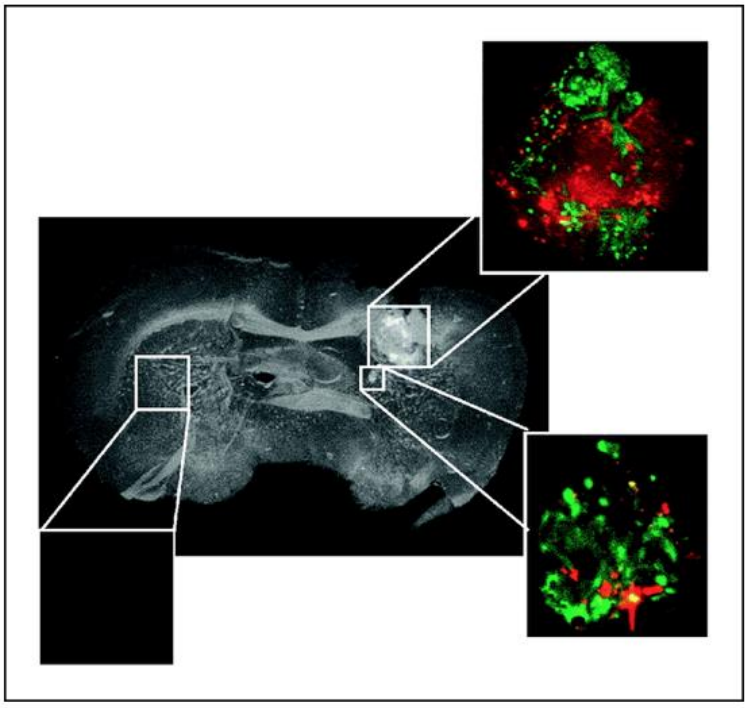


Figure 1.2: MSCs engraftment in a glioma model

Photomicrograph of section of brain showing hMSC (appearing red) selectively engrafting into a glioma (green) in the right hemisphere after injection into left sided carotid but not in normal brain tissue.

The concept of tumour tropism has been widely published [53] but the mechanism of migration is not fully understood. It is recognised that the tumour microenvironment secretes chemokines, cytokines, extra cellular matrix proteins and soluble tumour-driven factors such as SDF-1, TNFα and interleukin [54, 55],

simulating MSCs to home to and expresses conjugate receptors at the site of tumour [56].

The most extensively studied axis is that of CXCR4/SDF1 and the non-cognate, high affinity ligand CXCL12(MIF). Studies have shown that over-expression of CXCR4 on MSCs leads to increased MSC migration [57]. However knockdown of CXCR4 in experiments did not always mitigate MSC homing capacity [58], suggesting that this is not the only receptor responsible for homing.

It is this property, combined with ease of manipulation, that allows MSCs to be utilised as a 'Trojan horse', homing towards tumour cells to deliver anti-cancer therapies.

1.1.6 Recent clinical experience of MSCs

There are currently nearly 9000 clinical trials using MSCs registered on the National Institutes of Health clinical trials database (accessed February 2020) in the fields of immunomodulation and regeneration, as well as pharmaceutical vehicles for transporting therapeutics. The overarching outcome from all completed trials in MSCs is that they are safe, with no attributable dose limiting toxicities (DLTs).

MSCs have been shown to reduce inflammation by secreting paracrine factors including anti-inflammatory cytokines. Additionally, they are capable of transferring mitochondria to injured epithelial cells [59], which in pre-clinical trials has resulted in immunomodulation. Clinical trials utilising these properties include adult respiratory distress syndrome ARDS [59-61], sepsis [62], chronic obstructive pulmonary disease (COPD) [63] [64], and graft *vs* host disease (GVHD) [65] [66] [67]. The largest cohort of patient studies is in GVDH where over 200 patients have now been treated with MSCs with no reported significant MSC-related toxicities and positive disease-free outcomes.

MSCs have been studied in repair and regeneration, utilising both their anti-inflammatory immune modulatory properties as well as differentiation potential. Two such areas are osteoarthritis, a disease characterized by degeneration of the articular cartilage and, ultimately, joint destruction, and congestive cardiac failure where ischemia and infarction lead to myocyte death and subsequent fibrosis. MSCs have

also been trialed post-myocardial infarction [68-71] when chronic ischemic cardiomyopathy has been established [71] [72]. These trials have utilised allogeneic and autologous[73] bone marrow [74] and adipose-derived cells, harnessing capabilities of neo-angiogenesis for repair and regeneration. Results have not only shown MSCs to be safe but there have been promising results, especially in enhancing myocardial contractility.

1.1.7 MSCs in Clinical Cancer Trials

Treating advanced malignancy is challenging as dissemination and metastases render it a multi-organ disease. Many agents have been identified as attractive anti-cancer therapies for systemic delivery, however efficacy has been limited by short half-lives, often meaning high concentrations would be required for therapeutic effect, leading to toxic side effects. Delivering the agents directly to the tumour and providing long term stable delivery of that agent could ensure low doses and direct targeted anti-cancer effects [75]. By genetically modifying MSCs and harnessing their innate homing properties they can act as transporters for these anticancer agents. MSCs can not only transport these molecules to the site of disease but also protect them from inactivation via immune modulation mechanisms.

There are various methods of genetic modification. They can be broadly divided into viral and non-viral and are discussed in *Section 1.5: Lentiviral Vectors*.

Pre-clinical work has investigated using MSCs from a variety of sources, transfection methods for gene delivery, anti-cancer agents and tumor models [75]. Examples of these include human MSCs (h-MSCs) transduced to express INF-β for the treatment of melanoma, breast cancer [76] and glioma [52], MSCs modified using IL-12[77] to target cell proliferation in breast cancer, targeting angiogenesis with VEGFR-1[78], pigment epithelium-derived factor (PEDF) [79] or nitric oxide synthase [80] and through apoptotic pathways [51] or suicide genes [81]. Despite variations in methodology these trials have shown promising outcomes with strong safety data.

Even with this wealth of pre-clinical data there is a paucity of clinical trials and efficacy data in their use. Table 1.4 summarises the previous and ongoing clinical trials using genetically modified MSCs for the treatment of cancer. The overriding outcome of

these trials is that MSCs are safe with no dose limiting toxicities. There have been no trials to date combining MSCs with TRAIL.

Trial Team	Trial Design	Cancer Group	Cell Type	Therapy	Toxicity	Efficacy	Ref
Castro et al	Exploratory Compassionate IV delivery	Refractory Neuroblastoma	Autologous MSCs	ICOVIR-5 Engineered oncolytic adenovirus	Well tolerated, no DLTs.	1 patient showed complete remission at 3 years	[82]
Kim et al GX-051	Phase I, single centre, dose escalation Intratumoral delivery	Advanced head and neck	Allogenic BM-MSCs	Modified interleukin 12	Completed Phase I, safe. Moving to phase II		NCT 02079324
Olson et al	Phase I dose escalation Intraperitoneal delivery	Advanced ovarian	Allogenic BM-MSCs male donors only	Interferon beta	Completed phase I		NCT 02530047
Galanis et al,	Phase I/II Intraperitoneal delivery	Recurrent Ovarian	Adipose tissue derived MCSs	Oncolytic measles virus encoding thyroidal sodium iodide symporter (MV-NIS)	Recruiting, est complete 2021		NCT 02068794
TREAT-ME1 Apceth,	Phase I/II	Recurrent, metastatic gastrointestinal/ heptaopancreatobilary	Bone marrow derived MSCs	Suicide gene HSV-TK with pro drug gancyclovir	Phase I complete, feasible and well tolerated, moving to Phase II		[81, 83]

Table 1.4: Clinical trials using genetically modified MSCs in the treatment of cancer.

Most notable of these trials in cancer is the TREAT-ME1 trial, a first in class, phase I/II trial in patients with gastro-intestinal tumours. This trial utilised autologous human MSCs genetically modified to express Herpes-simplex-virus thymidine kinase

(HSVTK) working as a suicide gene, in combination with Ganciclovir, in a dose escalation study. Phase I is now complete and results show that these genetically modified MSCs were safe and tolerable, although there was no RECIST defined responders [83]. Phase II is in the recruitment stage.

TNF Related Apoptosis Inducing Ligand

TNF related apoptosis inducing ligand (TRAIL) or APO2 ligand is a type II transmembrane protein of the TNF death ligand superfamily. It has been found to cause selective apoptosis in cancer cells leaving healthy cells ineffective.

TRAIL is expressed in a variety of normal tissues, such as the placenta, kidney, and spleen. While the physiological function of TRAIL is not fully understood, it is believed to play a role in the control of autoreactive immune cells and immune surveillance [84].

1.1.8 Apoptosis

TRAIL induces apoptosis through use of death receptors expressed on the cell surface, via the extrinsic death pathway (*figure 1.3*). This contrasts with chemotherapy or radiotherapy which utilise the intrinsic death pathway.

TRAIL binds to one of five TRAIL receptors, TRAIL-R1 (DR4), TRAIL-R2 (DR5) TRAIL-R3/DcR1, TRAIL-R4/DcR2, and osteoprotegerin (OPG) [56, 85]. However only TRAIL-R1 and TRAIL-R2 are agonistic receptors, able to transmit the signal into the cell once TRAIL binds to the cell membrane. TRAIL-R3, TRAIL-R4 are able to bind to the ligand, however they lack the intracellular cytoplasmic domain, rendering them decoy receptors, unable to mediate the death signals. OPG is a low affinity receptor for TRAIL [86].

Once bound to these receptors TRAIL trimerises activating intracellular receptors, allowing binding of the adaptive molecule and recruitment of the FAS-activated death domain (FADD) to the intracellular death domain of the trimerised receptor. Procaspase 8 and 10, cellular FLICE-like inhibitory protein (cFLIP) and others form a

death-inducing signalling complex (DISC). Procaspase 8 and 10 are cleaved to form the active Caspase-8 and 10 in the DISC which in turn induces downstream activation of caspase cascade (caspase 3,6 and 7) and cellular apoptosis [84, 86].

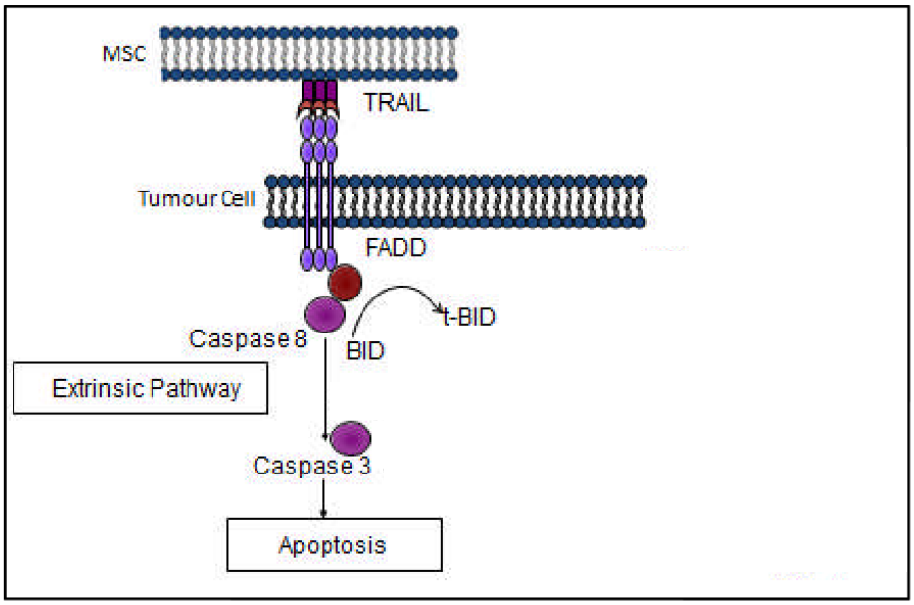


Figure 1.3: TRAIL induces apoptosis via the extrinsic pathway.

What makes TRAIL particularly noteworthy in cancer treatment is that it only induces apoptosis in transformed cancer cells with little or no toxicity to untransformed, 'healthy' cells[85], resulting in few 'off-target' adverse effects. The exact mechanism for this has yet to be well characterised; one theory is that untransformed cells express only decoy receptors while transformed cells express TRAIL-R1 (DR4) and TRAIL-R1 (DR5) [87]. However, others believe that the selective cytotoxicity occurs intracellularly suggesting it is a multifactorial mechanism.

TRAIL has been examined in two forms, recombinant TRAIL (rTRAIL) and monoclonal antibodies to TRAIL receptors. Therapeutic efficacy has been demonstrated in pre-clinical models of mesothelioma [88], lung cancer [6], breast cancer [89], myeloma [90] and glioma [91].

1.1.9 Previous Clinical Trials using TRAIL

The only recombinant form of TRAIL developed to date for clinical application is Dulanermin (also known as AMG-951). It constitutes the amino acids 114–281 of the extracellular domain of human TRAIL but lacks the N-terminal amino acids which help to anchor the human TRAIL to the cell membrane.

There have also been clinical trials using agonistic monoclonal antibodies to TRAIL receptors; these specifically target either TRAIL receptor 1 or TRAIL receptor 2.

These trials summarised below *(table 1.5 and 1.6*) have all shown excellent safety data with no DLTs attributed to TRAIL, however there has been less promising outcome data on tumour response.

Lack of efficacy may be due to a number of reasons; firstly the short half-life of rTRAIL, which is approximately 32 minutes *in vivo* [5] meaning repeated or high doses of rTRAIL would need to be administered to ensure prolonged therapeutic effect [92]. Even then the short half-life may not allow for a prolonged effect and high doses may result in toxicities. Secondly rTRAIL binds TRAIL-R3&R4 which, as discussed above, are thought to be decoy receptors thereby reducing its efficacy. Monoclonal antibodies to TRAIL overcome this by binding to TRAIL-R1 or R2 however their bivalent mode of receptor binding results in inefficient trimerisation which is required to activate the apoptotic pathway. Furthermore, they are specific to either TRAIL-R1 or R2 but not both. Cancer cells specifically express either TRAIL-R1 or R2 and if these are not matched then again there will be insufficient binding leading to reduced therapeutic effect

By modifying MSCs to express TRAIL many of these issues can be overcome. MSCs can act as vehicles, preferentially homing towards and incorporating into tumour stroma [93-95], delivering TRAIL directly to the site and providing sustained expression [96].

MSCTRAIL expresses full length human TRAIL on the cell surface which has been found to be superior to soluble, cytoplasmic expressed TRAIL at inducing cancer cell apoptosis [97]. This full-length trail targets both TRAIL receptors 1 and 2 which results in enhanced trimerisation and effective apoptosis.

Tumour Type	TRAIL form	Toxicity	Efficacy	Ref
Locally advanced recurrent or metastatic colorectal cancer	TRAIL-R2 agonist	No DLTs	Recommended phase II treatment dose. 2 patients had partial response	[98]
Solid tumours	Monoclonal antibody against TRAIL-R2	Well tolerated	Maximum dose identified. Some clinical activity in paediatric solid tumours 5 patients had stable disease	[99]
Advanced solid tumours	TRAIL-R2 antibody	Well tolerated 3 grade 3 DLTs at higher dose		[100]
Advanced NSCLC	TRAIL-R2 agonist	Well tolerated	Addition of conatumumab did not improve outcomes	[101]
Stage IIIb/IV NSCLC	TRAIL-R1 agonist	Well tolerated,	No improvement in response or disease control rates from addition of mapatumumab	[102]
Unresectable or metastatic pancreatic cancer	TRAIL-R2	Well tolerated		[103]

Table 1.5: Clinical trials utilising monoclonal antibodies to TRAIL receptors.

Tumour Type	Toxicity	Efficacy	Ref
Advanced solid tumours or non-Hodgkin lymphoma	2 SAEs	2 patients experienced durable partial response	[104]
NSCLC stage IIIb/IV or recurrent	Well tolerated. No occurrence of DLTs.	Anti- tumour activity was demonstrated 1 complete response, 13 partial response	[105]
Locally advanced, recurrent or metastatic colorectal	Well tolerated No DLTs		[106]
Advanced or recurrent NSCLC	Well tolerated	Addition of Dulanerim (rTRAIL) did not improve outcome	[107]
Relapsed B-cell non Hodgkin lymphoma	Well tolerated, no DLTs	Addition of Dulanerim (rTRAIL) did not lead to increased objective response. Study terminated early due to absence of efficacy in combination group	[108]
Relapsed or refractory multiple myeloma		Circulating permutated TRAIL single agent can elicit a response. Including 1/27 patients with near complete response	[109]
Relapsed or refractory multiple myeloma		Circulating permutated TRAIL plus TD improved overall response rate compared to CPT alone	[110]

Table 1.6: Clinical trials utilising rTRAIL

Lentiviral Vectors

There are a variety of methods used to genetically modify MSCs, these can be broadly divided into viral and non-viral.

Non-viral methods utilise physical and chemical methods of gene delivery [75]. The gene must either temporarily puncture the cell wall to gain access or use atonic lipids or polymers which form negatively charged particles that are taken up into the cell by endocytosis [75]. While this allows delivery of larger transgenes at a great scale, reducing the cost of manufacturing, there are a number of drawbacks, namely low transfection efficiencies and transient gene expression [111]

Viral vectors have the innate ability to invade a host cell wall and gain entry to the nucleus providing prolonged expression of the viral genome. Adenovirus, adeno-associated virus, retrovirus and lentivirus have all been trialled as forms of viral vectors to differing degrees of success.

MSCTRAIL consist of MSCs transduced with a lentiviral vector to express TRAIL. A lentiviral vector was used because it provided long term, stable gene expression. Thus, providing prolonged and reliable therapeutic effect once delivered to the target.

Lentiviruses are subtypes of retroviruses, they contain an RNA genome that is converted to DNA in the transduced cell by virally encoded reverse transcriptase [112].

The first clinical trial to use lentiviral vectors was in 2003. In this study, CD4+ lymphocytes from HIV-1 infected patients were harvested and transduced *ex vivo* with a lentiviral vector expressing an anti-sense gene against the HIV-1 envelope protein. There was no evidence of development of replication competent vector-derived HIV-1 and no evidence of insertional mutagenesis up to 3 years after administration [113].

Lentiviruses are inactive versions of the human immunodeficiency virus (HIV)-1, there is therefore a theoretical risk of the lentivirus being able to replicate independently once introduced into the host cell. In order to mitigate this risk, third generation lentiviruses have the genomes split onto different plasmids, retaining only sequences required for highly efficient packaging. They also possess self-inactivating (SIN) vectors resulting in an inability to carry out independent viral

replication due to non-sense functions. This combined with the genome modifications renders them replication incompetent [114]. Self-inactivating lentiviral vectors have been used in a number of clinical trials without any documented independent replication. The lentiviral vector, pCCL.CMV.TRAIL, used to make MSCTRAIL *(figure 1.4)* has been used previously in a trial in children with Wiskott-Aldrich syndrome. [115]

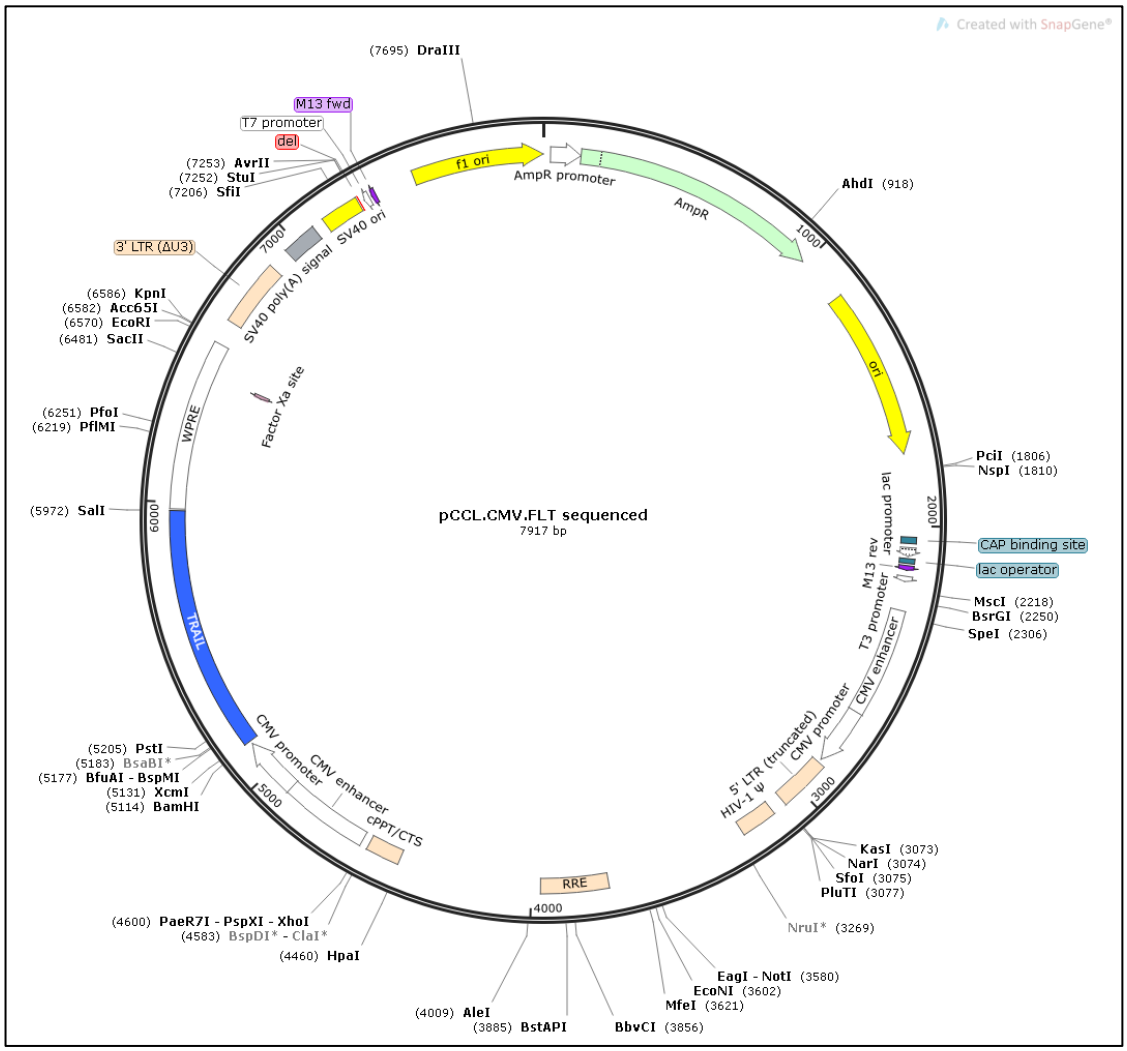


Figure 1.4: Map showing key features of the pCCL.CMV.TRAIL lentiviral vector used in the manufacture of MSCTRAIL.

MSCTRAIL

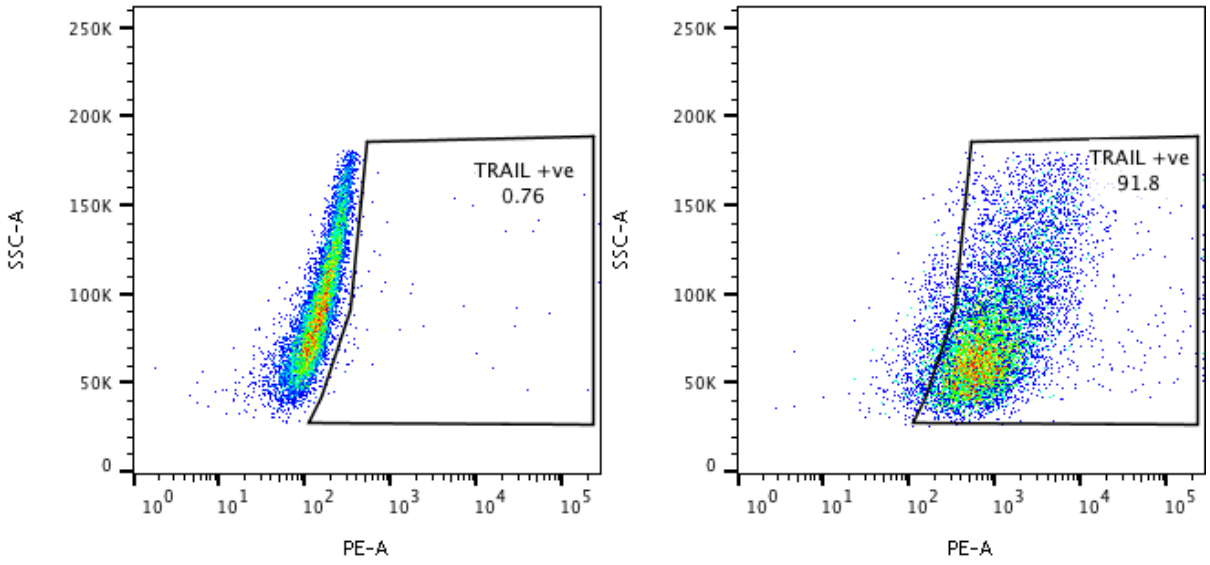
It has been shown that MSCs can be successfully modified to produce TRAIL (MSCTRAIL), that MSCTRAIL kills multiple cancer types *in vitro* and, when delivered in multiple murine cancer models, causes significant reduction in tumour growth [88] [6, 116]. Subsequently, data has demonstrated synergistic activity with chemotherapies and other therapeutic molecules.

This section will present the pre-clinical evidence supporting the use of MSCTRAIL in lung cancer. This work was completed and published before I started on the trial, figures and results are referenced and attributed accordingly.

A metastatic breast cancer cell line (MDAMB-231) was utilised by Loebinger et al (2009) in the below experiments to represent lung cancer because the team had experience using the cell line and had found it was easy to grow in a murine model. They found it developed a good established pulmonary metastatic model and so allowed for assessment on MSCTRAIL within a cancer of the lung model. MDAMB-231 was also sensitive to TRAIL in both populations and side populations[51].

1.1.10 MSCs can be transduced to express TRAIL

Loebinger et al (2009) [6] first demonstrated that bone marrow MSCs (BM-MSCS) could be successfully transduced with a lentiviral vector to express TRAIL [6]. Using flow cytometry, it can be seen that 91.8% of transduced MSCs express TRAIL.



Un transduced MSCs

Transduced MSCs

Figure 1.5: MSCs transduced with a lentiviral vector express TRAIL with 91.8% efficacy compared to MSCs not transduced [6].

1.1.11 MSCTRAIL induces apoptosis more effectively than recombinant TRAIL (RhTRAIL)

To demonstrate *in vitro* efficacy, they went on to co-culture MSCTRAIL with tumour cells labelled with fluorescent dye (Dil). Flow cytometry was used to measure apoptotic cell death of the tumour cells. Results showed that MSCTRAIL induced more tumour cell apoptosis than either untransduced MSCs or recombinant TRAIL (RhTRAIL) [6]

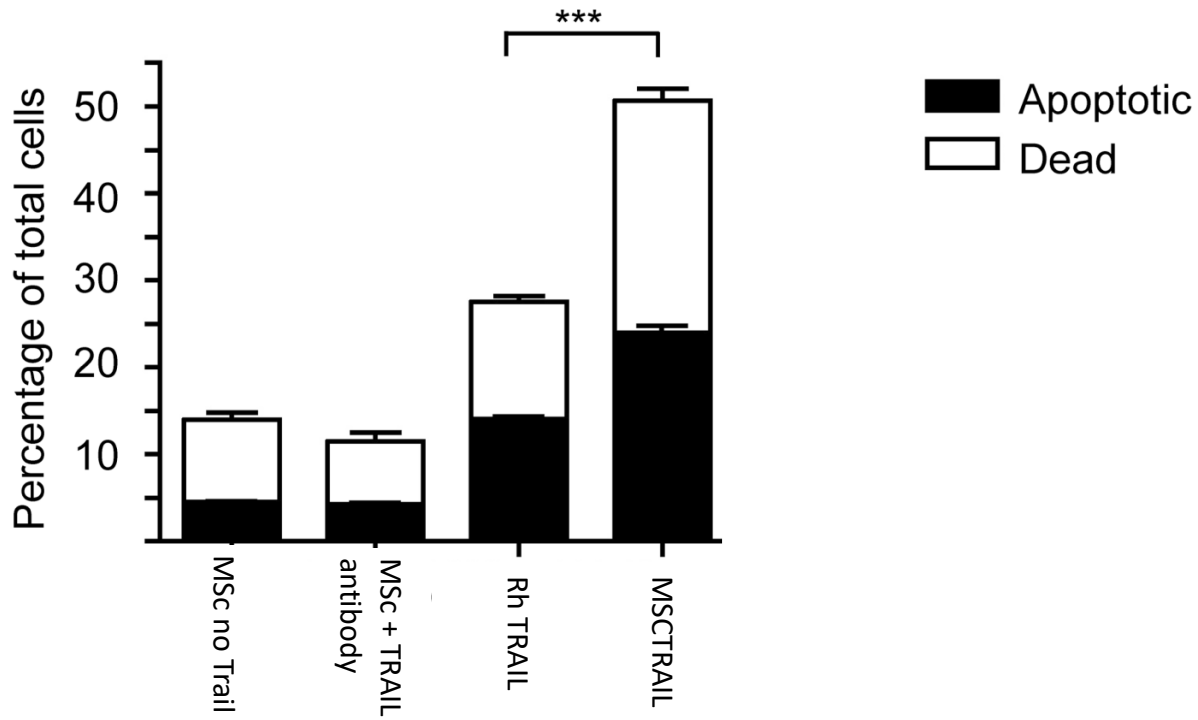


Figure 1.6: MSCTRAIL causes more tumour cell death in vitro than rhTRAIL or untransduced MSCs [6].

**** P<0.001*

1.1.12 MSCTRAIL homes towards lung metastases in vivo

To demonstrate *in vivo* homing Loebinger et al (2009) [6], grew tumours from a metastatic breast cancer cell line (MDAMB-231) in the lungs of immunodeficient mice (*figure 1.7 A&C*), DiI-stained MSCTRAIL was subsequently injected intravenously. 24hrs post injection, red MSCTRAIL can be seen to preferentially localise to lung metastases, engrafting and maintaining in the tumour environment compared to surrounding lung parenchyma *(figure 1.7 B&D)* [6].

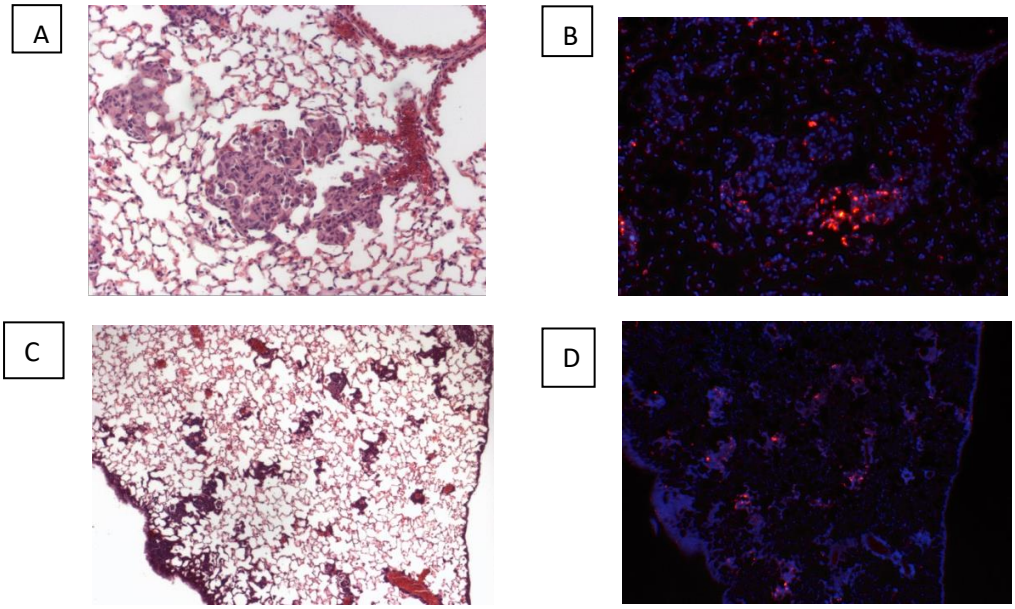


Figure 1.7: Section of murine lung containing metastasis with DiI-labelled MSCTRAIL localisation and engraftment

A and C, H&E contiguous sections magnification showing tumour metastasis within the lungs, 10; bar, 20Am. B and D show the corresponding samples after MSCTRAIL injection under fluorescent microscopy with DAPI nuclear counterstain ,showing red MSCS within the arears of tumour metastasis 4; bar, 60Am.[6]

1.1.13 MSCTRAIL reduces the size of tumour growth in vivo

To demonstrate *in vivo* tumour cell death Loebinger et al (2009) [6] injected tumour cells, MDAMB-231, of metastatic breast cancer lineage into NOD/SCID mice to produce lung metastasises. The mice were divided into 3 treatment groups with 8 mice in each with. In this experiment a Tet-on system element had been added to the lentiviral plasmid allowing the 'switching on' of TRAIL expression in transduced MSCs in the presence of Doxycycline. 1 treatment group did not receive any treatment, 1 received MSCTRAIL without Doxycycline (representing MSCs with no TRAIL expression) and 1 group received MSCTRAIL with Doxycycline (representing TRAIL expression) [6].

Therapy was administered on days 7,14, 21 and 28. At day 35 tumours were found in all (8/8) of the group which received no treatment and in all the MSCTRAIL without Doxycycline group (i.e. no TRAIL expression). In the MSCTRAIL plus Doxycycline

group (i.e. TRAIL expressing) three out of eight were tumour free (*figure 1.8A*). Upon harvest, the lung weight and metastases numbers per lung area, serving as a correlate for metastases load, were also lower in the MSCTRAIL plus Doxycycline group (*figure 1.8B*) [6].

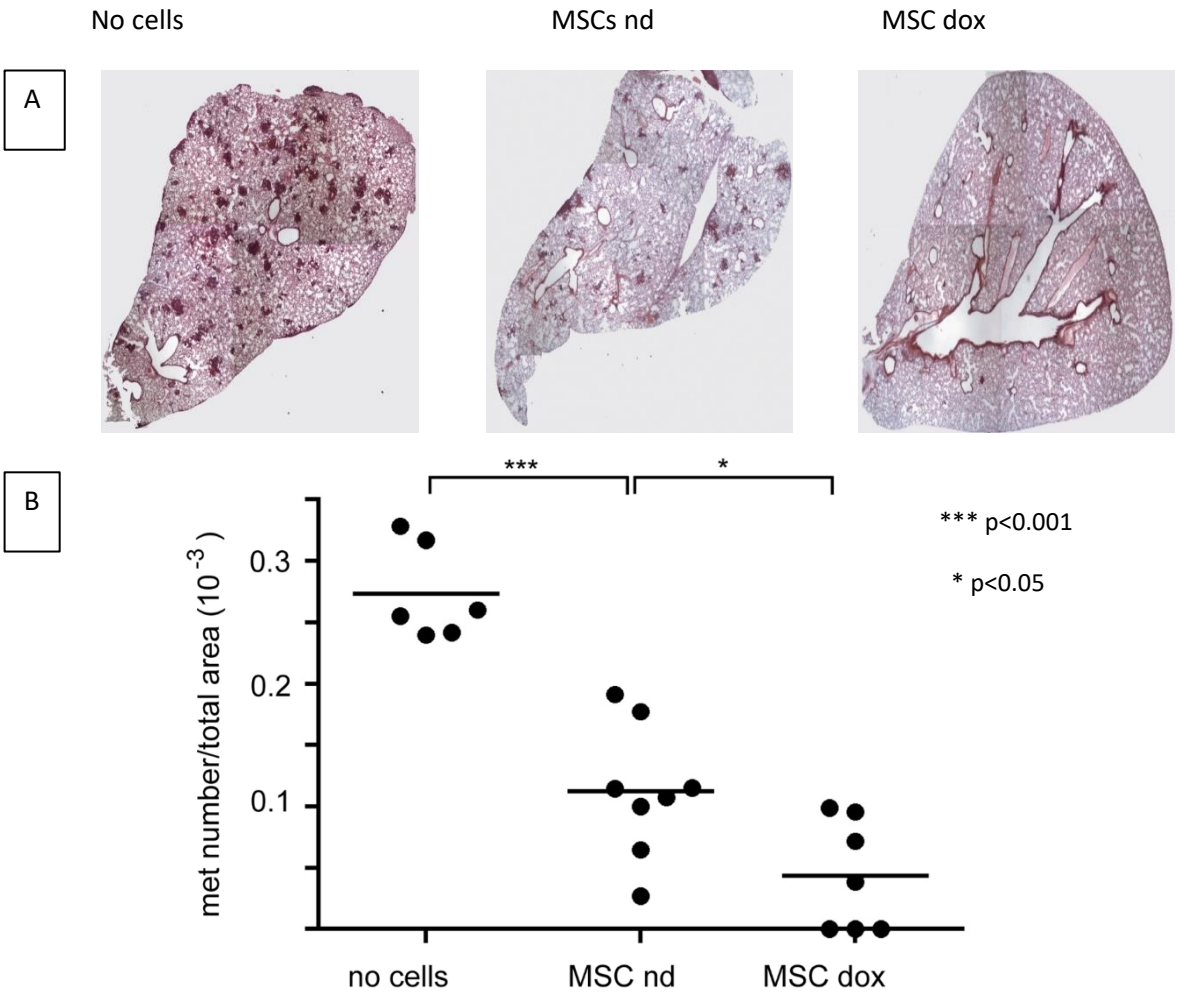


Figure 1.8: TRAIL expressing MSCs reduce growth of lung metastasis.

(nd= no Doxycycline, dox= Doxycycline)

*(A) Representative histology of lung lobes in the 3 experimental groups. TRAIL activation in MSCTRAIL resulted in elimination of metastasis in 3 of 8 mice (p=0.03). (B) Reduction in number of metastases per lung area was seen in both MSCTRAIL with and without Doxycycline but there was a further significant reduction between TRAIL activated (with Doxycycline) group compared to inactivated (***p<0.001, ** p<0.01, *p<0.05)[6].*

1.1.14 MSCTRAIL works synergistically with chemotherapy to cause apoptosis in cancer cells

Several chemotherapeutic agents including Cisplatin [117], SAHA (vorinostat) [118], Pemetrexed [119], Sunitinib [120], Etoposide [121], Doxorubicin [121], Bortezomib [122] have been shown to act synergistically with recombinant TRAIL (rTRAIL) *in vitro*. This synergy can also be exploited to treat TRAIL-resistant cancers.

To demonstrate this metastatic breast cancer (MDAMB-231) tumour cells were co-cultured with umbilical cord derived MSCTRAIL (UCMSC-TRAIL) Cisplatin and Pemetrexed. An Annexin V/DAPI apoptosis assay was carried out at 24 hours to quantify the tumour cell death.

The results below showed that there was significantly increased cancer cell apoptosis in the combination arm than in either arm in isolation.

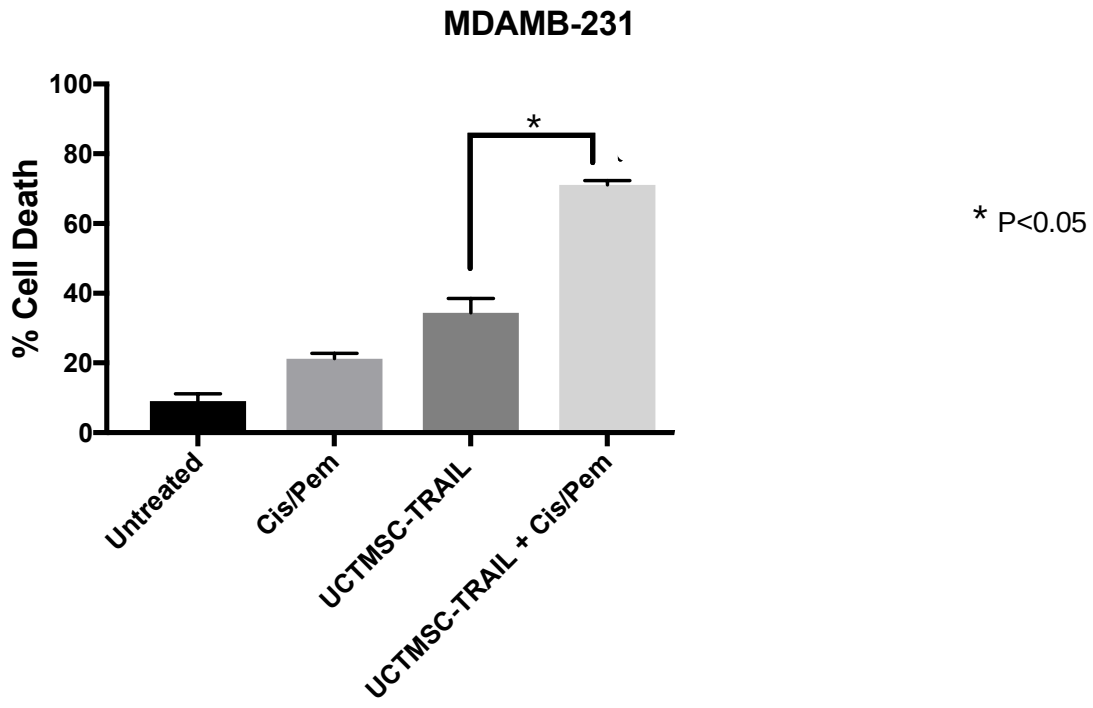


Figure 1.9: Combination of MSCTRAIL with chemotherapeutic agents Cisplatin and Pemetrexed (Cis/Pem) show increased in apoptosis of tumour cells

UCTMSC-TRAIL = Umbilical cord derived MSCs used in MSCTRAIL, Cis/Pem = Cisplatin and Pemetrexed

Unpublished data from the TACTICAL Investigators Brochure. Work carried out by Dr Ben Weil and Dr Krishna Kolluri and the TACTICAL team during the work up for change from bone marrow derived MSCs to umbilical cord.

A hypothesis for this is crosstalk between the extrinsic and the intrinsic apoptotic pathways, mediated by BID (*figure 1.10*). Chemotherapy upregulates the death receptor pathway by increasing caspase cleavage. When caspase-8 is activated, it cleaves BID to truncated BID (t-BID), which induces BAX/BAK to permeabilise mitochondria. This cross talk results in amplification of the apoptotic signals and increased tumour cell death. [123].

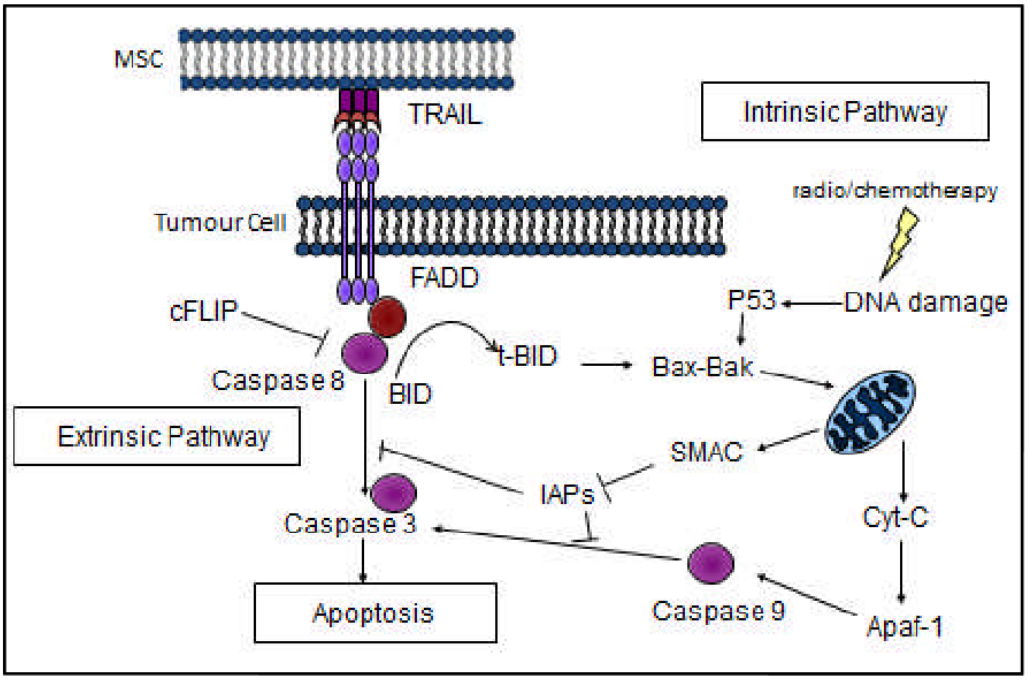


Figure 1.10: Hypothesis of synergy between chemotherapy and MSCTRAIL

This chapter has presented the current landscape of NSCLC with focus on advanced adenocarcinoma, highlighting the need for a novel targeted therapy. It has gone on to present a potential therapeutic option in the form of a cell and gene therapy, MSCTRAIL and the pre-clinical evidence to support its efficacy. Subsequent chapters will present the progress in moving this work from bench to bedside and deliver a first-in-human clinical trial.

Hypothesis

Mesenchymal stromal cells genetically modified to express TRAIL are safe and effective in the treatment of metastatic adenocarcinoma of the lung in combination with first line standard of care therapy.

1.1.15 Aims

This project sets out to determine if MSCTRAIL can be used as a novel treatment for metastatic adenocarcinoma of the lung in combination with first line standard of care in a First in Human clinical trial. My aims are therefore to:

1. Determine the safety of MSCTRAIL through a phase I/II clinical trial
2. Determine the efficacy of MSCTRAIL in combination with first line standard of care therapy
3. Examine the *in vivo* activity of MSCTRAIL through the use of a biomarker

2 Targeted Stromal Cells Expressing TRAIL as a therapy for lung cancer - The TACTICAL TRIAL

Background

As discussed in chapter 1 lung cancer is the leading cause of cancer death worldwide[2]. Current treatment options for advanced disease offer a limited survival benefit but can come with a heavy burden of side effects and toxicities. This highlights the need for novel therapies.

It is well recognised that MSCs can home to and incorporate into tumours or site of injury [51] and that TRAIL can induce selective cancer cell death, via the extrinsic death pathway, leaving healthy cells unaffected [84].

We have shown that MSCs can be transduced to express TRAIL and that MSCTRAIL will home towards cancerous cells and induce apoptosis in *in vitro* co-culture assays and *in vivo* in orthotopic lung metastasis murine model [6], resulting in regression of metastasises following intravenous delivery. Furthermore, we have demonstrated synergistic activity with other systemic anti-cancer therapies. The next step was to set up and run a first-in-human study of this novel advanced therapy to test safety and therapeutic efficacy.

The setup of a first-in-human study such as this presented a number of key challenges which needed to be overcome before patients could be enrolled.

- Manufacturing: No single facility was manufacturing a genetically modified cell bank to Good Manufacturing Practice (GMP) standards, to the size required for the trial. Furthermore, MSCTRAIL had not been manufactured anywhere for clinical use before.
- Regulatory approvals: These were required from numerous national bodies including Heath Research Authority, Medicines and Healthcare products Regulatory Agency (MHRA) and Research Ethics Committee (REC) as well as internal GMO safety committee and research ethics boards prior to trial initiation, each with rigorous evaluation processes.

- Designing the trial: Including trial protocol and accompanying documents such as patient information sheet and investigators brochure in the environments of a novel and unknown advanced therapeutic product.

A summary of the methods used, protocols designs, and justification of these choices is presented here. The full protocol as well as accompanying documentation can be found in the appendices.

Aims

The aim of this work was to investigate the clinical effect of MSCTRIL, to elucidate if MSCTRAIL is safe and if it can be used as a novel treatment for metastatic adenocarcinoma of the lung in combination with first line standard of care therapy in a first-in-human clinical trial.

We hypothesise that MSCTRAIL is a safe and effective treatment of metastatic adenocarcinoma of the lung in combination with first line standard of care therapy

Methods

The full TACTICAL protocol can be found in Appendix 1: The TACTICAL protocol v4

2.1.1 Funding

Funding for the trail was provided by the MRC DPFS scheme under the title: MSCTRAIL for lung cancer. It has the ClinicalTrials.gov Identifier: NCT03298763. The trial sponsor is University College London

Trial design

To ascertain the safety and efficacy of MSCTRAIL, a Phase I/ II First in Human clinical trial was designed and set up.

Standard of care (SOC) is defined in this chapter as the intravenous chemotherapeutic agents Cisplatin* (75mg/m^2) and Pemetrexed (500mg/m^2) and

delivered as per local policy. *Carboplatin can be used instead of Cisplatin if clinically appropriate at the discretion of treating clinician.

Subsequent alterations to this standard and the implications that had on the ongoing planning of the trial will be discussed in Chapter 3: Changing Paradigm of NSCLC Treatment.

Phase I is a single site dose de-escalation study using a modified Bayesian continual reassessment method (mCRM) to ascertain the safety of MSCTRAIL in combination with first line standard of care therapy (SOC) and to find the recommended Phase II dose (RP2D) of MSCTRAIL. Target accrual is 6-18 patients.

Patients with stage IIIB/IV metastatic adenocarcinoma of the lung, ECOG performance score 0-1 received standard of care therapy (SOC) on day 1 followed by MSCTRAIL on day 2 of a 21-day cycle for 3 cycles followed by a 4th cycle of SOC only (*figure 2.1).* They were monitored for adverse events and any dose limiting toxicities (DLTs) which would trigger a dose reduction.

Phase II is a multicentre randomised placebo control trial, patients with the same demographics as phase I, will receive SOC on day 1 and then be randomised (1:1) to receive MSCTRAIL, at the RP2D, or placebo on day 2 of a 21 day cycle for 3 cycles followed by a 4th cycle of SOC. Primary outcome will be tumour response rate by RECIST (v1.1) standards. Target accrual is 46 patients.

Patients in both phases will be followed up for up to 24 months, with ongoing care after the 4th cycle guided by their treating clinician.

	Cycle 1						Cycle 2						Cycle 3						Cycle 4					
Week	**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**		**12**	
Day	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Pemetrexed 500 mg/m^2, iv	•						•						•						•					
Cisplatin 75 mg/m^2, iv	•						•						•						•					
MSCTRAIL 4x10^8, 2x10^8 or 8x10^7 cells iv, depending on toxicities **Or placebo** (in phase II)		•						•						•										

Figure 2.1: TACTICAL Trial Treatment Schedule

2.1.2 Patient Eligibility

The inclusion and exclusion criteria for both phases are the same.

The full inclusion and exclusion criteria are listed in Appendix 1: The TACTICAL Protocol V4.

Key MSCTRAIL or trial specific eligibility criteria are:

- Inoperable stage IIIb/IV histologically /cytologically confirmed lung adenocarcinoma
- EGFR and EML4-ALK negative
- ECOG performance status of 0 or 1
- Negative pregnancy test for female patients of childbearing potential
- Adequate organ function

- No prior chemotherapy, hormonal therapy, radiotherapy (including palliative radiotherapy), immunotherapy or treatment with investigational drugs for advanced NSCLC
- No prior treatment with any cellular therapy
- No known pulmonary hypertension (WHO Class III or IV)
- No evidence of symptomatic brain metastases requiring treatment
- No venous thromboembolism within the last 6 months

These criteria aim to ensure homogeneity within the patient population, delivering a novel treatment in the safest treatment paradigm and allowing for any adverse effects or treatment gains to be correctly attributed.

All patients have the same histological subtype of lung cancer and advanced stage cancer. By excluding targeted tumour mutations, the patients are not being denied any treatment options that would otherwise be available for example TKI therapies for those with EGFR mutation.

ECOG performance score and adequate organ function is listed to ensure patients are fit enough for treatment, as defined by ESMO and NICE who offer systemic therapy to patients with a performance score 0-1. Adequate baseline organ function, measured through baseline blood tests allows rapid identification of adverse events.

There is no previous research around the use of cellular therapies in pregnancy or during conception however the trial treatment has been assessed through the Clinical Trial Facilitation Group as having a high risk of teratogenicity or fetotoxicity. All women of child-bearing potential (WOCBP) are screened for pregnancy and along with fertile men offered advice on appropriate contraception.

There is a theoretical risk of acute pulmonary embolism as the cells are being infused, however this has not been noted before in any MSC cell therapy trials. Patients with the known pulmonary hypertension or pulmonary embolism diagnosed in the last 6 months are excluded as they may be more predisposed to clot and mortality as a result of any embolism would be significantly higher.

Patients with known brain metastasis are excluded because there is little evidence that MSCs can cross the blood brain barrier[124] and their predicted life expectancy

is significantly reduced [125] making them less likely to reach trial end point for assessment.

These is no documented evidence of the effect of prior cellular therapies on current cellular therapy treatment, patients who have received them or recent prior treatment of the cancer are excluded to ensure clarity of result reporting.

Patients with intercurrent severe infections, active or infected wounds, recent myocardial infarction, known hepatitis or HIV are excluded as these concurrent comorbidities may increase the risk associated with trial participation or trial drug administration, or may interfere with the interpretation of trial results.

TACTICAL is being undertaken in the first line setting for both patient selection and clarity of response and on the background of strong pre-clinical data regarding the safety of MSCs, as discussed in chapter 1. Enrolling only patients who are treatment naive allows any toxicity or efficacy to easily attributed. It also hopes to ensure the patients are fit enough to undergo therapy and subsequent follow up.

Prior to registration into the trial patients were screened for eligibility and written informed consent was required before any trial specific screening investigations could be carried out. As well as full physical examination, review of the eligibility criteria, blood tests within 14 days, CT chest, abdomen and pelvis that must be within 28 days prior to registration/ randomisation to ensure an up to date baseline standard for RECIST reporting.

2.1.3 Justification of Tumour Type

Primary lung cancer has been chosen as the tumour type not only because of the wealth of pre-clinical knowledge and expertise held within our team but also because of the biodistribution of MSCs after delivery. The tracking and pharmacokinetics of MSCs after infusion is not well understood. Pre-clinical models using radiolabelled MSCs show accumulation of MSCs in the lungs, [126, 127] and that MSCs travel towards the lungs first becoming entrapped within them [128, 129] before homing to site of injury, even in subjects without lung injury.

By choosing a cancer group with the predominant site of injury with the lungs, there is an increased chance of positive outcomes as the maximum numbers of MSCTRAIL cells will reach the target site both through homing and post infusion kinetics.

A single tumour group within that was used to ensure homogeneity and clarity of results and ensured a single standard of care treatment regime. Adenocarcinoma was chosen over squamous cell because it represents a large proportion of those diagnosed, aiding recruitment in the shortest time frame.

2.1.4 Patient Assessments

When designing the frequency of patient assessments, it was important to balance both the needs of the trial and safety of the patient, allowing maximum capture of any events and early intervention if required, with quality of life for the patient. The toll of interventions and travel can be a heavy burden for some patients and could hinder quality of life in those with a reduced life expectancy.

For safety reasons reviews are more frequent in phase I compared to phase II.

To evaluate both the safety and efficacy of MSCTRAIL patients undergo blood sampling, computer tomography (CT) imaging and medical review with examination throughout and after completion of trial treatment. Trial treatment consists of 21day cycle for 4 cycles.

For purposes of this discussion a ‘trial review’ was carried out by a doctor on the trial team and consisted of:

- Clinical review
- Physical examination
- Vital signs, including heart rate, oxygen saturation, blood pressure.
- Adverse event (AE) and concomitant medication (conmed) check

Patient assessments during the trial were at the following times with additional visits not listed below for translational bloods to be taken:

Cycle 1,2,3,4 Day 1

- Trial review
- Confirmation of ECOG performance score
- Blood tests (can occur up to 72 hours before), full blood count (FBC), oncology profile including kidney and liver function, calcium, magnesium and CRP
- Urinalysis
- If WOCBP, a negative pregnancy test

If satisfactory they will receive chemotherapy

Cycle 1, 2 and 3, day 2

- Trial review
- ECG (pre and 4 hours post MSCTRAIL infusion)

If satisfactory they will receive MSCTRAIL infusion (or placebo in phase II)

Patients in phase I received a further assessment in cycle 1 on day 3

- Trial review
- ECG

Cycle 1, day 8 and day 15

- Trial review
- Blood tests, full blood count (FBC), oncology profile including kidney and liver function, calcium, magnesium and CRP

Patients in phase I will receive assessment in cycle 2 and 3, days 8 15

- Trial review

Cycle 4 day 21

- Trial review
- ECG
- Blood tests (can occur up to 72 hours before), full blood count (FBC), oncology profile including kidney and liver function, calcium, magnesium and CRP

After completion of trial treatment

- 6 weekly follow up for up to 24 months.

A CT Chest abdomen and pelvis with contrast was carried out at the following time points or within 7 days of that, below lists justification of each of these scans:

- Registration (or within 14 days)

To ensure eligibility for the trial, that patients have measurable disease, stage IIIB/IV and also provides a baseline from which further imaging can be compared

- Cycle 2, Day 14-21

To look for early changes or accelerated tumour growth at 6 weeks.

- Cycle 4 Day 21

To assess for tumour response rate at 12 weeks. As per trial outcomes and to guide ongoing patient care

- 6 weekly until radiological progression by RECIST criteria.

To assess for tumour response and time to progression. After this time patient care is decided by their treating Oncologist and not part of trial treatment.

Figure 2.2 summaries patients' visits in Phase I and is the TACTICAL patient information sheet which can be found in Appendix 2.

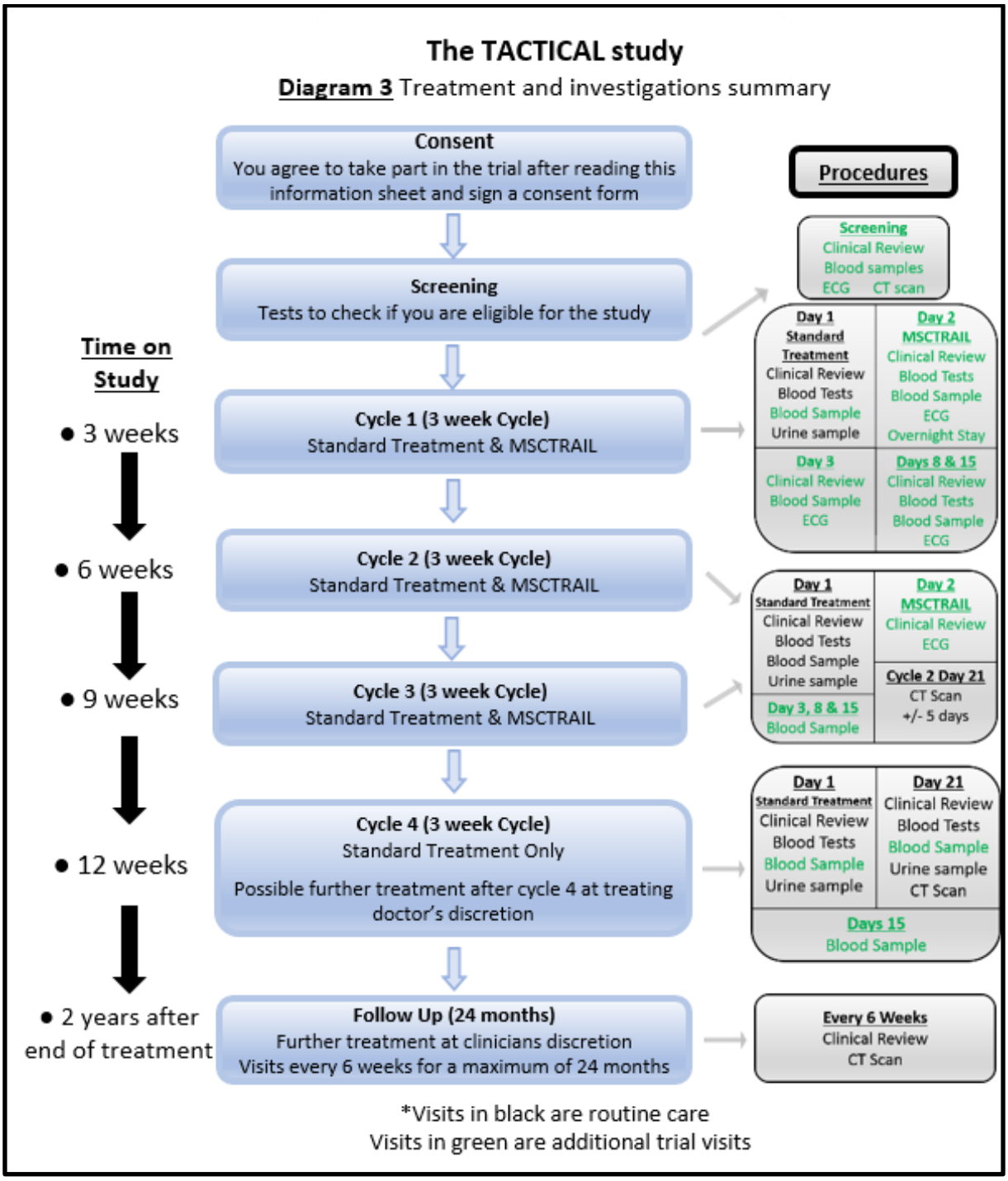


Figure 2.2: Summary of Patient Interventions from the Phase I Patient information Sheet

Assessment			Phase I Interventions														
	Pre Intervention		Cycle 1 Pemetrexed/Cisplatin & MSCTRAIL administration					Cycle 2 Pemetrexed/Cisplatin & MSCTRAIL administration			Cycle 3 Pemetrexed/Cisplatin& MSCTRAIL administration			Cycle 4 Pemetrexed/Cisplatin& MSCTRAIL administration			Follow Up
	Prior to registration	Within 14 days prior to registration	Day 1	Day 2	Day 3	Day 8	Day 15	Day 1	Day 2	Days 3, 8 and 15	Day 1	Day 2	Days 3, 8 and 15	Day 1	Day 15	Day 21	Every 6 weeks until 24 months after end of treatment
Interventions																	
Chemotherapy			x					x			x			x			
MSCTRAIL Infusion				x					x			x					
Examination/Investigation																	
Clinical Review			x	x	x	x	x	x	x		x	x		x		x	x
Physical examination		x	x	x	x	x	x	x	x		x	x		x		x	x
Vital signs **(1)**		x	x	x	x	x	x	x	x		x	x		x		x	x
ECG		x		x	x	x	x		x			x				x	
Weight		x						x			x			x			
ECOG status		x						x			x			x			
CT Scan	X									X						X	X
Laboratory tests																	
Haematology (FBC)		x	x			x	x	x			x			x		x	
Oncological Profile		x	x			x	x	x			x			x		x	
Urinalysis			x					x			x			x			
Translational research Sample			x	x	x	x	x	x	x	x	x	x	x	x	x		
Pregnancy test (if needed)		x	x					x			x						
Adverse event and Con Med collection		x	x	x	x	x	x	x	x		x	x		x		x	

Table 2.1: Phase I patient Interventions

Assessment	Pre Intervention		Phase II Interventions											Follow Up
			Cycle 1 SOC & ATIMP / Placebo					Cycle 2 SOC & MSCTRAIL / Placebo		Cycle 3 SOC & MSCTRAIL / Placebo		Cycle 4 Standard of care treatment		
	Prior to registration	Within 14 days prior	Day 1	Day 2	Day 3	Day 8	Day 15	Day 1	Day 2	Day 1	Day 2	Day 1	Day 21 of cycle 4	Every 6 weeks until 24 months post end of treatment
Interventions														
Chemotherapy			x					x		x		x		
MSCTRAIL / Placebo Infusion				x					x		x			
Examination/Investigation														
Clinical Review			x	x	x	x	x	x	x	x	x	x	x	x
Physical examination		x	x	x	x	x	x	x	x	x	x	x	x	x
Vital signs		x	x	x	x	x	x	x	x	x	x	x	x	x
ECG		x		x	x	x	x		x		x	x	x	
Weight		x						x		x		x		
ECOG status		x	x					x		x		x		
CT Scan	X								X				X	x
Laboratory tests														
Haematology (FBC)		x	x			x	x	x		x		x	x	
Oncological Profile		x	x			x	x	x		x		x	x	
Urinalysis			x					x		x		x		
Pregnancy test (if applicable)		x	x					x		x				
Adverse event and Con Med collection		x	x	x	x	x	x	x	x	x	x	x	x	

Table 2.2: Phase II patient interventions

2.1.5 Measuring Tumour Response

As detailed above patients underwent 6 weekly CT scans to measure response to treatment. CT scanning was reported using Response Evaluation Criteria in Solid Tumours (RECIST criteria v1.1). RECIST builds from the 1979 World Health Organisation (WHO) objective tumour response criteria[130] and aims to provide global standardisation on the reporting of scans of patients with cancer.

Target lesions (TL) are identified at baseline and changes in those lesions are measured at subsequent scans. Each scan is assigned a response depending on the status of the disease and this helps guide future treatment plans:

- 'CR' complete response
  All detectable tumour has disappeared, no new lesions, sustained at least four weeks, when confirmation is required.
- 'PR' partial response
  At least a 30% decrease in the sum of the longest diameter of target lesions with baseline as reference, no evidence of progression of non-target lesions and no new lesions.
- 'SD' stable disease
  Insufficient reduction in size of TL to qualify for PR nor sufficient increase to qualify for PD
- 'PD' progressive disease.
  At least a 20% increase in the sum of the longest diameter of target lesions, progression of a non-target lesion or a new lesion in comparison to nadir measurement of treatment.

Each CT scan will be programmatically assigned a RECIST (V1.1) visit response of CR, PR, SD or PD depending on the status of their disease compared to baseline and previous assessments. Progression of target lesions (TL) will be calculated in comparison to when the tumour burden was at a minimum (i.e. smallest sum of diameters previously recorded on study). In the absence of progression, tumour

response (CR, PR, SD) will be calculated in comparison to the baseline tumour measurements obtained before starting treatment. If a patient has had a tumour assessment, which cannot be evaluated, then the patient will be assigned a visit response of not evaluable (NE) unless there is evidence of progression in which case the response will be assigned as PD. If > 1/3 of lesions recorded at baseline are missing, then the TL response will be NE. However, if the sum of non-missing TL diameters would result in PD (i.e. if using a value of 0 for missing lesions the sum of diameters has still increased by > 20% or more compared to the smallest sum of diameters on study), PD takes precedence over NE. A visit response of CR will not be allowed if any of the TL data is missing.

Phase I

Phase I is a single centre accelerated dose de-escalation trial using a modified Bayesian continual reassessment method (*figure 2.3*).

The objective of this first-in-human phase of the trial is to determine the safety of MSCTRAIL when given in combination with standard of care therapy and ascertain the recommended phase II treatment dose (RP2D)

Patients will receive standard of care therapy (SOC) on day 1 followed by MSCTRAIL on day 2 of a 21-day cycle for 3 cycles followed by a 4^{th} cycle of SOC only.

The target accrual is 6-18. The first 3 patients, deemed cohort 1a, will receive $4x10^8$ cells of MSCTRAIL in combination with SOC for 3 cycles. For safety purposes treatment will be staggered by at least 21 days to ensure any dose limiting toxicities (DLT) are identified before a subsequent patient is treated.

If there are no DLTs a further 3 patients (cohort 1b) will receive the same dose of MSCTRAIL in combination with SOC. If there are no toxicities in all 6 patients this will be the recommended Phase II dose (RP2D) and will complete phase I.

However, if DLTs occur, the next cohort (cohort 2) of 3 patients will receive a reduced dose (either $2x10^8$ or $8x10^7$ cells). The dose will be decided by the independent data monitoring committee (IDMC) after evaluation of type and severity of toxicity. If there are no DLTs at this reduced dose a further 3 patients (cohort 2b) will receive the same reduced dose. If there are no toxicities in all 6 patients this will be the recommended Phase II dose (RP2D).

This will continue until a safe dose is established or it is not possible to find one.

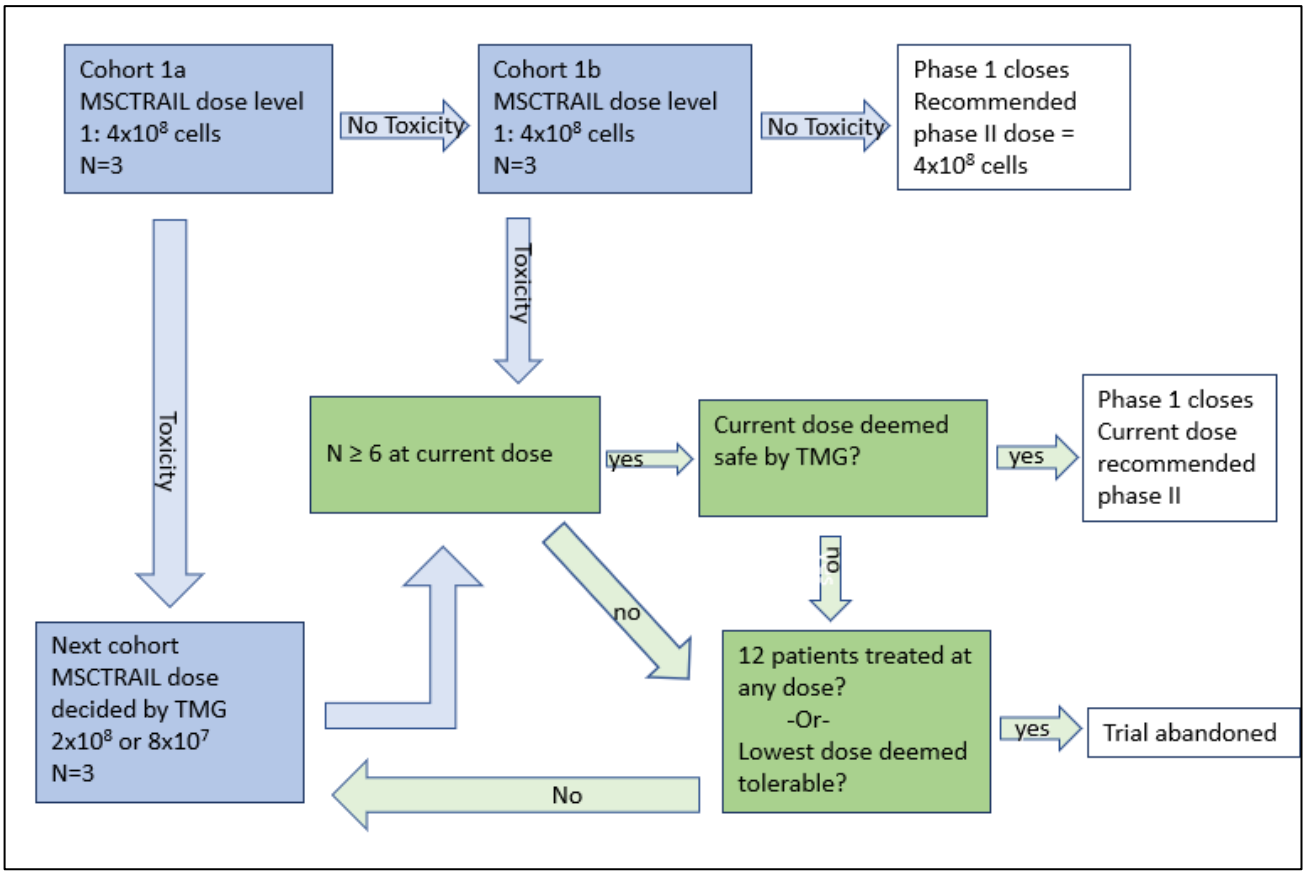


Figure 2.3: TACTICAL Phase I Trial Schema

2.1.6 Outcomes in Phase I

The objective of phase I is to ascertain the safety and tolerability of MSCTRAIL in combination with SOC therapy and hence identify the RP2D. The secondary objective is to assess the type and duration of treatment response, time to progression and survival in the treatment combination. To reflect this the trial end points are:

Primary Endpoints:

1. The incidence of dose limiting toxicities (DLT) within the first cycle of treatment
2. Determination of recommended Phase II dose (RP2D) of MSCTRAIL in combination with SOC as first line treatment for lung adenocarcinoma

Secondary Endpoints:

1. Frequency of adverse events within 3 cycles of treatment
2. Best overall response
3. Change from baseline in sum of target lesions
4. Duration of response
5. Progression free survival

For DLT analysis, the primary population for analysis will be the safety population (all patients who receive at least one dose of MSCTRAIL and one dose of SOC treatment). For the efficacy assessments, patients included will be those who are included in the safety population who also have evaluable tumour response (i.e. non missing baseline tumour assessments).

2.1.7 Defining Toxicities

Treatment related toxicities are captured throughout the trial by clinical assessment and recorded using the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) V5. This is a standard proforma used for recording adverse events in cancer clinical trials. It classifies and grades events or symptoms using a system from 1-5 (1-mild, 2- moderate, 3-severe, 4-life threatening, 5-death) and leads to more homogeneous and transparent reporting between clinicians and trial teams. After identification of an adverse event causality must be determined, it must be either related to or there is a reasonable possibility it is due to the trial treatment, or not related/ no reasonable possibility, to the trail treatment

The primary end point of the phase I is to evaluate the clinical tolerability of MSCTRAIL and describe any DLTs.

DLTs are defined within each trial protocol specifically and are thought to be side effects severe enough to prevent a dose increase or further doses at that level. They are assessed using CTCAE classification and must be attributable to MSCTRAIL. As

DLTs are trial specific they must encompass any predicted or theoretical severe side effects.

Within TACTICAL, DLTs were defined as any of the following MSCTRAIL related adverse events:

- Thromboembolic event ≥ CTCAE grade 4 within 48 hours of MSCTRAIL infusion
- New cardiac arrhythmias ≥CTCAE grade 4 requiring Direct Current (DC) cardioversion, ≥CTCAE grade 4 ventricular tachycardia, ventricular fibrillation or asystole within 4 hours of MSCTRAIL infusion
- Any other toxicity that results in a disruption of dosing schedule of more than 21 days not related to the chemotherapy
- MSCTRAIL related adverse event of grade 4 or higher that is assessed by the TMG to constitute a DLT

A DLT excludes:

- Alopecia of any grade
- Isolated laboratory changes of any grade without clinical sequelae or clinical significance
- Any chemotherapy or immunotherapy related adverse event

These events have been defined as DLTs because they are either events of particular interest or risk for MSCTRAIL or because they would be a severe enough reaction to consider dose changes.

Other events which occur are then labelled as, adverse event (AE), adverse reaction (AR), serious Adverse Event (SAE) or serious Adverse Reaction (SAR) and suspected Unexpected Serious Adverse Reaction (SUSAR) or Adverse Event of Special Interest. These are defined as:

Adverse Event (AE)

Any untoward medical occurrence, including signs, symptoms or abnormal finding in a patient treated on a trial protocol, which does not necessarily have a causal relationship with an MSCTRAIL.

Adverse Reaction (AR)

All untoward and unintended responses to MSCTRAIL. A causal relationship between an MSCTRAIL and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)

An adverse event or adverse reaction that results in death, is deemed 'life-threatening' at the severity they experienced it, required in-patient hospitalisation, results in persistent or significant disability or is otherwise deemed medically significant

Suspected Unexpected Serious Adverse Reaction (SUSAR)

An adverse event meeting the following criteria:

Serious – meets one or more of the SAE or SAR criteria of serious.
Related – assessed by the local investigator or sponsor as causally related to one or more elements of the trial treatment
Unexpected – the event is not consistent with the predicted adverse events.

Adverse event of special interest

An AE that is of particular interest to the Trial Management Group, even if it occurs outside the standard AE reporting timeframes for the trial. For TACTICAL this was defined as a thromboembolic event ≥ CTCAE grade 4 within 48 hours of MSCTRAIL infusion

Full details of these, along with how they will be reported, can be found in Appendix 1: The TACTICAL protocol

2.1.8 Bayesian Design and Sample Size

Statistics for this trial were devised in conjunction with Dr Graham Wheeler a medical statistician working at the Cancer Research UK and UCL Cancer trial Centre at UCL.

A Bayesian continual reassessment model allows a trial to take advantage of information accumulated as it progresses and modify key, pre-defined parameters in response to that information. In this trial the modification is the dose of MSCTRAIL and the information to be gathered is patient tolerability, measured as DLTs. [131]

The model is updated after each patient is treated and results are displayed as an empirical DLT rate for each dose with a credible interval to show uncertainty.

Patients will be assigned to dose levels in groups of 3, with the first patients being treated at the highest dose of MSCTRAIL, $4x10^8$. Based on the DLT outcomes of these patients, estimates of the probability of DLT will be calculated and the next 3 patients will receive the dose of MSCTRAIL with a probability of a DLT occurring that is less than but closest to target toxicity. The target toxicity is defined as 35%.

The recommended RP2D is the highest dose of MSCTRAIL administered that has an estimated risk of causing a DLT equal or closest to the target toxicity.

Using this model with the prior estimate for risk of DLT at the highest dose of 35%, if 6 patients receive this dose, with none of them experiencing a DLT, then the posterior mean estimate for the probability of DLT at this dose would be 2.8 % (95% credible interval of 0 – 23.4%).

This model was used because it is updated after each patient and so modifications can be made immediately, increasing patient safety. It is also not ‘memory less’ as the previous patient’s results remain within the model, unlike the traditional 3+3 design and it also allows for a target toxicity to be defined prior to commencing the trial. Thus, ensuring validity, maximum scientific efficiency and, most importantly, patient safety in a timely and cost-effective manner.

2.1.9 Analysis of Secondary Outcomes in Phase I

Phase I secondary outcomes are:

- Frequency of adverse events after the first cycle
- Best overall response,
- Change from baseline in sum of target lesions at 6 and 12 weeks
- Duration of response
- Progression free survival

Primary outcome of phase I investigates the frequency of DLTs but secondary outcomes will look further at the adverse events and other safety data. Data from all cycles of initial treatment will be combined in the presentation of safety data. The type, number of patients who experienced them and CTCAE grade of AE will be listed by patient and dose group. For patients who have a dose modification during treatment, all AEs (due to drug or otherwise) will be assigned to their initial dose group. Serious AEs will be summarised separately if a sufficient number occur.

Tumour response data will be listed and summarised by dose, if appropriate, using the response categories: Complete Response (CR), Partial Response (PR), Stable Disease (SD), Progressive Disease (PD) and Non-Evaluable (NE). In addition, the percentage of patients who have a confirmed PR or CR or have a visit response of SD that is at least 12 weeks after the first dose of study therapy will be summarised. Waterfall plots (bar charts) indicating the percentage change from baseline in sum of the diameters of TLs may be produced by dose level depending on how much data is obtained in patients with measurable disease at baseline. Duration of response will be summarised.

Progression Free Survival (PFS) is defined as the time from registration (or randomisation in phase II) to time of progression (as per RECIST V1.1) or time of death from any cause. Patients with no confirmed time of progression/death will be censored at the time that they were last confirmed as non-progressive/alive. PFS will be analysed using KM plots and will be presented along with median PFS time per dose level.

2.1.10 Starting dose of MSCTRAIL

The starting dose of MSCTRAIL was calculated using preclinical evidence on the efficacy of MSCTRAIL combined with safety data from other MSC therapy trials and the doses utilised in these other clinical trials using MSCs to treat lung diseases.

Extensive evidence from previous trials involving MSCs and TRAIL found them to be safe with no dose limiting toxicities described and it is therefore not expected that MSCTRAIL will cause significant toxicity.

Preclinical evidence shows there to be a dose dependent response on cancer cells by MSCTRAIL. Increased therapeutic efficacy, i.e. cancer cell death, was observed with an increasing ratio of MSCTRAIL cells without an identified maximum therapeutic dose or response plateaux *(figure 2.4).*

The dose of MSCTRAIL was therefore based on previous clinical trials and clinical safety balanced with the high manufacturing costs. Clinical trials using MSCs to treat lung diseases, have used doses ranging from 1 million cells/kg to 10 million cells/kg and with no significant adverse events attributed to the MSCs[59, 61, 63]. Allowing for the cost of manufacture and trial design of 3 doses, a starting dose of 400million cells was selected, this corresponds to a dose per body weight of 5 million cells/kg assuming an 80kg person. De-escalation doses correspond to 2.5 million cells/kg and 1 million cells/kg, all of which fall within the dose ranges already established to be safe.

By starting with this dose patients get the best affordable therapeutic option while, given previous experience, still maintain safety.

Given the high costs of a cellular therapy, cost efficacy is mitigated by utilising the dose de-escalation model. Patients are all treated at the highest dose and not with a perceived potentially ‘sub therapeutic doses’ as with a traditional dose escalation design, where a minimum of 3 patients would be treated before escalation to the highest or potentially therapeutic dose.

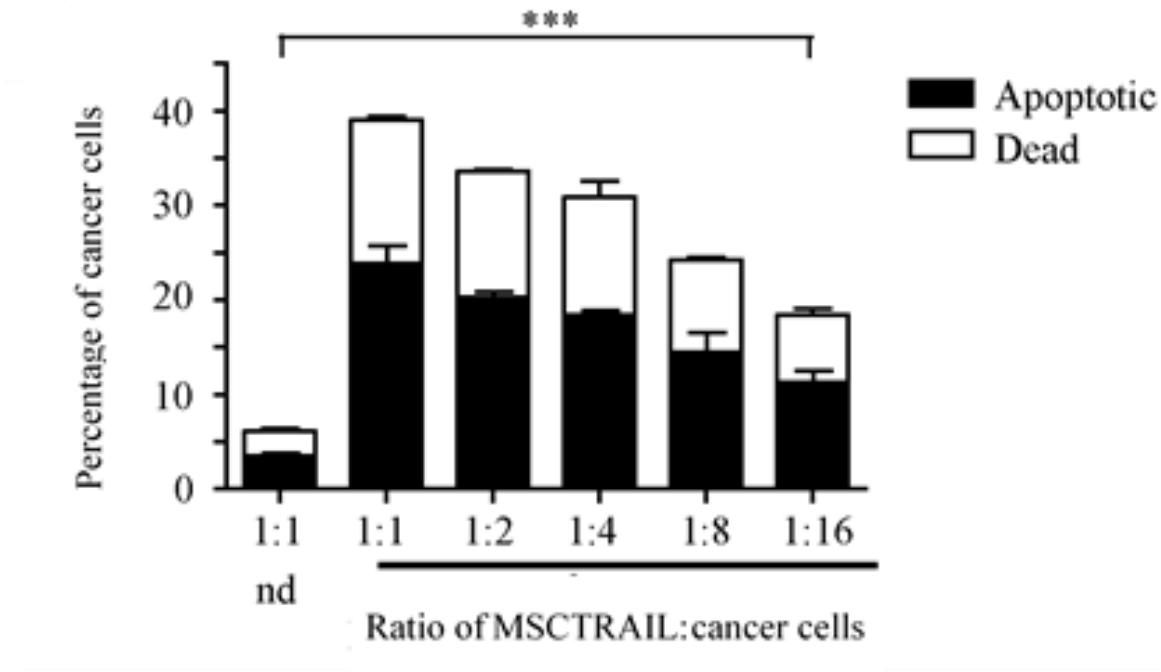


Figure 2.4: TRAIL-expressing MSCs induce cancer cell apoptosis in a dose dependent fashion with no identified therapeutic plateaux.

Nd= no doxycycline, a control
Figure from unpublished work carried out at Lungs for Living, UCL Respiratory prior to my work.

2.1.11 Translational samples

Patients were consented for the extraction and use of blood samples. The aim of this translational work was to ascertain if the activity of MSCTRAIL could be assessed through the use of a biomarker of apoptosis. Further details of this are found in chapter 5: TACTICAL Translation Results.

Samples will also be used to investigate the host response to a single dose of allogeneic MSC and the response to a re-challenge following multiple doses. The results from phase I will be used to optimise sample taking and methods prior to phase II and guide further on-going work in the placebo-controlled environment.

Samples were taken in phase I pre-chemotherapy and pre and post MSCTRAIL through all treatment cycles as well as on progression. The results observed will also be correlated to treatment response scans and patient outcome data.

Patient blood samples were taken on:

Cycles 1-3:

- Day 1
- Day 2: before and then 3 & 6 hrs post MSCTRAIL
- Day 3: 1 day post MSCTRAIL
- Day 8: 7 days post MSCTRAIL
- Day 15: 14 days post MSCTRAIL

Cycle 4:

- Day 1
- Day 15

On progression.

Up to 30ms of whole blood was obtained from patients following their written and verbal consent and transferred at ambient temperature to the laboratory within 2 hours. They were anonymised with a patient trial number and initials only and all samples were then assigned a sample number. Sample collection documentation can be found in Appendix III: TACTICAL Sample Collection Form.

Any samples remaining after these analyses were stored for future ethically approved research

Chapter 5: TACTICAL Translational Results discusses the aims, methods and results to date for this work.

Phase II

Phase II is a multicentre, randomised, blinded placebo control trial comparing MSCTRAIL at the RP2D in combination with SOC vs placebo in combination with

SOC. The aim is to investigate the safety and anti-tumour activity of MSCTRAIL compared to placebo in combination with SOC.

2.1.12 Trial design

Trial treatment, assessment and follow up will follow the pattern as discussed above with a full description available in Appendix 1: The TACTICAL protocol v4

Patients will be randomised 1:1 with 23 in each cohort (*figure 2.5*). Patient eligibility criteria is the same as phase I, detailed above, and recruiting sites will be UCLH (London) and The Christie Hospital (Manchester).

In the treatment arm, patients will receive SOC therapy on day 1 followed by MSCTRAIL on day 2 of a 21 day cycle for 3 cycle with a further 4th cycle of SOC only. In the placebo arm patients receive SOC therapy on day 1 followed by placebo on day 2 of a 21 day cycle for 3 cycles with a further 4th cycle of SOC only. After completion of the 4 cycles of trial treatment patients in both arms will revert to local standard of care as decided by their treating clinician. Patients will be followed up for a total of 24 months.

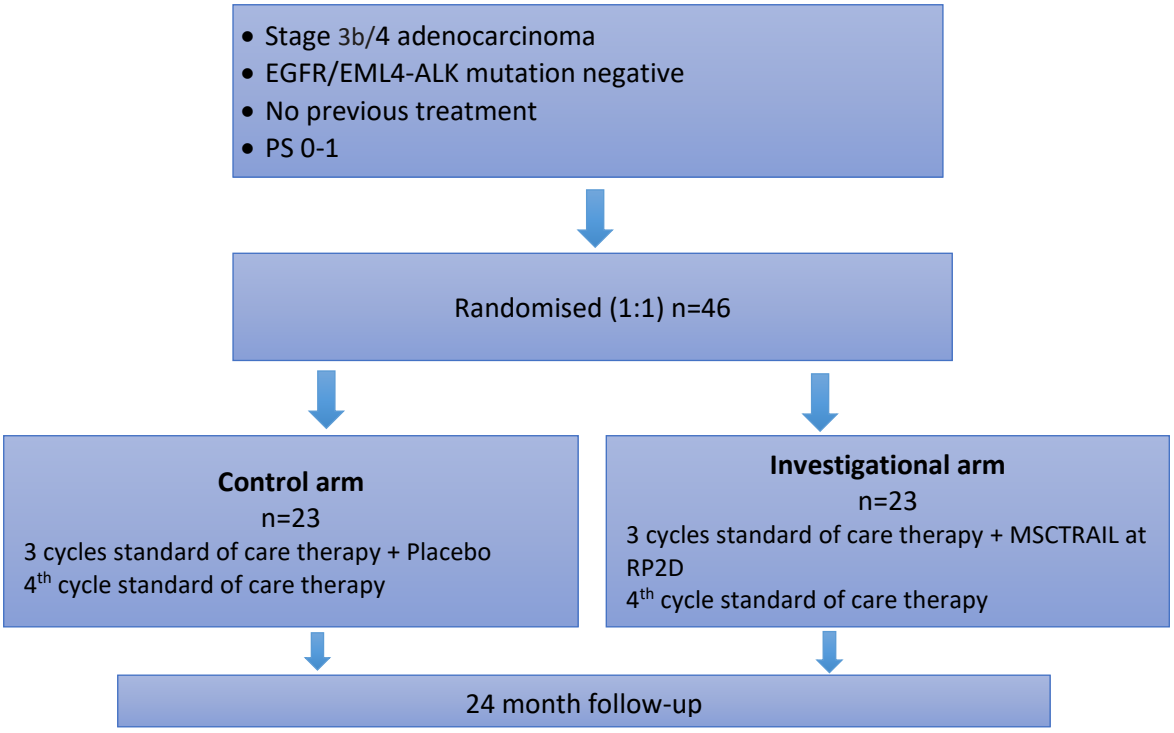


Figure 2.5: TACTICAL phase II trial schema

2.1.13 Blinding and Randomisation

Patients will be randomised 1:1 between the treatment and placebo arms using an online ‘sealed envelope’ software and stratified according to factors of:

- Performance status at baseline: 0 or 1
- Stage at time of diagnosis IIIB/C or IV

These parameters were chosen to ensure homogeneity between cohorts in terms of predicted disease natural history and life expectancy.

All members of the trial team and the patients will be blinded. While every effort has been made to make the placebo a similar product it is possible the administrating nurse, given their extensive experience, would be able to identify the product as a cell product however they work according to GCP and will maintain blinding to the patient and rest of trial team.

MSCTRAIL as a drug product is a viscous pale yellow colour on thawing and for safety reasons it must be handled and inspected for cell clumping. Untransduced MSCs cannot be used as the placebo because of the cost of MSCs and the unknown effects of delivering this product to patients. The placebo consisting of 50% phosphate buffered saline (PBS), 10% DMSO and 40% ZENALB® 4.5, identical excipients and volumes to the MSCTRAIL, but excluding MSCTRAIL cells.

To try to reduce the risk of this discrepancy a coloured translucent bag will also be placed over the products (MSCTRAIL and placebo) at the time of preparation in the clinical site’s cell therapy facility. However, it is paramount the administering nurse is still able to visualise the product for clumps and ensure it is thoroughly thawed. This method of covering the product is in keeping with practice in other blinded cell therapy trials [132] (ClinicalTrials.gov Identifier: NCT03042143, ClinicalTrials.gov Identifier: NCT02611609)

At all points, from administration to follow up, patients will be treated as if they have received MSCTRAIL by all members of the trial team. As there is no known antidote for MSCTRAILs any adverse events thought to be related to the administered product will be treated in line with standard guidelines and best supportive care.

Unblinding will occur in exceptional circumstances when a Serious Adverse Reaction occurs, and the treating investigator considers knowing the trial treatment would be in the patient's best interest. This will only be done after discussion with the PI and the Sponsor, unless it is an emergency.

This method will allow maintenance of strict experimental standards in the trial within the parameters of stringent patient safety and in line with.

2.1.14 Outcomes in phase II

The objective of phase II is to determine the anti-tumour efficacy of up to 3 doses of MSCTRAIL in combination with first line SOC in patients with metastatic lung adenocarcinoma. The secondary objectives are to assess the safety and tolerability of the treatment combination and the type and duration of treatment response, time to progression and survival in the treatment combination. To reflect this the trial end points are:

Primary Endpoints:

1. Tumour response at 12 weeks by RECIST (v 1.1) criteria after 12 weeks.

Secondary Endpoints:

1. Frequency of adverse events
2. Best overall response
3. Time to progression (TTP)
4. Change from baseline in sum of target lesions at 6 and 12 weeks
5. Tumour response at each time point
6. Duration of response
7. Progression free survival (PFS)
8. Overall survival (OS)

2.1.14.1 Population for analysis.

The primary population for analysis will be the efficacy/intent to treat (ITT) population defined as all patients randomised who receive at least one dose of protocol (randomised) study therapy (patients on the investigational arm must receive one dose of MSCTRAIL). For analysis of tumour response patients should also have evaluable tumour response (i.e. non missing baseline tumour assessments). The safety population will include all patients who receive protocol (randomised) study therapy.

Safety data will be summarised and all patients who receive at least one dose of MSCTRAIL will be included in the assessment of the safety profile (safety analysis set). Data from all cycles of initial treatment will be combined in the presentation of safety data. Adverse events (AEs) will be listed individually by patient and dose group (dose and schedule). For patients who have a dose modification during treatment, all AEs (due to drug or otherwise) will be assigned to the initial dose they received. The number of patients experiencing each AE will be summarised by the CTCAE grade. Serious AEs will be summarised separately if a sufficient number occur.

2.1.14.2 Analysis of Response

Response rate is defined as the percentage of patients who have a confirmed ‘CR’ complete response or ‘PR’ partial response prior to any evidence of progression. This primary end point allows for a small treatment population, has a defined time of 12 weeks allowing for early analysis compared with survival trials and at 12 weeks, tumour outcomes could be more attributed to the treatment intervention than if outcomes were measured at a point further away from when they received MSCTRAIL doses.

Tumour Response analysis will be calculated from response rates including CR, PR and SD. These will be used to generate confidence intervals in the investigational arm to generate best response and response for each visit. Waterfall plots indicating percentage change from baseline in sum of lesions will also be generated.

Progression Free Survival (PFS)

Is defined as the time from randomisation to progression as defined by RECIST (V1.1) criteria or time of death.

Time to Progression (TTP)

Is the time from randomisation to time of radiological progression as defined by RECIST (V1.1) criteria.

Overall Survival (OS)

Is the time from randomisation to the time of death irrespective of cause of death.

PFS, TTP and OS will be analysed using Kaplan-Meier (KM) plots and will be presented along with median PFS, TTP and OS times.

2.1.15 Statistical Modelling for Phase II

Statistics for this trial were devised in conjunction with Dr G Wheeler a medical statistical from Cancer Research UK and UCL Cancer trial Centre at UCL.

2.1.15.1 Justification of Sample Size

The sample size for phase II was based on the need to detect a difference in response rates between the treatment arms of 15%. This was calculated by comparing the standard response rates with target response rate in other recent NSCLC trials:

Standard response rates (complete or partial) in this population of patients with NSCLC on chemotherapy alone, defined as Cisplatin and Pemetrexed are in the region of about 25% [22, 23].

A target response rate of at least 41% is considered reasonable based on a review of recent Phase I/II studies in NSCLC and protocols published on the ISCRT website. Seto et al (2013) [133] powered for a more than doubling in response rates (from 25% up to 70%); Komiyama et al (2012) [134] a 15% improvement (10% vs

25%); Kurata et al (2012) [135] a difference of 15% (20% vs 35%) ; and Bral et al (2010) [136] reported response rates of 52%.

Statistical significance has to be weighed alongside the manufacturing costs of MSCTRAIL, detecting at least a 15% improvement from 25% to >41% is consistent with the type of effects anticipated in early phase II NSCLC trials.

Target accrual is 46, with an assumed 5% dropout 44 patients will be randomised 1:1 between MSCTRAIL treatment arm and placebo arm. Under a one-sided exact test at the 20% significance level we can detect a difference in response rate of at least 25% between treatment arms (i.e. 25% on control arm vs 50% on the investigational arm), with 80% power.

As well as evaluating if MSCTRAIL is more effective than placebo, the observed response rate to MSCTRAIL will also be compared with the historical control, a rate of ~25% but under much stricter type I and type II error criteria. To detect >15% improvement with MSCTRAIL and chemotherapy (i.e. to at least 41% response rate), at least 7 out of 23 patients are required to respond to MSCTRAIL. This assumes a power of about 90% and type I error (one sided) of 5%. The sample size method is based on using exact Binomial methods with approximate alpha.

Cell Manufacturing and Delivery

MSCs can be easily harvested from a number of sources, have a high capacity for proliferation, can be readily modified using viral vectors and are ‘immune privilege’ – expressing very low to no MHC class II[4]. This makes them not only ideal for the use as a cell therapy but also for large scale manufacture as an ‘off the shelf’ batched product can be made, stored and shipped to the patient as and when it is required without any delays caused by manufacturing. This is beneficial to the patient who does not need lengthy, toxic immune suppression or donor match and also in terms of reduced cost, which must be considered in this current economic climate.

MSCTRAIL is a novel product and had never been made before to GMP standards; the minimum standard required by a manufacturer to assure their product is of a sufficient standard for human use. Furthermore, there was no facility that was at the point of trial initial planning manufacturing a genetically modified cell product to the scale required for use in the whole of TACTICAL trial.

The manufacturing was under-taken by Professor Mark Lowdell and his team at the Centre for Cell, Gene and Tissue Therapeutics (CCGTT) at the Royal Free Hospital which holds a licence with the MHRA (MIA(IMP)11149) for the manufacture and release of advanced therapy medicinal products ATIMPs, including Gene Therapy products.

Much of the manufacturing process is held as confidential intellectual property. The section below is a therefore an overview of the manufacturing process, presentation of final drug product and how it was delivered.

2.1.16 Origin of the Cells

The initial trial design was to harvest bone marrow MSCs (BM-MSCs) from healthy donors with subsequent transduction and *ex vivo* expansion. However, it became clear this was not a viable route due to the logistics and costs of identifying donors and carrying out bone marrow aspirates, as well as the limited expansion potential

BM-MSCs. Umbilical cord derived MSCs (UCT-MSCs) were used instead and a full characterisation study of UCT-MSCs with validation of the switch was carried out by the CCGTT. They were found to be phenotypically comparable and met all International Society for Cellular Therapy (ISCT) criteria for MSC characterisation. Additionally, UCT-MSCs possess beneficial cell culture properties such as higher growth kinetics, harvest densities and delayed senescence permitting many more population doublings with significantly less donor-to-donor variability as the impact of donor age and lifestyle is mitigated.

Umbilical cord derived cells therefore presented a more reliable, safe, donor source and could be obtained by non-invasive means. The trial cells were procured from the Anthony Nolan Trust and passed through all the prerequisite infection tests prior to use.

MSCs were isolated from the cord, first by manual dissection, followed by digestion. These cells were seeded into cell culture flasks until 80-90% confluent then trypsinised and cryopreserved.

A total of 7 umbilical cords were used to procure the required MSCs making the final product an allogeneic pooled gene therapy Medicinal Product. Not only did this provide enough cells for the whole trial but also allowed the drug product to be defined as a ‘population’ by regulators and not an individual, requiring less repeated validation and reduced any effect of donor-donor variation.

2.1.17 Transduction

A lentiviral vector, pCCL.CMV.TRAIL (*figure 1.4*), was used to transduce MSCs with the aim of stable and constitutive expression of TRAIL. See section 1.5 for further details on the lentiviral vector. The lentiviral vector was manufactured by Cell and Gene Therapy, King’s (CGT-K) in the Rayne Cell Therapy Suite (RCTS) and the manufacture is carried out according to a Quality Assurance (QA) issued Batch Manufacturing Record (BMR) and associated Standard Operating Procedures (SOPs) with QC release testing including viral titration and infectious screening performed prior to use

Thawed MSCs were transduced with lentiviral particles to a multiplicity of infection (MOI) of 50 (i.e. 50 viral particles per MSC). This MOI ensures a high MSC transduction efficiency, as well as an adequate expression of TRAIL on the transduced cells, while not significantly affecting MSC growth kinetics and harvest densities. Transduction of MSCs with a TRAIL-expressing lentivirus does not affect their viability, phenotype, cell division, differentiation capability or tumour tropism[97].

2.1.18 Primary seed stock and Working cell stock

Transduced MSCs, now MSCTRAIL, are then pooled together to ensure homogeneity between doses as there is donor-donor variation between umbilical cords. Once pooled they are further expanded in a bioreactor before harvest, concentration, washing and cryopreserving in vials. This forms the primary seed stock (PSS).

PSS vials can then be thawed and again expanded in a further bioreactor to form the working cells stock (WCS) which is cryopreserved in bags. The final product is obtained from the expansion of cryopreserved WCS.

MSCTRAIL is packaged and cryopreserved as an aseptic product with an ISBT128 unique alphanumeric identifier.

2.1.19 Final drug product

MSCTRAIL is supplied in clear bags (phase I only) and once thawed is a turbid yellowish liquid, which should be free of particulates upon mixing/shaking with at least $2x10^6$ cells/ml and a maximum of 100mls per bag. The starting dose of MSCTRAIL is 4 $x10^8$ MSCTRAIL per dose so for the starting dose each patient requires 2 bags. This packaging allows for easier dose modifications if required as the initial dose reduction would be to $2x10^8$ cells, i.e. 1 bag.

MSCTRAIL cells are suspended in Dulbecco's phosphate buffered saline (DPBS) with a freezing medium consisting of dimethyl sulfoxide (DMSO) in ZENALB® 4.5

human albumin solution and stored in vapour phase dewars. Sterility, infectious and identity checks are carried out on the product per batch prior to QP release for clinical use.

2.1.20 Placebo

The placebo consists of phosphate buffered saline (PBS), DMSO and ZENALB® 4.5 human albumin solution with a maximum of 100ml per bag, identical excipients and volumes to the cell product but excluding MSCTRAIL cells.

The placebo will be cryopreserved and supplied in the same clear, individually labelled CryoMACS® freezing bags. Upon thawing, the appearance of the placebo is a clear yellowish liquid, which should be free from visible particulates

Figure 2.6: Photograph of thawed bag of MSCTRAIL prior to administration

2.1.21 Cell delivery

The method of delivery of MSCTRAIL was based around existing standard operating procedures (SOPS) for cell delivery as well as other cell therapy trials and tailored to the specific safety requirements and potential risk associated with this cellular product. The MSCTRAIL specific SOP for delivery can be found in Appendix IV: TACTCIAL Summary of Drug Arrangements (SoDA)

MSCTRAIL was delivered by intravenous infusion on day 2 of a 21 day cycle. Once the patient is registered in the trial and assessed as fit for treatment a prescription and request for MSCTRAIL is sent to CCGTT. The cells arrived by courier in a

cryoshipper at 09.00am on the day of use and were delivered directly to patient bedside. Administration was carried out by a senior nurse qualified in the delivery of cellular products who has undergone training in the trial, attended a site initiation visit and is on the delegation log. I, as an overseeing doctor, was present for all cell infusions.

The bags were thawed and infused one at a time to ensure no wastage of drug product. Thawing occurred in a water bath set at 30 degrees at the patient's bedside. The bags were visually inspected for clumps and then hung and infusion via a double lumen giving set with normal saline used to prime the line and flush the bag after administration (*figure 2.7*). Bags were inspected for clumping during infusion and agitated as needed.

Each bag of MSCTRAIL was administered over 20 - 30 minutes and infusion must be completed within 60 minutes of thawing to ensure patency and viability of the cells as DMSO in the freezing media is toxic to the cells. Infusion was carried out in the Apheresis centre at Macmillan Cancer Centre UCLH.

Patients were monitored closely, with bedside monitoring of oxygen saturations and heart rate and observations done every 15 minutes during the infusion, every 30 minutes for the 2 hours after and hourly thereafter until 4 hours post infusion. It was thought that any thromboembolic events would occur within first 24 hours post infusion with the highest risk being during or directly after as the cells flood the pulmonary vasculature. Observations monitoring consisted of:

- Temperature
- Heart Rate
- Blood pressure
- Respiratory rate
- Oxygen saturation level

Details of the infusion and patient observations were recorded in the 'Accountability and Monitoring Log', a copy of which can be seen in Appendix V.

Figure 2.7:MSCTRAIL delivery at patient bedside

Confidentiality, Data Protection and Trial Oversight

The trial was conducted through the UCL Cancer Research UK & UCL Cancer Trials Centre (UCL CTC). It was registered in accordance with the Data Protection Act 2018 and General Data Protection Regulation (EU)2016/679 (GDPR) with the Data Protection Officer at UCL.

Upon registration patients are assigned a unique trial number which will be used throughout this work. Data was stored in a secure manner and patient details were kept confidential. All work was and will be done in accordance with GCP and GDPR.

2.1.21.1 Informed consent

In consenting to the trial, patients are consenting to trial treatment, assessments, follow-up and data collection. All patients must be deemed to have capacity to give informed consent.

A patient information sheet was given to the patient and written informed consent obtained before entering in the study. A minimum of 24 hours was allowed for the patient to consider and discuss participation in the trial. A copy of this can be seen In Appendix II: TACTICAL Patient Information Sheet

Written informed consent on the current approved version of the consent form for the trial was obtained before any trial-specific procedures are conducted. The discussion and consent process was documented in the patient notes and their GP was informed of their decision to enter the trial.

2.1.21.2 Helsinki and GCP

The trial will be conducted in accordance with the World Medical Association Declaration of Helsinki entitled 'Ethical Principles for Medical Research Involving Human Subjects' (1996 version) and in accordance with the terms and conditions of the ethical approval given to the trial.

In conducting the trial, the Sponsor, UCL CTC and sites complied with all relevant guidance, laws and statutes, as amended from time to time, applicable to the performance of clinical trials. Full details of these can be found in Appendix 1: The TACTICAL protocol

2.1.21.3 Monitoring and Trial Oversight

Trial oversight and monitoring was conducted by UCL CTC in accordance with good practice. Further details of this and of oversight committees and independent data monitoring can be found in Appendix 1: TACTICAL protocol

2.1.21.4 Patient reimbursement.

Patients were not offered any monetary or other incentive for consenting into the trial. Reasonable travel costs and an overnight stay between days 1 and 2 were covered if requested.

This chapter has detailed the TACTICAL phase I and II protocol and trial outline including manufacturing and delivering MSCTRAIL. Chapter 3 will go on to detail the amendments needed to recruit and treat the first patient into the trial.

3 Changing Paradigm of NSCLC Treatment

In January 2019 NICE approved the combination of chemotherapy with Pembrolizumab in the first line setting for non-squamous NSCLC in patients with a tumour PD-L1 expression >1%.

Due to this changing paradigm the TACTICAL trial had to adapt as approvals had been sought and granted on the first line combination of Cisplatin and Pemetrexed with MSCTRAIL. Pre-clinical work, as detailed later in the chapter was carried out to investigate the combination of MSCTRAIL with immune checkpoint inhibitors. Subsequently ethics and regulatory approvals were sought to the protocol changes to introduce Pembrolizumab as standard of care therapy, administered as per NICE guidelines and local trust policy.

Background

Immunotherapy utilises the patient's own immune system to recognise and destroy cancer cells. Immune checkpoint inhibitors target the pathways that are being exploited by tumours to evade recognition and hence destruction [34]. They achieve this via T cell modulation [35], disrupting the physiological balance between receptors that activate and inhibit the immune system. The development of these therapies has heralded a milestone in the treatment of lung cancers which were previously thought to be poorly immunogenic.

One such pathway is that of programmed death receptor 1 (PD-1) and its ligand (PD-L1). The PD1 receptor down regulates excessive immune response and binding of it to the ligand (PD-L1) on tumour cells suppresses the host T cells, leading to evasion of the immune response and unregulated tumour growth.

Inhibition of this pathway by the therapeutics, immune checkpoint inhibitors, has been shown to be an effective therapy in the treatment of many cancers including NSCLC [8] *(figure 3.1*). These highly selective humanised monoclonal antibodies interrupt the pathway by binding to checkpoint signals, PD-1 or PD-L1, reigniting the

host anti-tumour immune response. To date, three such therapies have been approved by NICE for use in patients with NSCLC: Nivolumab (Opdivo; Bristol-Myers Squibb Company) and Pembrolizumab (Keytruda, Merck Sharp & Dohme), both targeting the PD-1 receptor, and Atezolizumab (trade name Tecentriq) which targets the ligand, PD-L1.

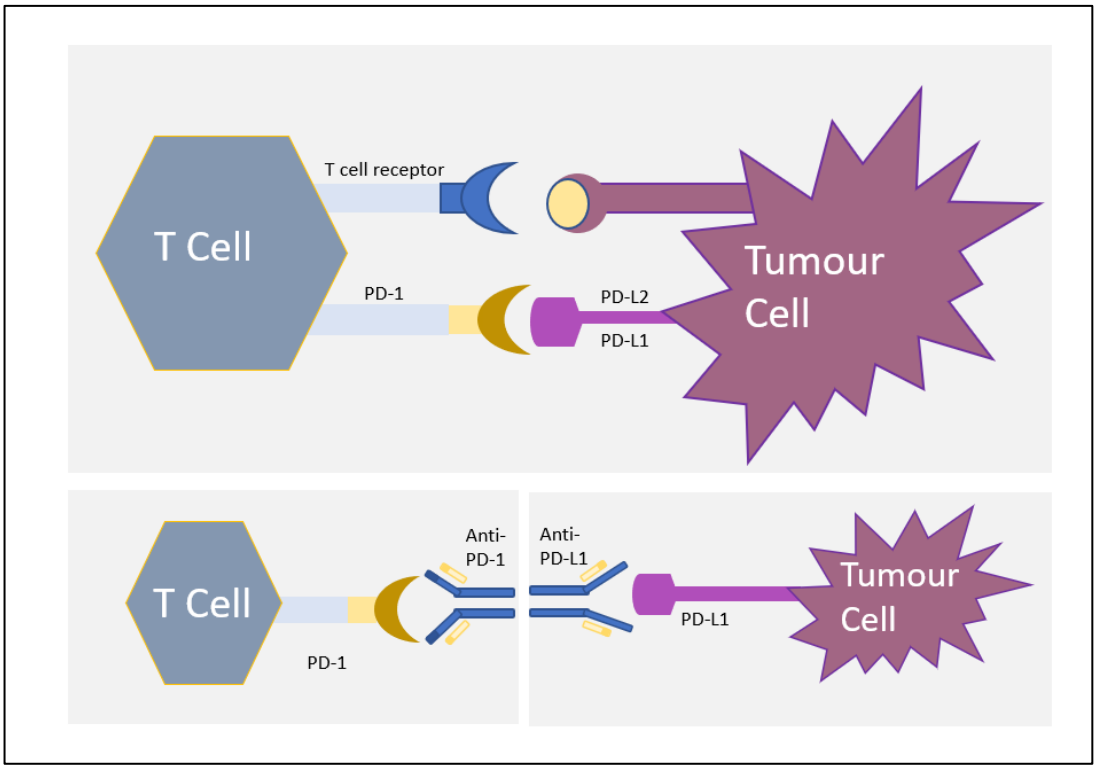


Figure 3.1: Immune check point inhibitors target and blocks the PD-1/PD-L1 pathway

T cells recognise and kill tumour cells through antigen recognition. Tumour cells evade this by blocking the T cell through the PD-1 and PD-L1 pathway. Immune checkpoint inhibitors bind to PD-1 or PD-L1 inactivating PD-1/PD-L1 pathway

A number of key studies have led to the approval of immune checkpoint inhibitors in NSCLC. Below is a summary of those relevant to advanced non-squamous NSCLC and the impact this had on the TACTICAL trial.

tKeynote042 [137] study initially that found in the second line setting that treatment with Pembrolizumab monotherapy led to significant improvement in OS over Docetaxel in metastatic NSCLC with PD-L1 expression of ≥1%. Furthermore this survival benefit was enhanced along with improved PFS in those with a PD-L1 expression of ≥50%. This led to the trial of Pembrolizumab in the first line setting; Keynote024 [138] demonstrated Pembrolizumab to have superior efficacy over chemotherapy, in a large phase III randomised control trial (RCT) in patients with previously untreated advanced NSCLC and a PD-L1 expression of ≥50% and no sensitising mutations. 305 patients were randomised to receive either Pembrolizumab or standard platinum doublet chemotherapy. Results showed improved objective response rate (ORR 45% versus 28%), progression-free survival [PFS, hazard ratio (HR) 0.5, 95% confidence interval (CI) 0.37–0.68, *P*<0.001] and overall survival (OS, HR 0.6, 95% CI 0.41–0.89, *P*=0.005) in the Pembrolizumab arm compared to the chemotherapy arm with safety and quality of life data also supporting the Pembrolizumab arm. Long term follow up of this cohort also showed continued positive outcomes with improved median OS (mOS) in those who received Pembrolizumab for 30 months versus 14 months in the chemotherapy arm.

These trailblazing studies led to NICE approval of Pembrolizumab monotherapy for first line treatment in advanced NSCLC for those whose tumour expressed ≥50% PD-L1 with no actionable driver mutations or contraindications.

Keynote189 [8] was pivotal in the introduction of Pembrolizumab for those who did not meet this PD-L1 benchmark. This large, phase III RCT compared the combination of Pemetrexed and a platinum-based drug plus either Pembrolizumab or placebo in patients with non-squamous NSCLC with any level of PD-L1 expression. mOS in the Pembrolizumab combination arm was 22.0 months (95% CI 19.5-25.2) versus 10.3 months (95% CI 8.7-13.7) in the chemotherapy placebo arm (HR 0.56, 95% CI 0.45-0.7 p<0.00001). The PFS data also favoured the Pembrolizumab combination with 8.8 months PFS in the Pembrolizumab and chemotherapy combination arm vs 4.9 months in the chemotherapy placebo arm (HR 0.48, 95% CI 0.40-0.58, P< 0.00001). Improvement in overall survival was seen across all PD-L1 categories that were evaluated, with similar adverse events reported in both cohorts.

Checkmate026 [139] investigated the use of Nivolumab vs platinum doublet therapy in previously untreated or recurrent NSCLC with PD-L1 expression >1%. Sub analysis demonstrated improved overall response rate (ORR) (47% versus 28% in the chemotherapy arm) and improved PFS (HR 0.62%, 95% CI 0.38-1.0) in those with a high tumour mutational burden (TMB)

IMpower150, IMpower132 and Impower130 provide evidence on the efficacy of Atezolizumab in advanced non-squamous NSCLC with Impower150 [140] showing improved PFS and OS in those receiving Atezolizumab in addition to Bevacizumab plus chemotherapy versus just bevacizumab plus chemotherapy alone.

Figure 3.2 below is adapted from the current NICE approved treatment algorithm for non-squamous NSCLC without actionable mutational drivers.

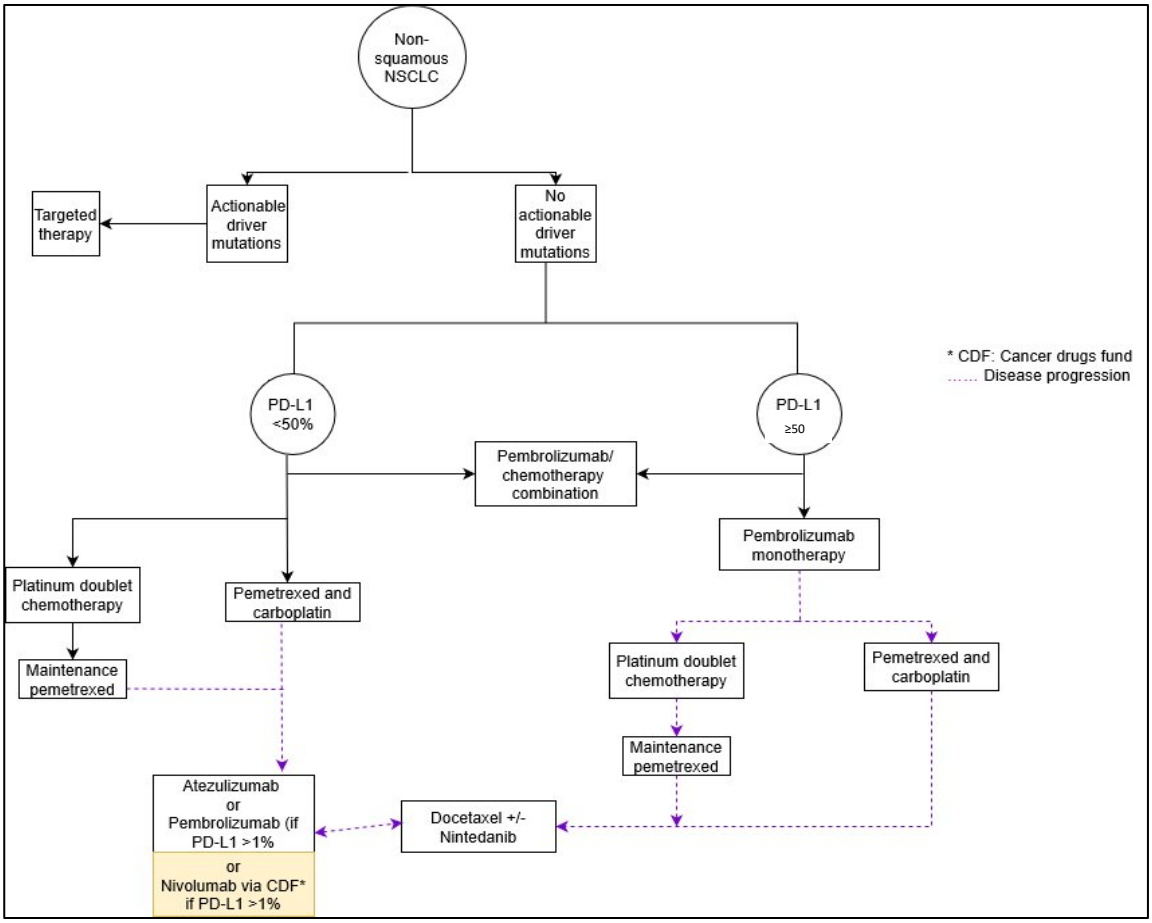


Figure 3.2: Treatment algorithm for advanced non squamous NSCLC without actionable driver mutations edited from NICE guidelines

Pre-clinical work

This work is unpublished and has been carried out in collaboration with Dr K Kolluri and Dr D Alrifai at Lungs for Living.

Given the novel nature of immune checkpoint inhibitors and MSCTRAIL there was no data on co-culture of the combination. Work was carried out to establish this and the preliminary *in vivo* data is summarised below.

Aim: To investigate the efficacy of tumour cell death with the combination of MSCTRAIL and anti-PD1 therapy in the presence of PBMCs.

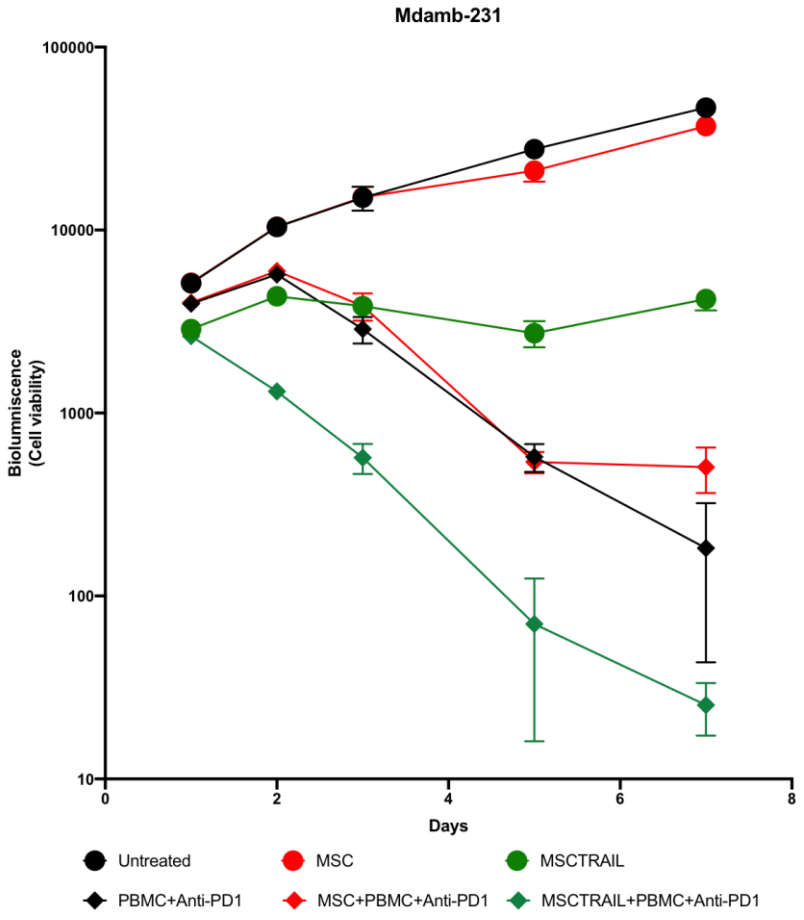
Results demonstrated that the combination of MSCTRAIL and PD1 inhibitor in the presence of peripheral blood mononuclear cells (PBMCs) caused increased apoptosis in cancer cells compared to when each treatment was given in isolation. Work to elucidate the mechanism of this as well as expand further are on-going and not included in the scope of this thesis.

3.1.1 MSCTRAIL in combination with a PD1 inhibitor, in the presence of PBMC.

To demonstrate the reduction of tumour cell viability when treated with MSCTRAIL in combination with PD1 inhibitor in the presence of PBMCs, MDAMB-231 metastatic breast cancer cells were transduced with lentiviral vectors expressing mStrawberry fluorescent protein and firefly luciferase. These MDAMB-231 cells were then treated with MSCs, MSCTRAIL and PD1 inhibitor in presence of PBMCs in isolation as well as combination. Luciferin was added to the wells prior to bioluminescent measurement.

Bioluminescent signal is a marker of viability of tumour cells and was measured at 1, 2, 3, 5 and 7 days following treatment.

The results showed a lower bioluminescent signal in the combination treatment group compared to the other treatment arms. This indicated that the combination of MSCTRAIL, PD1 inhibitor and PBMCs was more effective at causing tumour cell death than any of the other treatment arms.



Note: Y-axis in Log scale

Figure 3.3: Co-culture of MDAMB-231 cancer cell line expressing mStrawberry fluorescent protein and firefly luciferase with untransduced MSC, MSCTRAIL and in combination with anti-PD1 in presence of PBMCs to measure cell viability of the MDAMB-231 cells over time

The combination of MSCTRAIL and Anti-PD1 with PBMCs resulted in more cancer cell death than other treatment arms

The cells were also visualised by fluorescent microscopy following excitation with green light on day 5 (*figure 3.4*). On visual inspection under fluorescent microscope there is a reduced amount of mStrawberry florescent in the combination therapy arm (*figure 3.4 D)* compared to other arms reflecting a reduction in viable tumour cells in this group.

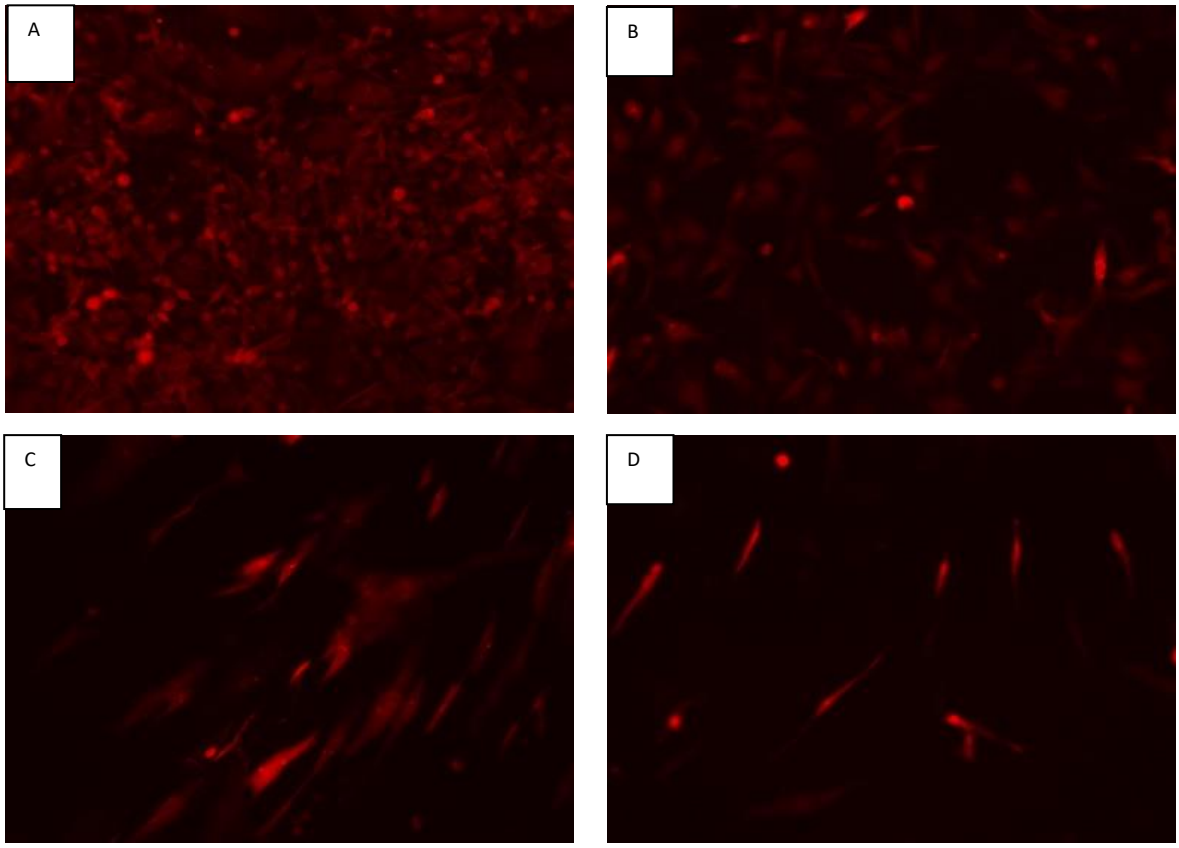


Figure 3.4: Representative sample of day 5 co-culture of MSCTRAIL and PD1 inhibitor and PBMCs with Luciferase expressing (Luc) MDAMB-231. Increased MDAMB-231 cell death. In the combination group.

A-D) Images under immunofluorescent microscopy
A) Untreated Luc MDAMB-231 cells B) MDAMB-231 cells with PD1 inhibitor (with PBMCs)
C) Luc MDAMB-231 cells with MSCTRAIL D) Luc MDAMB-231 cells with PD1 inhibitor (And PBMCs) and MSCTRAIL

To confirm the above results, an orthogonal assay was performed. MDAMB-231 cells were stained with a red florescent dye (DiI) and co-cultured with MSCTRAIL and PD1

inhibitor in presence of PBMCs. The cells were stained with Annexin V/DAPI to quantify the apoptosis by fluorescence-activated cell sorting (FACS) after 48 hours of co-culture. An increased percentage of apoptosed cells were seen in the combination group treated with MSCTRAIL and PD1 inhibitor in presence of PBMCs than with each treatment given in isolation *(figure 3.5).*

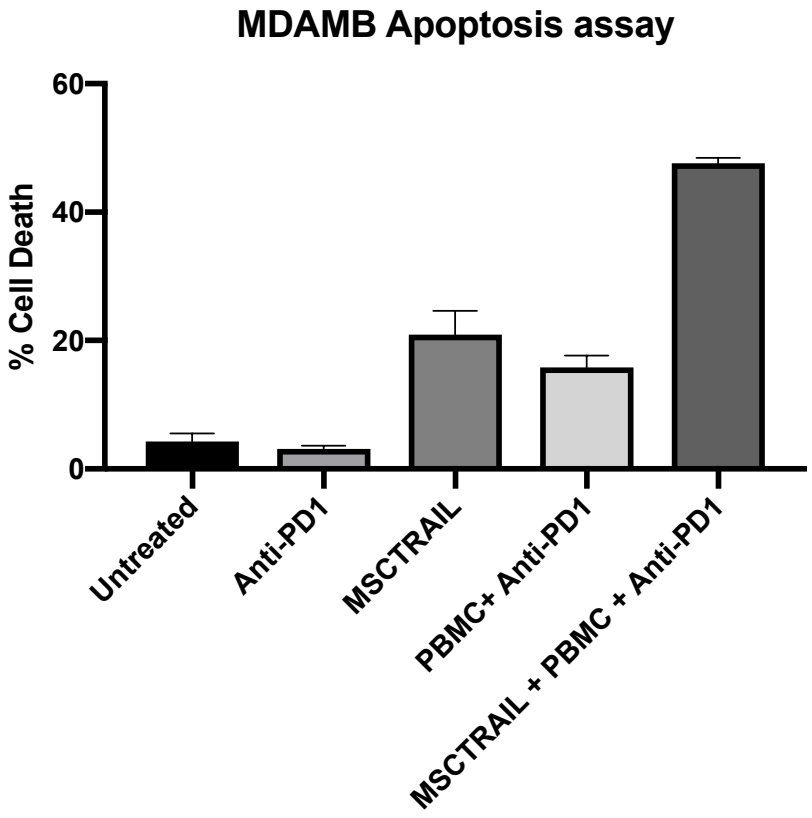


Figure 3.5 48 hour apoptosis co-culture assay of MDAMB-231 cells with MSCTRAIL, PD1 inhibitor+ PBMCs

There is maximum tumour cell death observed in the combination arm

These results suggest that the combination of MSCTRAIL and PD1 inhibitor, in presence of PBMCs leads to increases apoptosis in cancer cells.

Work to elucidate this mechanism is ongoing.

Substantial amendment

Following this pre-clinical work, the TACTICAL protocol was updated, and a substantial amendment was submitted with revised inclusion criteria to allow patients to receive Pembrolizumab as part of their SOC therapy.

Adapting the trial to include the use of Pembrolizumab allowed us to widen our patient cohort with patients receiving the up to date best practice first line treatment in combination with a novel therapy. Ensuring the role of MSCTRAIL can be evaluated in the context of this rapidly changing paradigm. Although the trial was originally opened without this amendment, all patients were ultimately recruited under these revised approvals.

3.1.2 Protocol amendments

The protocol was amended to reflect the implications of this change both from a trial perspective as well as safety.

'SOC' was still to be given on day 1 but re-defined as:

- Chemotherapy (Pemetrexed 500mg/m^2 and Cisplatin 75mg/m^2)
  and
- Immunotherapy (Pembrolizumab 200mg)

Safety measures pertinent to Pembrolizumab were added to the eligibility criteria as well as management. These can be found within Appendix 1: The TACTICAL protocol V4.

	Cycle 1						Cycle 2						Cycle 3						Cycle 4					
Week	**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**		**12**	
Day	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Pemetrexed 500 mg/m^2, iv	•						•						•						•					
Cisplatin 75 mg/m^2, iv	•						•						•						•					
Pembrolizumab 200mg, IV	•						•						•						•					
MSCTRAIL 4x10^8, 2x10^8 or 8x10^7 cells iv, depending on toxicities **Or placebo** (in phase II)		•						•						•										

Table 3.1: Amended TACTICAL patient treatment schedule

3.1.2.1 Changes to Statistical Modelling

The statistics for the trial were originally based on standard response rates to chemotherapy alone, which were in the region of about 25% [22, 23]**.** However this is higher in studies looking at the use of immune checkpoint inhibitors, where it can be observed at 44.8% in patients with PD-L1>50% receiving Pembrolizumab monotherapy[138] and 47.6% in patients receiving Pembrolizumab with Pemetrexed and platinum based chemotherapy[8]. As a result, a standard response rate of 45% was adopted. At the time of this change, the target accrual could not be increased due to the constraints of cost of manufacturing and time for patient accrual related to trial funding.

Target recruitment was therefore fixed at 46 in total and allowing for an assumed 5%, dropout rate, 44 patients will be randomised 1:1 between MSCTRAIL treatment arm and placebo arm. Under a one-sided exact test at the 20% significant level, we can detect a difference in response rate of at least 25% between treatment arms

with an 80% power i.e. a treatment response of 45% on the control arm versus 70% on the investigational arm (increased from 50% in the original trial design utilising chemotherapy alone).

This design allows recruitment of the same number of patients enabling the trial to run to the same timings and budget. However, increasing the target response rate in the investigational arm to 70% is quite high- but is an unavoidable pressure if the trial was to continue.

We will also continue to test whether the observed response rate in the MSCTRAIL arm is significantly different to a historical control rate of 45%, but under stricter type I and type II error criteria. To detect 25% improvement with MSCTRAIL and chemotherapy (i.e. to at least 70% response rate), at least 13 out of 22 patients are required to respond to MSCTRAIL. This assumes a power of about 90% and type I error (one sided) of 13%. The sample size method is based on using exact Binomial methods with approximate alpha.

3.1.2.2 Changes to Efficacy Reporting

Patients who receive immunotherapy will have scans reported by iRECIST. This is a modified version of the reporting system specifically for patient receiving immune therapy. It was introduced as the patterns of response seen in solid tumours on CT imaging in patients receiving immunotherapy were different to that previously recognised [141]. Scans were showing early changes which were being interpreted as progression but later found to have pronounced and prolonged responses, iRECIST allows for these observations. Responses assigned using iRECIST reporting have an ‘i’ prefix.

As iRECIST is commonly used in clinical trials for patients with solid tumours receiving immunotherapy it was adapted into the TACTICAL protocol alongside the amendment.

Ethics approvals

Timeline of approvals:

Date original MHRA approval: 19/02/18

Date original GTAC/REC approval: 16/03/18

A substantial amendment requires full approval by the ethics and regulatory bodies before it can be implemented.

- Submission to MHRA/HRA/REC 7.1.19
- MHRA acceptance 2.4.19
- HRA acceptance and permission to implement amendment 3.4.19
- Trial open to recruitment 3.5.19

Moving forward this amendment allowed for recruitment and treatment of the first patients in the TACTICAL trial. This chapter has summarised the changes that were required to allow this. Subsequent chapters will present the results to date for the TACTICAL trial.

4 TACTICAL Trial Results

Phase I of TACTICAL I is a single centre accelerated dose de-escalation trial using a modified Bayesian continual reassessment method. It opened on 05/03/19 and, following a series of delays, including manufacturing interruptions, protocol amendments and difficulties identifying an eligible patient, the first patient was recruited in June 2019.

To date four patients have been recruited and treated in the TACTICAL trial at the highest starting dose, $4x10^8$ cells. The first two patients (TAC-01 and TAC-02) received all three trial specified doses of MSCTRAIL in combination with standard of care therapy (SOC), one patient (TAC-03) received two doses of MSCTRAIL with SOC and one (TAC-04) received one dose of MSCTRAIL with SOC.

Following the identification of asymptomatic pulmonary embolisms (PE) in the first three patients an urgent safety review was carried out and a report to halt recruitment was submitted to MHRA on grounds of an urgent safety measure. This not only paused recruitment but stopped the administration of any further doses of MSCTRAIL. The patients continued in follow up but received SOC treatment only.

No DLTs were experienced by any patients during infusion or follow up. All patients have completed the assigned 4 cycles of trial treatment as detailed above and are now on maintenance in follow up.

All patients had a reduction in size of target lesions at 6 and 12 weeks. One patient had a reduction in keeping with stable disease (SD) and two showed a reduction in keeping with partial response (PR) by iRECIST criteria. One patient, TAC-01, however also had new lesions on 12 week scan in keeping with unconfirmed progressive disease (iUPD) (by iRECIST). Disease progression was confirmed (iCPD by IRECIST) on subsequent imaging 6 weeks later during maintenance therapy for this patient. They were found to have enlargement of the previously new lesions with new nodal disease as well as new brain lesions with a time to progression (TTP) of 141 days.

This chapter presents the clinical patient results including safety and efficacy.

Given the small patient cohort there is insufficient data to power statistical analysis.

Clinical Patient results

4.1.1 Patient recruitment

I identified patients initially during the biweekly UCLH MDTs and then screened their notes for eligibility, a copy of the screening log can be found in Appendix VII: TACTICAL Screening Log. If thought to be initially eligible I highlighted this to the team during the Oncology pre clinic meeting and in clinic they were asked if they would consider clinical trials and if so a patient information sheet (PIS) on TACTICAL was given. This was followed up by a telephone call more than 24 hours later and if the patient was happy to proceed, they were referred to the clinical research facility (CRF) for face to face review, trial counselling, eligibility confirmation and consenting. Patients were also referred to UCLH directly for consideration of clinical trials from surrounding hospitals. These patients were seen directly in the CRF and eligibility, counselling and consent begun from there.

Only 4 patients were approached and given a PIS and all 4 consented to be part of the trial.

Patients were assigned an alphanumerical trial number upon registration to ensure confidentiality in line with GCP and GDPR- TAC-XX.

All patients recruited were male with a median age at diagnosis of 65 (62-76) years old. Patients all had performance scores 0-1, with 50% PS0 and 50% PS1. All patients were current or ex-smokers with at least a 20 pack year history, (mean 43.75 pack years).

All patients had histologically diagnosed adenocarcinoma of the lung, 25% (n=1) stage IIIB, 75% (n=3) IVB, with no actionable driver mutations. 50% (n=2) had PDL1 expression 0% and 50% (50%) >1% expression.

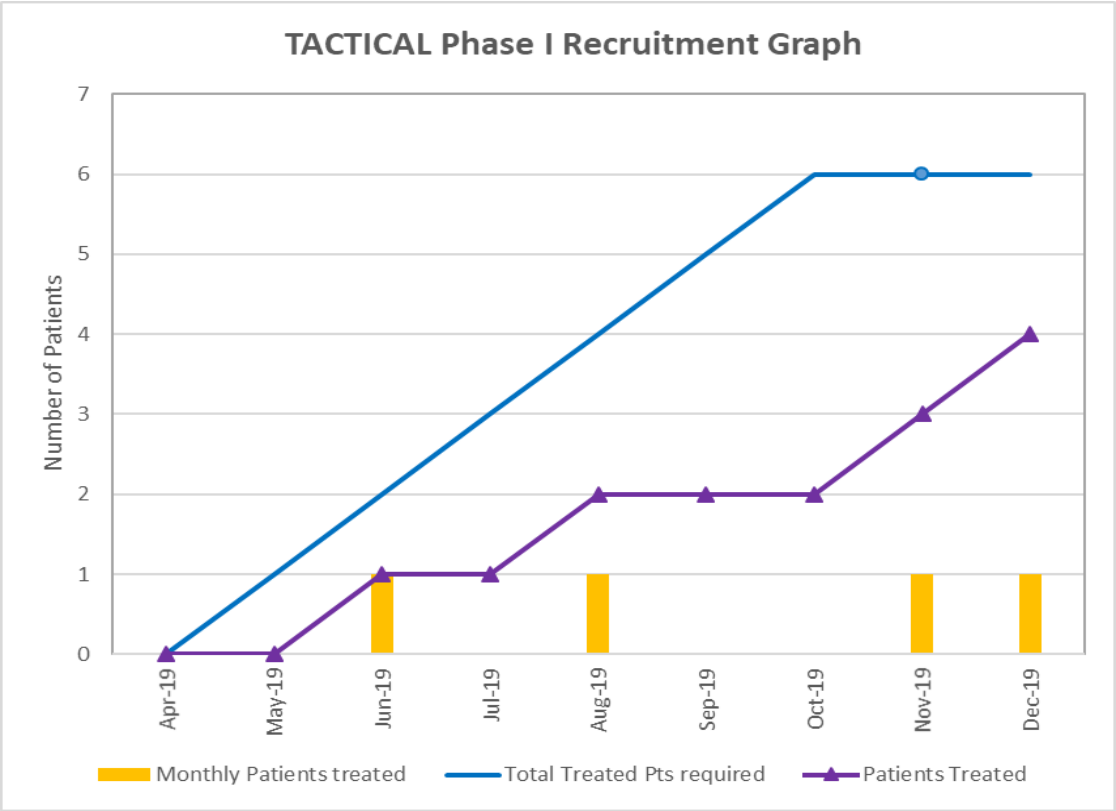


Table 4.1: TACTICAL phase I recruitment

	Median (range)
Age at registration (years)	65 (62-76)
Diagnosis to registration	1.9 months (1.2-2.3)
Gender	**N (%)**
Female	0 (0%)
Male	4 (100%)
ECOG performance status	**N (%)**
0	2 (50%)
1	2 (50%)
PDL1 Expression	**N (%)**
0%	2 (50%)
1-50%	1(25%)
>50%	1(25%)
Tumour Staging	**N (%)**
IIIb	1 (25%)
IVb	3 (75%)

Table 4.2: TACTICAL phase I patient demographics

4.1.2 TAC-01

TAC-01 is 63-year-old male patient who presented to his GP in March 2019 with a hoarse voice, sore throat and mild cough. There was no hemoptysis, but he experienced some mild breathlessness and an associated half a stone weight loss.

Past medical history of left femoral angioplasty in 1999 and hypertension. He took clopidogrel and atorvastatin.

The patient previously worked as a builder but was not currently working. He lived with his sister in a 4th floor flat and smoked 50 cigarettes a day and had done for 30 years (75 pack years) and drank occasional alcohol.

He was independent and could walk unrestricted on the flat and manage two flights of stairs- PS0.

TAC-01 was referred to ENT who diagnosed a vocal cord palsy and arranged a computer tomography (CT) scan of chest and neck. This revealed a large left upper lobe lung mass, bilateral hilar lymphadenopathy and supra clavicular lymph nodes. He was then referred to Respiratory who carried out a positron emission tomography scan (PET) and magnetic resonance imaging (MRI) of the head, results summarised in table 4.3.

Subsequent ultrasound (US) guided supra clavicular lymph node biopsy confirmed metastatic NSCLC in keeping with adenocarcinoma and he was staged as T2bN3M0 – stage IIIB. EGFR, Alk-ve, ROS-1 is negative. PD-L1 expression 10%

Date	Indication	Type of Scan	Target lesions (TL)	Sum TL	Non Target disease	Other
8.6.19	Baseline	CT CAP with Contrast	Pre carinal lymph node 2.3 cm Aortopulmonary lymph node 2.6 cm Left upper lobe spiculated lung mass 2.2 cm	7.1cm	Left supra hilar soft tissue. Mediastinal lymph nodes. Left sub clavicular fossa node. Small volume nodularity and septal thickening in the left upper lobe	Small hyperdense focus in the inferior aspect of the liver Small bilateral adrenal nodules. 1 cm nodule inferior to the hepatic flexure
4.5.19	Baseline	MRI head with contrast				No signs of intracranial malignant disease
4.5.19	Staging	PET-CT	FDG avid mass in the left upper lobe FDG avid left hilar, bilateral mediastinal and left supraclavicular fossa lymphadenopathy			

Table 4.3: TAC-01 Summary of baseline imaging

4.1.2.1 MSCTRAIL Infusions

TAC-01 tolerated treatment well, receiving all trial defined MSCTRAIL infusions on time. Treatment commenced on 26/06/19 with Pemetrexed, Cisplatin and Pembrolizumab followed $4x10^8$ MSCTRAIL cells day 2, 27/06/19. TAC-01 subsequently completed the stipulated trial treatment regimen without complication (MSCTRAIL dose 2 18/07/19 & dose 3 on 08/08/19).

1 AE occurred during the first infusion of MSCTRAIL (Grade 2 Hypoxia); a desaturation to 88% with associated chest tightness during, this occurred after the cannula had been flushed as it had become blocked during infusion. He recovered symptomatically with a return to baseline saturations within less than 1 minute without any intervention required.

During cycles 2 and 3 the patient suffered a number minor of transient desaturations. Every episode was asymptomatic, a reduction in saturation was seen on the monitor, they lasted less than 30 seconds with full resolution. There was no associated reflex tachycardia or increase in respiratory rate. Lowest recorded saturation 90%, not low enough to qualify for a CTCAE grading but noted for interest. ECGs post all infusion did not show any changes and observations were otherwise stable during infusion and for the 6 hours afterwards. No other adverse events occurred.

Cycle	Treatment	Dose	Date
1	Pemetrexed Cisplatin Pembrolizumab	500mg/m^2 IV 75mg/m^2 IV 200mg	26/06/19
	MSCTRAIL	$4x10^8$ Cells	27/06/19
2	Pemetrexed Cisplatin Pembrolizumab	500mg/m^2 IV 75mg/m^2 IV 200mg	17/07/19
	MSCTRAIL	$4x10^8$ Cells	18/07/19
3	Pemetrexed Cisplatin Pembrolizumab	500mg/m^2 IV 75mg/m^2 IV 200mg	07/08/19
	MSCTRAIL	$4x10^8$ Cells	08/08/19
4	Pemetrexed Cisplatin Pembrolizumab	500mg/m^2 IV 75mg/m^2 IV 200mg	28/08/19
5	Pemetrexed Pembrolizumab	500mg/m^2 IV 200mg	18/09/19
6	Pemetrexed Pembrolizumab	500mg/m^2 IV 200mg	16/10/19

Table 4.4: TAC-01 Summary of trial treatment timeline

4.1.2.2 Follow up Adverse Events

TAC-01 tolerated treatment well, he did not suffer any DLTs during or immediately after MSCTRAIL infusion. He experienced a number of grade 1-2 events related to other treatments or disease (*table 4.5*).

On 12 week, routine staging scan showed filling defects in the right lower lobe pulmonary artery branches in keeping with small pulmonary emboli. A grade 3 AE. Of note the patient reported mild breathlessness (grade 1) on 31/7/19 cycle 2 day 15. A CT pulmonary angiogram (CTPA) carried out on this day did not show any evidence of pulmonary or cause for the breathlessness which resolved without treatment.

The patient was reviewed clinically following this incidental finding. They were asymptomatic with no new shortness of breath, chest pain or palpitations and found to have a Hestia score of 0.

On clinical examination TAC-01 was haemodynamically stable, saturations and heart rate were maintained. An ECG did not show any new changes and subsequent transthoracic echo has also showed no evidence of right heart strain.

TAC-01 was informed of the findings of the CT and following appropriate counselling and education, commenced a treatment dose of LMWH (Enoxaparin subcutaneous 1.5mg/kg as per trust guidelines) on the same day. He has, to-date, not suffered any adverse events related to this treatment including major or minor bleeding or any sequalae related to pulmonary embolism.

Adverse event	Grade	Start Date	End Date	Related to MSCTRAIL	Related to standard treatment
Hypoxia	2	27-Jun-19	27-Jun-19	**Related**	Not Related
Non-cardiac chest pain	1	27-Jun-19	27-Jun-19	**Related**	Not Related
Rash maculo-papular	1	09-Jul-19	08-Aug-19	Not Related	Not Related
Watering eyes	2	10-Jul-19	Ongoing	Not Related	Related
Skin ulceration	1	19-Jul-19	08-Aug-19	Not Related	Related
Dyspnoea	1	31-Jul-19	31-Jul-19	Not Related	Not Related
Oropharyngeal thrush	1	14-Aug-19	17-Sep-19	Not Related	Related
Paraesthesia	1	02-Aug-19	29-Aug-19	Not Related	Not Related
Pulmonary embolism	3	18-Sep-19	ongoing	**Related**	Not related

Table 4.5: Summary of adverse events reported for TAC-01

4.1.2.3 Efficacy Results

TAC-001 initially showed a good response to treatment, 6 week scan showed stable disease (SD) by iRECIST criteria (sum of target lesions decreasing from 7.1cm to 6cm, 15.5% reduction). On the 12-week end of treatment scan, there had been further improvement in disease seen within the chest. However, an enlarging ischiorectal lesion was noted (unconfirmed progressive disease by iRECIST criteria). This was found to be FDG avid on PET and on subsequent CT scan after 6 weeks it had enlarged further in keeping with progressive disease (iCPD by iRECIST criteria). Biopsy of this lesion confirmed it was a metastasis from the original lung primary.

Of note during maintenance follow up the patient also experienced a transient episode of facial and scalp numbness; subsequent brain MRI imaging confirmed the presence of two parenchymal brain metastases. He was referred to neuro-oncology and received Gamma Knife treatment.

Table 4.6 below summarises the radiological imaging from baseline to progression for TAC-01.

Date	Indication	Type of Scan	Target lesions (TL)	Sum TL	Non-Target disease	Other	Outcome by iRECIST
8.6.19	Baseline	CT CAP with Contrast	Pre carinal lymph node 2.3 cm Aortopulmonary lymph node 2.6 cm Left upper lobe spiculated lung mass 2.2 cm	7.1cm	Left supra hilar soft tissue. Mediastinal lymph nodes. Left sub clavicular fossa node. Nodularity and septal thickening in the left upper lobe	Small hyperdense focus in the inferior aspect of the liver Small bilateral adrenal nodules. 1 cm nodule inferior to the hepatic flexure	
7.8.19	6 week response	CT CAP with Contrast	Pre carinal lymph node 1.6 cm Aortopulmonary lymph node 2.2 cm Left upper lobe spiculated lung mass 2.2 cm	6cm	Significant reduction in left supra hilar soft tissue and other mediastinal lymph nodes Left sub clavicular fossa node, absent Nodularity and septal thickening in the left upper lobe, has reduced.	Enhancing lesion in the left ischiorectal fossa slightly larger Nodule inferior to the hepatic flexure, much smaller	**iSD**
31.7.19	SOB	CTPA				No PE	
15.9.19	Left facial numbness	MRI head with contrast				At least 2 new parenchymal metastases, no significant mass-effect	
16.9.19	12 week response	CT CAP with Contrast	Precarinal lymph node 1.5 cm Aortopulmonary lymph node 2.2 cm Left upper lobe spiculated lung mass 2.2 cm	5.9cm	Significant reduction left suprahilar soft tissue other mediastinal lymph nodes, Left subclavicular fossa node, absent Nodularity and septal thickening in the left upper lobe, has reduced	Further increase in size left ischiorectal fossa nodule, right paracolic peritoneal nodule, retrocaval node. Right lower lobe pulmonary artery branches small pulmonary emboli.	**iUPD**
8.11.19	Response	CT CAP with Contrast	Precarinal lymph node 0.8 cm Aortopulmonary lymph node 2.2 cm Left upper lobe spiculated lung mass 2.3 cm	5.3cm	Left suprahilar soft tissue significant reduction Other mediastinal lymph nodes show significant reduction since Left subclavicular fossa node, absent	Further increase in size: peripancreatic lymph nodes, Right paracolic peritoneal nodule i Left ischiorectal fossa nodule Right lower lobe pulmonary emboli	**iCPD**

Table 4.6: TAC-01 Summary of imaging and efficacy

Note: iSD = Stable disease, iUPD= Unconfirmed Progressive disease, iCPD = Confirmed Progressive disease by iRECIST

4.1.2.4 TAC-01 Disease Progression

On 12 follow up response scan the patient was found to have new lesions in keeping with iUPD (by iRECIST) on subsequent imaging these lesions had increased in size with new nodal disease and new brain metastasis in keeping with confirmed progressive disease (iCPD by iRECIST). Time to progression and progression free survival is recorded as 141 days, 4.6 months.

Date of Registration	Date Progression confirmed	Mode of confirmation	Sites of progression	Time to progression
20-Jun-19	08-Nov-19	CT-Scan	Peripancreatic lymph nodes Right paracolic nodes **Left ischiorectal fossa nodule**	141 days

Table 4.7: TAC-01 Time to progression

4.1.3 TAC-02

TAC-02 is a 63-year-old male patient who initially presented to their GP in May 2019 with a cough productive with small volume heamoptysis.

He had other respiratory symptoms; with no breathlessness and regularly walked 3-miles per day. No chest pains or weight loss.

Chest X-ray showed a mass in the right lower lobe, so he was referred to hospital for review.

He has a history of spinal surgery for degeneration in 2012 and COPD.

Medication was tiotropium only.

The patient lives with his wife and son, he was an ex-smoker with a 50-pack year history and is still working as a teacher. PS0

CT Chest abdomen and pelvis (CT CAP) and subsequent PET-CT confirmed a 4.2cm right lower lobe mass with PET avid disease in the liver, lumbar vertebrae and right pubic bone staged at T2aN1M1c- stage IVB, baseline imaging is summarised in table 4.8.

CT guided biopsy of the lung mass confirmed metastatic adenocarcinoma of lung primary, EGFR, ALK, ROS-1 negative, PD-L1 60%

Date	Indication	Type of Scan	Target lesions (TL)	Sum TL	Non Target disease
29.7.19	Baseline	CT CAP with Contrast	Right lower lobe lung mass 4.2 cm	4.2cm	Right infrahilar lymph node. Small sub centimetre nodule apical segment right lower lobe Sclerotic bone lesions in the left acetabulum, L2 and right pubic bone
28.5.19	Staging	PET-CT	FDG avid 29 x 26 mm right lower lobe nodule Right lower lobe lymphadenopathy. FDG avid sclerotic bone metastases The focal FDG avid single liver lesion is in keeping with further metastasis		
5.6.19	Staging	MRI head			No space-occupying lesion. No evidence of leptomeningeal enhancement.

Table 4.8: TAC-02 Summary of baseline imaging

4.1.3.1 MSCTRAIL Infusions

TAC-02 tolerated treatment well receiving all trial defined MSCTRAIL infusions on time. Treatment commenced on 06/08/19 with Pemetrexed, Cisplatin and Pembrolizumab followed $4x10^8$ MSCTRAIL cells day 2, 07/08/19. TAC-02 subsequently completed the stipulated trial treatment regimen without complication (MSCTRAIL dose 2 28/07/19 & dose 3 18/09/19). He then continued on maintenance therapy. Summary of trial treatment can be seen in table 4.9.

He did not experience any infusion related DLTs but was also noted during MSCTRAIL infusions to experience minor, self-resolving, asymptomatic desaturations of less than 30 seconds in duration with the lowest recorded reading of 90%. This again did not meet the CTCAE grading threshold for desaturation but was noted for interest.

ECGs post all infusion did not show any changes and observations were otherwise stable during infusions and for the 6 hours afterwards. No other adverse events occurred.

Cycle	Treatment	Dose	Date
1	Pemetrexed Cisplatin Pembrolizumab	500mg/m^2 IV 75mg/m^2 IV 200mg	06/08/19
	MSCTRAIL	4x10^8 Cells	07/08/19
2	Pemetrexed Cisplatin Pembrolizumab	500mg/m^2 IV 75mg/m^2 IV 200mg	27/08/19
	MSCTRAIL	4x10^8 Cells	28/07/19
3	Pemetrexed Cisplatin Pembrolizumab	500mg/m^2 IV 75mg/m^2 IV 200mg	17/09/19
	MSCTRAIL	4x10^8 Cells	18/09/19
4	Pemetrexed Cisplatin Pembrolizumab	500mg/m^2 IV 75mg/m^2 IV 200mg	08/10/19
5	Pemetrexed Pembrolizumab	500mg/m^2 IV 200mg	31/10/19
6	Pemetrexed Pembrolizumab	500mg/m^2 IV 200mg	21/11/19
7	Pemetrexed Pembrolizumab	500mg/m^2 IV 200mg	14/12/19
8	Pemetrexed Pembrolizumab	500mg/m^2 IV 200mg	30/12/19
9	Pemetrexed Pembrolizumab	500mg/m^2 IV 200mg	27/1/20

Table 4.9: TAC-02 Summary of trial treatment timeline

4.1.3.2 Follow up and Adverse Events

TAC-002 did not suffer any infusion related AEs or DLTs during or immediately after treatment with MSCTRAIL. He experienced a number of minor AEs (grade 1-2) related to standard treatment and underlying disease and an episode of self-resolving grade 2 phlebitis at the MSCTRAIL canula site.

On their routine 6 week treatment response scan, reported on afternoon of 18/08/19, there filling defects noted in the main pulmonary arteries extending into the lower lobe pulmonary artery branches bilaterally in keeping with pulmonary emboli (PE), grade 3 AE thromboembolic event.

TAC-02 was reviewed clinically the same day following the reporting of the CT of incidental pulmonary embolism. They were asymptomatic with no new shortness of breath, chest pain or palpitations and found to have Hestia score of 0.

On clinical examination they were haemodynamically stable, saturations and heart rate were normal. An ECG did not show any new changes and a subsequent transthoracic echo also confirmed no right heart strain.

The patient was informed of the findings of the CT and, following appropriate counselling and education, commenced on treatment dose LMWH (Enoxaparin subcutaneous 1.5mg/kg as per trust guidelines) on the same day.

To date, there have been no adverse events related to either anticoagulant treatment, including major or minor bleeding, or PE related sequalae.

Adverse event	Grade	Related to MSCTRAIL	Related to standard treatment
Constipation		Not Related	Related
Gastroesophageal reflux	2	Not Related	Not Related
Phlebitis	2	**Related**	Not Related
Pulmonary Embolism	3	**Related**	Not Related
Hypophosphatemia	1	Not Related	Related
Fatigue	1	Not Related	Related

Table 4.10: Summary of adverse events reported for TAC-02

4.1.3.3 Efficacy Results

TAC-02 was shown to have positive outcomes on efficacy imaging: 6 week scan showed a reduction in target lesions in keeping with iSD by iRECIST criteria (sum of TL reduced from 4.2cm to 3.4cm, 19.1%). His 12 week scan showed a further reduction in keeping with iPR by iRECIST (sum of TL reduced from 4.2cm to 2.4cm, 42.8% reduction). This response has been maintained throughout maintenance therapy to date with the last scan (20.1.20) showing a target lesion that may represent scar tissue only.

Table 4.11 summarises efficacy response scans for TAC-02

Date	Indication	Type of Scan	Target lesions (TL)	Sum TL	Non Target disease	Other	Outcome by iRECIST
27.7.19	Baseline	CT CAP with Contrast	Right lower lobe lung mass 4.2 cm	4.2cm	Right infrahilar lymph node. Small nodule apical segment right lower lobe Sclerotic bone lesions in the left acetabulum, L2 and right pubic bone	Stable small indeterminate nodular medial limb left adrenal	
12.9.19	6 week response	CT CAP with Contrast	Right lower lobe lung mass 3.4 cm	3.4cm	Right infrahilar lymph node, similar in size, Small nodule apical segment right lower lobe, reduced Sclerotic bone lesions	Filling defects in the main pulmonary arteries extending to the lower lobe branches, in keeping with PE	iSD
24.10.19	12 week response	CT CAP with Contrast	Right lower lobe lung mass 2.4 cm	2.4cm	Right infrahilar lymph node, similar in size Small nodule right lower lobe, reduced Sclerotic bone lesions.	Persistent filling defects, in keeping with PE,	iPR
6.12.19	Response	CT CAP with Contrast	Right lower lobe lung mass 2.2 cm	2.2cm	Right infrahilar lymph node, similar in size, Small right lower lobe nodule, tiny difficult to see.	Persistent filling defects in keeping with PE, appearing less extensive	Maintained iPR
20.1.20	Response	CT CAP	Right lower lobe lung mass 1.7 cm Linear area of soft tissue at lesion site, indistinguishable from scarring.	1.7cm	Right infrahilar lymph node, similar in size. Small nodule right lower lobe remains tiny difficult to see. Sclerotic bone lesions. The left acetabulum lesion i difficult to see.	Persistent filling defects in keeping with PE, appearing less extensive	Maintained iPR

Table 4.11: TAC-02 Summary of imaging and efficacy

4.1.4 TAC-03

TAC-03 is a 68 year old male patient who initially presented to his GP in May with jaw aching, sinusitis, cough and wheeze.

His GP arranged a chest x ray which was abnormal and treated with a course of antibiotics. Follow up chest x ray was still abnormal, which led to referral to hospital for further investigations.

He had mild shortness of breath, which did not restrict activities, no weight loss but some dull shoulder pain.

Past history of hypercholesterolemia and gastroesophageal reflux. Medications were omeprazole and atorvastatin.

The patient lived with their wife and was an ex-smoker of 25 years with a 20 pack year history. He previously worked as a project manager but was retired and drove the school bus part time. He was fit and active, playing regular golf - PS0

He initially underwent an endobronchial ultrasound (EBUS) which confirmed metastatic adenocarcinoma of the lung. However, there was insufficient tissue for molecular testing, so he had a further CT guided biopsy of the scapular which confirmed EGFR, ALK, ROS -ve and a PDL1 expression of 0%.

Date	Indication	Type of Scan	Target lesions (TL)	Sum TL	Non Target disease	Other
6.11.19	Baseline	CT CAP with Contrast*	Tumour mass at right lung hilum extending into right lower lobe 7.1 cm	7.1	Mediastinal (subcarinal and left lower paratracheal) lymphadenopathy, metabolic active on recent PET scan but less than 1.5 cm. Bone metastases; sclerotic foci in right scapula and in T6 vertebra.	Atelectasis distal to tumour

Table 4.12: Summary of TAC-03 baseline imaging

**CT CAP reported was done for screening prior to registration and not in work up of diagnosis*

4.1.4.1 MSCTRAIL Infusions

TAC-03 tolerated treatment well, receiving all trial defined MSCTRAIL infusions on time. Treatment commenced on 12/11/19 with Pemetrexed, Carboplatin and Pembrolizumab followed by $4x10^8$ MSCTRAIL cells on day 2, 13/11/19. TAC-03 subsequently completed a further cycle of trial treatment before the trial was paused (MSCTRAIL dose 2 on 04/12/). He continued on SOC treatment of Carboplatin/Pemetrexed/ Pembrolizumab for a further cycle follow by SOC maintenance therapy. Summary of trial treatment can be seen in table 4.13.

He did not experience any infusion related DLTs or AEs, but it was noted that during MSCTRAIL infusions he also experienced some minor, self-resolving, asymptomatic desaturations of less than 30 seconds in duration with the lowest recorded reading of 90%.

ECGs post infusions did not show any changes and observations were otherwise stable during infusions and for the 6 hours afterwards. No other adverse events occurred

Cycle	Treatment	Prescribed Dose	Date
Trial Treatment			
1	Pemetrexed Carboplatin Pembrolizumab	500mg/m^2 IV 75mg/m^2 IV 200mg	12/11/19
	MSCTRAIL	$4x10^8$ Cells	13/11/19
2	Pemetrexed Carboplatin Pembrolizumab	500mg/m^2 IV 75mg/m^2 IV 200mg	3/12/19
	MSCTRAIL	$4x10^8$ Cells	4/12/19
3	Pemetrexed Carboplatin Pembrolizumab	500mg/m^2 IV 75mg/m^2 IV 200mg	31/12/19
4	Pemetrexed Carboplatin Pembrolizumab	500mg/m^2 IV 75mg/m^2 IV 200mg	22/1/20
Maintenance			
5	Pemetrexed Pembrolizumab	500mg/m^2 IV 200mg	12/2/20
6	Pemetrexed Pembrolizumab	500mg/m^2 IV 200mg	5/3/20

Table 4.13: TAC-03 Summary of trial treatment timeline

4.1.4.2 Follow up and Adverse Events

TAC-03 experienced a number of minor AEs (grade 1-2) related to standard treatment and underlying disease, summarised in table 4.14

He underwent routine 6-week treatment response scan on 19/12/19 and the report was expedited by the study team on 20/12/19. The CT CAP showed filling defects in both main pulmonary arteries, and lobar branches in both lungs in keeping with pulmonary emboli.

TAC-03 was reviewed on the same day, they were asymptomatic with no new shortness of breath, chest pain or palpitations and found to have a Hestia score of 0. On clinical examination they were haemodynamically stable, saturations and heart rate were within normal range.

An ECG did not show any new changes and subsequent transthoracic echo did not show any evidence of right heart strain. Bilateral lower limb dopplers did not show any evidence of distal thrombus in the lower limbs.

TAC-03 was informed of the findings of the CT and following appropriate counselling and education commenced on treatment dose LMWH (Enoxaparin subcutaneous 1.5mg/kg as per trust guidelines) on the same day.

As this was the third patient to have a pulmonary embolism identified an urgent safety review was carried out and subsequently a report to halt recruitment was submitted to MHRA on grounds of urgent safety measure. This not only halted recruitment but stopped the administration of any further doses of MSCTRAIL including TAC-03's third dose of MSCTRAIL.

The AEs were re graded at SAEs and as a relationship to MSCTRAIL could not be ruled out they were re classified as related to MSCTRAIL.

Adverse event	Grade	Related to MSCTRAIL	Related to standard treatment
Nausea	1	Not Related	Related
Insomnia	1	Not Related	Related
Fatigue	1	Not Related	Related
Epistaxis	1	Not Related	Related
Right Shoulder disease & rib pain	2	Not Related	Not Related
Dysuria	2	Not Related	Not Related
Dry eyes	1	Not Related	Related
Joint aches (Arthralgia)	1	Not Related	Not Related
Pulmonary Embolism	3	**Related**	Not Related
Mouth ulcers	1	Not Related	Related
Chest infection	2	Not Related	Not Related
Fatigue	2	Not Related	Related

Table 4.14:Summary of adverse events reported for TAC-03

4.1.4.3 Efficacy Results

TAC-03 was shown to have positive outcomes on efficacy imaging: 6 week scan showed a reduction in target lesions (TL) in keeping with iSD by iRECIST criteria (sum of TL reduced from 7.1cm to 6.5cm, 8.5% reduction). 12 week scan showed a further reduction but still in keeping with iSD by iRECIST (sum of TL reduced from 7.1cm to 6.4cm, 9.5% reduction.

Table 4.15 summarises efficacy response scans for TAC-03

Date	Indication	Type of Scan	Target lesions (TL)	Sum TL	Non Target disease	Other	Outcome by iRECIST
6.11.19	Baseline	CT CAP with Contrast	Tumour mass at right lung hilum extending into right lower lobe 7.1 cm	7.1	Mediastinal (subcarinal and left lower paratracheal) lymphadenopathy, metabolic active on recent PET scan but less than 1.5 cm. Bone metastases; sclerotic foci in right scapula and in T6 vertebra.	Atelectasis distal to tumour	
19.12.19	6 week response	CT CAP with Contrast	Tumour mass at right lung hilum extending into right lower lobe 6.5 cm	6.5cm	Mediastinal (subcarinal and left lower paratracheal) lymphadenopathy, have reduced. Bone metastases non CR non PD. Note increase in sclerosis in a lesion in L3, previously barely visible, probably reflecting post treatment change	Pulmonary emboli including in both main pulmonary arteries, and lobar branches in both lungs. No sign of right heart strain.	iSD
30.1.20	12 week response	CT CAP with Contrast	Tumour mass at right lung hilum extending into right lower lobe 6.4 cm	6.4	Mediastinal lymphadenopathy has reduced. Bone metastases, barely visible on baseline unchanged since last scan	There are persistent filling defects in the pulmonary arteries	iSD

Table 4.15: TAC-03 Summary of imaging and efficacy

4.1.5 TAC-04

TAC-04 is a 76 year old gentleman who initially presented to A&E with diarrhoea. During this review he underwent chest x-ray which was found to be abnormal.

He had no respiratory symptoms apart from a slight dry cough, no breathlessness, chest pain, hemoptysis or weight loss.

Past medical history of a stroke in 2007 at which point he was also diagnosed with hypertension, atrial fibrillation and hypercholesterolemia.

Medications were bendroflumethiazide, bisoprolol, omeprazole, atorvastatin, thiamine and rivaroxaban.

The patient lived at home with his wife and son. He was retired but previously worked as a painter decorator. He had stopped smoking 20 years before with a 30 pack year history. He was fit and active with a PS1.

Following on from the abnormal CXR a subsequent CT confirmed a 6cm spiculated mass in the right upper lobe abutting the fissure, right pleural effusion and pleural thickening with enlarged ipsilateral and mediastinal lymph nodes and a metastatic nodule in the left lower lobe with corresponding PET-CT uptake. Staged at T3N2M1b stage IVB

EBUS sampling confirmed metastatic adenocarcinoma. EGFR, ALK and ROS1 negative, PD-L1 0%.

Date	Indication	Type of Scan	Target lesions (TL)	Sum TL	Non Target disease	Other
12.12.19	Baseline	CT CAP with contrast	Right upper lobe lung mass 6 cm Left lower lobe lung nodule 1.6 cm Right hilar lymph node 1.6 cm Right lower paratracheal node 2.3 cm	11.5	Small lung nodules	Very small right pleural effusion and pleural thickening
30.0919	Staging	MRI head with contrast				No signs of intracranial malignant disease

Table 4.16: TAC-04 Summary of baseline imaging

4.1.5.1 MSCTRAIL Infusions

TAC-04 tolerated treatment well, receiving all trial defined MSCTRAIL infusions on time. Treatment commenced on 18/12/19 with pemetrexed, Carboplatin and Pembrolizumab then $4x10^8$ MSCTRAIL cells on day 2, 19/12/19. Following his first cycle the trial was paused for, he continued to receive 3 more treatment cycles of Carboplatin/Pemetrexed/ Pembrolizumab but without further MSCTRAIL infusions.

He did not experience any infusion related DLTs or AEs and was not noted to have any significant desaturations during infusion, lowest recorded saturation during infusion was 94%.

ECGs post infusion did not show any changes and observations were otherwise stable during infusion and for the 6 hours afterwards. No other adverse events occurred.

Cycle	Treatment	Dose	Date
1	Pemetrexed Carboplatin Pembrolizumab	500mg/m^2 IV SOC 200mg	18/12/19
	MSCTRAIL	$4x10^8$ Cells	19/12/10
2	Pemetrexed Carboplatin Pembrolizumab	500mg/m^2 IV 75mg/m^2 IV 200mg	8/01/2020
3	Pemetrexed Carboplatin Pembrolizumab	500mg/m^2 IV 75mg/m^2 IV 200mg	29/01/2020
4	Pemetrexed Carboplatin Pembrolizumab	500mg/m^2 IV 75mg/m^2 IV 200mg	25/02/2020

Table 4.17: TAC-04 Summary of trial treatment timeline

4.1.5.2 Follow up and Adverse Events

TAC-04 experienced a number of minor AEs (grade 1-2) related to standard treatment and underlying disease, summarised in table 4.18.

He has not experienced any AEs or DLTs related to MSCTRAIL. Following the identification of PEs within the other patients in trial he underwent a safety CTPA, this did not reveal any evidence of PE. Of note he was anticoagulated with rivaroxaban for atrial fibrillation and this had been continued throughout treatment.

Adverse event	Grade	Related to MSCTRAIL	Related to standard treatment
Dizziness	1	Not Related	Not Related
Fatigue	2	Not Related	Related
Hypophosphatemia	2	Not Related	Related

Table 4.18:Summary of adverse events reported for TAC-04

4.1.5.3 Efficacy Results

TAC-04 was shown to have positive outcomes on efficacy imaging: 6 week scan showed a reduction in target lesions in keeping with SD by iRECIST criteria (sum of TL reduced from 11.5cm to 8.2cm, 28.7% reduction). 12 week scan showed further reduction in keeping with PR by iRECIST criteria (sum of TL reduced from 11.5cm to 7cm, 39.0% reduction).

Table 4.19 summarises efficacy response scans for TAC-04

Date	Indication	Type of Scan	Target lesions (TL)	Sum TL	Non Target disease	Other	Outcome by iRECIST
12.12.19	Baseline	CT CAP with Contrast	Right upper lobe lung mass 6 cm Left lower lobe lung nodule 1.6 cm Right hilar lymph node 1.6 cm Right lower paratracheal node 2.3 cm	11.5	Small lung nodules	Very small right pleural effusion and pleural thickening	
24.12.19	Safety	CTPA				No PE	
29.1.20	6 week response	CT CAP with Contrast	Right upper lobe lung mass 3.6 cm Left lower lobe lung nodule 1.6 cm Right hilar lymph node 1.5cm Right lower paratracheal node 1.5 cm	8.2	Small lung nodules, non CR non PD.	Very small right pleural effusion and pleural thickening.	iSD
10.3.20	12 week response	CT CAP with Contrast	Line right upper lobe lung mass 3.7 cm Left lower lobe lung nodule 0.3 cm Right hilar node 1.6 cm Right lower paratracheal node 1.4 cm	7	Small lung nodules, non-CR non-PD.	None.	iPR

Table 4.19: TAC-04 Summary of imaging and efficacy

4.1.6 Efficacy Results from TACTICAL phase I

Four patients have been treated with at least one dose of MSCTRAIL. All patients were found to have a reduction in sum of target lesions in keeping with SD by iRECIST criteria at 6 weeks. All patients had a further reduction in sum of TLs at 12 weeks however 1 patient had new non target lesions in keeping with iUPD (N=1, 25%), 1 patient had a reduction in keeping with iSD (N=1 25%) and 2 had a greater than 30% reduction in sum of TL, iPR by iRECIST criteria (N=2 50%).

Changes in sum of target lesions are summarised in figure 4.21 and figure 4.22

1 patient (25%) has confirmed progression by iRECIST criteria with a time to a progression (TTP)/ progression free survival (PFS) of 141 days, 4.6 months.

Trial Number	6 week CT outcome by iRECIST	12 weeks CT outcome by iRECIST	% Change in Sum on TL at 12 weeks	Time to progression
TAC-001	iSD	iUPD	-17	141 days
TAC-002	iSD	iPR	-43	
TAC-003	iSD	iSD	-10	
TAC-004	iSD	iPR	-39	

Table 4.20: Summary of change in sum of TL from baseline to 6 weeks and 12 weeks

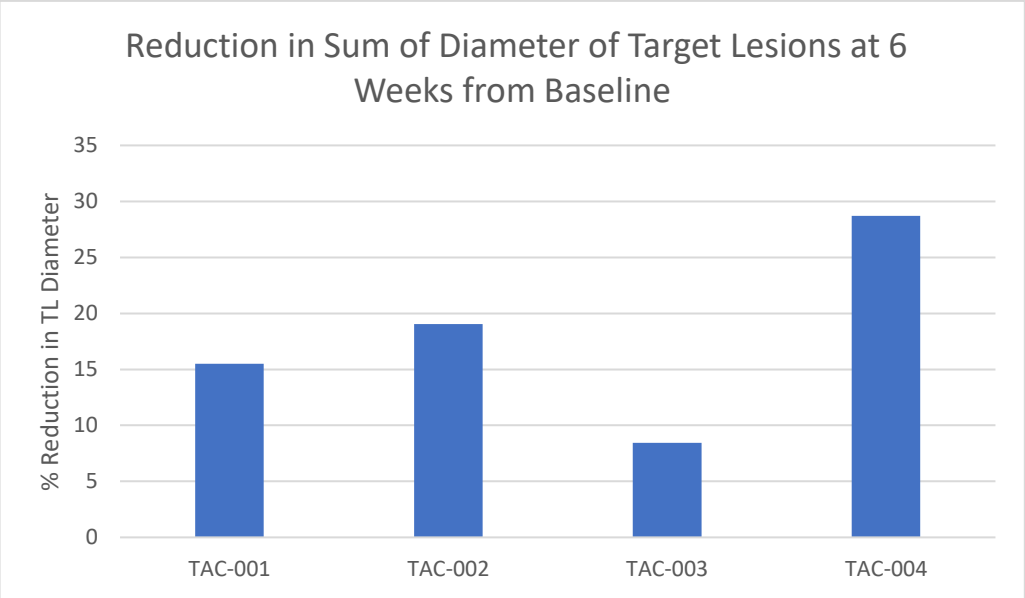


Figure 4.1: Bar chart to show percentage reduction in sum of target lesions baseline to 6 weeks

4 patients underwent 6 week CT efficacy scan. All patients had a reduction in sum of TLs at 6 weeks compared to baseline all in keeping with SD by iRECIST criteria

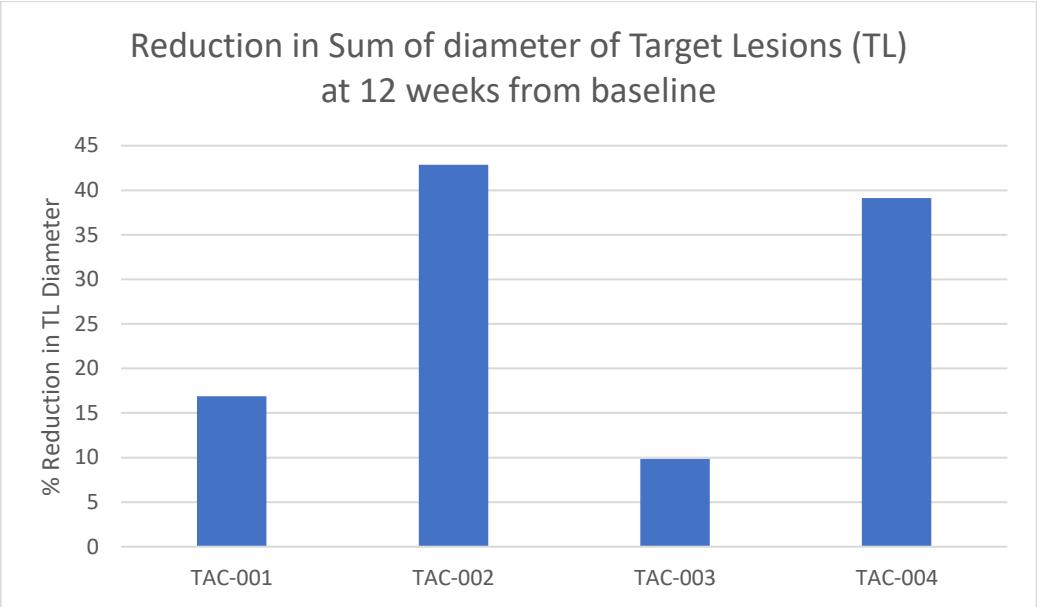


Figure 4.2: Bar chart to show percentage reduction in sum of target lesions baseline to 12 weeks

4 Patients underwent 12 weeks efficacy scans. 2 patients reached the threshold of iPR by iRECIST criteria of over 30% reduction.

4.1.7 Summary of AEs in phase I

An overall summary of AEs collected from registration until 21 days post the last dose of MSCTRAIL (C4D1) in phase I of TACTICAL can be seen in table 4.21.

The incidence of adverse events related to MSCTRAIL was low, only 5 AEs were related to MSCTRAIL, 1 patient experienced an episode of hypoxia and chest pain (CTCAE grade 2 and 1), 1 patient developed self-limiting phlebitis at the MSCRAIL canula site (CTCAE grade 2) and 3 patients experienced asymptomatic pulmonary embolism (CTCAE grade 3). These are discussed in more detail below.

Adverse event	Grade	N (%)
Hypoxia	2	1 (25%)
Non-cardiac chest pain	1	1 (25%)
Rash maculo-papular	1	1 (25%)
Watering eyes	2	1 (25%)
Dyspnoea	1	1 (25%)
Skin ulceration	1	1 (25%)
Oropharyngeal thrush	1	1 (25%)
Constipation	1	1 (25%)
Gastroesophageal reflux	1	1 (25%)
Phlebitis	2	1 (25%)
Pulmonary Embolism	3	3 (75%)
Hypophosphatemia	2	2 (50%)
Fatigue	2	4 (100%)
Nausea	1	1 (25%)
Insomnia	1	1 (25%)
Epistaxis	1	1 (25%)
Right Shoulder disease & rib pain	2	1 (25%)
Dysuria	1	1 (25%)
Dizziness	1	1 (25%)
Mouth ulcers	1	1 (25%)
Chest infection	2	1 (25%)

Table 4.21: Summary of adverse events reported in Phase I of TACTICAL

Adverse events recorded between registration and end of cycle 4 for patients who had received at least 1 dose of MSCTRAIL in the TACTICAL trial.

TAC-01 experienced a CTCAE grade 2 hypoxia with chest tightness during the first infusion which was related to flushing of the canula. It was noted that patients experienced transient desaturations during infusion. While these do not qualify upon the CTCAE grading of adverse events, as the desaturation did not reach the 88% threshold, it is interesting to note the occurrence.

All patients were asymptomatic, the drop-in saturations were noted on the bedside monitor with no reflex tachycardia or change in respiratory rate. Every event lasted less than 30 seconds with full resolution back to baseline saturations or higher. The desaturations did not seem to correlate to a time during the infusion or in the 1st bag

preferentially over the 2nd. In response to desaturations the nurse administering reduced the rate of infusion but continued the infusion. This phenomenon has not been reported in other MSC cell therapy trials although the asymptomatic nature of patients and severity not reaching the CTCAE grading may have led to underreporting.

Possible mechanism for these desaturations may include transient flooding of the pulmonary vasculature by MSCs causing a reduction in gas transfer. Or indeed MSC aggregation or formation of micro thrombi given their propensity for adherence and clumping. Therefore, the reduction in infusion rate leads to improvement in gas transfer as concentration of cells in the narrow vasculature reduces. The hypoxia rapidly resolves because the cell bolus passes through the vasculature during infusion or due to the rate reduction reducing the concentration.

This phenomenon of hypoxemia has been observed in patients with leukostasis where high cell count leads to increased viscosity and vascular obstruction[142]. Also in patients with sickle cell disease in an acute chest crisis where pulmonary vascular occlusion occurs due to either bone marrow embolisation, sickled erythrocytes or microthrombi result in hypoxia and chest pain[143]. However, the transient nature of the desaturations, lack of co-existing signs or symptoms including chest pain may point to alternative mechanism.

Miller et al (2020) [144] very recently reported on the use of hMSCs in an Ovine model of ARDS and veno-venous ECMO. They found that the sheep who received hMSCs had an increase in trans-membrane oxygenator pressure gradients and on post mortem hMSCs were found within the disordered vasculature, suggesting that it was the know plastic activity[39] of MSCs adhering to the vessel walls contributing to impaired oxygenation.

As with all peripheral monitoring there are limitations to the accuracy of the monitoring systems. Bedside monitors estimate venous oxygen saturations utilising photoplethysmography (PPG); this uses an algorithm derived from the ratio of absorbance of red and infrared light by haemoglobin to calculate pulse oximetry during periodic blood volume changes [145]. Therefore, variations in periodic blood volume can interfere with the accuracy of readings. For example, during breathing[146], or if there are changes in intrathoracic pressure which are

transmitted from the great veins in the thorax to smaller peripheral veins or if there is any degree of respiratory modulation[147]. The infusion of intravenous infusion of fluids has also been reported to induced intensity variations in recordings and hence alter readings[148]. Belhaj et al (2017) [149] recently compared PPG-derived peripheral venous oxygen saturations directly with venous saturation measured from co-oximetry blood samples in healthy subjects. They found not only was there significant differences between PPG monitoring and central monitoring but that respiratory modulations of the PPG signal were observed, leading to variations in readings. This evidence may suggest that the peripheral monitoring of patients may be over estimating desaturations and they may indeed be a reflection of physiological variations in period blood volume changes and not true episodes of hypoxia.

4.1.7.1 Pulmonary Embolism (PE)

TAC-01,02 and 03 were all incidentally found to have PEs during trial treatment. They were all asymptomatic, hemodynamically stable with Hestia scores of 0. ECGs did not reveal any abnormality including evidence of right heart strain.

All the patients have since undergone ECHO to assess for evidence of right heart strain as a result of the pulmonary embolism, all have been found to be within normal limits.

To date, there have not been any adverse events related to anticoagulant treatment including major or minor bleeding or the pulmonary embolism.

Given the high risk of PEs in the cohort they were not initially reported as serious adverse events for TAC-01 and TAC-02 however, when a pattern emerged with a third patient this was re-reviewed. Given the potential harm or even mortality thromboembolism can cause an urgent safety review was organised with the trial management group (TMG). The implications and actions as a result of this, subsequent impact and safety measures instigated are detailed below

Discussion

Following review of emerging evidence, it was felt that MSCTRAIL may be the possible cause of PEs. Due to the medical significance and possible link to the experimental treatment these events were deemed SUSARs, an urgent safety report was submitted to the MHRA with an amendment to pause the trial to recruitment and halt administration of further doses while investigation was carried out.

A review into the possible causes was led, it looked at whether the cells were aggregating during infusion which could be leading to clots as well as reviewing the literature on the risk of PEs in this cohort and pro-thrombotic nature of MSCS.

Following these reviews, the next steps were to introduce measures to mitigate any risk to further patients recruited and then re-submit to regulatory and ethics boards to re-commence the trial.

4.1.7.2 Risk of PE in Patients with Lung Cancer

It is well recognised that patients with lung cancer are in a pro-thrombotic state [150] and are at a higher risk of thromboembolism, including pulmonary embolism. This is also independently increased in patients with advanced cancer, adenocarcinoma histology [151-153], within the first 6 months of diagnosis and in those receiving chemotherapy, with literature quoting the risk of PE in lung cancer as up to 65% [154-159], six to eight times more likely than cancer free controls at 12 months [154]. Interestingly a recent review by Li et al (2018) [156] highlighted the prevalence of PEs identified incidentally on treatment response evaluation. They found evidence for between 29-63% of PEs identified in lung cancer patients being diagnosed in this way [160-162].

This increased risk is likely to be multifactorial with mechanisms of vascular endothelial damage, stasis of blood flow, and hypercoagulability all contributing factors. It has been shown that patients with lung cancer are hypercoagulable because they have a decreased clot formation time due to high levels of fibrinogen [163], increased prothrombin fragment 1 + 2 and higher levels of thrombin- activatable fibrinolysis inhibitor immunologic activity[164] or raised tissue factor (TF), the initiator

of the clotting cascade [165]. With advancing stage lung cancer there can be found to be an even more accelerated clot time with higher plasminogen activator inhibitor (PAI-1) level compared to those with early stage disease [163]. Chemotherapy agents, including Cisplatin and Carboplatin have been shown to increase procoagulant activity on endothelial cells, again contributing to risk of PE [166, 167] (correlation between risk of PE and Cisplatin-based chemotherapy (HR, 1.51; 95% CI: 1.12-2.36 [168]).

ASCO, in conjunction with the independent academic working group, The International Initiative on Thrombosis and Cancer, recent published guidelines on the prophylactic anticoagulation of cancer patients in view of the high incidence of venous thromboembolism in this cohort. They recommended prophylactically anticoagulating patients, in the absence of bleeding risk, with a Khorana Risk Score (a thrombosis risk scoring system) [169] of ≥2 where 1 point can be attributed if the patient has lung cancer alone. This highlights the VTE risk as well as the potential role for prophylactic anticoagulation in this cohort.

4.1.7.3 Risk of PE following treatment with MSCs

There is no definitive link between the administration of intravenous MSCs and formation of pulmonary embolism and no other clinical trials involving the use of MSCs have reported a high incidence of PE related to the MSCs.

Prior to the trial, it was postulated, that a theoretical risk was that during infusion the cells could aggregate in the pulmonary vasculature leading to thrombosis. However, it was felt if this was to occur it would be within the first 24 hours, i.e. upon infusion as the MSCs flood the pulmonary vasculature.

However, while there is no clinical evidence of increased thrombosis there are *in vitro* and *in vivo* studies that suggest MSCs exert a procoagulant effect medicated by increased levels of tissue factor (TF) [170] that can initiate coagulation[171], secretion of procoagulant microvesicles[172] or direct enhancement of platelet deposition[173].

In vitro there is evidence of significant measurable levels procoagulant activity (PCA) in human adult liver-derived mesenchymal progenitor cells, bone marrow mesenchymal stem cells, placenta-derived decidual stromal cells, adipose and

umbilical cord derived MSCs [173-177]. High levels of PCA are linked to high levels of cell surface expressed Tissue Factor (TF) [170]. While TFs alone do not cause coagulation when they bind with activated platelets via a mechanism involving P-selectin glycoprotein ligand-1 they initiate coagulation[178]. TF also has roles in adhesion, migration, inflammation, and cell signalling,

It must also be noted that handling conditions and growth media can affect PCA and hence changes in TF expression over culture time [171]. Furthermore, Netsch et al, showed that MSCs inhibit the activation and aggregation of platelets in platelet-rich plasma and whole blood, reducing platelet activation and acting instead as anticoagulants.

In *in vivo* models it was observed that injection of high dose human bone marrow derived, adipose derived, human adult liver-derived progenitor cells or porcine derived bone marrow MSCs led to acute respiratory and circulatory failure[173, 176, 179, 180]. On post-mortem examination study of the animals there was evidence of obstruction by micro-thrombi within organs. The doses used in these studies ranged from $50x10^6$ cells/kg to $160x10^6$cells/kg, significantly higher than the starting dose of TACTICAL which corresponds to around $5x10^6$ cells/kg in an 80kg person. Three of these studies went on to show that by reducing the dose, to ranges between $7.5x10^6$cells/kg and $5x10^6$ cells/kg, these symptoms were not noted, reducing adverse events and increasing survival. Again, it has been shown that TF could play an important role in the activation of coagulation by MSC infusions[181]. Coppin et al (2019) also went on to show that administering heparin to rats receiving human adult liver-derived progenitor cells reduced their thrombotic risk [180]. Yet In Millier et al's (2020) ovine model 6 out of 7 of the sheep who received $3x10^8$ hMSCs endobronchialy were found to have pulmonary artery embolism on post mortem [144] despite the use of heparin, although they do not comment on the rate in the control group, it clearly highlights the need for further investigation into the pro-coagulable effects of MSCs.

4.1.8 Next steps

Following the temporary suspension of the trial, a number experiments were carried out to investigation if there was a link between MSCTRAIL and the formation of PEs and, if so, how to overcome this to ensure patients safety before restarting the trial. Two possible mechanisms of thrombus formation were postulated: spontaneous aggregation of the cells during infusion, and the prothrombotic effect of MSCs as discussed above.

The work to investigate the prothrombotic effect of MSCs is ongoing and beyond the scope of this thesis, however, methods to overcome this potential effect and ensure safety are to be instigated and are discussed below.

In order to investigate spontaneous aggregation experiments were carried out. The aims of these were:

1. To investigate if there is spontaneous cell aggregation upon thawing the medicinal drug product;
2. To investigate if the giving set filters cell aggregates;
3. To investigate the propensity for aggregation throughout the drug product administration protocol;
4. To investigate the time needed for cell aggregation and determine if dilution can mitigate aggregation.

The experiments were carried out by Dr Ben Weil and Dr Krishna Kolluri at the Centre for Cell Gene and Tissue Therapeutics (CCGTT). The MSCTRAIL drug product was thawed, as per the clinical trial protocol, in a water bath at 37°C. An identical giving set, filter, and gauge needle was used to match the trial protocol as closely as possible

Initially on reviewing a freshly thawed bag of cellular product, no spontaneous aggregates were visible to the naked eye, in keeping with records from the patient infusions.

The giving set used for administration has a standard blood filter of 200µm, as used in all cell infusions. Review of this filter post infusion demonstrated it had prevented the passage of larger aggregates (> 200µm), without impacting the infusion flowrate.

Using a mock infusion set up with the same flow rate, giving set and cannula as those used for patient administration, serial samples were collected at multiple time points and examined microscopically for aggregates. There was no evidence of cell aggregation in any of the samples collected, at any time point during the infusion process.

Finally, to investigate the time needed for cell aggregation, the samples collected from the above experiment were incubated at room temperature in a static plate and then imaged. It was noted that after 15 minutes, small aggregates could be visualised. However, if these samples were diluted 1:1 then aggregation was prevented figure 4.3.

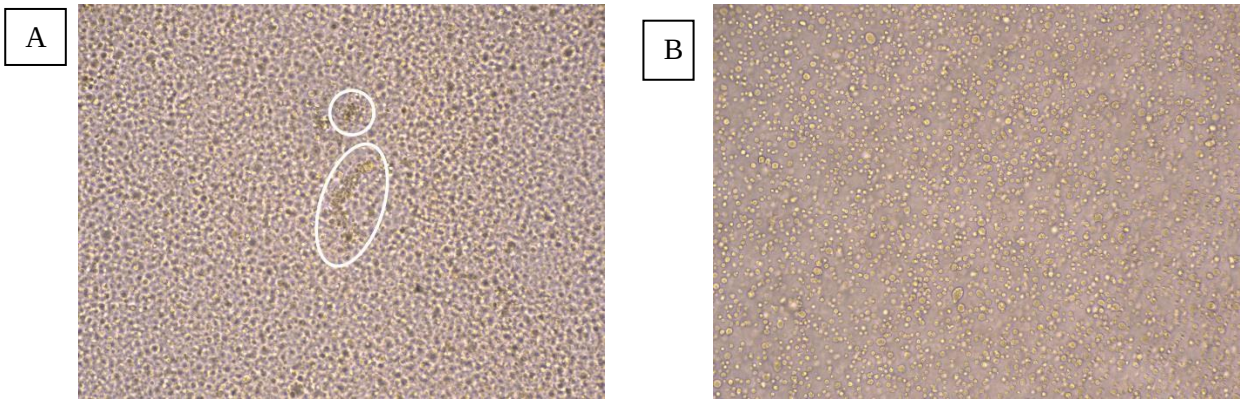


Figure 4.3: Static incubation of MSCTRAIL

(A) Static incubation for 15mins following administration protocol, small aggregates (circled) can be seen. (B) Subsequent dilution of the sample 1:1 show dispersion without aggregate formation (magnification, x10)

The system described above does have some design limitations and is not physiologically relevant to the infusion of MSCTRAIL cells, which are in constant fluidic motion and are diluted and mixed with blood upon administration, however, it does suggest that increased dilution may prevent cell aggregation during infusion.

Thawed MSCTRAIL samples were also tested using a peristaltic infusion pump at 2.5ml/minute for the infusion procedure. The pump was found to have no impact upon either aggregation or cells viability.

Following these experiments, it was concluded that in order to prevent the formation of cell aggregates, the following preventive measures can be followed:

1.) Reduce the dose of cells given to the patient to reduce the cell concentration;
2.) Dilute the cells with saline solution, prior to administration;
3.) Administer the cell suspension at a set flowrate of 2.5ml/minute. A standard peristaltic pump can be used to achieve this consistent set flowrate.

4.1.9 Substantial trial amendment

Following a pause to the trial and submission of an urgent safety report to MHRA a substantial amendment had to be made to the trial protocol before the trial can be re-opened. Changes were made to ensure the safety of future patients recruited into the TACTICAL trial and to mitigate the risk of further PEs. This was balanced with both the efficacy of the drug product and the restraints of a costly clinical trial.

Following extensive discussions, weighing up clinical and pre-clinical evidence alongside the opinions of experts, the following changes have been made to the protocol. Reasoning and justification of these changes follow.

4.1.10 Changes to the TACTICAL Protocol

1) Dose de-escalation to the next dose level ($2x10^8$) MSCTRAIL.
2) Addition of a Computerised Tomography Pulmonary Angiogram (CTPA) at the end of the 1st cycle of trial treatment.
    - If PE is identified during trial treatment (either on CTPA after C1 or on CT scan already required after C2), patients will not have further MSCTRAIL doses.
3) Addition of mandated anticoagulation with Low Molecular Weight Heparin (LMWH) at prophylactic dose throughout trial treatment (cycles 1 to 4, until the end of treatment 12 week CT scan confirms absence of PE)
4) Changes to MSCTRAIL delivery:

- MSCTRAIL infusion bags to be diluted with saline to a volume of 100mls prior to infusion
- Speed of infusion reduced to 2.5 mls/minute via peristaltic pump.
- Minimum infusion time of 40 minutes

4.1.10.1 Dose de-escalation to the next dose level ($2x10^8$) MSCTRAIL.

While the identification of PEs did not fall within the remit of a DLT they did represent serious adverse events. The patients already treated in the TACTICAL trial did not suffer any serious sequala as a result of these events. However, given the potential causative link between MSCTRAIL and PEs and the potential risk to life that PEs carry, the safest option is to de-escalate. The de-escalation dose as per the protocol is to $2x10^8$ cells, a reduction in half from the original dose.

As the cells were manufactured in bags containing $2x10^8$ cells the patients will now receive one bag instead of two. We will de-escalate to the next dose cohort, (*figure 4.4*), a further 6 patients will need to be treated at this dose for it to represent the RP2D. As per starting the trial the initial 3 patients will be treated, 21 days apart, if no DLTs are observed, following TMG and IDMC review, a further 3 can be recruited and treated.

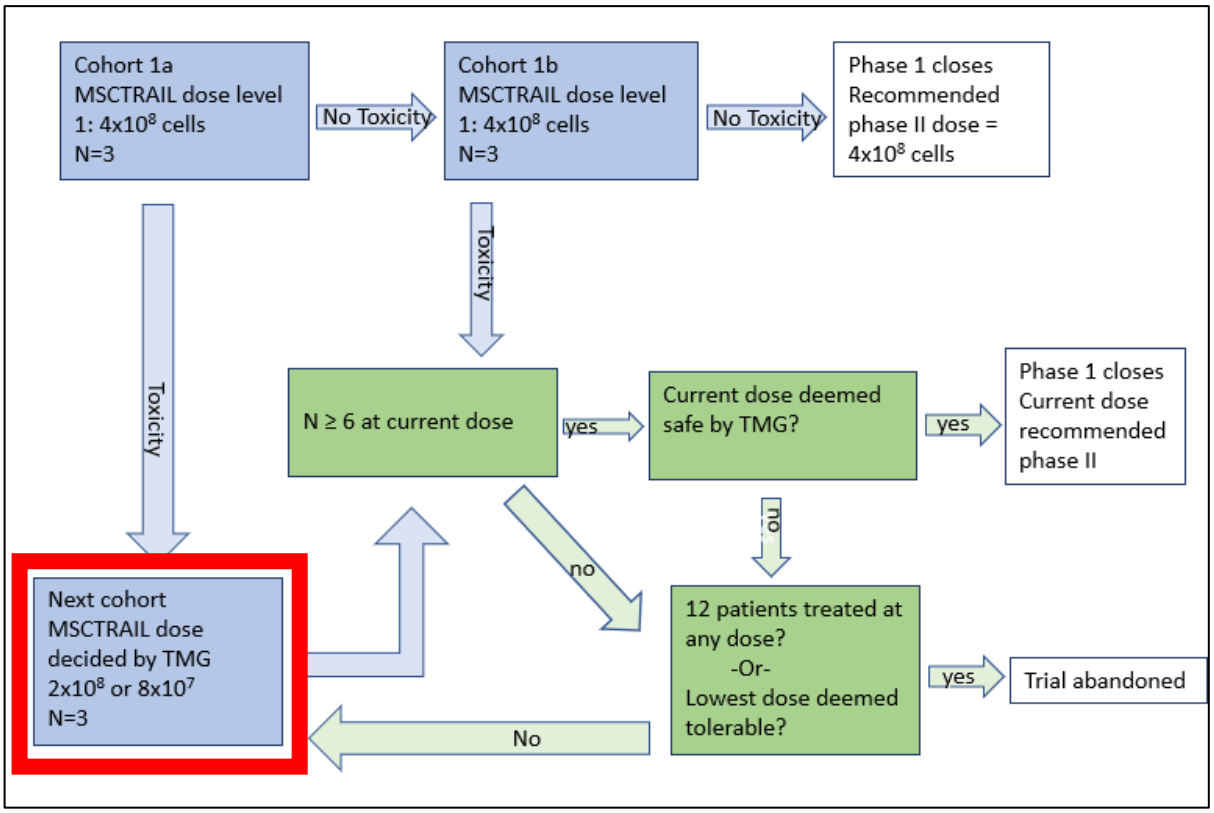


Figure 4.4: TACTICAL Phase I Trial Schema

4.1.10.2 Addition of a CTPA after completion of cycle 1

CTPA assessment has been added on day 15 (+/- 5 days) of the 1st cycle of treatment.

The previous protocol stipulated a treatment response CT CAP with contrast (at 6 weeks) prior to the 3rd cycle of treatment, however earlier imaging will allow earlier detection of PEs. Given that the previous PEs have been incidentally detected on routine scans with no patients showing any clinical signs or symptoms then clinical review and examination are insufficient to screen for PE. The addition of this CTPA will allow a prompt identification of any PE that develops after a single cycle of MSCTRAIL even if the patient is asymptomatic leading to the instigation of early therapeutic interventions as needed.

If a PE is detected at either the initial CTPA or the CT prior to the 3rd cycle the patient will not receive further doses of MSCTRAIL but will continue with best practice clinical care.

4.1.10.3 Addition of Low Molecular Weight Heparin (LMWH)

Prior to entering the trial patients will need to consent to the use of subcutaneous LMWH from the beginning to the end of trial treatment, i.e. cycle 1 day 1 to cycle 4 day and once the 12-week scan confirm the absence of PE.

As discussed above there is evidence to support the prophylactic anticoagulation of high risk cancer patients[182]. MSC human trials have not been associated with PEs though there is *in vivo* and *in vitro* evidence that untransduced MSCs may be thrombogenic[171]. There is also evidence; *In vivo* this can be overcome with the use of heparin or reducing cell concentrations [180]. After seeking haematological advice, the protocol was altered to add low dose prophylactic anticoagulation, in the form of LMWH. This will reduce the risk of thromboembolic events in our patient cohort without increasing the risk of further serious adverse events.

A recent Cochrane review by Di Nisio et al (2012) [183] to assess the efficacy and safety of primary thromboprophylaxis in ambulatory cancer patients receiving chemotherapy concluded that primary thromboprophylaxis with LMWH reduced the risk of symptomatic VTE by roughly half (RR, 0.54; 95% CI, 0.38 to 0.75) compared with no thromboprophylaxis. LMWH had no significant impact on 1-year mortality (RR, 0.93; 95% CI, 0.80 to 1.09) or risk of major bleeding (RR, 1.44; 95% CI, 0.98 to 2.11). This evidence is supported by 2 meta-analysis, which focus specifically on lung cancer patients, that found the use of LMWH reduced the risk of thromboembolic event by approximately half [184, 185] and that there was no statistically significant association between the use of LMWH and major bleeding [184] or total bleeding episodes[185]

The safety of LMWH use as a prophylactic is well established even in the longer term setting [186-188] with no association found with increased major bleeding. Its use is supported by many key bodies including NICE who recommended it for all high-risk medical inpatients, including oncology patients, surgical patients and in pregnancy to reduce the risk of venous thromboembolism.

4.1.10.4 Changes to MSCTRAIL delivery

It is recognised that MSCs and MSCTRAIL cells can be adherent to each other and form aggregates. This aggregation may have been the cause of the embolism as cells clump and form a bolus within the pulmonary vasculature. The work presented above shows that cell aggregates were not found in the giving set or bag upon delivery, but there are limitations to this system. There was evidence however that when they were not agitated and in a well the cells form aggregates but that these could be dispersed when the cells were held in a greater dilution.

$2x10^8$ MSCTRAIL cells were diluted in 30mls of solute for the first patient cohort. This allowed for a rapid administration, minimal storage space as well as minimal dose of DSMO administration to the patient. However, for the next cohort this will be increase to a volume of 100mls. The cells are administered via a closed loop double giving set (*figure 2.7*), with normal saline attached to prime the line and wash the bag. It is therefore possible to increase the volume for administration by adding saline to the cell bag before infusion using this system without contamination to the bag or cells. This method was favored because it ensured the volume of DSMO remained at a minimum and allowed the use of product already made up in storage. Increasing the volume of dilution will ensure the cells are less concentrated, reducing the risk of aggregation and also delivering a lower concentration of cells to the small capillaries again reducing the risk of clumping and micro thrombus.

The speed of infusion has also been reduced to 2.5mls/min for a minimum duration of 40mins and maximum of 60min. This is now also to be given through a pump which will ensure homogeneity between patient administrations. Reducing the speed will reduce the concentration of cells delivered per time again reducing the risk of cell to cell aggregation and micro thrombus.

4.1.11 Re-opening TACTICAL

By introducing early surveillance for PEs and reducing risk through MSCTRAIL dilution and slower delivery, along with prophylactic anticoagulation we hope to re-open the trial in a safer paradigm.

A substantial amendment was submitted on 26.2.20. We received approval from ethics and regulatory bodies to implement these changes on 01.04.20 however due to the COVID-19 climate all clinical trials are halted to recruitment.

5 TACTICAL Translational Results

5.1.1 Background

One of the key barriers to translation of a novel stem cell product is understanding its in-human pharmacodynamics; the investigation of how and when it works and if there is a host immunological response to the administration of a third party allogeneic agent.

Understanding the host response to a novel therapy could lead to the identification of a biomarker that could be utilised to guide treatment and give an indication of patient response. Early identification of treatment response can be vital in cancer treatment. It allows for a change in treatment before there is a significant accumulation of toxicities. Currently, therapeutic response is determined by imaging, namely CT scans at 6 or 12 weeks. If a biomarker, which can predict response within the first few days after treatment could be identified it could allow for these treatment changes, dramatically altering the management of patients care.

Phase I of the TACTICAL trial is a single arm dose finding trial. Blood samples taken during phase I will allow us to investigate a range of serum blood markers that may give an early indication of the treatment effects. We will also investigate if there are patterns or a peak timing of action for the combination of MSCTRAIL and SOC which may guide when an appropriate sample could be taken in clinical practice.

Completion of Phase I will optimise the blood screening methods in preparation of phase II; a placebo-controlled trial. In phase II we will be able to compare any responses seen in those receiving MSCTRAIL to placebo in the setting of SOC therapy.

This chapter will present some of the translational work carried out to date on the phase I blood samples and the plan of work moving forward.

All blood samples were obtained following ethics and regulatory approvals and with patients' formal written consent.

5.1.2 Aim

The aim of this work was to examine the *in vivo* anti-cancer activity of MSCTRAIL through the use of a biomarker of cell death.

Question 1: Are there identifiable patterns of circulating markers of cell death and could these indicate the peak timing of treatment action occurring in the combination of MSCTRAIL and SOC?

Question 2: Can circulating markers of cell death be used as a biomarker of treatment efficacy.

Question 3: Using this preliminary phase I data can we delineate the optimum time for patient blood sampling for phase II.

5.1.3 Circulating marker of apoptosis

TRAIL induces apoptosis in cancer cells via the extrinsic death pathway, measuring the levels of apoptosis could thereby provide a biomarker for *in vivo* MSCTRAIL activity. If this biomarker mirrors patient treatment response it may provide a minimally invasive method of monitoring tumour activity or as other have postulated, be used for predictive importance after treatment [189]

5.1.3.1 Cytokeratin 18

Cytokeratins are proteins belonging to the intermediate filament (IF) family. They are required for maintenance of the cytoskeletal architecture. They are exclusively present in epithelial cells and are released upon the breakdown of the cell membrane in cell death. As a result cytokeratins have been utilised as circulating blood markers of apoptosis in epithelial cell-originated malignancies such as breast[190], laryngeal[191], colonic cancer[192], gastric cancer[193], bladder cancer[194], biliary tract and NSCLC [107, 195]. In these trials cytokeratin 18 (CK18) has shown the most promise. Levels of CK18 fragments have also been found to be higher in patients with lung cancer, compared to either benign lung disease or healthy control subjects and in those with a higher tumour burden [196, 197]. Possible mechanisms include the high turnover of cells in a malignant environment.

During early and intermediate apoptosis CK18 is cleaved by caspases to yield caspase-cleaved CK18 (ccCK18). During caspase cleavage a neoepitope is formed, the M30 epitope. This epitope is specific to ccCK and so serves as an identifiable marker for apoptosis[198]whereas, M65 represents an epitope present on both intact and caspase cleaved CK18, and therefore serves as a marker for total cell death independent of death mode (necrosis or apoptosis) (*figure 5.1).* By combining the two you can ascertain the degree or contribution of apoptotic cell death in the total tumour cell death. High M30:M65 ratios indicate predominance of apoptosis where as low M30 compared to M65 indicates necrosis.

This biomarker has previously been utilised in a phase Ia trial of rTRAIL (Dulanermin) in solid tumours and a phase II trial of Dulanermin. in combination with other chemotherapeutic agents, in advanced NSCLC [107, 195]. Conclusions from the phase II trial suggested it could be used as a potential pharmacodynamic marker of Dulanermin activity.

Apoptosis is not unique to MSCTRAIL, chemotherapy induces apoptosis via the intrinsic death pathway. Preclinical and clinical pharmacodynamic studies have shown that Cisplatin induced cell death begins at around 8-11 hours post infusion [199] and significantly increases 1 to 3 days after chemotherapy administration[200]. As discussed in section 1.6.5 it is thought that there is synergistic induction of cell death when MSCTRAIL is combined with other chemotherapies. This may be due to cross-talk between the extrinsic and the intrinsic apoptotic pathway that results in amplification of the apoptotic signals and increased tumour cell death [123]. To date there has been no published investigation on the effect of PDL-1 inhibitors on circulating markers of cell death.

With this knowledge blood samples will be taken in phase I pre-chemotherapy and pre and post MSCTRAIL infusion through all treatment cycles as well as on progression. This will allow identification of patterns of biomarker changes and for optimisation ahead of phase II -comparing MSCTRAIL to placebo. The outcomes will also be correlated to treatment response acquired by CT imaging taken at 6 and 12 weeks from baseline.

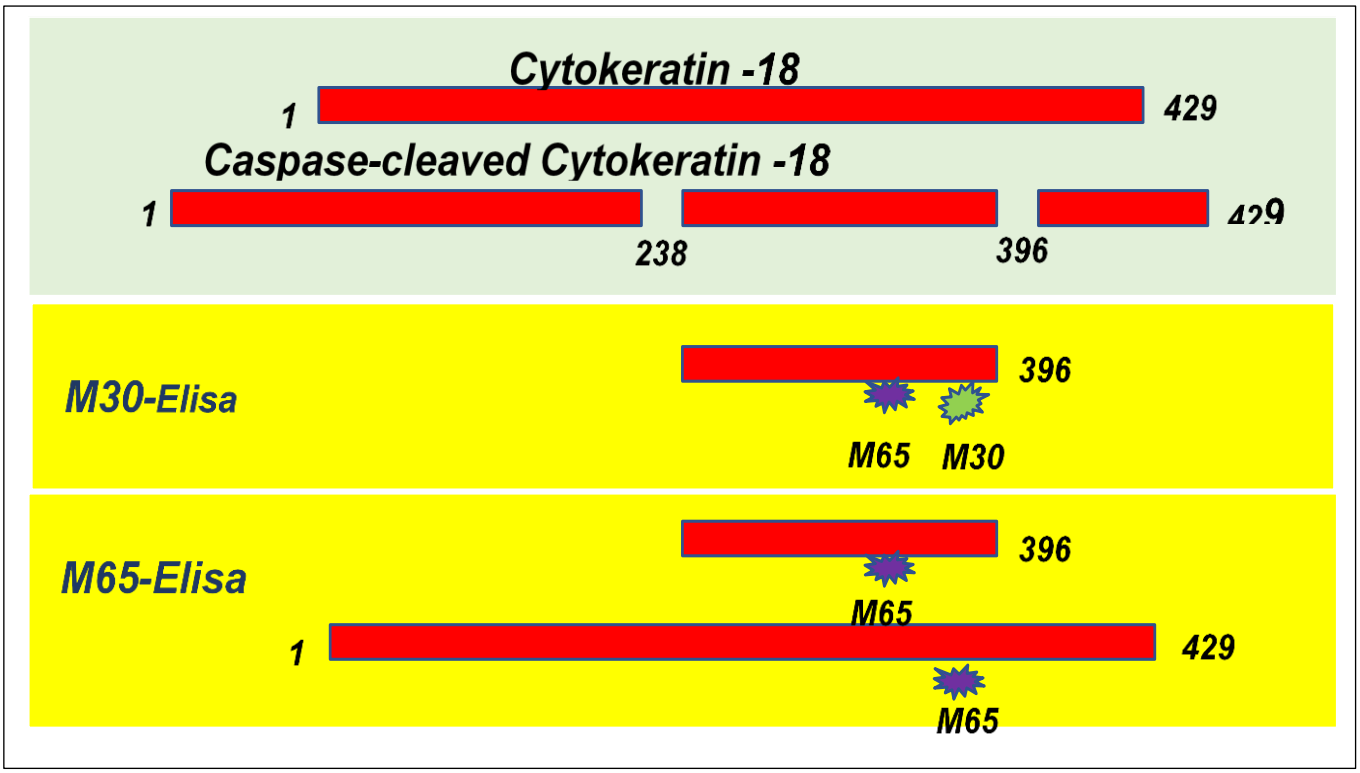


Figure 5.1: Cytokeratin 18 cleavage and generation of M30 epitope

M30 and M65 Elisa assays can be used to measure contribution of apoptotic cell to total cell death.

5.1.4 Methods

This work is unpublished and has been carried out in collaboration with Rebecca Graham at Lungs for Living, UCL Respiratory.

Patient blood samples were taken on:

Cycles 1-3:

- Day 1: pre standard of care treatment
- Day 2: pre and then 3 & 6 hrs post MSCTRAIL treatment
- Day 3: 1 day post MSCTRAIL
- Day 8: 7 days post MSCTRAIL
- Day 15: 14 days post MSCTRAIL

Cycle 4:

- Day 1 pre standard of care treatment
- Day 15

On Progression.

Whole blood samples were obtained using ethylenediaminetetraacetic acid (EDTA) sample tubes from patients following their written and verbal consent and transferred at ambient temperature to the laboratory within 2 hours. They were anonymised with a patient trial number and assigned a unique sample number. Collection details were recorded on the Translational Blood Sample Form (appendix III) and then transferred to an electronic log which detailed the time and place of origin, tracking of the samples as well as details of processing and storage.

5.1.4.1 Extraction of Plasma from Whole Blood

For the extraction of plasma, whole blood samples were centrifuged at 300G for 10 mins at 4 ˚C, then using a Pasteur pipette, supernatant was removed prior to being centrifuged at 1000G at 4˚C, transferred to cryovials and stored in 1-2ml aliquots at -80 ºC for subsequent apoptosis assay.

5.1.4.2 Measurement of Circulating Values M30/M65

The M30 Apoptosense ELISA and the M65® ELISA assay from Previva have been previously utilised in clinical trials [201-205]. The M30 Apoptosense® ELISA assay specifically measures the level of caspase-cleaved CK18 fragments (ccK18) containing the K18Asp396 neo-epitope. Whereas, the M65® ELISA assay measures total soluble CK18, the M65 epitope, released from dead cells (necrotic and apoptotic). Measurements by the M65® ELISA therefore represent total cell death by any cause and M30 Apoptosense representing apoptosis induced cell death.

The procedure was performed under dedicated Good Clinical Laboratory Practice conditions. Samples were processed in batches per patient and plated in triplicate. M30 and M65 plates for each sample were carried out at the same time to reduce wastage of the samples through freeze thawing.

Assays were carried out as per the manufacturer's instructions using serum samples extracted from whole blood and stored as detailed above. Briefly, using the 96 well plate provided 25µl of each standard, controls and undiluted samples were added to each well. 75 µl of diluted conjugate solution was added, M30 or M65 corresponding to relevant plate. Plates were incubated on a plate shaker at 600 rpm at room temperature. After 4 hours for M30 and 2hours for M65 the plate was washed 5 times with 250 µl of premade wash solution. Following this 200 µl of 3,3', 5, 5'-Tetramethylbenzadine (TMB) was added and the plates were incubated in darkness for 20 minutes at room temperature. 50 µl of stop solution was then added before the plates were shaken for 10 seconds. Absorbance was then read at 450nm in a microplate reader after 5 minutes.

Analysis was carried out using GraphPad Prism by plotting a standard best fit cubic curve, from known concentrations vs. measured absorbances. M30 and M65 levels were expressed as U/L.

5.1.5 Results

Blood samples were obtained from all 4 patients enrolled in the TACTICAL trial so far at the defined time points within the cycles. Samples were only obtained from the cycles in which the patients received MSCTRAIL and on progression for TAC-01. In total 68 samples were obtained, 24 from TAC-01, 23 for TAC-02, 14 for TAC-03 and 7 for TAC-04.

All were obtained, sampled, processed and stored as per methods described above and logged and anonymised accordingly.

Part 1: Are there identifiable patterns within the circulating markers of apoptosis and could these indicate the peak timing of treatment action occurring in the combination of MSCTRAIL + SOC?

This work sought to establish if a peak in cell death could be determined during treatment and if this varied following repeated doses of MSCTRAIL + SOC.

5.1.5.1 TAC-01

TAC-01 recorded the highest baseline pre-treatment (C1D1) measure of CK18 (492 U/L) compared to the other patient samples which ranged from 84U/L to 170U/L this may reflect ongoing necrosis or tumour burden.

CK18 (M65) peaked on D2, 3hours post MSCTRAIL in cycle 1 (662 U/L), D3 24 hours post MSCTRAIL on cycle 2 (498 U/L) and D8 on cycle 3 (479U/L). The peak values decrease through the cycles and occur at a later time points which may represent a decreasing effect, possibly suggestive of developing resistance to treatment leading to PD as this result was not observed in the other patients.

Whereas, caspase cleaved CK18 (ccCK18, M30) was 74U/L at baseline and peaked on D2 6h hours post MSCTRAIL (135U/L) in cycle 1, D3 24h post MSCTRAIL (118U/L) in cycle 2 and D8, 7 days post MSCTRAIL (156 U/L) cycle 3 (*Figure 5.2*).

TAC-01 was found to have disease progression (iCPD by iRECIST criteria) at 141 days, and a sample was taken at this time point. CK18 (M65) was found to be 444U/L and M30 was 100U/L. Although these values are returning to baseline this patient's end of treatment values are still higher than the baseline or end of treatment values when compared to the other patients.

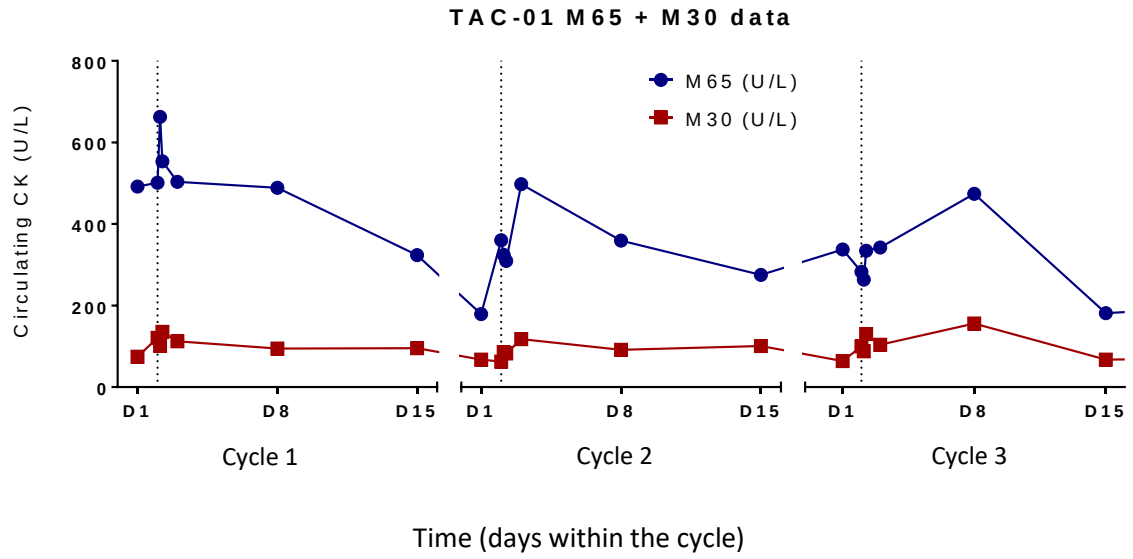


Figure 5.2: ELISA assay results of measurement of circulating CK, M65 and M30 over time in 3 cycles for patient TAC-01.

Dotted line represents treatment with MSCTRAIL. TAC-01 received SOC therapy on day 1 and MSTRAIL of day 2 of a 21 cycle for 3 cycles. Cycle 4 they received SOC treatment alone and progression was seen at day 141 (not shown)

5.1.5.2 TAC-02

TAC-02 had the lowest pre-treatment (C1D1) baseline CK18 (M65) at 84 U/L. While it rose rapidly on treatment, peaking in cycles 1 and 2 on D2 3 hours post MSCTRAIL (242U/L and 379 U/L respectively) and in cycle 3 on D3, 24h post-MSCTRAIL (346U/L). These peak values were still lower than the peak values of all other patients which ranged from 479U/L to 740 U/L.

ccCK18 (M30) peaked on D15 in cycle 1, and in cycle 2 and on D3 in cycle 3, 24h post-MSCTRAIL (*figure 5.3*).

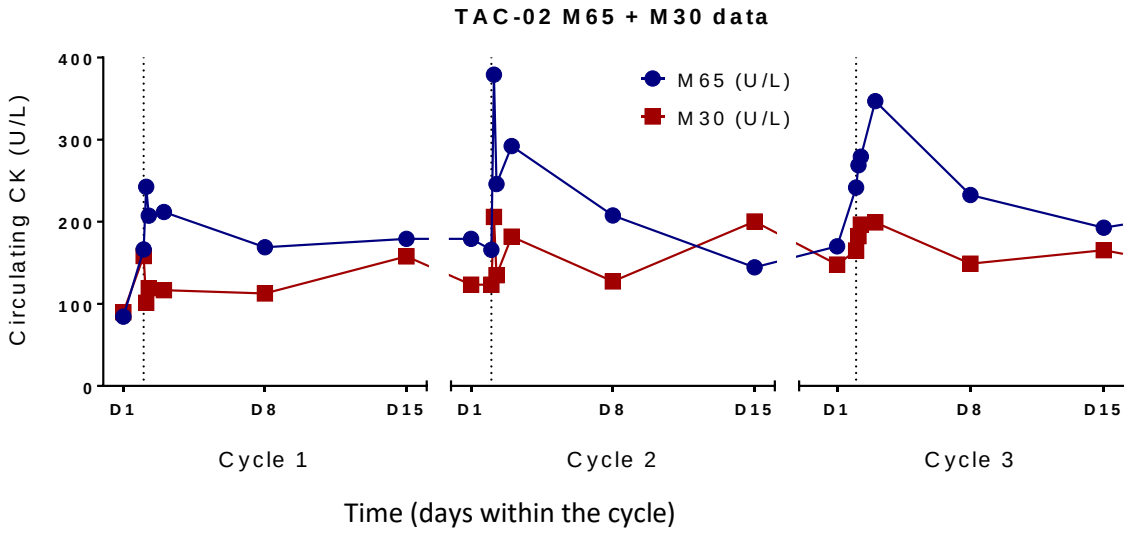


Figure 5.3:ELISA assay results of measurement of circulating CK, M65 and M30 over time in 3 cycles for patient TAC-02.

Dotted line represents treatment with MSCTRAIL. TAC-02 received SOC therapy on day 1 and MSTRAIL of day 2 of a 21 cycle for 3 cycles. Cycle 4 they received SOC treatment alone (not shown)

5.1.5.3 TAC-03

TAC-03 had a baseline CK18 (M65) of 104U/L at D1 cycle 1. In this patient the value of the peak increased from cycle 1 to cycle 2 but the peak was evident at the same time point on D3, 24 hours after MSCTRAIL infusion (582U/L and 740U/L respectively). The peak in cycle 2 was the highest recorded value for M65 seen for all patients.

The value for ccCK18 (M30) at baseline was 42U/L, the peaks also increased in value from cycle 1 to 2. However, the highest M30 was seen in cycle 1 on D15 (124U/L), and on D2 at 3hours post MSCTRAIL on cycle 2 (160U/L) (*figure 5.4*)

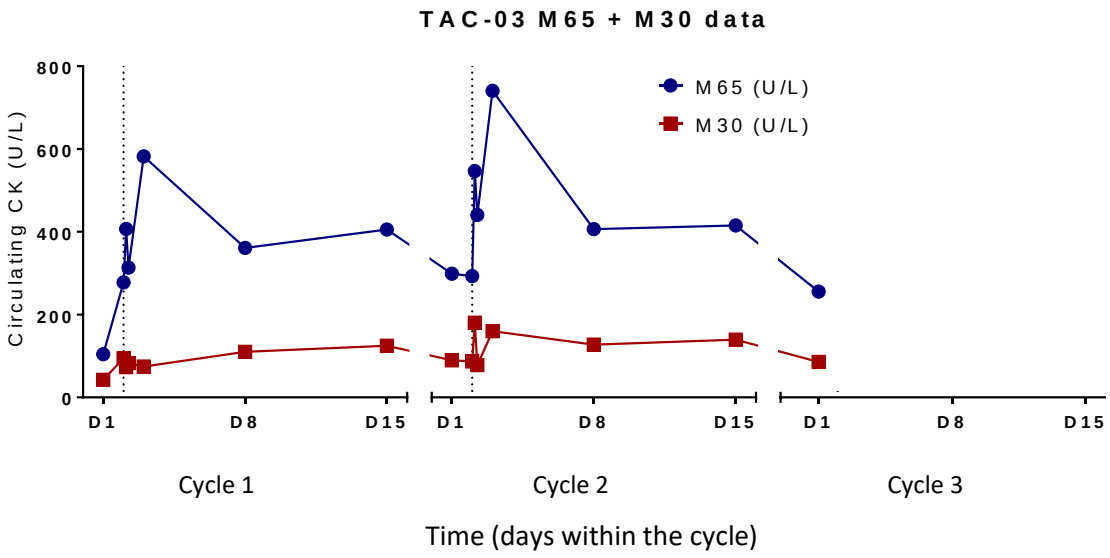


Figure 5.4: ELISA assay results of measurement of circulating CK, M65 and M30 over time in 2 cycles for patient TAC-03

Dotted line represents treatment with MSCTRAIL. TAC-03 received SOC therapy on day 1 and MSTRAIL of day 2 of a 21 cycle for 2 cycles. Cycle 3& 4 they received SOC treatment alone (translational bloods not taken)

5.1.5.4 TAC-04

TAC-04 had a baseline CK18 (M65) of 170 U/L at D1 cycle 1. However, the patient only received 1 dose of MSCTRAIL before the trial was paused and so results are only shown for cycle 1 where M65 peaked on D3, 24h post MSCTAIL infusion (587 U/L).

ccCK18 (M30) was 201U/L at baseline and peaked on D2, 6h post infusion in cycle 1 (256U/L) (*figure 5.5*) the highest values for baseline and peak seen in any patient, which may reflect high levels of cell apoptosis and sensitivity to treatment.

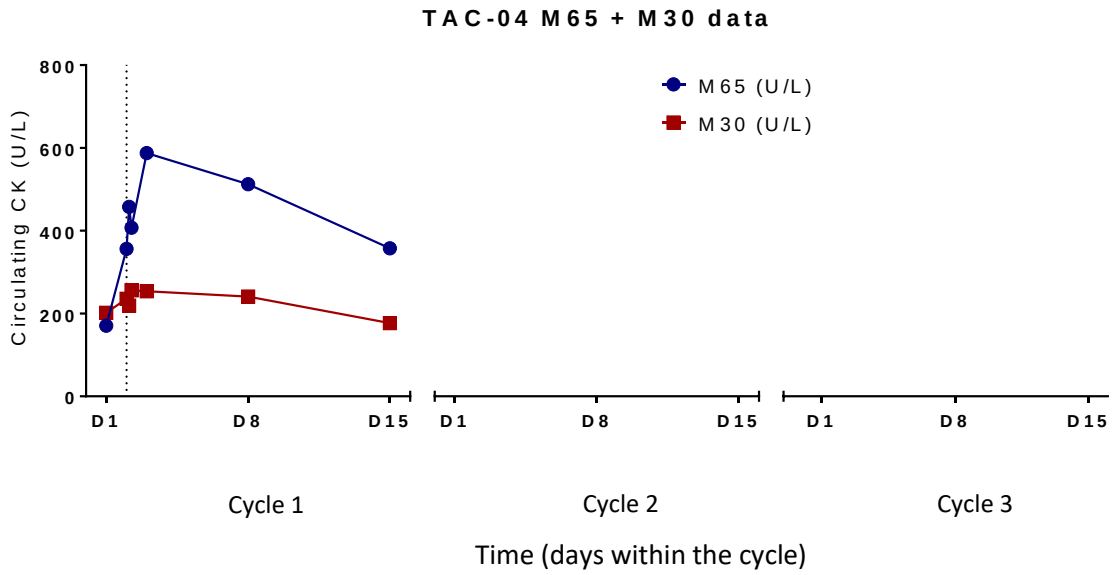


Figure 5.5: ELISA assay results of measurement of circulating CK, M65 and M30 over time in 1 cycle for TAC-04

Dotted line represents treatment with MSCTRAIL. TAC-04 received SOC therapy on day 1 and MSTRAIL of day 2 of a 21 cycle for 1 cycle. Cycle 2,3& 4 they received SOC treatment alone (translational bloods not taken)

Questions 2: Can circulating markers of apoptosis be used as a biomarker of treatment efficacy

In order to ascertain if there was a correlation between CK18 levels and treatment response the percentage change in CK18 (M65) was calculated at varying time points, this was compared to the percentage change in sum of target lesions (TL) obtained from CT images at 6 and 12 weeks. It was hypothesized that the percentage change in the sum of TLs would correlate positively to percentage change in circulating values of M65.

A Pearson product-moment correlation coefficient in GraphPad Prism software was used to assess the relationships.

No correlation or linear relationship was shown between the change in the sum of TLs at 6 weeks from baseline compared to change in M65 value at the end of cycle 1 from pre-treatment (r=0.024, n=4, p=0.97) (*figure 5.6A*). There was also no correlation or linear relationship between the change in the sum of TLs at 6 weeks compared to change in M65 value at the end of cycle 2 from pretreatment (r= -0.439, n=3, p=0.710). Scatterplots summarise these results (*figure 5.6B*).

Due to insufficient data, cycle 3 and 4 and comparisons to 12 week scan was not investigated.

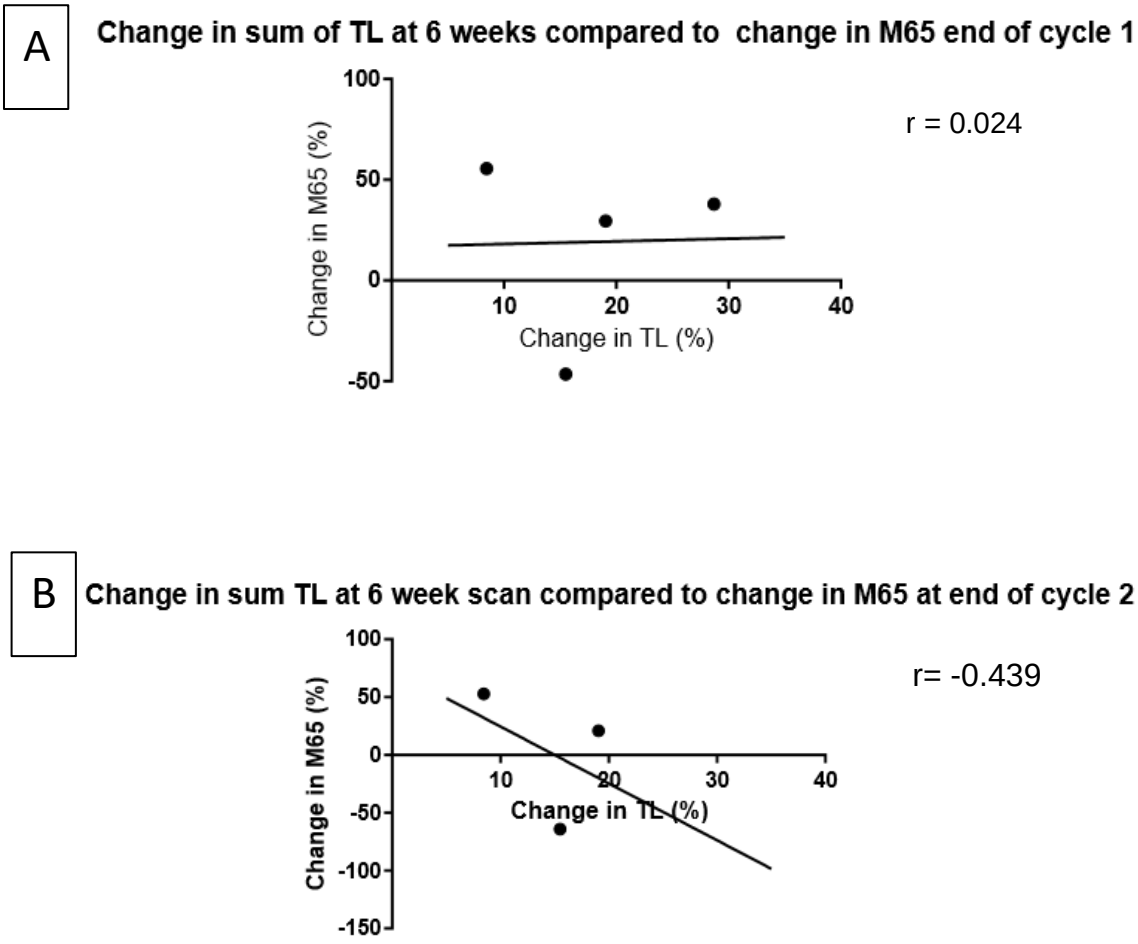


Figure 5.6: Percentage change in sum of target lesions (TLs) from baseline to 6 weeks compared to percentage change in M65 for Phase I patients after cycle 1 & 2

*(A) Scatterplot representing the non-linear relationship between change in TL at 6 weeks from baseline compared to change in M65 at the end of cycle 1 from baseline, no correlation is seen (*r=0.024*). (B) Scatterplot representing the non-linear relationship between change in TL at 6 weeks from baseline compared to change in M65 at the end of cycle 2 from baseline, no correlation is seen (*r= -0.439*).*

Next the percentage change in the sum of the TLs on CT at 6 weeks and 12 weeks from baseline was compared to the maximum recorded percentage change in M65 from pre-treatment level. Again, no correlation or linear relationship in percentage change in the sum of TLs at 6 weeks from baseline compared to maximum recorded change in M65 (r=-0.2010, n=4, p=0.79) and no correlation or linear relationship in percentage change in sum of TLs at 12 weeks compared to maximum recorded

change in M65 (r=0.035, n=4, p=0.965). Scatterplots summarise these results (*figure 5.7*).

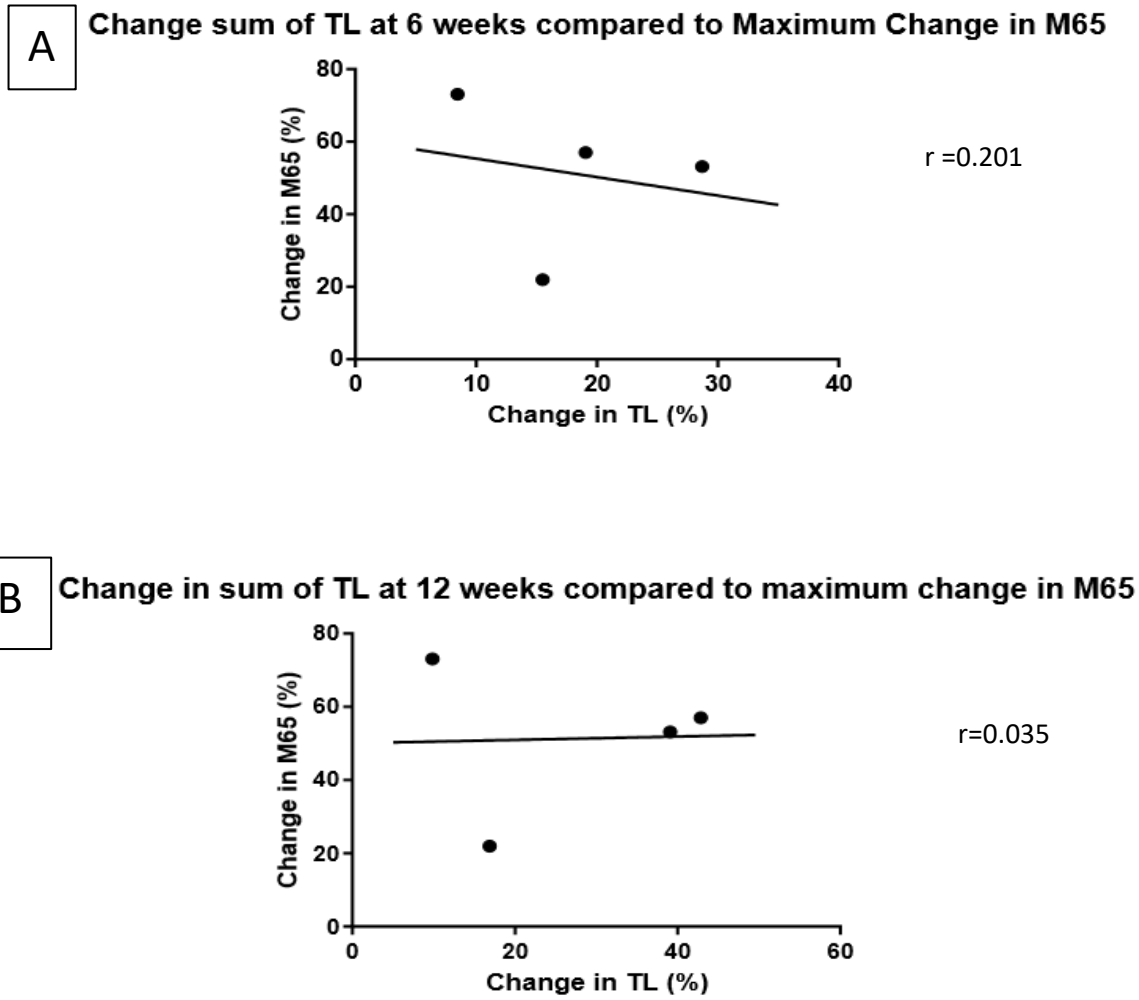


Figure 5.7: Percentage change in Sum of Target Lesions (TLs) from baseline to 6 weeks and 12 weeks compared to maximum percentage change in M65 for Phase I patients

*(A) Scatterplot representing the nonlinear relationship between change in sum of TL at 6 weeks compared to maximum change in M65 (*r=-0.2010*). (B) Scatterplot representing the nonlinear relationship between change in sum of TL at 12 weeks compared to maximum change in M65 (*r=0.035*)*

5.1.6 Discussion

As with other similar studies[196, 205] there was wide variation in the levels of circulating markers of cell death (M30 and M65) between patients but mean levels (212U/L) were in keeping with lung cancer studies [196, 197].This variation may reflect the unique patient responses to treatment or that spontaneous, treatment independent, cell death is unique to the individual.

These results have shown that given the combination of treatment, cell death measured by circulating CK18 is highest early in the treatment cycle. Allowing for the small sample number gathered, our assay detected that levels rise after initial treatment and peak between day 2 and day 3 of the cycle corresponding to 1 day after receiving SOC or between 3 hours and 24 hours after receiving MSCTRAIL.

Preclinical and clinical pharmacodynamic studies have shown that cisplatin induced cell death, as measured by circulating CK18 , begins at around 8-11 hours post infusion [199] and significantly increases 1 to 3 days after chemotherapy administration[200].

Our results therefore support these observations and it can be postulated that, as has been seen in *in vitro* models, MSCTRAIL works synergistically with chemotherapy through cross-talk between the extrinsic and the intrinsic apoptotic pathways (*figure 1.10*). Chemotherapy is given first and initiates cell death via apoptosis, upregulating the death receptor pathway. When MSCTRAIL is given there is further amplification of the apoptotic signals and increased tumour cell death. In order to test this hypothesis, I propose to continue CK18 biomarker analysis in phase II TACTICAL. This placebo-controlled setting will allow us to elucidate if there is increased cell death, measured by circulating CK18, in the MSCTRAIL plus SOC arm compared to chemotherapy/immunotherapy alone.

There has been no published data on the effect of PDL-1 inhibitors on CK18. While there is significant variation in patient results, the values observed in this study are similar to those seen by Ulukaya et al (2007) for patients with lung cancer[196]. Ulukaya et al (2007) measured circulating M30 in patients with lung cancer. NSCLC patients received Cisplatin plus Gemcitabine or Vinrelbine and patients with SCLC received Cisplatin plus Etoposide. At 24 hours post treatment they found a mean

M30 of 165UL, in the TACTICAL trial this mean was 104U/L. In order to remove any confounding information from the addition of MSCTRAIL the pre-treatment value on D2 was used for comparison. It could therefore be shown that Pembrolizumab is not causing an increase in cell death, measured here as the apoptotic marker M30 early on within the cycle.

Although the variation in the results obtained within our small cohort may reflect that some patients were responding to Pembrolizumab and others were not. Further work should be done to investigate the effect of immune check point inhibitors on CK18.

From the results of the cohort taken to date it was shown that there was no patterns or correlations between the change in levels of cell death, measured by CK18 (M65) and patient response, measured as the change in the sum of the target lesions from baseline. This may be due to the small cohort numbers as we only had complete data from 2 patients (all 4 cycles) and therefore in the larger phase II setting patterns correlations may become more apparent. The inclusion of TAC-01 who had progression may also be an obscuring factor as CK18 levels can also correspond to tumour burden.

These results also showed that there was no correlation between CK18 (M65) and tumour response as measured at 6 or 12 weeks. However, this may be due the choice of comparator. There has been insufficient time to investigate values in comparison to progression free survival (PFS) or overall survival (OS) however observationally TAC-01 had the highest baseline value for CK18 (M65) compared to the other patients and also experienced early progression, which may indicate that they are likely to also have a reduced OS. This has been shown in studies involving both lung cancer patients[196, 197, 206] and other tumour groups, such as testicular [204], pancreatic [205, 206] gastric [207] and colorectal cancer [208] where higher recorded levels of circulating markers of cell death, (either M30 or M65) correlate to poorer prognosis with reduced PFS or OS. The high baseline value may also reflect a high tumour burden with increased tumour necrosis. Ustaalioglu et al (2012) postulate that it is tumour necrosis, driven by hypoxia that leads to the treatment resistance and poor prognosis[197] as high baseline CK18 (M65) could be due to high tumour necrosis.

Further work should be done when progression and survival data is available to compare levels of M65 to time to progression and overall survival.

Limitations of this work must also be noted in the timings. While the timings were strictly maintained at 3 hours and 6 hour post MSCTRAIL the chemotherapy agents are given over the course of day 1 and not at the same timing for each patient. As the patient receives 3 agents, and this has to be done within a busy trials unit, the timing of dosing per patient over the day may vary. There were also variations in the timing of when the dose of cells were administered and hence the time since chemotherapy due to difficulties with courier transport of cells. These limitations could not be avoided in the setting of clinical practice, however modifications to the sample collection form will be made to record the times the chemotherapeutics and cells are given.

5.1.7 Future work.

Upon the trial re-opening blood samples will continue to be collected in phase I in the plan described above. This will add further evidence to the described questions of, if there are identifiable patterns in circulating markers of cell death and can these markers of cell death be used as a biomarker for treatment efficacy. In addition, I plan to investigate if there is a reduction in cell death measured by circulating CK as a result of the dose reduction of MSCTRAIL cells given. This will allow us to not only evaluate the extent of cell death due to MSCTRAIL but if there is a dose dependent treatment response.

Once phase I is complete we will move onto phase II. We will continue to assess cell death via circulating markers of apoptosis (CK18). This phase will compare the levels in the placebo arm to the MSCTRAIL arm which will allow us to see the added effect of MSCTRAIL to the treatment combination and if those changes can be correlated to treatment response.

Work to date has shown that changes in CK18 levels occur early in the treatment cycle therefore samples will continue to be taken at the same time points:

D1: pretreatment,

D2: pre and post MSCTRAIL

D3: Post treatment, post MSCTRAIL

D15: end of cycle

On progression

By obtaining samples at these points we can delineate the peak timing of cell death and compare MSCTRAIL treated patients to non MSCTRAIL treated results in the setting of the therapeutic combination. We can also further investigate if there is any correlation between changes in CK18 and tumour response, PFS and OS. Highlighting if can CK18 can be used as an early biomarker of treatment response.

6 Exploring MSCTRAIL in Other Tumour Groups

TRAIL has been found to have *in vitro* and *in vivo* efficacy in a number of different tumour models including, mesothelioma [88], lung cancer [6], breast cancer [89], myeloma [90] and glioma [91]. The next step was therefore to explore the therapeutic potential of MSCTRAIL in other cancer groups. Sage et al (2014) investigated the use of MSCTRAIL in malignant pleural mesothelioma (MPM) [88] showing evidence of both homing as well as reduction in size of mesothelioma xenograft models when delivered intravenously. Further work carried out in our lab also provided evidence for TRAIL sensitivity in tumours with loss-of-function mutations in BRCA associated protein-1 (BAP1). This suggested that BAP1 mutation could act a treatment biomarker, allowing stratification of those patients with MPM who may be more likely to respond to MSCTRAIL treatment. Using this data, we moved forward and were successful in our application for funding for a phase IIa trial of MSCTRAIL in BAP1 mutated MPM.

This chapter will present the rationale for MSCTRAIL in MPM, discussing first the current therapeutic landscape, highlighting the desperate need for a novel targeted therapy, then the pre-clinical evidence for MSCTRAIL in MPM. Finally, the proposed protocol and trial design for the STRATEGIC trial: a randomised, blinded, placebo-controlled trial assessing the difference in anti-tumour activity between MSCTRAIL and standard of care (SOC) first line chemotherapy versus placebo and SOC first line chemotherapy in BAP1 mutated MPM.

Malignant Mesothelioma

Malignant mesothelioma is a rare, insidious, fatal cancer. It most commonly affects the monolayer of mesothelial tissue making up the pleura but can also arise in the peritoneum, pericardium and rarely the tunica vaginalis of the testis [209]. Global it is attributed to 30,000 deaths yearly[210].

Approximately 80% of all cases worldwide are linked to direct exposure to asbestosis fibres [211] and it is recognised to have extensive latency period of

around 20 – 50 years between exposure and disease. Patients most often present later in life, peak incidence of cases is found to be in patients in their 80's, and it is more common in men than women. Signs and symptoms relate to pleural invasion and accumulation of fluid in the pleural space leading to breathlessness, chest pain and weight loss. Given the latency period, difficulties with diagnosis and often slow onset of symptoms, patients typically present late, with advanced disease.

Systemic treatment options are limited with few randomised clinical trials and little evidence for surgery or radiotherapy. There is currently only one licenced first line regime in the UK with no second line option outside trials. Much of the management therefore revolves around symptom control to maximise quality of life.

With insidious onset and few effective therapies, the overall median survival in the UK in just 9.5 months from diagnosis and the 5 year survival rate is only 2%[212]. This highlights the need for effective novel therapies.

6.1.1 Aetiology and Pathophysiology

As mentioned above MPM is most commonly caused by exposure to asbestos and this link was first established in the 1960s [213] although as far back as 1899 the negative effects of mining asbestos were noted [214]. Large scale mining of this silicate material began worldwide in the late 19th century rending it a cancer primarily of occupational origin and predominantly in the labouring male work force. Today males make up 83% of those diagnosed [215]. Prevalence is still noted in woman as the fibres can be carried on clothes as well as the background environmental exposure from asbestos use in insultation and electrical work, the more common aetiology in the UK today.

Asbestos licensing regulations were introduced in the UK in the 1980s and the mining of it banned in 1999. This led to decline in mortality rates in the UK [216, 217] however other countries such as China [218] and Canada [219] continue to mine and use asbestos which, combined with the recognised 'lag time', contributes to an ongoing global rise in incidence and mortality rates.

The pathophysiology of asbestos induced mesothelioma is still not fully understood but there are several well recognised mechanisms. Initially the long, fine asbestos fibres are inhaled, adhering to the cell surface, this is turn induces an inflammatory response leading to chronic inflammation and malignant transformation within cells [220]. Three possible therories as to the contributing mechanism of this malignant transformation have been proposed; firstly that of DNA damage by reactive oxygen species (ROS) generated by the mesothelial cells and macrophages which have been exposed to asbestos [221, 222]. Secondly, accumulation of carcinogens and hazardous material due to asbestosis' ready ability to absorb proteins and chemicals [223]. Finally, as a result of cytokines and growth factors, including high-mobility group box 1 (HMGB1) and tumour-necrosis factor-α (TNF-α) released by asbestos-exposed mesothelial cells and macrophages [224-226], which induce inflammation and cell DNA damage.

Other possible causes of MPM have also been identified but remain controversial. These include prior radiotherapy [227-229], other mineral fibres such as erionite, fluoro-edenite, balangeroite and carbon nanotubes [230] and a link between simian virus 40 (SUV 40) [231, 232] although this has only been identified in animal models and is yet to be proved in humans.

More recently rare clustering of cases has been noted in some families and a link has been made between germline mutations in the gene encoding BRCA1 associated protein-1 (BAP1) and the incidence of a syndrome of cancers which include MPM and uveal melanoma [233, 234]

6.1.2 Diagnosing Malignant Pleural Mesothelioma

Patients with MPM typically present later in life and with a poorer performance status (PS). The average age of diagnosis in the UK is 83 years [212]. This is due to the long latency period between exposure and developing the disease, on average being 40 years for pleural and 46 years for peritoneal mesothelioma [235].

Symptoms of disease present with insidious onset and are most commonly chest pain, breathlessness and weight loss although there may also be systemic unrest including fevers, anorexia and general malaise or indeed the finding of fluid in the

pleural space termed pleural effusion. Although many cases are still identified incidentally and are asymptomatic at presentation.

Patients should then be investigated with chest x ray followed by more detailed imaging such as CT in pleural phase and PET-CT. Imaging can reveal a thickened irregular pleura, pleural effusion, rib invasion or lymphadenopathy.

Sampling of pleural effusions is recommended for therapeutic and diagnostic purposes, although the diagnostic yield of pleural fluid is often cited as low as 30-60% [236].

If a definitive diagnosis has not been obtained by sampling pleural fluid a biopsy should be obtained either via thoracoscopy (local anaesthetic thoracoscopy or video assisted thoracoscopic surgery (VATS)) or if there are focal areas of abnormal pleura with no pleural effusion or the patient is unsuitable for thoracentesis an image guided needle biopsy is recommended [212]. A biopsy is considered the gold standard measure of diagnosis.

Definitive diagnosis in MPM is challenging for a number of reasons; firstly, the patient cohort, as described above at the point of presentation they are often of a poorer PS rendering invasive investigations and subsequent treatment inappropriate and/or unwanted. Secondly the disease itself, MPMs are heterogenous not only in location but in morphological appearance, classically displaying diffuse intra-tumour heterogeneity [211]. A single point biopsy may resemble both malignant and benign areas or indeed only benign characteristics when other spot biopsies from the same patient are definitely malignant. MPM also has the ability to mimic a wide range of other epithelial and sarcomatoid malignancies [212]. These factors can contribute to diagnostic challenges, often leading to prolonged time to diagnosis and repeated diagnostic methods.

Once a biopsy is obtained immunohistochemical markers can be useful to distinguish MPM from other cancers but there is no characteristic protein that offers a 100% sensitivity or specificity. Calretinin, cytokeratin 5/6, Wilms' tumour 1 (WT-1), mesothelin and D2-40 are positive mesothelial markers [237, 238] and so help guide diagnosis. Mutation of BAP1 and deletions in P16 using fluorescence in situ hybridization (FISH) are also becoming more commonly utilised [239, 240].

6.1.3 Classification and Staging of MPM

The WHO Classification of Tumours of the Pleura clearly defines the histological criteria for diagnosing both malignant and benign pleural tumours [237]. MPM can be divided into epithelioid (accounting for 50-60%), sarcomatoid (10%) and biphasic (30-40%) [241] but there are a great many other morphologic subtypes including tubulopapillary, papillary, micropapillary, trabecular, solid, and pleomorphic[237]. Unlike lung cancer, where the subtype can alter treatment, all types of MPM receive the same treatment regime. However, histological subtype does have an impact on the likely natural course of the disease and patient outcomes. Patients with sarcomatoid and biphasic tumours have significantly poorer survival time compared to patients with epithelioid. A consideration when designing a clinical trial to ensure heterogeneity between cohorts.

As with lung cancers, MPM is staged using the TNM staging system established by the International Mesothelioma Interest Group and the International Association for the Study of Lung Cancer. While it has useful prognostic significance, especially when surgery is considered as it describes the extent of tumour invasion into the surround tissue, it does not take into account the extent of disease spread throughout the chest cavity.

6.1.4 Current treatment

Treatment options for MPM remain limited with a heavy burden of toxic side effects. The UK National Mesothelioma Audit 2018 (audit period 2014-2016) reported only 51% of patients diagnosed with MPM had received any anticancer treatment, of that only 40% received chemotherapy [242]. This is in a large part due to the poor therapeutic options but also due to the performance, functional status and preferences of the patient cohort. Currently there is only a single regime licensed for first line treatment that offers modest improvements in overall survival; measured in

only a few months, no licensed second line therapy and no biomarker to guide those who are more likely to respond.

First line chemotherapy, as approved by NICE, for patients with a PS 0-1 is the combination doublet therapy of Cisplatin with the anti-folate agent Pemetrexed. A 3 month survival benefit has been demonstrated over single agent Cisplatin (median overall survival was improved from 9.3 months in cisplatin only arm to 12.1 months in doublet arm (HR 0.77, p=0.020)) [243-245]; although Carboplatin, a less toxic agent, can be used with similar outcome results [246].

A number of other combination and single agent regimes have been trialled including Mitomycin C, Vinblastine and Cisplatin combination or Vinorelbine. However, these have not demonstrated significant survival benefit. [212]. The addition of Bevecizamab, an anti - vascular endothelial growth factor (VEGF) monoclonal antibody to other chemotherapy agents, namely Cisplatin and Pemetrexed, has seen some improvement in overall survival in clinical trials compared to Cisplatin and Pemetrexed alone (mOS 18·8 months [95% CI 15·9–22·6] in the Bevecizamab combination arm vs 16·1 months [14·0–17·9]; hazard ratio 0·77 [0·62–0·95]; p=0·0167) in the Cisplatin, Pemetrexed arm)[247]. While this has led to it being licensed in some countries it is yet to be so in the UK. Possible reasons for this include the strength and confidence in the late stage trial, it demonstrated an unusually high survival in the control arm compared to baseline statistics as well as high reported toxicities.

For those patients who progress on first line chemotherapy the advancing disease and worsening PS often makes them unfit for second line chemotherapy and there is currently no licensed second line treatment option. Clinical trials should be considered in those fit enough i.e. still PS 0-1.

Radiotherapy has a role in its use in pain management but has not been shown to offer any survival advantage as a treatment modality.

Surgical intervention has remained controversial since its introduction in the 1950s. Trials are ongoing to ascertain the benefit (NCT02040272) but early large scale clinical trials demonstrated poorer patient outcomes [248]. The aim of surgery is that of debulking tumour mass to control fluid accumulation, reducing pulmonary restriction or to attempt to achieve a complete macroscopic resection. There are

four main surgical procedures offered, as defined by The International Association for the Study of Lung Cancer's Staging and Prognostic Factors Committee; Partial pleurectomy (PP): partial removal of parietal and/ or visceral pleura for diagnostic or palliative purposes but leaving gross tumour behind. Pleurectomy/Decortication (PD): parietal and visceral pleurectomy to remove all gross tumour without resection of the diaphragm or pericardium. Extended Pleurectomy/Decortication (EPD): parietal and visceral pleurectomy, with the goal of complete macroscopic resection, with resection of the diaphragm and/or pericardium as required.
Extra pleural Pneumonectomy (EPP): en bloc resection of the parietal pleura, pericardium, diaphragm, lung and visceral pleura [249].

Currently none of these techniques are recommended beyond the trial setting with evidence to suggest that some may even lead to poorer survival outcomes [212] and that achieving complete macroscopic clearance is near impossible.

The mainstay of intervention for mesothelioma patients revolves around symptom relief, namely the management of recurrent pleural effusions and pain resulting from pleural infiltration. Recurrent drainage either via temporary or more permanent drains or pleural catheters have allowed patients to have care delivered in the community which can have a huge impact on patients' quality of life.

Exploratory work and clinical trials investigating the role of immune checkpoint inhibition, arginine inhibition and targeting of genomic alterations such as BAP1 show some promise but are yet to offer definitive results.

6.1.5 MSCTRAIL for MPM

The pre-clinical work detailed below was carried out prior to my starting on the project and is references accordingly.

Sage et al (2014) demonstrated that IV delivery of MSCTRAIL significantly reduced tumour growth in a murine mesothelioma model [88]:

Luciferase (Luc) expressing MPM tumour cells were injected into NOD/SCID mice on day 0 ad allowed to grow for 5 days. The mice were then divided into treatment groups, first receiving phosphate buffered solution (PBS), second MSCs and the

third 1×10^6 MSCTRAIL all delivered intravenously on days 5, 9, 12, 15 and 18. The growth of the tumour was tracked longitudinally (*figure 6.1A*) and a significant reduction in the tumour size in the MSCTRAIL treatment group was seen compared to other treatment groups, this was quantified by measuring bioluminescent signal and lung weight (*figure 6.1 B&C*) [88].

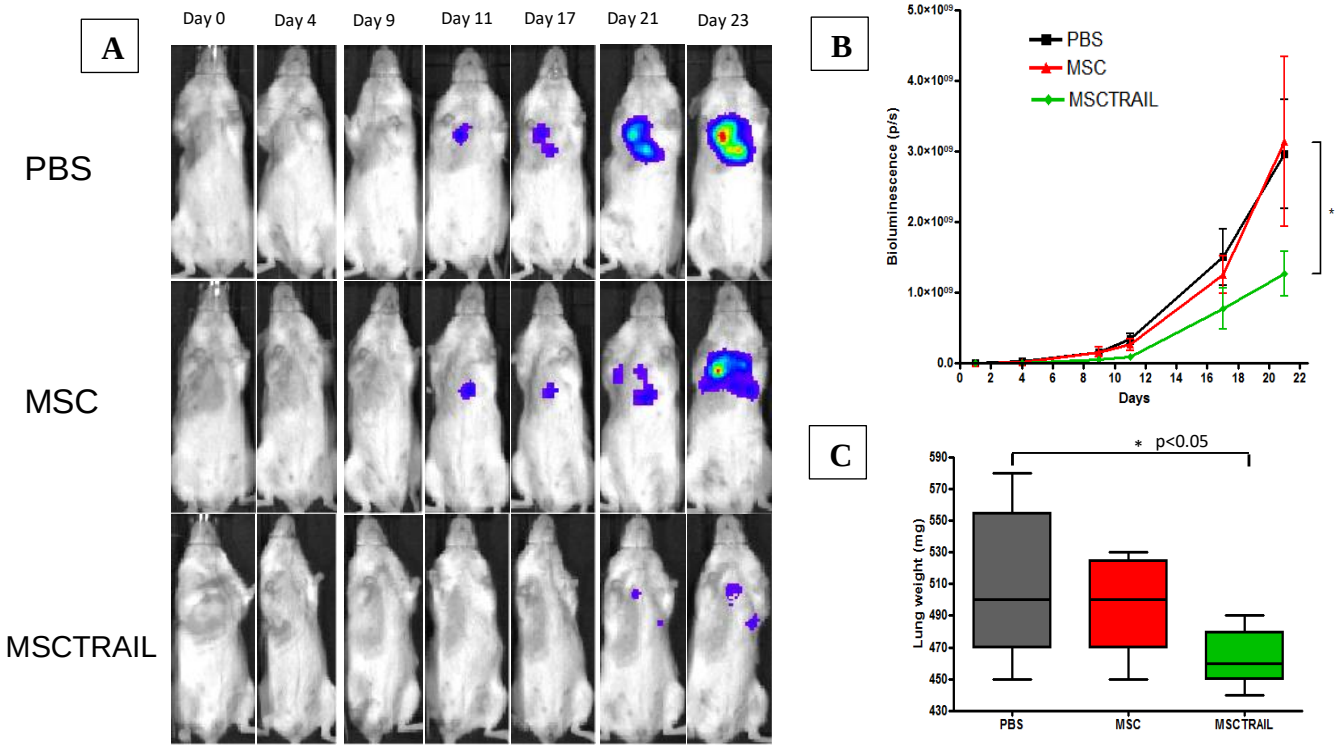


Figure 6.1: MSCTRAIL reduces the growth of tumour when delivered intravenously

(A) IVIS images of representative animals from each experimental group showing reduced bioluminescent signal in animals treated with intravenous MSCTRAIL. (B) Line graph to demonstrate a reduction in total photon count (p/s) seen in animals in the intravenous MSCTRAIL-treated group (c) Box plot showing reduction in lung weights with intravenous MSCTRAIL treatment compared with all other treatment groups (p<0.05) [88]

Statistical analysis was performed using GraphPad Prism V.4 (GraphPad Software). In vivo experiments with multiple groups were analysed using repeated measures ANOVA.

In 2018 Kolluri et al [250] provided further evidence to support the use of MSCTRAIL in MPM as they identified a potential biomarker to stratify those tumours that were more likely to respond the MSCTRAIL therapy. They demonstrated in *in vitro*, *in vivo*

and *ex vivo* models that a subset of cell harboring loss-of-function mutations in BRCA associated protein-1 (*BAP1*), which are frequently seen in MPM [237] demonstrated heightened sensitivity to the TRAIL [250] (*figure 6.2).* While the mechanism of this is still being investigated it highlighted the possibility that this drug-sensitising genomic alteration could be used to identify which patients were more likely to respond to a TRAIL treatment, heralding a personalised targeted therapy for this devastating condition.

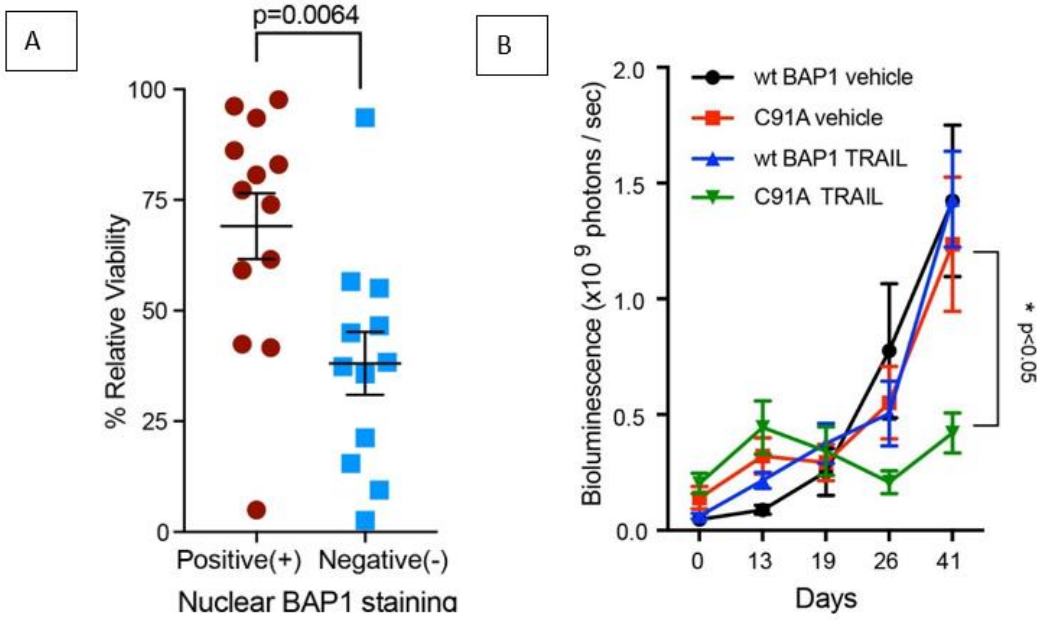


Figure 6.2: Loss of functional BAP1 leads to TRAIL sensitivity[250].

(A) *Mean cell viability in human early passage MPM cell lines 3 days after treatment with rTRAIL in BAP1 positive and negative cell lines. (B) Bioluminescence, as a marker of tumour burden, over time in MPM tumour xenografts after injection of rTRAIL (Wt = BAP1 wild type C91A = inactive BAP1 mutant)*

BAP1 belongs to the ubiquitin C-terminal hydrolase subfamily of deubiquitinating enzymes that are involved in the removal of ubiquitin from proteins. It has been associated with the development of MPM since 2011 when germline mutations were associated with familiar clustering of MPM p53 [234]. Genomic sequencing data initially suggested a prevalence of between 20-30% [251-253] however loss of

expression of BAP1 detected using immunohistochemistry (IHC) can be identified in up to 67% of MPM [254]. Loss of function mutations in BAP1 have also been identified in uveal melanoma [255], clear cell renal carcinoma [256], cholangiocarcinoma [257], breast carcinoma [258] and even 1% of NSCLC [259].

MPM is a rare and devastating disease without a definitive cure. Current treatment options only offer a limited survival benefit and are licensed for first line use only. This data demonstrates that a novel agent has been identified with a potential biomarker to stratify those likely to respond. The next exciting step was to move this into the clinical field with a phase IIa clinical trial.

The STRATEGIC Trial

The potential role of MSTRAIL as a targeted therapy for MPM in those which show a BAP1 loss of function mutation has been demonstrated pre-clinically where it has been shown not only that MSCTRAIL reduces MPM tumour growth in *in vivo* and in v*itro* models when delivered intravenously [88] but that cells harbouring mutations in BAP1 conferring sensitivity to TRAIL [250]. With loss of expression of BAP1, detected using immunohistochemistry, found in just under 70% [254] of MPM.

Using the TACTICAL phase I safety data we plan a randomised, blinded, placebo-controlled trial of MSCTRAIL in combination with first line chemotherapy in BAP1 mutated malignant pleural mesothelioma - The STRATEGIC trial.

The aim of this work is to assess the difference in anti-tumour activity in BAP1 mutated MPM between MSCTRAIL and chemotherapy versus placebo and chemotherapy as a first line treatment.

6.1.6 Funding and ethics

In June 2019 my team and I were successful in an application for a grant from Innovate UK in collaboration with UCL Technology fund in the competition Investment accelerator: Innovation in precision medicine.

Trial Design

STRATEGIC is a multicentre, randomised, blinded, placebo-controlled phase IIA trial comparing MSCTRAIL and first line standard of care chemotherapy versus placebo and first line standard of care chemotherapy in BAP1 mutated MPM.

Standard of care (SOC) is defined, as per NICE guidelines, as the intravenous chemotherapeutic agents Cisplatin* (75mg/m^2) and Pemetrexed (500mg/m^2) and delivered in accordance with local policy.

*Carboplatin can be used instead of cisplatin if clinically appropriate at the discretion of treating clinician.

Patients will be randomised (1:1) via blocked stratification between the intervention and control arms. The intervention arm will receive SOC on day 1, followed by MSCTRAIL on day 2 of a 21day cycle for 3 cycles (cycle 1-3) followed by up to 3 further cycles of chemotherapy only (cycles 4-6) as guided by their treating physician in line with SOC.
Patients in the control arm will receive SOC on day 1 and placebo on day 2 of a 21 day cycle for 3 cycles (cycle 1-3) followed by up to 3 further cycles of chemotherapy only (cycles 4-6) as guided by their treating clinician and in line with SOC.

After completion of the 4-6 cycles all patients will continue with a SOC therapy as appropriate.

The dose of MSCTRAIL will be RP2D calculated from safety data accrued in phase I of the TACTICAL study.

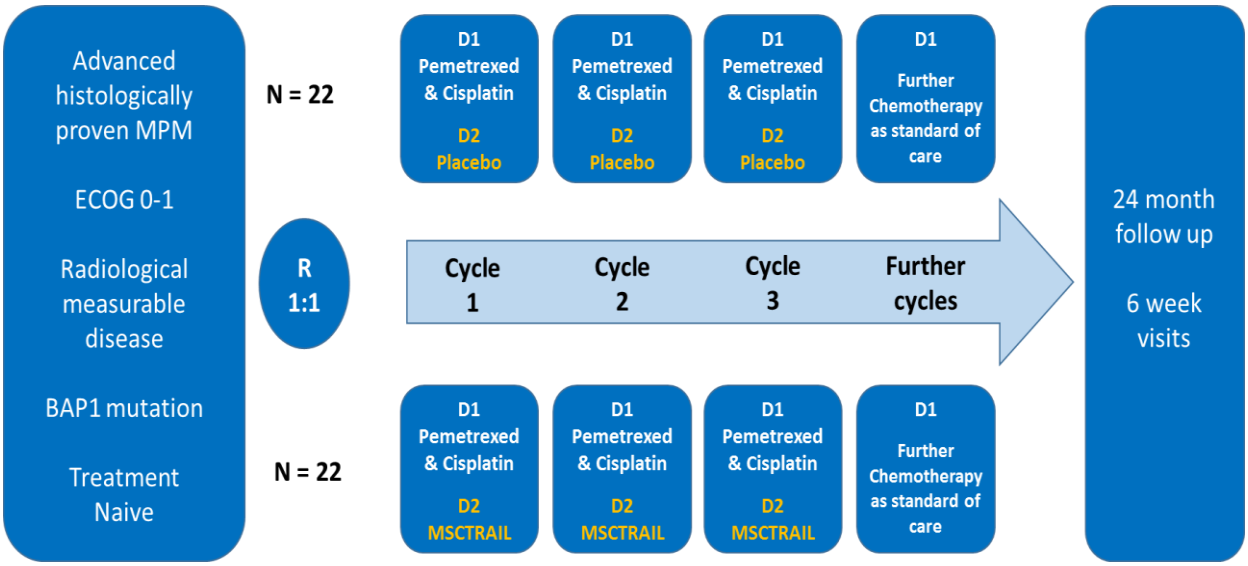


Figure 6.3: STRATEGIC Trial Schema

21 day cycle	Cycle 1		Cycle 2		Cycle 3		Cycle 4*		Cycle 5*		Cycle 6*	
Day	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Pemetrexed + Cisplatin*/	•		•		•		•		•		•	
MSCTRAIL/ Placebo		•		•		•						

Table 6.1: STRATEGIC trial treatment schedule.

**Patients will receive 4-6 cycles of chemotherapy as guided by their treating clinician in line with SOC*

6.1.7 Eligibility criteria

The full inclusion and exclusion criteria are listed in Appendix VI: STRATEGIC Protocol (draft V1) and MSCTRAIL specific criteria are discussed in Chapter 2 and

align with the TACTICAL protocol. A summary of those more specific to STRATEGIC and MPM are listed below.

Key eligibility criteria are:

- Histologically confirmed MPM
- Loss of nuclear expression of BAP1 using immunohistochemistry
- ECOG performance status of 0 or 1
- Inoperable, unresectable disease
- Documented radiological measurable disease in at least 1 site as measured by revised modified RECIST criteria (v1.1) in MPM within 28 days prior to randomisation.

These criteria aim to ensure homogeneity within the patient population, delivering this novel treatment in the safest treatment paradigm and allowing for any side effects or treatment gains to be correctly attributed.

It is well recognised that histological subtypes of MPM offer different prognoses; epithelioid (associated with relatively high survival), sarcomatoid (poorest survival), and biphasic/mixed (intermediate survival) [260]. In order to mitigate, this all subtypes will be included but will be stratified at randomisation.

Patients with a PS 0-1 will be included as that correlates with the NICE guidelines on the use of Pemetrexed in MPM, ensuring the trial falls within SOC.

6.1.8 Outcomes

The primary objective of this trial is to assess the difference in anti-tumour activity when MSCTRAIL is used with chemotherapy in BAP1 mutated MPM patients compared to chemotherapy alone.
The secondary outcome is to assess the type and duration of treatment response and the safety and associated toxicity of MSCTRAIL in combination with SOC and the impacts this has on the patients' quality of life. To reflect this the trial end points are:

Primary Endpoints:

1. Best response by revised modified RECIST criteria (v1.1) in MPM

Secondary Endpoints:

1. Tumour response at each assessment point
2. Change from baseline in sum of target lesions at each assessment point
3. Duration of response
4. Progression free survival
5. Overall survival
6. Frequency of adverse events
7. Quality of Life

6.1.8.1 Modified RECIST vs RECIST Criteria

Modified RECIST criteria (v1.1) for MPM (mRECIST) will be utilised for disease assessment in CT scans as opposed to RECIST V1.1 because of the unique morphological and growth patterns of MPM. mRECIST maintains the same stringent categorical response criteria established by RECIST V1.1 but has adapted the tumour measurement from a single unidimensional value to tumour thickness perpendicular to the chest wall or mediastinum with a limitation on the number of measurement sites.

MPM most commonly grows as a covering or ‘rind’ on the surface of the pleura and not classically the bidimensional lesions seen in other cancers. RECIST V1.1 relies on a reduction in the longest diameter of these bidimensional lesions. As MPM does not grow as a spherical lesion measurement of the longest axis and subsequent reduction in this is therefore not an accurate reflection of tumour response. mRECIST instructs user to select numerous sites within the scan and from these measure tumour thickness (perpendicular to the curve of the pleura). Six measurement sites should be selected in two positional at three separate levels at least 1 cm apart.

There are limitations as with all response criteria, namely interobserver variability especially when considering the angular orientation of the measurement. However, it

is suggested that images of where the measurements were taken from are stored so they can be referenced back to at the next scan point.

mRECIST was first introduced in 2004 but more recently updated to version 1.1 in 2018 [261]. It is endorsed by the International Association for Study of Lung Cancer (IASLC) [262] although they caveat that emphasis should be placed on refining further novel volumetric quantification. It is now a well-recognised outcome tool and recommended for use in clinical trials, and despite limitations mRECIST, offers a more accurate reflection of the response of this heterogenous tumour to treatment.

6.1.8.2 Primary Outcome

Best response can be defined as the proportion of response-evaluable patients who achieve a PR or CR as their best observed mRECIST outcome from baseline to time of disease progression or end of follow-up. It was chosen as the primary end point because it gives a clear early indication of disease activity, given that in this patient group remission is unlikely and relapse is almost inevitable. The outcome can be measured early in comparison to overall survival as it relies on time to progression and not death, benefiting an early phase trial as it allows more rapid progression to larger later stage trials. Best response is being utilised over 12 week response as in TACTICAL because patients may receive, as is standard of care, between 4-6 cycles of treatment so a specific treatment endpoint cannot be defined.

Statistical Modelling for STRATEGIC

Statistics for this trial were devised in conjunction with Dr G Wheeler a medical statistician from Cancer Research UK and UCL Cancer trial Centre at UCL.

6.1.9 Sample Size

The sample size for STRATEGIC is based on the need to detect a difference in response rate between the treatment arms of 25%. This was calculated by comparing the standard response rates observed in other MPM trials with a reasonable target

response rate that would make the combination of MSCTRAIL plus Pemetrexed and Cisplatin worthy of further investigation in a large phase III randomised controlled trial.

Patients will be randomised 1:1 between the MSCTRAIL plus chemotherapy (Pemetrexed plus Cisplatin) arm and chemotherapy alone. Previous studies have shown a best response rate (BRR; defined as the proportion of patients having either a partial or complete response to treatment according to modified RECIST v1.1 in MPM as their best response[261]) to Pemetrexed plus Cisplatin in this population of about 40% [243]. To detect a 25% improvement with MSCTRAIL plus chemotherapy (i.e. detecting at least a 25% improvement from 40% to 65%) with 80% power in a randomised two-arm trial, we require 44 patients overall (22 per arm). This controls the one-sided type I error rate at 20%, using a chi-square test to compare the response rates per treatment arm.

6.1.10 Data Analysis

The primary population for analysis will be the intention to treat (ITT) population defined as all patients who receive at least one dose of protocol study medication and have evaluable baseline tumour measurements.

6.1.10.1 Primary endpoint:

The primary endpoint for STRATEGIC is best response rate (BRR) by revised modified RECIST (mRECIST) (v1.1) in MPM[261].

At each visit patients will be assigned a response of complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD) as per the mRECIST v1.1 in MPM. If a patient has a non-evaluable tumour assessment, then the patient will be assigned a response of not evaluable (NE) unless there is evidence of progression in which case the response will be assigned as PD. If > 1/3 of lesions recorded at baseline are missing, then the target lesion (TL) response will be NE. However, if the sum of non-missing TL diameters would result in PD (i.e. if using a value of 0 for missing lesions the sum of diameters has still increased by > 20% or

more compared to the smallest sum of diameters on study), PD takes precedence over NE. A visit response of CR will not be allowed if any of the TL data is missing.

Based on the response assessments, the BRR, defined as the proportion of response-evaluable patients who achieve a PR or CR as their best observed mRECIST outcome from baseline to time of disease progression or end of follow-up, will be calculated as a percentage in each arm. As well as reporting of the absolute difference in BRRs between treatment arms, a chi-square test will be used to compare the BRR in the MSCTRAIL plus chemotherapy arm to that of the chemotherapy-alone arm to evaluate the strength of evidence that the BRR in the MSCTRAIL plus chemotherapy arm is greater than that of the chemotherapy-alone arm. A breakdown of separate best response percentages by best response category and treatment arm will also be tabulated.

6.1.10.2 Secondary endpoints:

Tumour response at each assessment point

Response rates at each disease assessment point will be reported as percentages with exact confidence intervals per treatment arm. Absolute differences in response rates at each time point and best response will also be computed with exact 95% confidence intervals.

Change in sum of target lesions from baseline at each assessment point

Change in sum of TLs from baseline will be measured at all disease assessment visits where such data are collected. Mean change per timepoint will be computed in each treatment arm, and absolute differences will be computed with 95% confidence intervals. Waterfall plots (bar charts) indicating the best percentage change in sum of TLs from baseline will also be produced.

Duration of response

Duration of response (DoR) will be computed from the time of first response either PR or CR, whichever occurs first. to progression or death from any cause. Patients who are responding to treatment and subsequently lost to follow-up (i.e. no PD and alive at last visit) will be censored at the time they were last confirmed as PR or CR.

Median DoR will be calculated per treatment arm, and the associated Kaplan-Meier (KM) curves will be plotted.

Progression free survival

Progression Free Survival (PFS) is defined as the time from randomisation to time of progression (as per mRECIST) or time of death from any cause. Patients with no confirmed time of progression/death will be censored at the time that they were last confirmed as non-progressive/alive. PFS will be analysed using KM estimates of median PFS per treatment arm and plotted on a KM plot. If there is no reason to reject the assumption of proportional hazards for PFS between treatment arms, a Cox Proportional Hazards (PH) model will be used to estimate the hazard ratio for PFS, and adjusted hazard ratios will also be derived (adjusting for any stratification factors in the randomization process). Otherwise, a suitable alternative will be used (e.g. weighted KM test, weighted log-rank test). PFS rates (along with 95% confidence intervals) will be presented at 3, 6, 9 and 12 months.

Overall survival

Overall survival (OS) is defined as the time from randomisation to death from any cause. Patients with no confirmed time of death will be censored at the time they were last observed in the study/recorded as alive. OS will be analysed using KM estimates of median OS per treatment arm and plotted on a KM plot. If there is no reason to reject the assumption of proportional hazards for OS between treatment arms, a Cox PH model will be used to estimate the hazard ratio for OS, and adjusted hazard ratios will also be derived (adjusting for any stratification factors in the randomization process). Otherwise, a suitable alternative will be used (e.g. weighted KM test, weighted log-rank test). OS rates (along with 95% confidence intervals) will be presented at 3, 6, 9 and 12 months.

Frequency of adverse events

Adverse event (AE) analysis will include all patients who receive at least one dose of their respective trial treatment (MSTRAIL or placebo). The number of patients experiencing each AE will be graded by the NCI Common Terminology Criteria for Adverse Events (CTCAE). For each reported AE, the number of patients who

experience each grade as their maximum grade will be reported, and lower grades (i.e. grades 1 and 2) may be combined. Serious AEs will be reported separately or summarised in a table if a sufficient number occur.

Quality of life

The impact of the treatment on a patient's quality of life will measured using the EORTC QLQ-C30 (version 3) questionnaire.

The Constitution of the World Health Organization (WHO) defines quality of life (QoL) as an individual's perception of life, values, objectives, standards, and interests in the framework of culture. QoL assessments reflect how a treatment impacts an individual's cognitive, emotional, and social functioning beyond survival or physiological responses. It can capture symptoms not always reflected in adverse events recordings.

EORTC QLQ-C30 (version 3) is a validated 30 point questionnaire designed for use in patients with cancer developed by the European Organization for Research and Treatment. It is composed of 5 multi item scales (physical, role, social, emotional and cognitive functioning) and 9 single items (pain, fatigue, financial impact, appetite loss, nausea/vomiting, diarrhoea, constipation, sleep disturbance and quality of life). By utilising this questionnaire, we can see the impact our novel treatment has on a patient's life and hence suitability for universal use.

6.1.11 Blinding and Randomisation

STRATEGIC is a randomised, blinded, placebo-controlled trial. Patients will be randomised 1:1 between the intervention and control arm. Online 'sealed envelope' software will be used for randomisation/MSCTRAIL allocation (and unblinding).

Patients will be randomised via blocked stratification and stratified according to the following factors:

- Performance status at baseline: 0 or 1
- Histology at point of diagnosis: biphasic and sarcomatoid Vs epithelioid

Patient randomisation will be performed prior to commencement of any trial treatment/intervention.

Stratifying for PS and histology aims to balance the treatment groups for known factors that influence prognosis or treatment responsiveness with the aim of reducing type I error. As discussed in the introduction all histological groups of MPM receive the same treatment. However, the known outcomes, responses and survival between these groups can vary with epithelioid having a better prognosis than non-epithelioid subgroups. Stratifying for this therefore aims to homogenise the cohorts.

STRATEGIC is a blinded trial. Therefore, all members of the trial team including the patient will be blinded to knowing if they are receiving placebo or MSCTRAIL.

The placebo will be the same product used in TACTICAL phase II consisting of the ATIMP excipient without MSCTRAIL, in a cryobag of consistent fill volume with the drug product.

Unblinding will only be done in exceptional circumstances when a Serious Adverse Reaction occurs, and the treating investigator considers knowing what the patient has received would be in the patient's best interest. As there is no known antidote to MSCTRAIL then all patients will be treated as if they have received the drug product and as per local guidelines and best supportive care.

As discussed in section 2.7.6 the delivering nurse may be able to discern the contents of the product, they are administering but will refrain from informing any member of the team or the patient.

6.1.12 Patient assessments

Pre-randomisation assessments and assessments during treatment will follow the same pattern as TACTICAL phase II. They are detailed in section 2.4.3 and are summarised below in table 6.2.

Following completion of trial treatment, patients will be followed up for a maximum of 24 months.

They will have follow-up visits 6 weekly and CT scans 3 monthly until there is evidence of disease progression on CT by revised modified RECIST criteria (v1.1) in MPM. Follow up visits will consist of the same review as per TACTICAL phase II:

- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest)
- Clinical review and physical examination, adverse events (AE) and concomitant medication (Con Med) check

Assessment																
			STRATEGIC													
	Pre Intervention		Cycle 1 Pemetrexed/Cisplatin & MSCTRAIL or placebo					Cycle 2 Pemetrexed/Cisplatin & MSCTRAIL or placebo		Cycle 3 Pemetrexed/Cisplatin & MSCTRAIL or placebo		Cycle 4 Pemetrexed/ Cisplatin	Cycle 5 Pemetrexed/ Cisplatin	Cycle 6 Pemetrexed/ Cisplatin	Follow Up
Days	Prior to registration	Within 14 days prior to registration	1	2	3	8	15	1	2	1	2	1	1	1	Every 6 weeks until 24 months post end of treatment
Interventions															
Pemetrexed /Cisplatin			x					x		x		x	x	x	
MSCTRAIL or Placebo				x					x		x				
Examination/Investigation															
Clinical Review			x	x	x	x	x	x	x	x	x	x	x	x	x
Physical examination		x	x	x	x	x	x	x	x	x	x	x	x	x	x
Vital signs		x	x	x	x	x	x	x	x	x	x	x	x	x	x
ECG		x		x	x	x	x		x		x				
Weight		x						X		x		x	x	x	
ECOG status		x	x	x				X	x	x	X	x	x	x	

Assessment			STRATEGIC												
	Pre Intervention		Cycle 1 Pemetrexed/Cisplatin & MSCTRAIL or placebo					Cycle 2 Pemetrexed/Cisplatin & MSCTRAIL or placebo		Cycle 3 Pemetrexed/Cisplatin & MSCTRAIL or placebo		Cycle 4 Pemetrexed/ Cisplatin	Cycle 5 Pemetrexed/ Cisplatin	Cycle 6 Pemetrexed/ Cisplatin	Follow Up
Days	Prior to registration	Within 14 days prior to registration	1	2	3	8	15	1	2	1	2	1	1	1	Every 6 weeks until 24 months post end of treatment
CT Scan	X								X (D14-21)			X (D14-21)		X (D14-21)	x
Laboratory tests															
Haematology (FBC)		x	x			x	x	x		x		x	x	x	
Oncological Profile		x	x			x	x	x		x		x	x	x	
Urinalysis			x					x		x		x	x	x	
Pregnancy test (if applicable)		x	x					x		x		X	x	X	
Adverse event and Con Med collection		x	x	x	x	x	x	x	x	x	x	x	x	x	

Table 6.2: STRATEGIC patient interventions

Progress

Safety data from phase I of TACTICAL is required before the STRATEGIC starting dose can be obtained and ethics approval sought. Given the ongoing revised timetables we aim to open early 2021.

Mesothelioma is a known orphan disease, given its prevalence of 0.45/10,000 in the UK. To aid the path to commercialisation for MSCTRAIL I have been working towards applying for Orphan Drug Designation for the use of MSCTRAIL in Mesothelioma. Obtaining this will significantly benefit the commercialisation of MSCTRAIL as amongst other things it allows, seven years of marketing exclusivity, tax credits of the qualified clinical research costs, waiver of Prescription Drug User Fees.

STRATEGIC aims to show that MSCTRAIL is safe and effective when given in combination with SOC treatment for BAP1 mutated MPM. This will not only pave the way for a novel, targeted therapy for a devastating and life-limiting cancer but may also open the door for the use of MSCTRAIL in other tumour groups.

7 Discussion

It has long been recognised that lung cancer is the leading cause of cancer death world-wide[2] and despite current advances in targeted therapies this remains the case[3]. The treatment options for advanced disease, in the form of radio and chemo-therapeutics offer some overall and progression free survival but patients do eventually progress. These treatments also carry a heavy burden of, at times, intolerable side effects. This highlights the real need for a novel therapeutic option.

A targeted cell and gene therapy, MSCTRAIL, has been presented as that therapeutic option. There is strong pre-clinical evidence for the use of mesenchymal stromal cells (MSCs) genetically modified to express TRAIL in the treatment of cancer[6, 51, 88], specifically lung cancer. I have demonstrated the setup, trial design and adaptations required for the opening of a phase I/II trial- TACTICAL. TACTICAL aims to investigate the safety and efficacy of MSCTRAIL in combination with first line standard of care (SOC) therapy for advanced adenocarcinoma of the lung.

TACTICAL has recruited and treated 4 patients and successfully delivered 9 doses of MSCTRAIL to those patients. The first 2 patients receiving all 3 doses, the 3rd patient received 2 doses and the 4th patient received 1 dose. At the12 week efficacy evaluation CT scans 1 patient had new lesions in keeping with unconfirmed progressive disease (iUPD), 1 patient was shown to have stable disease (SD) and 2 patients had partial response (by iRECIST criteria). All patients remain alive and there were no DLTs experienced.

The first 3 patients were incidentally found to have asymptomatic pulmonary embolisms (PEs) on 6 or 12 week CT scans which led to a serious adverse event review. The trial was temporarily paused while investigation of causality could be conducted, and subsequent safety measures evaluated. Amendments were then made to the protocol before resubmission for approvals. These substantial amendments now have full regulatory and ethics approvals and the trial will re-start recruiting.

The current cohort number does not allow for any comparative conclusions on the efficacy of MSCTRAIL in combination with SOC therapy for advanced

adenocarcinoma to be made. But the set up already completed and ongoing recruitment into the TACTICAL trial will provide an approved platform to examine this efficacy question. Moving forward, completion of phase I and opening of the larger placebo-controlled phase II trial will also provide further evidence of MSCTRAIL efficacy in comparison to placebo.

The safety of MSCTRAIL remains a key unknown issue, clearly the occurrence of pulmonary embolism is a significant concern. The cohort of patients treated within TACTICAL are at high risk of PEs, not only does the presence of lung cancer render them in a pro-thrombotic state [150] but advanced disease, histological diagnosis of adenocarcinoma, receiving chemotherapy and being within 6 months of diagnosis have all also been shown to be independent risk factors for venous thromboembolism [151-153]. However, with the first 3 patients all having been found to have PEs further investigation was warranted. As discussed in chapter 4 there is *in vivo*[173-177] and *in vitro* [144, 179, 180] evidence that MSCs may be pro-coagulable and it has been hypothesised that this may be driven by number of causes including increased levels of tissue factor (TF) [170] that can initiate coagulation[171], secretion of procoagulant micro vesicles[172] or direct enhancement of platelet deposition[173]. However, previously there has not been any clinical evidence to support this. It may be postulated that because previous trials did not employ such a rigorous CT scanning regime these incidental findings were not seen, or that method of delivery employed in the initial infusions in TACTICAL led to an increased propensity for the cells to aggregate, forming micro thrombi, as seen in pre-clinical work presented.

Patient safety however remains paramount and at the forefront of any clinical trial. Therefore, in order to move forward adaptations, detailed in section 4.1.19, have been made to the protocol to try to ensure the safety of future TACTICAL patients. With ethics and regulatory approvals now in place the trial can re-start recruitment and answer key questions regarding both the safety and efficacy of MSCTRAIL.

Translational work investigating markers of apoptosis has shown that, given the combination of treatment, cell death measured by circulating CK18 is highest early in the treatment cycle. There is currently no clear pattern between patient clinical response and changes in those markers. There may be correlation between patients who experience early progression or poorer survival outcomes and high baseline

values of CK18, as has been seen in similar studies [204-206] [207] [208], but further data is required to draw these conclusions.

This work has shown that MSCTRAIL may provide a safe and effective treatment for not only advanced metastatic lung cancer but other devastating cancers. However, further evidence in required. The on-going work of both the TACTICAL and STRATEGIC trials aim to provide this.

8 Future Directions

Barriers to Translation

The landscape of medical therapies has seen a revolutionary boom in cell and gene therapy over the past 20 years. With increasing scale and marketable 'off-the-shelf' potential the industry is projected to be worth over £20 billion by 2022. In the UK in 2016 cell and gene trails made up 37% of all cell therapy research [263]. These cells are attractive entities for therapeutics because of their innate ability to migrate to site of disease, potential to provide sustained benefit by proliferation and act as transporters through genetic modification thus acting as both the 'drug' and the 'device'[264].

While the development and trial of cell and gene products seems to be moving forward the majority of previous clinical trials have not progressed beyond phase II; there remain many barriers to translation for this promising field including large scale reproducible manufacture of cells, unknowns of pharmacokinetics or dynamics and ultimately the longevity and journey of the cells within the patient. However, elucidating this knowledge is challenging because the delivery and migration of cells through the body cannot be visualised or measured by any traditional methods.

An effective and translational tracking technology could give valuable feedback on implantation success, migration and cell longevity. This in turn would reveal patient-specific responses, insight on mechanism of action, and routes for optimising delivery and retention. We aim to gain this knowledge by using TACTICAL as a backdrop and radiolabelling MSCs before delivery to 3 patients.

8.1.1 Radiolabelling

8.1.1.1 Background

As discussed above one of the barriers to translation is the paucity of understanding of the distribution, kinetics and survival of MSCs after intra-venous delivery and transplantation into patients. This knowledge would provide valuable feedback on

implantation success, migration and cell longevity. Which in turn would reveal patient-specific responses, insight on mechanism of action, and routes for optimising delivery and retention not just pertinent for this therapy but for all developing cellular therapies.

To try to understand this better we plan an additional sub-study as part of phase II of TACTICAL to implement a new labelling and imaging technology using ^{89}Zirconium (^{89}Zr) with Positron Emission Tomography (PET).

Direct labelling of cells with radioactive tracers detected by nuclear imaging platforms provides a sensitive way of tracking cells non-invasively. ^{111}Indium (^{111}In)-oxine has been routinely used as a white blood cell labelling agent [128], however it has clinical limitations; ^{111}In is a gamma emitter with a 2.8 day half-life that can be detected by single photon emission computer tomography (SPECT) imaging. For direct labelling ^{111}In requires a chelator such as oxine, to transport the radioactive metal across the cell membrane, where it rapidly dissociates and binds to cellular proteins and DNA[265] . However, due to the inherent properties of ^{111}In radioactive decay and the dose needed for high-resolution SPECT imaging, the intracellular radiation dose of ^{111}In can lead to rapid cytotoxicity and leakage of the tracer by cell lysis[266].

^{89}Zr a positron emitter with a slightly longer half-life than ^{111}In (3.3 days), which can be detected by PET and may provide a novel option to overcoming the many limitations of ^{111}In-SPECT cell tracking. SPECT imaging has a lower sensitivity and resolution than PET imaging and it thereby requires much higher doses of radioactivity per cell than PET.

^{89}Zr has been shown to exhibit a lower cell toxicity than other radioactive direct cell labelling agents [267, 268] and requires the chelator oxine for rapid and effectively up take by cells after a brief incubation. However, ^{89}Zr-oxine has not be used in a clinical cell therapy trial before.

8.1.1.2 Pre-Clinical Data

Preclinical work by Patrick et al (2020). has demonstrated that the ^{89}Zr-oxine cell labelling technique is well-tolerated by MSCTRAIL across a range of ^{89}Zr doses up to and above those needed for clinical imaging[269].

The study showed that there was no evidence of DNA damage or cell stress response and that cellular phenotype and therapeutic functional efficacy was retained post ^{89}Zr labelling. PET imaging after labelling and delivery of the cells into a mouse lung cancer model showed successful delivery of the cells to the lungs. Furthermore, that they could be tracked quantitatively in 3 dimensions using PET imaging for approximately 1 week, with signal assessed by bioluminescence imaging indicating viable cells (figure 8.1).

^{89}Zr-oxine toxicity was comparable to that of ^{111}In-oxine at equivalent doses and proliferation rates of MSCTRAIL were unaltered over a wide dosing range with ^{89}Zr-oxine[269]. Human dosimetry estimates were produced using simulations and preclinical biodistribution data for mouse to human extrapolation.

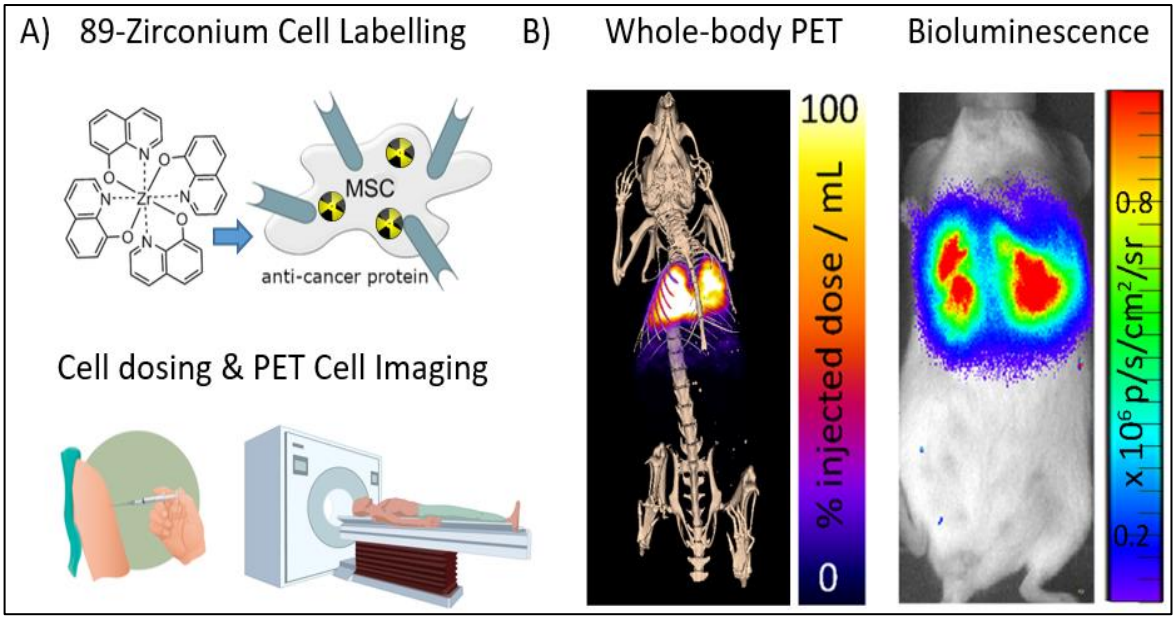


Figure 8.1: Work up and proposed application of 89-Zr labelling

A) Schematic showing the work flow of clinical ^{89}Zr-oxine cell labelling, administration and PET scanning, B)Whole body PET maximum projection image of a mouse 1 hour after intravenous injection of 1x10^{6} ^{89}Zr-MSCTRAIL cells and the corresponding bioluminescence image showing are mainly within the lung and are viable at this time point (PET and bioluminescence images modified from Patrick et al (2020) [269].

Moving forward into clinical practise we now propose to deliver ^{89}Zr labelled MSCTRAIL (^{89}Zr-MSCTRAIL) to 3 patients as a sub-study to the TACTICAL trial. This will follow a similar design to TACTICAL, with the same patient eligibility criteria, but ^{89}Zr-MSCTRAIL will be administered in cycle 1 and 3 with un-labelled MSCTRAIL given in cycle 2. Doses will be followed by whole body PET imaging at multiple time points. Images will be analysed to determine cell location and blood samples will be taken to determine blood pool tracer activity. This data will allow us to track the cells journey and better understand their bio-distribution. By delivering a 2nd ^{89}Zr labelled dose we will also be able to see the effect of repeated doses of MSCTRAIL and if there is any alteration in cells pharmacodynamics which may indicate an acquired host response to the cells.

8.1.2 Clinical Trial Design

This sub-study is in the early stages of development, the preliminary pre-approval trial plan and protocol design are discussed below.

The study will be carried out at a further site which utilises their expertise in both nuclear imagine and trial deliver. It also ensures clarity for patient eligibility as patients from that site will only be eligible for the sub-study and not the randomised placebo-controlled phase II arm.

Primary Objectives:

- To map the journey of MSCTRAIL after intravenous delivery.

Secondary Objective

- To assess the effect of repeated doses of MSCTRAIL after intravenous delivery.

The statistical endpoints to reflect these will be

- Differences in Standardised Uptake Values (SUVs) between tumour and normal lung tissue
- Differences in distribution in SUVs between cycle 1 and cycle 3.

Translational work will investigate the donor-specific cellular and humoral immune responses as well as blood pool tracer activity from injection of ^{89}Zr.

Analysis of SUVs in tumour and normal lung tissue

Per-patient SUVs will be calculated for each tissue type and plotted against time of scan; this will be done for both cycle 1 and cycle 3 separately. Mean and standard errors of SUV differences will be presented. The non-parametric Wilcoxon signed-rank test may be used at each scan time point to compare the distribution of SUVs between tissue type.

Analysis of SUV distributions between cycle 1 and cycle 3

SUV data is structured such that measurements taken at different time points from the same individual may be correlated. SUVs per patient and cycle in the tumour sites will be plotted over time and mean (and standard error) differences in SUVs between cycles at each time point will be presented. To estimate the difference in SUVs measured in cycle 1 and cycle 3, a mixed-effects modelling approach may be used to account for the dependency between observations.

Limitations

Only three patients will receive radiolabelled infusions. Therefore, findings obtained from formal statistical test procedures should be interpreted with caution. Results should be interpreted as an observational study.

8.1.2.1 Trial Treatment

Patient eligibility will be the same as TACTICAL phase II and detailed in section 2.4.1, including stage IIIb/IV adenocarcinoma of the lung, PS 0-1 with no targetable driver mutations.

'SOC' will continue to be given on day 1 and defined as: Chemotherapy (Pemetrexed 500mg/m^2 and Cisplatin 75mg/m^2) and Immunotherapy (Pembrolizumab 200mg)

The dose and safety of MSCTRAIL will be confirmed in phase I of TACTICAL.

Within the TACTICAL trial patients receive standard of care therapy on day 1 followed by MSCTRAIL on day 2 of a 21 day cycle for 3 cycles with CT imaging at 6 weeks and 12 weeks, reported by iRECIST criteria. In this sub-study, we propose to delivery instead Zirconium labelled MSCTRAIL on day 2 in cycle 1 and cycle 3 with unlabelled MSCTRAIL cells in cycle 2, again on day 2 (*figure 8.2*). Patients will then undergo serial whole body PET imaging after infusion of Zr^{89}-MSCTRAIL, within cycle 1 and 3 on:

- Cycle day 2- day of administration of Zr^{89}-MSCTRAIL
- Cycle day 3- 24 hours post administration of Zr^{89}-MSCTRAIL
- Cycle day 5- 3 days post administration of Zr^{89}-MSCTRAIL
- Cycle Day 9- 7 days post administration of Zr^{89}-MSCTRAIL

The timing of these scans has been based on preclinical, murine work. It was seen that cells labelled with ^{89}Zr-oxine can be tracked for around 7 days with maximum visibility within the first 24-48hours. As this is a first-in-human study we therefore propose to track the cells for the duration of this visibility to gain maximum information on initial journey and implantation success and then longitudinal retention of cells.

If there is insufficient signal seen on the PET scan done 7 days after administration of Zr^{89}-MSCTRAIL in the first patient we plan to move the final scan forward to 5 days post Zr^{89}-MSCTRAIL.

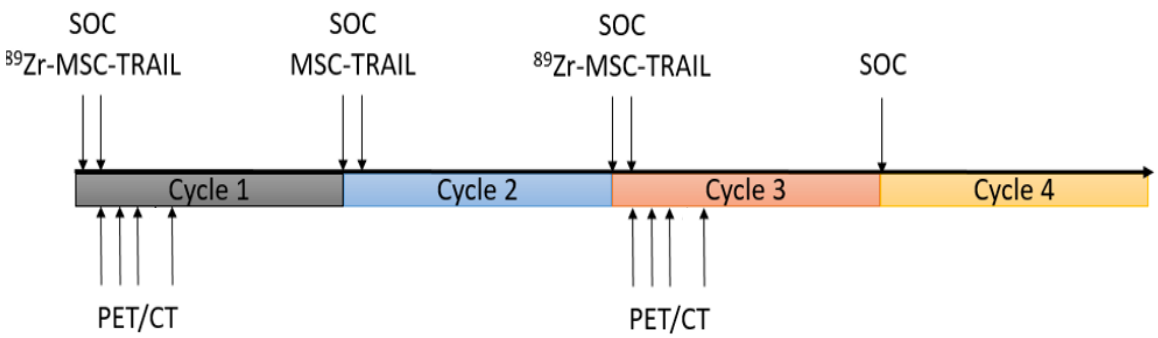


Figure 8.2: Proposed Radiolabelling Trial Schema

8.1.3 Progress

The GMP method, FDA approval and subsequent production of ^{89}Zr-oxine for cell labelling has been supported by an 18 month Confidence in Collaboration, London Advanced Therapies grant (CiC012) which started in Dec 2019 and is a collaboration between UCL, King's College London and Bart's Queen Mary's.

In December 2019 we were successful in our application to the JP Moulton Charity Trust ("Whole-body ^{89}Zirconium PET Imaging of a Genetically Modified Mesenchymal Stem Cell Therapy: First-in-Man Study") for the funding for the GMP scale up and of the ^{89}Zr-oxine cell labelling process and clinical delivery of ^{89}Zr labelled MSCTRAIL as part of the TACTICAL trial. We aim to open the trial early 2021 following the completion of TACTICAL phase I and alongside TACTICAL phase II.

With this first in man labelling trial we hope to break down the barriers of translation for a novel cell and gene therapy. It will provide vital information on journey and fate of the cellular therapies after human intravenous administration; broaden the therapeutic landscape of cell and gene therapies and pave the way to larger phase III trials and commercialisation

9 Statement of Contribution

This work could not have been carried out without the considerable support and advice from all the members of the TACTICAL team as well as my mentors and supervisors.

Preclinical work detailed in chapter 1 was carried out prior to my starting this work and is referenced and attributed accordingly.

The design of the TACTICAL trial was undertaken with Dr Beth Sage, Professor Sam Janes and Dr Martin Forster. My role was initially to collaborate the Dr Sage in the writing of the protocol and planning of the study. After her move away from UCL I took the work forward, completing the preparatory work and bringing about the trial.

I took the lead on key documents, including the investigators brochure, patient information sheet, summary of drug arrangements, infusion procedures and clinical report forms. This led to submission to internal and external regulatory authorities which was done in conjunction with colleagues at the Cancer Research UK Clinical Trials Centre.

I led the process of site set up, liaising between the Clinical Research Facility and the McMillian Cancer centre, writing patient pathways and SOPs for cellular administration.

The pre-clinical work detailed in chapter 3 regarding the work up of MSCs in combination with immune check point inhibitors was done in collaboration with Dr Krishna Kolluri and Dr Doraid Alrifai. I had minimal prior lab experience but through this was able to learn about tissue culture of both MSCs and Cancer cells, extraction of PBMCs from whole blood samples, measuring bioluminescence as well as flow cytometry. Using this data, I drafted the request for substantial amendment as well as the changes to the protocol.

I, with Dr Martin Forster was responsible for patient recruitment, screening and consent. Patients were recruited from the UCLH and referred to the clinical research facility (CRF) for the trial. I was present for all cell infusions at the McMillian Cancer

centre and trial treatment. I reviewed patients on the CRF during trial treatment and follow up.

The translational work was undertaken in collaboration with Rebecca Graham. Due to the number of samples we both carried out the processing of the samples for storage and the Cytokertin18 assays in order to complete it in a timely way.

I, with the support of the STRATEGIC team applied for funding for the STRATEGIC trail but the pre-clinical work investigating MSCTRAIL in MPM and BAP1 loss of function in MPM was carried out prior, it is references and attributed accordingly. I have led in the writing of the STRATEGIC protocol, it is currently in draft form awaiting review from members of the working team prior to full ethics and regulatory approvals which will be done with the help of colleagues at the Cancer Research UK Clinical Trials Centre.

The radiolabelling project has been ongoing since before I began this work. The preclinical work was conducted by Dr Tammy Kalber and Dr Stephen Patrick and their colleagues at the Centre for Advanced Biomedical Imaging (CABI) UCL and is references accordingly. I worked with them and others in the successful application for funding and continue to work on the complex issues of bringing this to in-human trial, leading on the clinical aspects.

I have had much guidance and advice at all stages from my supervisors and mentors, mentioned and acknowledged throughout this thesis.

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TACTICAL

Targeted stromal cells expressing TRAIL as a therapy for lung cancer

Trial Sponsor:	University College London
Trial Sponsor reference:	UCL/14/0453
Trial funder:	Medical Research Council
Funder reference:	MR/M015831/1
Clinicaltrials.gov no:	NCT03298763
EUDRACT no:	2015-005526-18
CTA no:	20363/0383/001-0001

Protocol version no:	4.0
Protocol version date:	14th Feb 2019

Protocol v4.0 14/02/2019 Authorisation signatures:

Name & Role:	Signature:	Date authorised:
Chief Investigator: Professor Sam Janes Professor of Respiratory Medicine		
For the Sponsor:		
[redacted] Director / Deputy Director, UCL CTC		
[redacted] Trial Group Lead, Advanced Therapies		

Please note: This trial protocol must not be applied to patients treated outside the TACTICAL trial. Cancer Research UK & UCL Cancer Trials Centre (UCL CTC) can only ensure that approved trial investigators are provided with amendments to the protocol.

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Cancer Research UK & UCL Cancer Trials Centre
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Bilyana Popova	Senior Trial Coordinator	UCL CTC
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1. PROTOCOL SUMMARY

1.1. Summary of Trial Design

Title:	TArgeted stromal Cells expressing TRAIL as a therapy for lung CAncer
Short Title/acronym:	TACTICAL
EUDRACT no:	2015-005526-18
Sponsor name & reference:	University College London 14/0453
Funder name & reference:	Medical Research Council MR/M015831/1
Clinicaltrials.gov no:	NCT03298763
Design:	Phase I: Single site dose de-escalation design with a modified Bayesian continual reassessment method (mCRM) to estimate the recommended Phase II dose (RP2D) of MSCTRAIL in combination with first line standard of care therapy (SOC) Phase II: Multicentre, randomised double blinded placebo controlled trial comparing MSCTRAIL at the RP2D and first line standard of care therapy versus placebo and first line standard of care therapy
Overall aim:	To evaluate the safety and anti-tumour activity of MSCTRAIL in addition to standard of care therapy in metastatic Non-small cell lung cancer (NSCLC) patients in a Phase I/II clinical trial
Name of Advanced Therapy Investigational Medicinal Product (ATIMP)	MSCTRAIL
Primary endpoint:	**Phase I:** 1. The incidence of dose limiting toxicities (DLT) within the first cycle of treatment 2. Determination of recommended Phase II dose (RP2D) of MSCTRAIL in combination with SOC as first line treatment for lung adenocarcinoma **Phase II:** Tumour response rate by RECIST (v1.1) criteria after 12 weeks (iRECIST if patient received pembrolizumab)
Secondary endpoints:	**Phase I:** • Frequency of adverse events within 3 cycles of treatment • Best overall response • Change from baseline in sum of target lesions

	• Duration of response • Progression free survival **Phase II:** • Frequency of adverse events • Best overall response • Change from baseline in sum of target lesions • Tumour response at each time point • Duration of response • Progression free survival • Time to Progression • Overall survival
Exploratory Biological Studies:	• Investigate the relationship between circulating biomarkers of apoptosis and safety and efficacy parameters • Evaluation of circulating biomarkers and enumeration of circulating tumour cells (CTCs) and circulating tumour DNA (ctDNA) • To assess how the recipient immune system affects the therapeutic efficacy of donor allogeneic MSCTRAIL using immune cells isolated from peripheral blood samples • Assess whether specific tumour mutations may predict response to treatment
Target accrual:	Phase I: minimum of 6 patients, maximum of 12 patients Phase II: 46 patients
Inclusion & exclusion criteria:	**Inclusion criteria** • Inoperable stage IIIb/IV histologically /cytologically confirmed lung adenocarcinoma • EGFR and EML4-ALK mutation negative • Patients with unmeasurable but evaluable disease can be included in the phase I study, but disease must be measurable to be included in the phase II study. • ECOG performance status of 0 or 1 • Life expectancy of at least 12 weeks • Age at least 18 years • Adequate haematological status: o Haemoglobin 100g/L or greater o Neutrophil count ≥1.5 x 10^9/L o Platelets ≥100 x 10^9 /L • Adequate organ function: o Bilirubin ≤1.5 x ULN

	○ ALT or AST ≤ 2.5 x ULN in the absence of liver metastases(≤5 x ULN is acceptable with liver metastases) ○ Creatinine clearance ≥ 60 ml/min (Cockcroft-Gault or EDTA) • Negative pregnancy test for female patients of child bearing potential. • Male subjects and women of child bearing potential must agree to use a highly effective method of birth control • Ability to understand and provide written informed consent • Ability to comply with the requirements of the protocol **Exclusion criteria** • Prior chemotherapy, hormonal therapy, radiotherapy (including palliative radiotherapy), immunotherapy or treatment with investigational drugs for advanced NSCLC. • Prior treatment with any cellular therapy • Any surgical procedure in the previous 6 weeks prior to registration/randomisation • Known respiratory failure with baseline resting SpO2<88% • Long term oxygen therapy • Known WHO Class III or IV pulmonary hypertension • Active infection requiring systemic therapy • Active or infected wounds • Vaccination with any live attenuated vaccine within 30 days prior to trial registration/randomisation during dose administration and for 90 days after last dose • Subject has known sensitivity/allergy to any of the trial drugs to be administered during the trial. • Any contraindication to the administration and use of cisplatin, pemetrexed,vitamin B12 or folic acid if the patient is to receive chemotherapy (cisplatin and pemetrexed) • Prior malignancy other than NSCLC (except if the tumour was a non-melanoma skin tumour that has been completely excised or in situ cervix carcinoma), unless have been treated

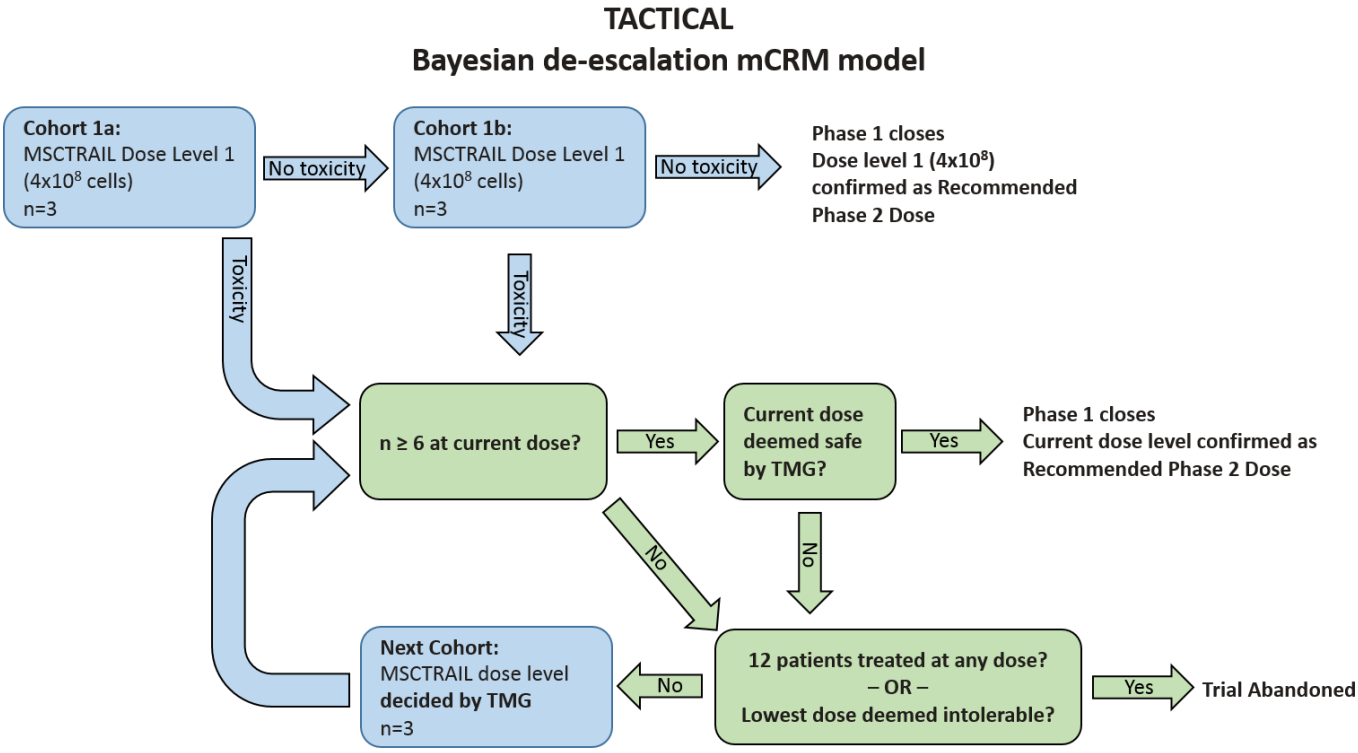
	with curative intent with no evidence of disease for > 3 years • Evidence of symptomatic brain metastases requiring treatment • Myocardial infarction, or unstable or uncontrolled disease or condition related to or impacting cardiac function (e.g., unstable angina, congestive heart failure [New York Heart Association > class II]) within 1 year of enrolment • Venous thromboembolism within the last 6 months • Known hepatitis B or C infection, human immunodeficiency virus (HIV)-positive patients • Pregnant women or those who are breast feeding • Other medications, severe acute/chronic medical or psychiatric condition, or laboratory abnormality that may increase the risk associated with trial participation or trial drug administration, or may interfere with the interpretation of trial results, and in the judgment of the investigator would make the patient inappropriate for entry into this trial Extended exclusion criteria if patient is to receive pembrolizumab: • Diagnosis of immunodeficiency or is receiving systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior the first dose of study drug. • Active autoimmune disease that has required systemic treatment in past two years (that is, with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg, thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment and is allowed. • History of (non-infectious) pneumonitis that required steroids or current pneumonitis.
Number of sites:	Phase I – 1 site Phase II – 2-4 sites

Treatment summary:	**Phase I:** • SOC treatment, day 1 Pemetrexed 500mg/m^2 and Cisplatin 75mg/m^2 Or Pemetrexed 500mg/m^2 and Cisplatin 75mg/m^2 and Pembrolizumab 200mg IV Or Pembrolizumab 200mg IV • MSCTRAIL cells day 2 MSCTRAIL is to be administered on day 2 of cycles 1-3 at the dose specified by the Cancer Trials Centre (CTC) at the time of registration. There will be up to three cohorts. The first cohort (3 patients) will receive dose level 1. If no dose limiting toxicities are observed then the cohort will be expanded (further 3 patients) who will also receive dose level 1. If dose limiting toxicities are recorded then the second cohort will receive dose level 2. Further, lower dose cohorts are planned only in case of dose limiting toxicities: • Dose Level 1: 4x10^8 cells (per cycle) • Dose Level 2: 2x10^8 cells (per cycle) • Dose Level 3: 8x10^7 cells (per cycle) o Each patient will receive 3 cycles of MSCTRAIL with SOC therapy (if no toxicity is noted) followed by a 4th cycle of SOC therapy only (without MSCTRAIL). o Each cycle is 21 days in duration *After completion of the four cycles of trial treatment, the patients will revert to local standard of care therapy as decided by their treating clinician.* **Phase II:** **Control arm** • SOC treatment, day 1 Pemetrexed 500mg/m^2 and Cisplatin 75mg/m^2 Or Pemetrexed 500mg/m^2 and Cisplatin 75mg/m^2 and Pembrolizumab 200mg IV Or Pembrolizumab 200mg IV • Placebo , day 2

	○ Patients will receive 3 cycles of placebo with SOC treatment followed by a 4th cycle of SOC treatment only (without placebo) ○ Each cycle is 21 days in duration **Investigational arm** • SOC treatment, day 1 Pemetrexed 500mg/m^2 and Cisplatin 75mg/m^2 Or Pemetrexed 500mg/m^2 and Cisplatin 75mg/m^2 and Pembrolizumab 200mg IV Or Pembrolizumab 200mg IV • MSCTRAIL cells day 2 (at dose established in Phase I) ○ Patients will receive 3 cycles of MSCTRAIL with SOC therapy (if no toxicity is noted) followed by a 4th cycle of SOC therapy only (without MSCTRAIL) ○ Each cycle is 21 days in duration *After completion of the four cycles of trial treatment, the patients will revert to local standard of care therapy as decided by their treating clinician.*
Duration of recruitment:	Phase I: 12 months Phase II: 24 months
Duration of follow up:	Phase I: maximum 24 months follow-up per patient Phase II: maximum 24 months follow-up per patient
Definition of end of trial:	The end of trial will be 2 years after the last patient in phase II has reached the end of trial treatment

1.2. Trial Schema

Phase I



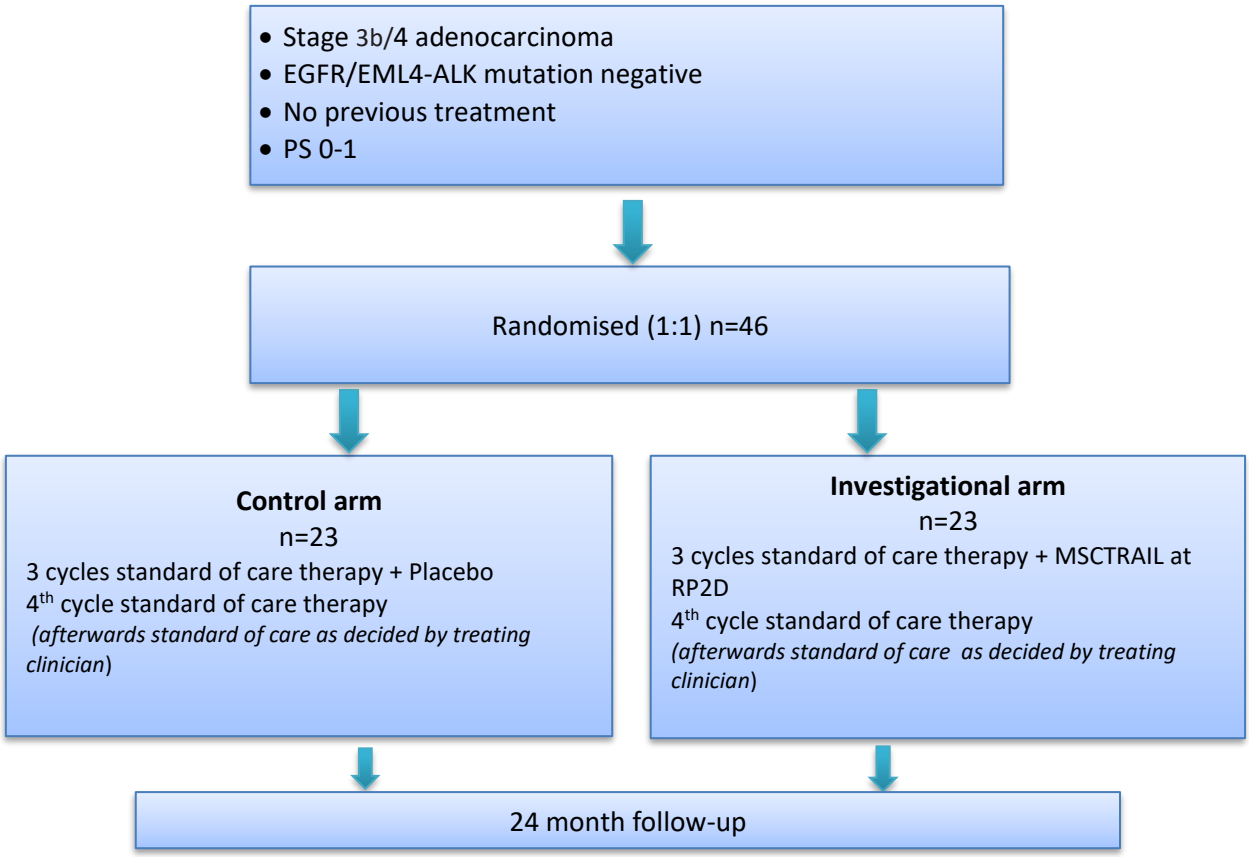
Phase I is a first-in-human, accelerated dose de-escalaton study: 3 patients, deemed cohort 1a, will receive $4x10^8$ cells of MSCTRAIL in combination with first line standard of care treatment (SOC) as defined by NICE guidelines (chemotherapy with pemetrexed and cisplatin and or immune therapy with pembrolizumab)for 3 cycles, followed by a 4th cycle with SOC only (without MSCTRAIL). After the 4 cycles are completed patients will continue with a standard of care therapy as decided by their treating clinician.

If there are no dose limiting toxities (DLT) a further 3 patients (cohort 1b) will receive the same dose of MSCTRAIL in combination with SOC as described above. If there are no toxicities in all 6 patients this will be the recommended Phase II dose (RP2D).

If patients in cohort 1 have DLTs then cohort 2 will receive a reduced dose of MSCTRAIL decided by the Trial Management Group (either $2x10^8$ or $8x10^7$ cells), if there are no DLTs after 3 patients, a further 3 patients will receive the same dose. If there are no toxicities in all 6 patients this will be the recommended Phase II dose (RP2D).

This pattern will continue until the RP2D is discovered. At this point the Trial Management Group (TMG) and Independent Data Monitoring Committee (IDMC) will review the data from phase I, and decide if the trial can proceed to phase II with all subsequent patients receiving the RP2D. The TMG and IDMC will also advise whether any changes to the trial conduct are needed before the start of phase II in which case a substantial amendment addressing their suggestions will be submitted for approval.

Phase II



Phase II is a randomised, placebo controlled study comparing MSCTRAIL at the RP2D* and SOC versus placebo and SOC therapy

Patients will be randomised 1:1 between the intervention and control arm. Patients in the intervention arm will receive SOC on day 1 followed by MSCTRAIL at the RP2D on day 2. This schedule will be repeated after 21 days for 3 cycles. Afterwards patients will have a 4th cycle with SOC only (without MSCTRAIL).

Patients in the control arm with receive SOCon day 1 and placebo on day 2. This will be repeated after 21 days for up to 3 cycles. Afterwards patients will have a 4th cycle with SOC only (without placebo).

After completion of the 4 cycles all patients will continue with a SOCtherapy as decided by their treating clinician.

** recommended Phase II dose*

2. INTRODUCTION

2.1. Background

Over 45,000 people are diagnosed with lung cancer in the UK per year. It is the leading cause of cancer death worldwide and is responsible for over 35,000 deaths per year in the UK alone. Over 70% of patients present with advanced disease and cannot be cured with current treatments and for these patients 1 year survival is approximately 15%. About 80% of patients will be diagnosed with non-small cell lung cancer (NSCLC) and of these approximately 40% will have adenocarcinoma. Of those patients with lung adenocarcinoma a small number will have tumours with gene mutations either in the epidermal growth factor receptor (EGFR) or rearrangements in the anaplastic lymphoma kinase (ALK) gene. Patients with tumours exhibiting these oncogenic drivers are offered treatment with molecular therapies targeted to these aberations.

In recent years there has been a rapid advancement in the use of immune therapy in the treatment of cancer.

Immune therapy utilizes the patients own immune system to recognize and destroy cancer cells. Immune checkpoint inhibitors target the pathways that are by exploited by tumours to evade recognition and hence destruction [2]. They achieve this via T cell modulation and hence activating the host immune response to cancer cells [3].
One such pathway is that of of programmed death receptor (PD-1) and its ligand (PD-L1). PD1 receptor down regulates excessive immune response and binding of it to the PD-L1 ligand on tumour cells causes T cell suppression and evasion of the immune response.

Inhibition of this pathway has been shown to be effective in the treatment of metastatic NSCLC [4]. To date, four immune checkpoint pathway inhibitors have been approved by the United States Food and Drug Administration (FDA) for use in patients with NSCLC: nivolumab and pembrolizumab, both targeting the programmed cell death-1 (PD-1) receptor, as well as atezolizumab and durvalumab, targeting the anti-programmed death-ligand 1 (PD-L1).

Original NICE guidelines on the use of immune checkpoint inhibitors as first line treatment of NSCLC approved the use of pembrolizumab (Keytruda, Merck) in patients in whom the percentage of tumours cells with membranous PD-L1 staining in 50% or greater as a single agent first line treatment. However recent studies have shown there may be a role for pembrolizumab in those patients whom PD-L1 expression is less than 50% [4-6]. KEYNOTE-042 recruited 1274 patients with a PD-L1 expression of greater than 1% and randomised them to receive pembrolizumab alone vs invetigator's choice combination chemotherapy; pembrolizumab significantly improved survival including those with PD-L1 expression of 1% or greater [6]. This benefit is driven by activity in patients with tumours express PD-L1 >50% and has not led to a change in NICE guidance.

KEYNOTE-189 was a global, double-blind, placebo controlled phase 3 trial of 616 patients, comparing chemotherapy (pemetrexed and platinum based drug) plus either pembrolizumab or placebo in patients with untreated NSCLC with no PD-L1 threshold

restriction. The primary end points OS and PFS were met, with the pembrolizumab arm recording a significant OS improvement at 12-months of 69.2% compared with 49.4%, with benefit seen across all PD-L1 groups [4]. This has led to updated NICE guidance to include platinum, pemetrexed and pembrolizumab as first line therapy for fit patients with NSCLC, independent of PD-L1 status, via the Cancer Drugs Fund.

The use of this combination approach is associated with response rates of around 50% and novel therapies are still needed.

2.2. Mesenchymal Stromal Cells

Mesenchymal stromal cells (MSCs) are defined by the International Society for Cellular Therapy (ISCT) as being adherent to tissue culture plastic under standard culture conditions, expressing the cell surface markers CD105, CD73 and CD90 and lacking expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules. In addition, they must be capable of differentiating into adipocytes, osteoblasts and chondroblasts under the correct experimental conditions [8]. MSCs can be isolated from multiple sources including bone marrow, umbilical cord, adipose tissue and dental pulp and they have a number of properties that make them good delivery vehicles for a targeted anti-cancer therapy. Firstly they have a unique ability to preferentially migrate to multiple tumour types following systemic delivery [9]. Secondly, they express the major histocompatibility complex class I (MHC class I) but lack MHC class II and the co-stimulatory molecules CD80, CD40 and CD86 [10] meaning that injection of allogeneic MSCs into immunocompetent patients can be done safely without the risk of rejection.

As a result scientists had been interested in modifying MSCs to use them as a vehicle for cell therapy without the complications surrounding immunomodulation. This allows for the development of a bank of allogeneic cells that can be stored and used ‘off the shelf’ without the need for tissue type matching or long term immunosuppressive treatment. Standardised preparations of MSCs are being used in many clinical trials including chronic obstructive pulmonary disease [11], graft vs host disease (GvHD) [12] and Crohn’s disease [13] without the need for human leukocyte antigen (HLA) matching.

In addition to their tumour homing properties MSCs can be easily harvested and expanded [14] in vitro. They are readily modifiable with viral vectors and provide long – term gene expression without affecting their phenotype [15] [16]. This characteristic has already been used to carry some anti-cancer therapies including interferon-β [17, 18] and interleukin-12 [19]. We have modified MSCs to carry a cancer specific pro-apoptotic molecule using a lentiviral vector [20-22].

MSCs were first described in the 1970s by Friedenstein et al [23] and since then there has been an increasing drive to use them therapeutically. There are over 800 clinical trials using MSCs registered on the National Institutes of Health clinical trials database and an increasing proportion of these are using genetically modified MSCs (http://www.clinicaltrials.gov; accessed April 2017). A systematic review and meta analysis of the safety of cell therapy with mesenchymal stromal cells was carried out by Lalu et al in 2012; they looked at 36 studies including 1012 patients with cells being

delivered intravscularly, no significant safety signals were identified concluding MSC therapy appeared safe [24] (for more detailed information please see the investigators brochure).

2.3. TNF-Related Apoptosis Inducing Ligand

TNF-related apoptosis inducing ligand (TRAIL) is an anti-cancer therapy which causes apoptosis selectively in tumour cells whilst leaving healthy cells unaffected. TRAIL therapy has been explored in the form of recombinant protein and monoclonal antibodies to TRAIL receptors in phase 2 clinical trials with good safety and tolerability data but a comparative failure in efficacy [25, 26]. The targeted delivery of TRAIL with MSCs overcomes some of the problems encountered with these therapies. Recombinant TRAIL is delivered intravenously and has a short pharmacokinetic half-life of 32 minutes meaning repeated high-dose systemic delivery is required to produce a local anti-tumorigenic effect. It is also a shortened soluble form of the protein which has been shown to be less effective in triggering cancer cell apoptosis 4 [22]. Monoclonal antibodies have a prolonged receptor specific high-affinity binding, but there are concerns over treatment specificity as not all tumours express the same TRAIL receptors. MSC-targeted TRAIL has a significantly better half-life than recombinant TRAIL. In addition the use of MSCs enables TRAIL delivery directly to the tumours, particularly useful given that most patients with lung cancer will have multiple tumours. Further information regarding TRAIL physiology and mechanism can be found in the investigators brochure.

2.4. MSCTRAIL

We have successfully modified MSCs with a lentiviral vector to produce cells that have long term stable TRAIL expression (MSCTRAIL). MSCTRAIL kills multiple cancer types in vitro and works synergistically with existing chemotherapy agents including cisplatin and pemetrexed which are used first line for the treatment of lung adenocarcinoma. When delivered to an animal model of lung metastases 30% of tumours are eliminated and there is a significant reduction in tumour volume in the remaining 70% [17]. We and others have subsequently shown efficacy in a range of murine tumour models both within the lungs and other organs [21] and that the efficacy is synergistic with chemotherapies and other molecules affecting the death pathways [26].

As this therapy has never been used in humans, there are no known adverse events. There are a number of theoretical concerns over the use of both MSCs and lentiviral vectors but none of these have been observed in humans. Lentiviral vectors are commonly based on the human immunodeficiency (HIV)-1 virus which is a pathogenic virus in humans. This has given rise to a number of theoretical concerns which have largely been addressed with advances in vector design. One of the earliest viruses used to deliver genetic therapies were retroviruses and the main concern about using these was the possibility of incorporation errors such as host gene activation. Retroviruses have a predilection to insert into the promoter regions of proto-oncogenes potentially resulting in oncogenic mutations. This is thought to be the mechanism behind the

development of leukaemia's that were seen within a clinical trial for severe combined immunodeficiency syndrome (SCID) ([27]). Lentiviruses do not have this predilection to insert into oncogenes and therefore the risk of insertional mutagenesis is significantly less [28].

Another concern in using a modified HIV virus is the risk of it being able to replicate independently once introduced into the host cell. To reduce this risk the virus contains self-inactivating (SIN) vectors making the likelihood of independent transcription extremely unlikely [29]. In addition, key enzymes involved in the replication of viruses have been modified rendering it replication incompetent [30]. Since these modifications were made lentiviral vectors have been used in a number of clinical trials with no reports of adverse events. The lentiviral vector used in our therapy has been previously used in children with a rare immune deficiency and has shown excellent safety [31].

One of the defining properties of MSCs is their ability to undergo self- renewal and asymmetric expansion. Because of these inherent cellular properties there is some concern that MSCs themselves have either the potential to undergo malignant change or to enhance the proliferation of malignant cells. Whilst there have been in vivo studies showing that systemically injected murine MSCs result in the formation of osteosarcomas due to karyotype abnormalities [32] this does not occur with human MSCs. To date MSCs have been used in over 400 clinical trials in the last 10 years for treatment of a wide range of diseases and there have been no long term adverse events reported [33].

The aim of this trial is to manufacture and deliver a novel non-toxic tumour targeted therapy that will work synergistically with current therapies to improve response rates and overcome tumour resistance.

3. TRIAL DESIGN

This is a first-in-human single-site phase I dose de-escalation study and multi-centre phase II double blind randomised, placebo controlled trial of MSCTRAIL in combination with standard of care therapy for metastatic non-squamous non-small cell lung cancer. The overall aim of the trial is to determine the safety and preliminary efficacy of repeated doses of MSCTRAIL when delivered in combination with standard of care therapy.

In the phase I study, patients will receive SOC as defined by NICE guidelines and local policy (chemotherapy with 75mg/m^2 cisplatin and 500mg/m^2 pemetrexed and/or immune therapy with pembrolizumab 200mg IV or pembrolizumab 200mg IV alone) on day one followed by MSCTRAIL cells on day 2 by intravenous infusion. This constitutes one cycle of treatment. Each patient will receive 3 cycles of this treatment at 21 day intervals. After this, patients will receive a 4th cycle SOC therapy without MSCTRAIL cells.

After completion of the four cycles of trial treatment, the patients will revert to local standard of care as decided by their treating clinician..

During the phase II study patients will be randomised to either the intervention or the control arm of the study. All patients in both arms will receive SOC on day one of treatment. Patients randomised to the intervention arm will receive the recommended dose of MSCTRAIL from Phase I on day 2 by intravenous infusion whilst those in the control arm will receive a placebo on day 2 by intravenous infusion. This is a double blind trial and patients will not know whether they are receiving MSCTRAIL or a placebo. Each patient will receive 3 cycles of this treatment at 21 day intervals. After this, patients will receive a 4th cycle of SOC without MSCTRAIL/placebo.

After completion of the four cycles of trial treatment, the patients will revert to local standard of care as decided by their treating clinician.

Phase I:

This is a first-in-human, single site, accelerated, dose de-escalation design with a modified Bayesian continual reassessment method (mCRM) to estimate the recommended phase II dose (RP2D) of MSCTRAIL in combination with SOC. This is a dose de-escalation design whereby the first cohort of three patients will receive SOC on day 1 followed by the highest dose of MSCTRAIL, $4x10^8$ cells, on day 2.

Dose selection

Our pre-clinical data has shown a dose–dependent increase in therapeutic efficacy with increasing cell death as the ratio of MSCTRAIL:cancer cells increased [20] (see figures 8 and 9 in Investigator Brochure). We have not identified a maximum therapeutic dose and therefore our clinical dose has been determined by balancing existing safety and efficacy data with the practicalities and costs of cells manufacture.

Other clinical trials using MSCs to treat lung disease have tested doses ranging from 1 million cells/kg to 10 million cells/kg all of which have shown no significant adverse events related to the MSC therapy [34-36]. Assuming a body weight of 70 kg there is data to support the safety of doses between 70 million and 700 million cells and to date a maximum tolerated dose has not been established.

We have selected a starting dose of 400 million cells which corresponds to a dose per body weight of 5 million cells/kg assuming an 80 kg person. Other doses chosen within the dose de-escalation design correspond to approximately 2.5 million and 1 million cells/kg both of which fall within the dose ranges already established to be safe. Starting with the maximum dose allows immediate testing of a dose we believe to be both safe and therapeutic in a timely and cost efficient manner.

The primary endpoint is determination of safety and recommended phase II dose of MSCTRAIL. This will be established by evaluating the rate of dose limiting toxicities (DLT) during the first cycle of MSCTRAIL treatment.

DLT information is included in section 8.7.

DLTs will be managed according to section 8.3.1. If a patient has a DLT after the first or second MSCTRAIL doses then that individual patient may receive further cycles with a reduced MSCTRAIL dose following evaluation and advice by the by the TMG (and IDMC if needed) and agreement with sponsor. If 2 patients within a single cohort have DLTs then all MSCTRAIL doses will be de-escalated as described in section 8.3.1. Patients will continue to receive first line SOC for up to 4 cycles on trial (and up to 2 further cycles as part of standard care) unless they have chemotherapy or immune therapy related toxicities requiring chemotherapy or immune therapy dose reduction in line with current local clinical guidelines as described in section 8.4.2.

If there are no DLTs within the first cohort then a subsequent expansion cohort will receive the same regimen of first line SOC therapy and MSCTRAIL and data from this expansion cohort will be used to determine the recommended phase 2 dose (RP2D). Between 6 and 12 patients will be enrolled into phase I of the trial depending on the number of cohorts assessed.

Phase II:

This is a multicentre, randomised, placebo controlled trial comparing MSCTRAIL at the RP2D and first line SOC standard of care therapy versus placebo and first line SOC therapy.

Patients will be randomised 1:1 between the intervention and control arm. Patients entering the **intervention arm** will receive SOC therapy on day 1 followed by MSCTRAIL at the RP2D on day 2. This schedule will be repeated after 21 days for 3 cycles. After this, patients will receive a 4th cycle of SOC therapy without MSCTRAIL.

Patients in the **control arm** with receive SOC therapy on day 1 and placebo on day 2. This will be repeated after 21 days for up to 3 cycles. After this, patients will receive a 4th cycle of SOC therapy without placebo.

After completion of the 4 cycles of trial treatment patients in both arms will revert to local standard of care as decided by their treating clinician.

3.1. Trial Objectives

3.1.1. Primary objective

Phase I

- To determine the safety and recommended phase II dose of MSCTRAIL when given in combination with standard of care therapy for patients with metastatic lung adenocarcinoma.

Phase II

- To determine the anti-tumor efficacy of up to 3 doses of MSCTRAIL in combination with first line SOC in patients with metastatic lung adenocarcinoma.

3.1.2. Secondary objective

- To assess the safety and tolerability of the treatment combination (in both phase I and phase II).
- To assess the type and duration of treatment response, time to progression and survival in the treatment combination (in both phase I and phase II).

3.1.3. Exploratory Biological Study Objectives

- Investigate the relationship between circulating biomarkers of apoptosis and safety and efficacy parameters.
- To evaluate circulating biomarkers and enumerate circulating tumour cells (CTCs) and circulating tumour DNA (ctDNA)
- To assess how the recipient immune system affects the therapeutic efficacy of donor allogeneic MSCTRAIL using immune cells isolated from peripheral blood samples.
- Assess whether specific tumour mutations may predict response to treatment

3.2. Trial Endpoints

3.2.1. Primary endpoint:

Phase I:

- Occurrence of DLTs within the 1st cycle of MSCTRAIL treatment
- Determination of the RP2D

Phase II:

- Tumour response rate at 12 weeks by RECIST(v1.1) criteria (iRECIST if patient received pembrolizumab)

3.2.2. Secondary endpoints:

Phase I:

- Frequency of adverse events within 3 cycles of treatment
- Best overall response
- Change from baseline in sum of target lesions at 6 and 12 weeks
- Duration of response
- Progression free survival (PFS)

Phase II:

- Frequency of adverse events
- Best overall response
- Time to progression (TTP)
- Change from baseline in sum of target lesions at 6 and 12 weeks
- Tumour response at each time point
- Duration of response
- PFS
- Overall survival (OS)

3.3. Trial Activation

UCL CTC will ensure that all trial documentation has been reviewed and approved by all relevant bodies and that the following have been obtained prior to activating the trial:

- Health Research Authority (HRA) approval, including Gene Therapy Advisory Committee (GTAC) approval
- Clinical Trial Authorisation (CTA) from the Medicines and Healthcare products Regulatory Agency (MHRA)
- **‘**Adoption’ into NIHR portfolio
- Adequate funding for central coordination
- Confirmation of sponsorship
- Adequate insurance provision
- Notification of premises (Trial Site) to Health and Safety Executive (HSE)
- Local approval from the Genetic Modification Safety Committee (GMSC)

4. SELECTION OF SITES/SITE INVESTIGATORS

4.1. Site Selection

In this protocol trial 'site' refers to a hospital where trial-related activities are conducted.

Sites must be able to comply with:

- Trial treatments, imaging, clinical care, follow up schedules and all requirements of the trial protocol
- Access to emergency care
- Requirements of the UK Policy Framework for Health and Social Care Research, issued by the Health Research Authority, the Medicines for Human Use (clinical trials) Act (SI 2004/1031), and all amendments
- Advanced Therapy Medicinal Products Regulation (1394/2007/EC) and SI 2010/1882, and all amendments
- Data collection requirements, including adherence to Case Report Form (CRF) submission timelines as per section 11.3 (Timelines for Data Return)
- Biological sample collection, processing and storage requirements
- ATIMP handling and storage requirements (refer to Summary of Drug Arrangements [SODA])
- Monitoring requirements, as outlined in protocol section 14 (Trial Monitoring and Oversight) and trial monitoring plan
- Any conditions of the Genetic Modification Safety Committee (GMSC) approval
- Archiving requirements in accordance with Regulation 1394/2007 on Advanced Therapy Medicinal Products and Human Tissue (Quality and Safety for Human Application) Regulations (SI 2007/1523)

4.1.1. Selection of Principal Investigator and other investigators at sites

Sites must appoint an appropriate Principal Investigator (PI), i.e. a health care professional authorised by the site to lead and coordinate the work of the trial on behalf of the site. Co-investigators must be trained and approved by the PI. All investigators must be medical doctors and have experience of treating lung cancer. The PI is responsible for the conduct of the trial at their site and for ensuring that any amendments are implemented in a timely fashion. If a PI leaves/goes on a leave of absence, UCL CTC **must be informed promptly** and a new PI identified and appointed by the site.

4.1.2. Training requirements for site staff

All site staff must be appropriately qualified by education, training and experience to perform the trial related duties allocated to them, which must be recorded on the site delegation log.

CVs for all staff must be kept up-to-date, signed and dated and copies held in the Investigator Site File (ISF). A current, signed copy of the CV with evidence of GCP training (or copy of GCP certificate) for the PI must be forwarded to UCL CTC upon request.

GCP training is required for all staff responsible for trial activities. The frequency of repeat training may be dictated by the requirements of their employing institution, or 2 yearly where the institution has no policy, and more frequently when there have been updates to the legal or regulatory requirements for the conduct of clinical trials.

4.2. Site initiation and Activation

4.2.1. Site initiation

Before a site is activated, the UCL CTC trial team will arrange a site initiation with the site which the PI, lead laboratory contact (responsible for receipt, storage and release of the ATIMPs) and site research team must attend. The site will be trained in the day-to-day management of the trial and essential documentation required for the trial will be checked.

Site initiation will be performed for each site by site visit. Re-initiating sites may be required where there has been a significant delay between initiation and enrolling the first patient, as per monitoring plan.

4.2.2. Required documentation

The following documentation must be submitted by the site to UCL CTC prior to a site being activated by the UCL CTC trial team:

- Trial specific Site Registration Form (identifying relevant local staff)
- Relevant institutional approvals (e.g. local NHS permission) and GMSC approval
- A completed site delegation log that is initialled and dated by the PI (with all tasks and responsibilities delegated appropriately)
- Completed site contacts form (with contact information for all members of local staff)
- A signed and dated copy of the PI's current CV (with documented up-to-date GCP training, or copy of GCP training certificate)
- Trial specific prescription

In addition, the following agreements must be in place:

- a signed Clinical Trial Site Agreement (CTSA) between the Sponsor and the relevant institution (usually an NHS Trust/Health Board)

4.2.3. Site activation letter

Once the UCL CTC trial team has received all required documentation and the site has been initiated, a site activation letter will be issued to the PI, at which point the site may start to approach patients.

Following site activation, the PI is responsible for ensuring:

- adherence to the most recent version of the protocol
- all relevant site staff are trained in the protocol requirements
- appropriate recruitment and medical care of patients in the trial
- timely completion and return of case report forms CRFs (including assessment of all adverse events)
- prompt notification and assessment of all serious adverse events
- that the site has facilities to provide **24 hour medical advice** for trial patients

5. INFORMED CONSENT

Sites are responsible for assessing a patient's capacity to give informed consent.

Sites must ensure that all patients have been given the current approved version of the patient information sheet, are fully informed about the trial and have confirmed their willingness to take part in the trial by signing the current approved consent form.

Sites must assess a patient's ability to understand verbal and written information in English and whether or not an interpreter would be required to ensure fully informed consent. If a patient requires an interpreter and none is available, the patient should not be considered for the trial.

The PI, or, where delegated by the PI, other appropriately trained site staff, are required to provide a full explanation of the trial and all relevant treatment options to each patient prior to trial entry. During these discussions, the current approved patient information sheet for the trial should be discussed with the patient.

A **minimum of twenty four (24) hours** must be allowed for the patient to consider and discuss participation in the trial.

Written informed consent on the current approved version of the consent form for the trial must be obtained before any trial-specific procedures are conducted. The discussion and consent process must be documented in the patient notes.

Site staff are responsible for:

- checking that the current approved version of the patient information sheet and consent form are used
- checking that information on the consent form is complete and legible
- checking that the patient has initialled all relevant sections and signed and dated the form
- checking that an appropriate member of staff has countersigned and dated the consent form to confirm that they provided information to the patient
- checking that an appropriate member of staff has made dated entries in the patient's medical notes relating to the informed consent process (i.e. information given, consent signed etc.)
- following registration (phase I) or randomisation (phase II), adding the patients' trial number to all copies of the consent form, which should be filed in the patient's medical notes and investigator site file
- following registration (phase I) or randomisation (phase II), giving the patient a copy of their signed consent form, patient information sheet, and patient contact card

The right of the patient to refuse to participate in the trial without giving reasons must be respected. All patients are free to withdraw at any time. Also refer to section 15 (Withdrawal of Patients).

6. SELECTION OF PATIENTS

6.1. Screening Log

A screening log must be maintained and appropriately filed at site. Sites should record all potentially eligible patients screened for the trial and the reasons why they were not registered (phase I) or randomized (phase II) in the trial if this is the case. The log must be sent to UCL CTC when requested.

For pre-registration or pre-randomisation evaluations refer to section 9.1.

6.2. Patient Eligibility

There will be no exception to the eligibility requirements at the time of registration (phase I) or randomization (phase II). Queries in relation to the eligibility criteria must be addressed prior to registration/randomisation. Patients are eligible for the trial if all the inclusion criteria are met and none of the exclusion criteria applies.

Patients' eligibility must be confirmed by an investigator who is suitably qualified and who has been allocated this duty, as documented on the site staff delegation log, prior to registering/randomising the patient. Confirmation of eligibility must be documented in the patients' notes and on the registration/randomisation CRF.

Patients must give written informed consent before any trial specific screening investigations may be carried out. Refer to section 9.1 (Pre-registration/randomisation Assessments) for the list of assessments and procedures required to be performed prior to entry into the trial.

6.2.1. Inclusion criteria

1. Inoperable stage IIIb/IV histologically/cytologically confirmed lung adenocarcinoma
2. EGFR mutation and EML4-ALK translocation negative
3. Patients with evaluable but unmeasurable disease can be included in the phase I study, but disease must be measurable (CT scan must be within 28 days of randomisation) to be included in the phase II study
4. ECOG performance status of 0 or 1
5. Life expectancy of at least 12 weeks
6. Age at least 18 years
7. Adequate haematological status:
    a. Haemoglobin ≥100g/L
    b. Neutrophil count ≥1.5 x 10^9/L
    c. Platelets ≥100 x 10^9 /L
8. Adequate organ function:
    a. Bilirubin ≤1.5 x ULN
    b. ALT or AST ≤2.5 x ULN (≤5 x ULN is acceptable with liver metastases)
    c. Creatinine clearance ≥ 60 ml/min (Cockcroft-Gault or EDTA)
9. Negative pregnancy test for female patients of child bearing potential.

10. Male patients and female patients of child bearing potential must agree to use a highly effective method of birth control for the duration of the trial and for 12 months after the last trial treatment administration.
11. Ability to understand and provide written informed consent
12. Ability to comply with the requirements of the protocol

6.2.2. Exclusion criteria

1. Prior chemotherapy, hormonal therapy, radiotherapy (including palliative radiotherapy), immunotherapy or treatment with an investigational drug for advanced NSCLC.
2. Prior treatment with any cellular therapy
3. Any surgical procedure in the previous 6 weeks prior to registration/ randomisation
4. Known respiratory failure with baseline resting SpO2 <88%
5. Long term oxygen therapy
6. Known WHO Class III or IV pulmonary hypertension
7. Active infection requiring systemic therapy
8. Active or infected wounds
9. Vaccination with any live attenuated vaccine within 30 days prior to trial registration/randomisation
10. Subject has known sensitivity to any of the trial drugs to be administered during the trial.
11. Any contraindication to the administration and use of cisplatin, pemetrexed, vitamin B12 or folic acid if patient is to receive chemotherapy (cisplatin and pemetrexed)
12. Prior malignancy other than NSCLC (except if the tumour was a non-melanoma skin tumour that has been completely excised or in situ cervix carcinoma), unless have been treated with curative intent with no evidence of disease for > 3 years
13. Evidence of symptomatic brain metastases
14. Myocardial infarction, or unstable or uncontrolled disease or condition related to or impacting cardiac function (e.g., unstable angina, congestive heart failure [New York Heart Association > class II]) within 1 year of enrolment
15. Venous thromboembolism within the last 6 months
16. Known hepatitis B or C infection, human immunodeficiency virus (HIV)-positive patients
17. Pregnant women or those who are breast feeding
18. Other medications, severe acute/chronic medical or psychiatric condition, or laboratory abnormality that may increase the risk associated with trial participation or trial drug administration, or may interfere with the interpretation of trial results, and in the judgment of the investigator would make the patient inappropriate for entry into this trial

Extended exclusion criteria if patient is to receive Pembrolizumab:

19. Diagnosis of immunodeficiency or receiving systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior the first dose of study drug.
20. Active autoimmune disease that has required systemic treatment in past two years (that is, with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg, thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment and is allowed.
21. History of (non-infectious) pneumonitis that required steroids or current pneumonitis.

6.3. Pregnancy and birth control

6.3.1. Pregnancy and birth control

Definition of women of childbearing potential (WOCBP) and fertile men

A woman of childbearing potential (WOCBP) is a sexually mature woman (i.e. any female who has experienced menstrual bleeding) who:

- Has not undergone a hysterectomy or bilateral oophorectomy/salpingectomy
- Is not postmenopausal (a post-menopausal woman is a female who has not had menses at any time in the preceding 12 consecutive months without an alternative medical cause)
    - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with two FSH measurements in the postmenopausal range is required.
    - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrolment.

- Has not had premature ovarian failure confirmed by a specialist gynaecologist

A man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy.

6.3.2. Risk of exposure to trial treatment during pregnancy

The risk of exposure to trial treatment has been evaluated using the safety information available in the IB for MSCTRAIL and individual Summaries of Product Characteristics (SPCs) for pemetrexed, cisplatin, carboplatin and pembrolizumab .

Pemetrexed, cisplatin, carboplatin and pembrolizumab may be toxic and have genetically damaging effects. The risks to an embryo or foetus from exposure to MSCTRAIL are currently unknown.

Overall, the trial treatment has been assessed as having a high risk of teratogenicity/fetotoxicity* and genotoxicity.

Women of child bearing potential and sexually mature males are advised respectively not to become pregnant or father a child during the treatment, and for up to 6 months after treatment. Contraceptive measures as detailed in 6.3.4 are recommended.

*** refer to Clinical Trial Facilitation Group (CTFG) recommendations**

6.3.3. Pregnancy testing

All female participants who are WOCBP must undergo a pregnancy test by urinary beta HCG at screening and immediately pre-treatment if there is a delay of > 7 days between trial entry and first treatment administration.

Pregnancy tests will be repeated prior to each MSCTRAIL/placebo infusion for WOCBP.

6.3.4. Contraceptive Advice

Requirement for female patients*:*

All female participants who are WOCBP must consent to use one of the following methods of highly effective contraception from registration before the first administration of treatment until 6 months post last treatment administration. Methods with low user dependency are preferable, particularly where introduced as a result of participation in the trial.

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[1]:
    - oral
    - intravaginal
    - transdermal
    - progestogen-only hormonal contraception associated with inhibition of ovulation[1]:
        - oral (e.g. desogestrel)
        - injectable
        - implantable[2]
- intrauterine device (IUD)[2]
- intrauterine hormone-releasing system (IUS)[2]
- bilateral tubal occlusion[2]
- vasectomised partner[2,3]
- sexual abstinence[4]

1. Hormonal contraception may be susceptible to interaction with the IMP/NIMP, which may reduce the efficacy of the contraception method.
2. Contraception methods that are considered to have low user dependency.
3. Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success.
4. Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

Requirement for male patients with female partners who are pregnant or WOCBP*:*

Due to the risk of genotoxicity and/or risk to the foetus from exposure to seminal fluid:

- Male patients (including male patients who have had vasectomies) must consent to use condoms with female partners who are WOCBP or partners who are pregnant, during treatment and until 6 months post last treatment administration.
- Male patients must also advise their female partners who are WOCBP regarding contraceptive requirements as listed for female patients who are WOCBP.

For female and male patients (where applicable):

The method(s) of contraception used must be stated in the patient medical notes and CRFs. Where applicable the medical notes of male participants should include a statement that the female partner has been informed about contraception advice.

6.3.5. Action to be taken in the event of a pregnancy

Female patients:

If a female patient becomes pregnant

- prior to initiating treatment, the patient will not receive trial treatment unless they elect to have a termination (please note, in such instances, termination must be the patient's own choice)
- during treatment, the patient will be withdrawn from further trial treatment and, if they consent to pregnancy monitoring, followed up until pregnancy outcome
- after the end of the treatment, but during the pregnancy at-risk period (6 months post last trial treatment), the patient will be followed up until pregnancy outcome if they consent to pregnancy monitoring.

Male patients:

If a female partner of a male patient becomes pregnant between the patient's treatment start and 6 months after the end of treatment, the male participant can continue with the study (if applicable) whilst their female partner will be followed up if they have given consent to pregnancy monitoring.

Notification to UCL CTC – refer to Pregnancy Report Processing (see Pharmacovigilance sub-section)

6.3.6. Long Term Infertility

Owing to the possibility of pemetrexed and cisplatin treatment causing irreversible infertility and the unknown effects of pembrolizumab and MSCTRAIL, genetic consultation is recommended if a patient wishes to have children after ending the treatment. Men are advised to seek counselling on sperm storage before starting trial treatment.

6.3.7. Lactation

Pemetrexed and cisplatin is excreted in human milk, and adverse reactions on the child cannot be excluded. It is not known if pembrolizumab and MSCTRAIL are secreted in human milk. Women treated with trial treatment therefore must not breastfeed.

7. REGISTRATION AND RANDOMISATION PROCEDURES

For phase I of the protocol patients will be registered onto the trial.

For phase II of the protocol (placebo controlled) patients will be randomised.

7.1. Registration (Phase I)

Phase I of the trial aims to establish the recommended MSCTRAIL dose when given in combination with standard of care therapy in metastatic adenocarcinoma patients.

Patient registration will be undertaken centrally at UCL CTC and this must be performed prior to commencement of any trial treatment. Pre-registration evaluations should be carried out at sites as detailed in section 9.1 (Pre-registration/randomisation Assessments).

Following pre-treatment evaluations, confirmation of eligibility and consent of a patient at a site, the registration form must be fully completed and faxed to UCL CTC (contact numbers listed in the table further below). The faxed registration form will be used to confirm patient eligibility at UCL CTC. If further information is required UCL CTC will contact the person requesting registration to discuss the patient and request updated forms to be faxed.

Once eligibility has been confirmed a trial number and a 'MSCTRAIL dose cohort' will be assigned for the patient and this number and MSCTRAIL dose should be added to the form by the site.

UCL CTC will fax/e-mail confirmation of the patient's inclusion in the trial, their trial number and MSCTRAIL dose to the PI, main contact and ATIMP manufacturer.

7.2. Randomisation (Phase II)

Phase II of the trial is randomised, placebo controlled, double blinded trial, comparing the combination of MSCTRAIL with standard of care therapy (intervention arm) to standard of care therapy and placebo (control arm). Patients are randomised 1:1 between the intervention and control arm. An interactive web-based randomisation system (IWRS) will be used for randomisation/ATIMP bag code allocation (and unblinding). Information on how to randomise and use the IWRS will be provided in the ISF for phase II of the study and training will be given as needed.

Patients will be randomised via blocked stratification. Patients will be stratified according to the following factors:

- Performance status at baseline: 0 or 1
- Stage at time of diagnosis IIIB or IV

Patient randomisation (phase II only) must be performed prior to commencement of any trial treatment/intervention. Pre-randomisation evaluations should be carried out at sites as detailed in section 9.1 (Pre-registration/randomisation Assessments).

Following pre-treatment evaluations, confirmation of eligibility and consent of a patient at a site, the randomisation form must be fully completed prior to randomisation using the

IWRS. The IWRS will assign a patient trial number and the 'blinded code' for the bags with the ATIMP (MSCTRAIL or placebo) to be used for administration to this patient. A confirmation email of the patient's inclusion in the trial, their trial number and 'blinded code' will be sent to the PI, main contacts (including staff at the cellular therapy laboratory where the ATIMP/placebo are stored prior dispensing) and UCL CTC.

In the unlikely event of difficulty completing randomisation, sites should telephone UCL CTC as soon as possible during office hours.

Registration/Randomisation telephone number:	[redacted]
Registration/Randomisation fax number:	[redacted]
UCL CTC Office hours:	09:00 to 17:00 Monday to Friday, excluding Bank Holidays

Once a patient has been registered/randomised onto the trial they must be provided with the following:

- A copy of their signed consent form and patient information sheet
- A patient contact card. Site contact details for 24 hour medical care must be added to this card and patients advised to carry this with them at all times while participating in the trial

7.3. Initial Trial Treatment Supply

Pemetrexed, cisplatin* and pembrolizumab are Non Investigational Medicinal Products (NIMPs) and are to be supplied from Hospital Commercial Stock.

**Carboplatin can be used for patients who do not tolerate cisplatin treatment. Carboplatin is a NIMP for the study and will be provided (if needed) from Hospital Commercial Stock.*

The ATIMP, MSCTRAIL, (and the placebo, for phase II) will be released from the manufacturer, Royal Free Hospital London, Centre for Cell and Gene Tissue Therapeutics (CCGTT), delivered to participating sites and stored in their relevant cellular therapy laboratory until required for patient infusion, if applicable.

Refer to Summary of Drug Arrangements for details of supply of MSCTRAIL/placebo for the trial.

8. TRIAL TREATMENT

For the purpose of this protocol, the IMPs are MSCTRAIL and placebo (in phase II).

Pemetrexed, cisplatin (and carboplatin, if used) and pembrolizumab are standard therapies for NSCLC and have been classified as NIMPs (provided from hospital stock).

8.1. Investigational Medicinal Product

- **MSCTRAIL:** mesenchymal stromal cells genetically modified to express TRAIL (TNF-related apoptosis inducing ligand).

The Investigational Medicinal Product in this trial is an advanced therapy investigational medicinal product (ATIMP) as defined in EU Directive 2001/83/EC as amended by Directive 2009/120/EC. Specifically, the ATIMP is a Gene Therapy Product as defined in Article 2 1 (a) of the ATMP Regulation (Regulation (EC) No.1394/2007). The ATIMP is manufactured at the Centre for Cell, Gene & Tissue Therapeutics (CCGTT) located within the Royal Free London NHS Foundation Trust, Pond Street, London, NW3 2QG. The CCGTT holds a license with the MHRA (MIA(IMP)11149) for the manufacture and release of ATIMPs, including Gene Therapy products. MSCTRAIL is not currently licensed anywhere in the world.

- **Placebo**

The placebo for this trial consists of the same excipients as the ATIMP without the active drug product; a solution of 50% Dulbecco's Phosphate-Buffered Saline (DPBS), 10% Dimethly sulfoxide (DMSO) and 40% Human Albumin Serum (HAS) at a concentration of 4.5%. The volume of placebo will be consistent to that of the ATIMP. The placebo is also manufactured at the CCGTT (details listed above).

Details on the manufacturing process including flow diagrams are listed in the IMPD and IB.

Presentation of ATIMP

The drug product consists of passage 4 (P4) MSCTRAIL containing the defined cell dose for a patient in 1-2 bags. Each bag contains a maximum of 100ml with at least $2x10^6$ MSCTRAIL per ml.

Presentation of placebo

The placebo consists of the ATIMP excipient without MSCTRAIL, in a cryobag of consistent fill volume with the drug product.

Source of ATIMP, Manufacture, Storage and Distribution

The final product is manufactured to GMP standards by expansion of a Working Cell Stocks (WCS) of MSCTRAIL. Umbilical cords (the starting material for the drug substance/WCS) are procured by the Anthony Nolan under a service level agreement with the CCGTT. MSCs are isolated and expanded, before being transduced to express the therapeutic protein TRAIL (TNF-related apoptosis inducing ligand). In order to

increase cell yield and genetic variability of the ATIMP, transduced cells from multiple umbilical cord tissue (UCT) donors are pooled together. All tissues are screened for mandatory infectious disease markers (2006/17/EC).

8.1.1.1. Manufacture of the primary seed stock and working cell stock

Upon arrival to the CCGTT, UCT is digested, filtered, then seeded into a T175 cell culture flask. MSCs adhere to the plastic and are expanded for a maximum of 14 days before harvesting for either direct transduction, or cryopreserved for transduction at a later date. The MSC isolation process is repeated for each UCT donor (5-10 units in total).
To transduce MSCs, a lentiviral vector encoding TRAIL was manufactured at the Cell and Gene Therapy Laboratory, at Kings College London. This vector is used to transduce either thawed Passage 0 (P0) MSCs, or fresh Passage 1 (P1) MSCs in T175 cell culture flasks at the CCGTT.
MSCTRAIL from multiple donors are pooled together, before being seeded into a multilayer bioreactor for cell expansion. Confluent cells are harvested and cryopreserved in vials to form the Primary Seed Stock (PSS). Each individual vial of PSS contains at least $2x10^6$ cells per ml.
To manufacture the Working Cell Stock (WCS), PSS vials are thawed and again seeded into a multilayer bioreactor. Upon confluency, cells are harvested and cryopreserved in bags to form the WCS. Each individual bag of WCS contains enough cells to seed a bioreactor for manufacture of the ATIMP.
The PSS then WCS will be manufactured before the clinical trial commences. IMP manufacture will occur periodically from WCS completition. If a second WCS is required, this will be manufacturered concurrently.

8.1.1.2. Manufacture of the final product (MSCTRAIL) and placebo (for phase II only)

The final product (MSCTRAIL) is manufactured from the WCS. One bag of WCS is thawed and seeded into a multilayer cell expansion bioreactor. As with PSS and WCS manufacture, upon confluency, the cells are harvested and cryopreserved in bags to form the ATIMP.
The ATIMP will contain $4x10^8$ MSCTRAIL, split between 1 or 2 bags with a maximum of 100ml per bag. If toxicity is noted during cohort 1, the volume of ATIMP delivered will be reduced accordingly with the dose de-escalation to $2x10^8$ cells. The following batch of the ATIMP will be manufactured and bagged to contain the new dose.
For phase II only, placebo doses will be produced alongside the ATIMP. Each placebo consists of the identical cryopreservation solution as used with the ATIMP (10% DMSO, 50% DPBS and 40% HAS at 4.5%), but with no MSCTRAIL present. The volume will be consistent to the ATIMP.
Both the ATIMP and placebo are packaged and cryopreserved as aseptic products, labelled with Eudralex volume 4 annex 13 compliant trial labels incorporating an ISBT128 unique alphanumeric identifier.

Following manufacture, all ATIMP and placebo doses are stored in vapour phase nitrogen dewars or mechanical freezer between -135°C and -196°C until transportation

to the site for patient admnistration. ATIMP and placebo units are shipped by an approved courier directly to the treating site for patient infusion. Bags are transported inside a cryoshipper which holds temperature between -135°C and -196°C for up to 10 days.

8.1.2. Handling of the ATIMP/placebo at site

Once the ATIMP/placebo arrives at the clinical site, staff will verify the temperature logger, which includes a “temperature excursion” alarm set to a predefined range
The ATIMP will be stored in the cellular therapy lab until requested for patient treatment, the ATIMP is then transported to the ward by a designated member of staff.
Further details of the ATIMP/placebo release, shipment, storage, handling and accountability can be found in the IB and the Summary of Drug Arrangements.

8.1.3. IMP/placebo Administration Details

The ATIMP/placebo will be thawed, using a temperature monitored water bath at 37°C. The target MSCTRAIL/placebo dose will be administered as an intravenous injection by a trained member of staff. Infusion must be done over 20-30 minutes and must be within 60 minutes of thawing. The end user will confirm the identity of the participant and the information on the labels to ensure the correct ATIMP and correct dose has been sent for administration.

Further details of the ATIMP/placebo administration can be found in the Summary of Drug Arrangements**.**

8.1.4. Accountability and Traceability of ATIMP

In compliance with the ATMP regulations (120/2009/EC), the clinical site (PI or delegate) must ensure that there is a system in place allowing for full traceability of ATIMP received from the manufacturer and administered to participants. Details regarding accountability/traceability of the ATIMP and associated documents are listed in the SODA present in the investigator site file (ISF) for the study. The requirement for the tissue establishment, manufacturer and investigator/clinical trial site(s) to retain their part of the traceability information will be set-out in the relevant contractual agreements.

Please refer to the Annex of the European Commission document ‘Detailed guidelines on good clinical practice specific to advanced therapy medicinal products’ for minimum data set to be kept by each party. See section 16.2.1 for further information on traceability data retention and archiving.

8.2. Non Investigational Medicinal Products (NIMPs)

Pemetrexed, cisplatin and pembrolizumab are standard treatment for patients with advanced/metastatic lung cancer and are given at standard doses in the trial. All trial patients must be treated according to the protocol schedule in order to isolate the effects of MSCTRAIL.

Cisplatin and pemetrexed are both licensed for use as first line treatment in locally advanced and metastatic adenocarcinoma of the lung and will be supplied directly from hospital pharmacy.

Carboplatin can be used instead of cisplatin if clinically appropriate at the discretion of treating clinician. Carboplatin will be given at the standard of care dose and supplied from hospital stock.

Pembrolizumab as monotherapy is indicated for the first-line treatment of metastatic non-small cell lung carcinoma (NSCLC) in adults whose tumours express PD-L1 with a ≥ 50% tumour proportion score (TPS) with no EGFR or ALK positive tumour mutations. Pembrolizumab in combination with pemetrexed and platinum chemotherapy, is indicated for the first-line treatment of metastatic non-squamous NSCLC in adults whose tumours have no EGFR or ALK positive mutations. Pembrolizumab will be given at the standard of care dose and supplied from hospital stock.

8.3. Treatment Summary

8.3.1. MSCTRAIL

The initial dose for cohort 1a (3 patients) in the phase I study will be $4x10^8$ cells given on day 2 of each 21-day cycle and will be delivered for 3 cycles. If there are no dose limiting toxities (DLT) a further 3 patients (cohort 1b) will receive the same dose. If there are no toxicities in all 6 patients this will be the recommended phase II dose (RP2D).

If patients in cohort 1 have DLTs (as detailed in the 'Guidelines for dose de-escalation in the 1st cohort' table section 8.4.1) then cohort 2 will recieve a reduced dose of MSCTRAIL decided by the Trial management group (either $2x10^8$ or $8x10^7$ cells), if there are no DLTs after 3 patients, a further 3 patients will receive the same dose. If there are no toxicities in all 6 patients this will be the recommended phase II dose (RP2D).

At the end of each cohort, the TMG and IDMC will review the trial data and decide whether the cohort should be expanded or dose de-escalated based on the DLTs observed. If 6 patients have received the same dose and the next 3 patients are to be recommended to this dose, then this dose will become the RP2D.

At this point, the TMG and the IDMC will review the phase I data and decide if the trial can proceed to phase II with all subsequent patients receiving the RP2D. The TMG and IDMC will also advise whether any changes to the trial conduct are needed before the start of phase II in which case a substantial amendment addressing their suggestions will be submitted for approval before patients are treated on phase II.

8.3.2. Pemetrexed and cisplatin

Pemetrexed ($500mg/m^2$) and cisplatin ($75mg/m^2$) will be given as separate intravenous infusions on the first day of the 21 day cycle for 4 cycles. To be given as per local site policy and SPC. Carboplatin (AUC 5 or according to local protocol) can be used instead of cisplatin if clinically appropriate at the discretion of treating clinician.

Patients must receive adequate antiemetic treatment and appropriate hydration prior to and/or after receiving cisplatin as per local policy.

Pre-medication to reduce the incidence and severity of skin reactions and toxicity from pemetrexed (e.g. corticosteroids, vitamin supplementation) should be given as per local policy.

8.3.3. Pembrolizumab

Pembrolizumab will be given as an intravenous infusion on the first day of the 21 day cycle for a 4 cycles. To be given as per local site policy and SPC.

	Cycle 1						**Cycle 2**						**Cycle 3**						**Cycle 4**					
Week	**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**		**12**	
Day	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Standard of care treatment Pemetrexed& Cisplatin or Pemetrexed, Cisplatin & Pembrolizumab or Pembrolizumab	•						•						•						•					
MSCTRAIL $4x10^8$, $2x10^8$ or $8x10^7$ cells iv, depending on cohort		•						•						•										

**Carboplatin can be used (according to local policy) at the discretion of the treating clinician*

8.4. Trial Treatment Details

8.4.1. Phase I:

All patients will be evaluated using criteria defined in section 9 and summarised in appendix 2. In addition patients should be monitored immediately prior to the ATIMP infusion, every 15 minutes during the infusion, every 30 minutes for the 2 hours after the infusion and hourly thereafter until 4 hours post infusion. Monitoring should include:

- Temperature
- Pulse*
- Blood pressure
- Respiratory rate
- Oxygen saturation level*

* Heart rate and Oxygen saturation should be monitored continuously throughout infusion

Monitoring for pemetrexed and cisplatin/carboplatin will be done as per local policy guidelines.

Patients enrolled in phase 1 will receive:

- Standard of care (SOC) treatment, day 1
  Pemetrexed 500mg/m^2 and Cisplatin 75mg/m^2- iv
  Or
  Pemetrexed 500mg/m^2 and Cisplatin 75mg/m^2 and Pembrolizumab 200mg- iv
  Or
  Pembrolizumab 200mg - iv
  - MSCTRAIL (dose depending on cohort) - iv, day 2

MSCTRAIL is to be administered on day 2 of cycles 1-3 at the dose specified by the CTC at the time of registration which will be detailed on the patient registration confirmation form. MSCTRAIL should be administered as an intravenous infusion on the second day of each 21-day cycle. MSCTRAIL is administered over 20 - 30 minutes and infusion must be completed within 60 minutes of thawing.

Patients will receive 3 cycles of standard of care therapy and MSCTRAIL, followed by 4th cycle of standard of care therapy only (but no MSCTRAIL). Each cycle is 21 days in duration.

After completion of the four cycles of trial treatment, patients will revert to local standard of care as decided by their treating clinician.

Guidelines for dose de-escalation in first cohort

Dose de-escalation decisions based on DLT outcomes for patients in the first cohort are shown below. Subsequent decisions for de-escalation will be based on recommendations from the dose-toxicity model (based on all DLT outcome data collected, rather than only the last three patients observed) and other recommendations from the Trial Management Group.

Number of patients with DLTs (out of first 2 patients treated in first cohort)	**Action for 3rd patient**	**Number of patients with DLTs if 3rd patient to be treated**	**Action for next cohort**
0 out of 2 patients	Continue with current dose	-	-
1 out of 2 patients	Include 3rd patient at current dose	1 out of 3 patients	Continue with current dose
		2 out of 3 patients	De-escalate to lower dose
2 out of 2 patients	Go to lower dose	-	-

Criteria for DLTs are defined in section 8.7 (Dose Limiting Toxiciteis)

8.4.2. Phase II:

All patients in both the placebo and investigational arm will be evaluated using criteria defined in section 9 and summarised in appendix 3. In addition patients should be monitored immediately prior to the MSCTRAIL/placebo infusion, every 15 minutes during the infusion, every 30 minutes for the 2 hours after the infusion and hourly thereafter until 4 hours post infusion. Monitoring should include:

- Temperature
- Pulse*

- Blood pressure
- Respiratory rate
- Oxygen saturation level*

* Heart rate and Oxygen saturation should be monitored continuously throughout infusion

Control arm

- Standard of care (SOC) treatment, day 1
  Pemetrexed 500mg/m^2 and Cisplatin 75mg/m^2- iv
  Or
  Pemetrexed 500mg/m^2 and Cisplatin 75mg/m^2 and Pembrolizumab 200mg- iv
  Or
  Pembrolizumab 200mg - iv
- Placebo – iv, day 2

Investigational arm

- Standard of care (SOC) treatment, day 1
  Pemetrexed 500mg/m^2 and Cisplatin 75mg/m^2- iv
  Or
  Pemetrexed 500mg/m^2 and Cisplatin 75mg/m^2 and Pembrolizumab 200mg- iv
  Or
  Pembrolizumab 200mg - iv
- MSCTRAIL - at recommended phase II dose (RP2D) - iv, day 2

MSCTRAIL/placebo is to be administered on day 2 of cycles 1-3. MSCTRAIL/placebo should be administered as an intravenous infusion over 20 - 30 minutes on the second day of each 21-day cycle. The infusion must be completed within 60 minutes of thawing.

In both arms, patients will receive 3 cycles of SOC therapy and MSCTRAIL/placebo, followed by 4th cycle of SOC therapy only (but no MSCTRAIL/placebo). Each cycle is 21 days in duration.

After completion of the four cycles of trial treatment, the patients will revert to local standard of care as decided by their treating clinician.

	Cycle 1						**Cycle 2**						**Cycle 3**						**Cycle 4**					
Week	**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**		**12**	
Day	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Standard of care treatment Pemetrexed& Cisplatin or Pemetrexed, Cisplatin & Pembrolizumab or Pembrolizumab	•						•						•						•					

MSCTRAIL RP2D dose or Placebo		•						•						•								

** Carboplatin can be used (according to local policy) at the discretion of the treating clinician*

8.5. Dose Modifications

If a dose delay is necessary for a particular drug then the treatment cycle for all other drugs should also be delayed.

8.5.1. MSCTRAIL

If a single patient suffers DLTs due to MSCTRAIL following dose 1 or 2 then their subsequent dose will be reduced to the next dose cohort. If 2 patients at a specific dose suffer DLTs then the next patient will receive the de-escalated lower dose as per section 8.4.1.

Patients may temporarily delay treatment for up to an additional 21 days from the last dose if they experience adverse events that require a dose to be delayed. MSCTRAIL may also be delayed if there is a delay in SOC but SOCis still to be given.

If MSCTRAIL is held because of adverse events for $>$ 21 days beyond the last scheduled dose, then the patient will be discontinued from MSCTRAIL treatment and will return to standard clinical care which may include further chemotherapy and/or immunotherapy. In the cases where patients have received at least one dose MSCTRAIL, they will remain within the trial for the purposes of follow-up and data analysis unless they explicitly withdraw consent.

If a patient discontinues chemotherapy and/or immunotherapy either due to side effects or poor tolerability which was solely attributed to the chemotherapy/immunotherapy then they can continue in the cycle and receive MSCTRAIL as planned.

8.5.2. Pemetrexed and cisplatin and pembrolizumab

Dose adjustments at the start of a subsequent cycle should be based on pre-cycle blood results and according to local policy. Treatment may be delayed for up to 21 days to allow sufficient time for recovery. Upon recovery, patients should be re-treated at a dose according to local policy.

If there is a clinical need patients can be switched from cisplatin to carboplatin as per local site policy and remain within the trial.

8.6. Management of Adverse Events

8.6.1. MSCTRAIL

There are currently no known adverse events related to MSCTRAIL. A potential but previously unseen adverse events is a thromboembolic event related to MSCTRAIL infusion.

8.6.2. Placebo

There are currently no known adverse events related to the placebo.

8.6.3. Pemetrexed and cisplatin/carboplatin

The most commonly reported undesirable effects related to pemetrexed are bone marrow suppression manifested as anaemia, neutropenia, leucopenia, thrombocytopenia; and gastrointestinal toxicities, manifested as anorexia, nausea, vomiting, diarrhoea, constipation, pharyngitis, mucositis, and stomatitis. Other undesirable effects include renal toxicities, increased aminotransferases, alopecia, fatigue, dehydration, rash, infection/sepsis and neuropathy. Rarely seen events include Stevens-Johnson syndrome and toxic epidermal necrolysis.

The most frequently reported adverse events of cisplatin/carboplatin are haematological (leucopenia, thrombocytopenia and anaemia), gastrointestinal (anorexia, nausea, vomiting and diarrhoea), ear disorders (hearing impairment), renal disorders (renal failure, nephrotoxicity, hyperuricaemia) and fever. Serious toxic effects on the kidneys, bone marrow and ears have been reported in up to about one third of patients given a single dose of cisplatin; the effects are generally dose-related and cumulative.

Adverse events related to pemetrexed and cisplatin/carboplatin should be managed as per local policy.

8.6.4. Pembrolizumab

The most commonly reported side effects, which are reported in >10% of NSCLC patients receiving pembrolizumab are decreased appetite, nausea, thrombocytopenia, constipation, vomiting, dyspnea, cough, rash, arthralgia, back pain, pruritus. Less commonly patients suffer with haematological conditions (anaemia, leukopenia, thrombocytopenia) loss of appetite, dry eyes and mouth, headaches, flu like symptoms, liver tyroid and kidney disruption.

However adverse events associated with pembrolizumab may also represent an immunologic etiology. These immune related AEs may occur shortly after the first dose or several months after the last pembrolizumab dose and may affect more than one body system simultaneously. Early recognition and initiaition of treatment is critical to reduce complications. Based on existing clinical study data, most immune related AEs are reversible and can be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected immune related AEs, investigators should ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation.
Immune related AEs previously reported following pembrolizumab treatement include pneumonitis, diarrhoea/colitis, nephritis and renal dysfunction, AST / ALT elevation or increased bilirubin, myocarditis.

Thyroid disorders can occur at any time during treatment. Patients should be monitored for changes in thyroid function (at start of treatment, periodically during treatment, and as clinically indicated) and for clinical signs and symptoms of thyroid disorders.

Adverse events related to pembrolizumab should be managed as per local policy however early recognition and swift treatment is recommended. Treatment should be based on CTCAE v5.0 grade and guidelines for that treatment as given below.

CTCAE grade	**Management**
1	• Supportive treatment • Increased monitoring of symptoms • Exclude infection • Patient education
2	As per grade 1 but in addition: • Withhold immunotherapy until toxicity has resolved to grade 1 or less • Consider oral steroids if persistent symptoms >5 days
3	• Supportive therapy • Commence intravenous steroids (typical dose 1–2 mg/kg methyprednisolone) • If not resolving within 48 hours consider addition of other immunosuppressants (e.g. infliximab, mycophenolate) • Consider system specific investigations (e.g. colonoscopy) • Seek expert opinion of relevant specialist • Investigate and treat infection • Withhold immunotherapy, consider restarting if toxicity grade 1 or less on individual basis • Steroids will need to be tapered over 3–6 weeks
4	• As for grade 3 but permanently discontinue immunotherapy

Specific treatments for immune related toxicities include:

Gastrointestinal conditions:
Initially grade 1 and 2 diarrhoea supportive care with antidiarrheal medications, oral hydration and electrolyte supplements, grade 3-4 consider IV methylprednisolone and if perforation is found or symptoms not settling consider surgical opinion or other forms of immunosuppression.

Skin conditions:
Mild skin toxicity is managed supportively with emollients, steroid creams (1% hydrocortisone) and antihistamines. Grade 3 or 4 toxicity may require evaluation by a dermatologist and treatment with high dose corticosteroids.

Endocrine conditions:
Grade 1 or 2 endocrine toxicity may be monitored without cessation of therapy and hormone replacement therapy instituted where appropriate. Higher grades require advice of expert opinion, direct hormone replacement and high dose steroids.

Pulmonary conditions:
Pneumonitits has been reported with the use of immune check point inhibitors. Grade 1 pneumonitis (asymptomatic radiological changes) may be monitored with no change in immunotherapy treatment. For grade 2 toxicity, immunotherapy therapy should be withheld and oral steroids commenced. For grade 3 or 4 toxicity, a more aggressive approach is required with hospitalisation and review by a respiratory physician, together with high dose intravenous steroids.

8.7. Dose Limiting Toxicities

The phase I primary endpoint is determination of safety and recommended phase II dose of MSCTRAIL. This will be established by evaluating the rate of dose limiting toxicities (DLT) within the first cycle of MSCTRAIL treatment.

A DLT is defined as any of the following MSCTRAIL related adverse events:

- Thromboembolic event ≥ CTCAE grade 4 within 48 hours of MSCTRAIL infusion
- New cardiac arrhythmias ≥CTCAE grade 4 requiring Direct Current (DC) cardioversion, ≥CTCAE grade 4 ventricular tachycardia, ventricular fibrillation or asystole within 4 hours of MSCTRAIL infusion
- Any other toxicity that results in a disruption of dosing schedule of more than 21 days not related to the chemotherapy
- MSCTRAIL related adverse event of grade 4 or higher that is assessed by the TMG to constitute a DLT

A DLT excludes:

- Alopecia of any grade
- Isolated laboratory changes of any grade without clinical sequelae or clinical significance
- Any chemotherapy or immunotherapy related adverse event

DLTs will be managed according to section 8.3.1. If a patient has a DLT after the first or second MSCTRAIL doses then that individual patient may receive further cycles with a reduced MSCTRAIL dose following advice by the TMG and agreement with sponsor. If 2 patients within a single cohort have DLTs then all MSCTRAIL doses will be de-escalated as described in section 8.3.1. Patients will continue to receive SOC therapy for up to 4 cycles (on trial) unless they have toxicities requiring dose reduction in line with current local clinical guidelines as described in section 8.4.2.

If there are no DLTs within the first cohort then a subsequent expansion cohort will receive the same regimen of SOC therapy and MSCTRAIL and data from this expansion cohort will be used to determine the recommended phase II dose (RP2D). Between 6 and 12 patients will be enrolled into phase I of the trial depending on the number of cohorts assessed.

8.8. Management of Overdoses, Trial treatment error, or Occupational Exposure

Overdose

Administration of a quantity of a trial treatment, either per administration or cumulatively, which is in excess of the protocol specified dose. The dose can either be evaluated as overdose by the trial team at site or by the Sponsor upon review.

Overdoses should be reported on an incident report (see section 13.1). Any adverse events resulting from an overdose should be reported as an SAE (see section 12.2.2 for reporting procedures).

8.8.1. MSCTRAIL

There is currently no specific treatment in the event of an overdose with MSCTRAIL and the symptoms of overdose are not known. In cases of suspected overdose, trial treatment should be withheld and supportive care initiated.

8.8.2. Cisplatin/carboplatin

Overdoses can be expected to cause the toxic effects described in the Summary of Product Characteristics (SPC), but to an exaggerated degree. Adequate hydration and osmotic diuresis may help reduce the toxicity of cisplatin if administered promptly following overdose.

In case of overdose direct effects on the respiratory centre are possible, which might result in life threatening respiratory disorders and acid base equilibrium disturbance due to passage of the blood brain barrier. An acute overdose may result in renal failure, liver failure, deafness, ocular toxicity (including detachment of the retina), significant myelosuppression, untreatable nausea and vomiting and/or neuritis. An overdose may be fatal.

There is no specific antidote in the event of an overdose of cisplatin/carboplatin. Even if haemodialysis is initiated 4 hours after the overdose it has little effect on the elimination of cisplatin/carboplatin from the body following a strong and rapid fixation of drug to proteins.

Treatment in the event of an overdose consists of general supportive measures.

8.8.3. Pemetrexed

Overdoses can be expected to cause the toxic effects described in the SPC, but to an exaggerated degree. Reported symptoms of overdose include neutropenia, anaemia, thrombocytopenia, mucositis, sensory polyneuropathy, and rash. Anticipated complications of overdose include bone marrow suppression as manifested by neutropenia, thrombocytopenia, and anaemia. In addition, infection with or without fever, diarrhoea, and/or mucositis may be seen.

In the event of suspected overdose, patients should be monitored with blood counts and should receive supportive therapy as necessary. The use of calcium folinate/folinic acid in the management of pemetrexed overdose should be considered.

8.8.4. Pembrolizumab

Overdoses can be expected to cause the toxic effects described in the SPC, but possibly to an exaggerated degree. In case of overdose, patients must be closely monitored for signs or symptoms of adverse reactions, and appropriate symptomatic treatment instituted.

Trial treatment error

Any unintentional error in prescribing, dispensing, or administration of a trial treatment while in the control of a healthcare professional or consumer. The error can be identified either by the trial team at site or by the Sponsor upon review.

Trial Treatment errors should be reported on in incident report (see section 13.1). Any adverse events resulting from a medication error should be reported as an SAE (see section 12.2.2 for reporting procedures).

Occupational exposure

Exposure to a trial treatment as a result of one's professional or non-professional occupation. Occupational exposure should be reported on an incident report form (see section 13.1).

8.9. Supportive Care

Full supportive care measures should be offered to treat any emerging toxicities. Supportive care measures including those directed at controlling symptoms resulting from disease progression are allowed. Concomitant medications or therapy to provide adequate care may be given as clinically necessary. Details of the following concomitant medication will be captured on the trial CRF: pre-medication prior to ATIMP/placebo infusion (if given), any supportive medication given for patients who develop allergic/infusional reactions after ATIMP/placebo administration, any support medication patient may need to have for their cancer.

Localised radiation therapy to alleviate symptoms such as bone pain is allowed provided that the total dose delivered is in a palliative range according to institutional standards and does not involve a target lesion(s) utilised for response determination.

Permitted treatments:

- Corticosteroids both oral and intravenously delivered
- Radiotherapy for symptom control such as bone pain or potential airway occlusion from tumour
- Endobronchial interventions for potential airway occlusion
- Any form of analgesia
- Antihistamines

- Anti-emetics
- Blood product transfusions as clinically indicated
- Antibiotics for treatment of infection
- Granulocyte colony stimulating factor (G-CSF) as clinically indicated

Prohibited treatment for patient's receiving pembrolizumb:

- Live vaccines during dose administration and for 90 days after last dose
- Prolonged therapy (> 7 days) with systemic glucocorticoids for any purpose other than to modulate symptoms
- Immunotherapy not specified in this protocol

The use of any natural/herbal products or other non prescribed products should be discouraged.

Upon registration, and while the patient remains on study treatment, patients must not be given any concurrent cancer therapy, including cytotoxic agents, biological response modifiers (including cytokines), hormonal therapy (used specifically for cancer treatment), or any other investigational agents.

8.10. Contraindications

There are no known contraindications for MSCTRAIL/placebo.

Refer to the SPC for the brand of pemetrexed, cisplatin/carboplatin and pembrolizumab used at site for listed contraindications.

8.11. Pharmacy/ATIMP storage facility Responsibilities

MSCTRAIL (and placebo in phase II) will be delivered to participating sites who will be responsible for receipt, storage and issue of the ATIMP/placebo (as applicable) and will comply with the SODA. The management of MSCTRAIL (and placebo in phase II) at participating sites is the responsibility of the PI.

MSCTRAIL (and placebo in phase II) supplied for the TACTICAL trial are for TACTICAL patients only and must not be used outside the context of this protocol.

8.11.1. ATIMP accountability

The accountability for the MSCTRAIL/placebo at the trial site is the responsibility of the PI, who may delegate this responsibility to appropriately qualified personnel. The delegation of duties must be recorded on the site staff delegation log. The responsible person will ensure that MSCTRAIL/placebo is used only in accordance with this protocol and that appropriate records are maintained.

The PI (or delegated qualified personnel) must maintain records for the MSCTRAIL/placebo, which includes receipt, issuing, unused MSCTRAIL/placebo, storage conditions and destruction of unused MSCTRAIL/placebo. Template accountability logs will be supplied.

Copies of completed ATIMP/placebo accountability logs must be submitted to UCL CTC for all trial patients at the end of treatment or upon request. Also refer to section 14.2 (Central Monitoring).

Refer to the Summary of Drug Arrangements for details.

8.11.2. Accountability of pemetrexed, cisplatin and pembrolizumab

Accountability for drugs that are Non-investigational Medicinal Products (NIMPs), such as pemetrexed, cisplatin (and carboplatin, if used) and / or pembrolizumab are performed according to institutional guidelines.

8.11.3. Temperature Excursions

All temperature excursions outside the storage conditions specified in the Summary of Drug Arrangements must be reported to UCL CTC as per the 'Procedure for Reporting Temperature Excursions' (see Investigator Site File)

Upon identifying an excursion:

- all affected MSCTRAIL/placebo stock must be quarantined IMMEDIATELY
- the 'Notification of Temperature Excursion' form must be completed and e-mailed to ctc.excursions@ucl.ac.uk or faxed to 020 7679 9871.

Please note that UCL CTC must be informed immediately if a patient has been administered drug affected by a temperature excursion.

8.12. Unblinding

Unblinding must only be done in exceptional circumstances when a Serious Adverse Reaction occurs **and** the treating investigator considers knowing the trial treatment would be in the patient's best interest. This should only be done after discussion with the CI and the Sponsor, unless it is an emergency.

If an event causes concern, the dose of the trial drug should be delayed, reduced or discontinued, as appropriate, and the event treated. Patient safety will not be compromised by acting as though the patient were on active drug, whether or not this is in fact the case.

If a patient is unblinded and found to be on MSCTRAIL, they will be allowed to resume treatment if in the opinion of the clinician they have recovered and are likely to derive benefit from continuing treatment. Treatment may only be resumed after discussion with the CI and UCL CTC and with appropriate dose reductions if applicable (as per protocol).

Trial specific 'Unblinding instructions' will be provided to participating sites prior to the start of phase II as part of the ISF for the study.

8.13. 24 Hour/Out-of-Office Hours Emergency ATIMP-Specific Advice

Site staff will provide all registered patients with the current approved version of the patient contact card for the trial. Site staff will need to add the name of the subject, trial

number, the investigator contact details, on-call 24 hour medical care contact number. Patients must be reminded to carry the card at all times whilst participating in the trial. Refer to the 'Site Procedure for Issuing patients with emergency contact details and out-of-hours arrangements for drug or medical advice' for TACTICAL present in the ISF.

8.14. Clinical Management after Treatment Discontinuation

The management and subsequent treatment of patients who withdraw consent or stop protocol treatment early due to toxicity or any other reason, will be left to the discretion of the clinician and cease regular trial-specific follow-up.

All patients who have received at least one dose of MSCTRAIL, irrespective of the above, will be followed-up until 24 months after end of trial treatment (see section 9.2 and 9.4) for survival, safety and disease status.

Also refer to sections 9 (Assessments) and 15 (Withdrawal of Patients) for further details regarding treatment discontinuation, patient withdrawal from trial treatment and withdrawal of consent to data collection.

9. ASSESSMENTS

Please also see Schedule of Events table for phase I in Appendix 2 and for phase II in Appendix 3.

9.1. Pre-registration/randomisation Assessments for both phase I and phase II patients

Patients must have histologically/cytologically confirmed adenocarcinoma of the lung with incurable disease. Patients must give written informed consent **before** any trial specific screening investigations may be carried out. However, with the exception of blood samples for circulatory biomarkers, it is expected that all other eligibility and baseline investigations would fall within routine pre-treatment investigations for this patient population.

All screening procedures must be performed prior to registration/randomisation.

- Histological / cytological confirmation of adenocarcinoma
- EGFR and ALK testing
- CT scan - chest, abdomen pelvis to document baseline disease status using RECIST version 1.1 (within 28 days prior to registration/ randomisation)

The following to be performed within 14 days prior to registration:

- Complete physical examination and medical history
- Cancer signs and symptoms
- Height and weight
- Assessment of ECOG performance status (see appendix 4)
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest)
- ECG
- Full blood count – including haemoglobin, RCC, white cell count with differentials, platelets, neutrophils
- Oncological profile – including sodium, potassium, urea, creatinine, bilirubin, ALP, AST or ALT, lactate dehydrongenase, albumin, total protein, calcium, magnesium, glucose, CRP and Thyroid function tests (T4 and TSH) if patient received pembrolizumab
- Coagulation screen (PT, APTT and INR)
- Assessment of renal function (GFR) – EDTA or Cockcroft-Gault formula
- Adverse event and concomitant medication check
- If female and of childbearing potential, a negative pregnancy test
- Procurement of the archival diagnostic FFPE block

9.2. Phase I - Assessments during Treatment

Cycle 1, Day 1

- Clinical review and physical examination
- ECOG Performance status
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest)
- Full blood count – including haemoglobin, white cell count, platelets, neutrophils
- Oncological profile – including sodium, potassium, urea, creatinine, bilirubin, ALP, AST or ALT, albumin, total protein, calcium, magnesium, CRP
- Urinalysis
- If WOCBP, a negative pregnancy test
- Blood sample for translational research (section 10 and appendix 1; refer to the Laboratory manual for details on sample processing/sending)
- Adverse event and concomitant medication check

Bloods, urinalysis and pregnancy test do not need repeating if done within 72 hours prior to day 1.

Cycle 1, Day 2

- Limited clinical review and physical examination
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest; heart rate and oxygen saturation monitored continuously throughout infusion)
- ECG (pre & 4h post-dose)
- Blood samples for translational research: before MSCTRAIL infusion, 3 and 6 hours post infusion(section 10 and appendix 1; refer to the Laboratory manual for details on sample processing/sending)
- Adverse event and concomitant medication check

Cycle 1, Day 3

- Limited clinical review and physical examination
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest
- ECG
- Blood samples for translational research (section 10 and appendix 1; refer to the Laboratory manual for details on samples processing/sending)
- Adverse event and concomitant medication check

Cycle 1, Day 8 and Cycle 1, Day 15

- Limited clinical review and physical examination
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest
- ECG
- Full blood count – including haemoglobin, white cell count, platelets, neutrophils
- Oncological profile – including sodium, potassium, urea, creatinine, bilirubin, ALP, AST or ALT, albumin, total protein, calcium, magnesium, , CRP
- Blood samples for translational research(section 10 and appendix 1; refer to the Laboratory manual for details on samples processing/sending)
- Adverse event and concomitant medication check

Cycle 2, Day 1

- Limited clinical review, physical examination and weight
- ECOG Performance status
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest
- Full blood count – including haemoglobin, white cell count, platelets, neutrophils
- Oncological profile – including sodium, potassium, urea, creatinine, bilirubin, ALP, AST or ALT, albumin, total protein, calcium, magnesium, CRP
- Urinalysis
- Blood sample for translational research (section 10 and appendix 1; refer to the Laboratory manual for details on sample processing/sending)
- Adverse event and concomitant medication check
- If WOCBP, a negative pregnancy test

Bloods, pregnancy tests and ECOG performance status can be performed up to 72 hours prior to cisplatin/pemetrexed infusion.

Cycle 2, Day 2

- Limited clinical review and physical examination
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest; heart rate and oxygen saturation monitored continuously throughout infusion)
- ECG (pre & 4h post-dose)
- Blood samples for translational research: (before MSCTRAIL infusion, 3 and 6 hours post infusion(section 10 and appendix 1; refer to the Laboratory manual for details on samples processing/sending).
- Adverse event and concomitant medication check

Cycle 2, Days 3, 8 and 15

- Blood samples for translational research (section 10 and appendix 1; refer to the Laboratory manual for details on samples processing/sending).

Cycle 2, Day 14-21

- CT scan – chest, abdomen, pelvis (r) – RECIST v1.1 (iRECIST if patient received pembrolizumab)

Cycle 3, Day 1

- Limited clinical review, physical examination and weight
- ECOG Performance status
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest)
- Full blood count – including haemoglobin, white cell count, platelets, neutrophils
- Oncological profile – including sodium, potassium, urea, creatinine, bilirubin, ALP, AST or ALT, albumin, total protein, calcium, magnesium, CRP
- Thyroid function tests (T4 and TSH) if patient received pembrolizumab
- Urinalysis
- Blood sample for translational research (section 10 and appendix 1; refer to the Laboratory manual for details on sample processing/sending)
- Adverse event and concomitant medication check
- If WOCBP, a negative pregnancy test

Bloods, pregnancy tests and ECOG performance status can be performed up to 72 hours prior to cisplatin/pemetrexed infusion.

Cycle 3, Day 2

- Limited clinical review and physical examination
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest; heart rate and oxygen saturation monitored continuously throughout infusion)
- ECG (pre & 4h post-dose)
- Blood samples for translational research: before MSCTRAIL infusion, 3 and 6 hours post infusion(section 10 and appendix 1; refer to the Laboratory manual for samples processing/sending)
- Adverse event and concomitant medication check

Cycle 3, Days 3, 8 and 15

- Blood samples for translational research(section 10 and appendix 1; refer to the Laboratory manual for samples processing/sending)

Cycle 4, Day 1

- Limited clinical review, physical examination and weight
- ECOG Performance status
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest
- Full blood count – including haemoglobin, white cell count, platelets, neutrophils
- Oncological profile – including sodium, potassium, urea, creatinine, bilirubin, ALP, AST or ALT, albumin, total protein, calcium, magnesium, CRP
- Urinalysis
- Blood samples for translational research(section 10 and appendix 1; refer to the Laboratory manual for samples processing/sending)
- Adverse event and concomitant medication check

Bloods and ECOG performance status can be performed up to 72 hours prior to cisplatin/pemetrexed infusion.

Cycle 4, Day 15

- Blood samples for translational research(section 10 and appendix 1; refer to the Laboratory manual for samples processing/sending).

Cycle 4 Day 21

- Limited clinical review and physical examination
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest
- ECG
- Full blood count – including haemoglobin, white cell count, platelets, neutrophils
- Oncological profile – including sodium, potassium, urea, creatinine, bilirubin, ALP, AST or ALT, albumin, total protein, calcium, magnesium, CRP
- Thyroid function tests (T4 and TSH) if patient received pembrolizumab
- Adverse event and concomitant medication check (only adverse events deemed causally related to the ATIMP will be recorded in the trial CRFs after the last MSCTRAIL cycle)
- CT scan – chest, abdomen pelvis (at 12 weeks ie Cycle 4, Day 21- within 5 days) – RECIST v1.1. (iRECIST if patient received pembrolizumab)

After completion of the four cycles of trial treatment, patients may continue with further standard of care treatment as decided by their clinician and assessments will be according to local policy (these investigations will not be part of the trial). They will therefore continue to receive the appropriate monitoring for this therapy including thyroid function tests 6-8 weekly if they are on pembrolizumab.
Patients will continue to be followed up for the trial as detailed in section 9.3 below.

9.3. Phase I - Assessments during Follow-up

Follow-up period will continue for a maximum of 24 months after the end of trial treatment. Patients will have follow-up visits and CT scans 6 weekly until there is evidence of disease progression on CT RECIST v1.1 (iRECIST if patient received pembrolizumab). Once there is disease progression patients will have 6 weekly follow-up visits and 3 monthly CT scans up to 12 months and then a further scan at 18 and 24 months.

Follow-up visits consist of:

- Clinical review and physical examination
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest
- CT scan – chest, abdomen pelvis (6 weekly until evidence of disease progression then 3 monthly up to 1 year and a further scan at 18 and 24 months)

All efforts should be made by the Site to contact the patient's GP to assess their condition, if a patient fails to attend a clinic or cannot be followed up at site. .

9.4. Phase II - Assessments during Treatment

Cycle 1, Day 1

- Clinical review and physical examination
- ECOG Performance status
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest)
- Full blood count – including haemoglobin, white cell count, platelets, neutrophils
- Oncological profile – including sodium, potassium, urea, creatinine, bilirubin, ALP, AST or ALT, albumin, total protein, calcium, magnesium, CRP
- Urinalysis
- If WOCBP a negative pregnancy test
- Adverse event and concomitant medication check

Bloods, pregnancy tests and ECOG performance status can be performed up to 72 hours prior to cisplatin/pemetrexed infusion.

Cycle 1, Day 2

- Limited clinical review and physical examination
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest; heart rate and oxygen saturation monitored continuously throughout infusion)
- ECG (pre & 4h post-dose)
- Adverse event and concomitant medication check

Cycle 1, Day 3

- Limited clinical review and physical examination
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest
- ECG
- Adverse event and concomitant medication check

Cycle 1, Day 8 and Cycle 1, Day 15

- Limited clinical review and physical examination
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest
- ECG
- Full blood count – including haemoglobin, white cell count, platelets, neutrophils
- Oncological profile – including sodium, potassium, urea, creatinine, bilirubin, ALP, AST or ALT, albumin, total protein, calcium, magnesium, CRP
- Adverse event and concomitant medication check

Cycle 2, Day 1

- Limited clinical review, physical examination and weight
- ECOG Performance status
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest
- Full blood count – including haemoglobin, white cell count, platelets, neutrophils
- Oncological profile – including sodium, potassium, urea, creatinine, bilirubin, ALP, AST or ALT, albumin, total protein, calcium, magnesium, CRP
- Urinalysis
- Adverse event and concomitant medication check
- If WOCBP, a negative pregnancy test

Bloods, pregnancy tests and ECOG performance status can be performed up to 72 hours prior to cisplatin/pemetrexed infusion.

Cycle 2, Day 2

- Limited clinical review and physical examination
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest; heart rate and oxygen saturation monitored continuously throughout infusion)
- ECG (pre & 4h post-dose)
- Adverse event and concomitant medication check

Cycle 2, Day 14-21

- CT scan – chest, abdomen pelvis at 6 weeks – RECIST v1.1 (iRECIST if patient received pembrolizumab)

Cycle 3, Day 1

- Limited clinical review, physical examination and weight
- ECOG Performance status
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest
- Full blood count – including haemoglobin, white cell count, platelets, neutrophils
- Oncological profile – including sodium, potassium, urea, creatinine, bilirubin, ALP, AST or ALT, albumin, total protein, calcium, magnesium, CRP
- Thyroid function tests (T4 and TSH) if patient received pembrolizumab
- Urinalysis
- Adverse event and concomitant medication check
- If WOCBP, a negative pregnancy test

Bloods, pregnancy tests and ECOG performance status can be performed up to 72 hours prior to cisplatin/pemetrexed infusion.

Cycle 3, Day 2

- Limited clinical review and physical examination
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest; heart rate and oxygen saturation monitored continuously throughout infusion)
- ECG (pre & 4h post-dose)
- Adverse event and concomitant medication check

Cycle 4, Day 1

- Limited clinical review, physical examination and weight
- ECOG Performance status

- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest
- Full blood count – including haemoglobin, white cell count, platelets, neutrophils
- Oncological profile – including sodium, potassium, urea, creatinine, bilirubin, ALP, AST or ALT, albumin, total protein, calcium, magnesium, CRP
- Urinalysis
- Adverse event and concomitant medication check

Bloods and ECOG performance status can be performed up to 72 hours prior to cisplatin/pemetrexed infusion.

Cycle 4 Day 21

- Limited clinical review and physical examination
- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest
- ECG
- Full blood count – including haemoglobin, white cell count, platelets, neutrophils
- Oncological profile – including sodium, potassium, urea, creatinine, bilirubin, ALP, AST or ALT, albumin, total protein, calcium, magnesium, CRP
- Thyroid function tests (T4 and TSH) if patient received pembrolizumab
- Adverse event and concomitant medication check (only adverse events deemed causally related to the ATIMP will be recorded in the trial CRFs after the last MSCTRAIL/Placebo cycle)
- CT scan – chest, abdomen pelvis (at 12 weeks ie Cycle 4, Day 21 within 5 days) – RECIST v1.1. (iRECIST if patient received pembrolizumab)

After completion of the four cycles of trial treatment, patients may continue with further standard of care treatment as decided by their clinician and assessments will be according to local policy (these investigations will not be part of the trial).They will therefore continue to receive the appropriate monitoring for this therapy including thyroid function tests 6-8 weekly if they are on pembrolizumab.
Patients will continue to be followed up for the trial as detailed in section 9.5 below.

9.5. Phase II - Assessments during Follow-up

Follow-up period will continue for a maximum of 24 months after the end of trial treatment.

Patients will have follow-up visits and CT scans 6 weekly until there is evidence of disease progression on CT RECIST v1.1 (iRECIST if patient received pembrolizumab). Once there is disease progression patients will have 6 weekly follow-up visits and 3 monthly CT scans up to 12 months and then a further scan at 18 and 24 months.

Follow-up visits consist of:

- Vital signs, including heart rate, oxygen saturation, blood pressure and pulse (blood pressure and pulse to be measured after 2 minutes supine rest)
- Clinical review and physical examination
- CT scan – chest, abdomen pelvis (6 weekly until evidence of disease progression and then 3 monthly until 12 months with further scan at 18 and 24 months)

All efforts should be made by the Site to contact the patient's GP to assess their condition, if a patient fails to attend a clinic or cannot be followed up at site.

10. TRANSLATIONAL AND EXPLORATORY RESEARCH

Phase I only

Where patients have consented, blood and tissue samples will be collected throughout the study for analysis in research laboratories. Planned analyses are outlined below. Any samples remaining after these analyses will be stored for future ethically approved research. Samples will be taken, stored and subsequently processed and analysed according to laboratory standard operating procedures.

Up to 30mls of blood will be taken in cycles 1,2 and 3 at the following timepoints:

- Day 1
- Day 2, pre MSCTRAIL, 3 and 6 hours post MSCTRAIL
- Day 3
- Day 8
- Day 15

Up to 30mls of blood will be taken in cycle 4 on:

- Day 1
- Day 15

Further blood sample for analysis will be taken at the time of disease progression.

1. Biomarkers of apoptosis

Markers of apoptosis will be taken before treatment and at pre-determined times following treatment. These studies will provide evidence that MSCTRAIL is able to induce apoptosis which is the mechanism by which TRAIL works.

Using this analysis we will also determine whether circulating blood markers obtained by minimally invasive venepuncture could provide less invasive ways of monitoring therapeutic efficacy. This could potentially help guide treatments in the future as tumour response could be gauged from simple tests which can be carried out in the outpatient setting.

Blood will be collected in EDTA tubes and transferred to the central laboratory (Lungs for Living Research Centre, UCL, London) for processing and storage at -80°C until subsequent analysis by M30 and M65 ELISA for cleaved and full-length CK18. This biomarker of apoptosis has previously been successfully used both with the use of rTRAIL (Dulanermin) and in NSCLC in a phase Ia trial of solid tumours and a phase II study of efficacy and safety of Dulanermin combined with other chemotherapeutic agents in advanced NSCLC [37, 38].

2. Donor immune response to allogeneic MSCTRAIL therapy

We will also investigate whether there is an anti-donor immune response to MSCTRAIL and its subsequent effect on therapeutic efficacy. By collecting patients blood and

analysing the different types of white cells present before and after therapy we can examine both the response to single doses of allogeneic MSC and to re-challenge with multiple doses.

3. Collection of archival tissue:

Background

Baseline formalin-fixed paraffin-embedded (FFPE) tissue blocks will be collected from patients if there is supplementary tissue remaining following the diagnostic process. Assessment of archival tissue will predominantly involve validated immunohistolchemical (IHC) assays to evaluate markers of disease response. Additional analysis may include gene sequencing or further studies which may aid in the research of non-small cell lung cancer.

Sample Collection and Processing

A representative paraffin block from the primary lung tumor or from nodal deposit or metastasis will be identified by the local pathologist following all required diagnostic testing.

4 Further ethically approved Research Studies:

With the remaining blood samples we plan to carry out ethically approved translational research. This may include circulating tumour cell capture, staining and storage. This will allow for pharmacodynamic biomarker and genetic analysis. Samples for this will be included in the blood taken on day 1 and 3 in cycle 1 and day 1 only in cycle, 2, 3 and 4 and a further sample will be taken upon disease progression.

Analysis	Sample	Time points	Research laboratory and main contact
• **Biomarkers of apoptosis** • **Donor immune response to allogeneic MSCTRAIL therapy** • **Future ethically approved research**	Up to 30ml blood in EDTA	**Cycles 1-3:** • Day 1 • Day 2 (before MSCTRAIL) • Day 2: 3 & 6 hrs post MSCTRAIL • Day 3: 1 day post MSCTRAIL • Day 8: 7 days post MSCTRAIL • Day 15: 14 days post MSCTRAIL **Cycle 4:** • Day 1 • Day 15 **Sample on progression**	*Dr Alice Davies* [redacted]
Collection of archival tissue	Diagnostic formalin-fixed paraffin-embedded (FFPE) block from the primary lung tumor or from nodal deposit or metastasis	**Screening**	*Dr Alice Davies* [redacted]

Detailed information on sample handling, storage and shipping are provided in the Laboratory Manual in the Investigator Site File (ISF).

11. DATA MANAGEMENT AND DATA HANDLING GUIDELINES

Data will be collected from sites on version controlled case report forms (CRFs) designed for the trial and supplied by UCL CTC. Data must be accurately transcribed onto trial CRFs and must be verifiable from source data at site. Examples of source documents are hospital records which include patient's notes, laboratory and other clinical reports.

Where copies of supporting source documentation (e.g. autopsy reports, pathology reports, CT scan images) are being submitted to UCL CTC, the patient's trial number must be clearly indicated on all material and any patient identifiers removed/blacked out prior to sending to maintain confidentiality.

11.1. Completing Case Report Forms

All CRFs must be completed and signed by staff who are listed on the site staff delegation log and authorised by the PI to perform this duty. The PI is responsible for the accuracy of all data reported in the CRF.

All entries must be clear, legible and written in ball point pen. Any corrections made to a CRF at site must be made by drawing a single line through the incorrect item ensuring that the previous entry is not obscured. Each correction must be dated and initialed. Correction fluid must not be used.

The use of abbreviations and acronyms should be avoided.

11.2. Missing Data

To avoid the need for unnecessary data queries CRFs must be checked at site to ensure there are no blank fields before sending to UCL CTC (unless it is specifically stated that a field may be left blank). When data are unavailable because a measure has not been taken or test not performed, enter "ND" for not done. If an item was not required at the particular time the form relates to, enter "NA" for not applicable. When data are unknown enter the value "NK" (only use if every effort has been made to obtain the data).

11.3. Timelines for Data Return

CRFs must be completed at site and returned to UCL CTC as soon as possible after the relevant visit and within 2 weeks (phase I) and 4 weeks (phase II) of the patient being seen. Data required for intra-patient and inter-patient dose decisions by the TMG/IDMC will be required to be returned to UCL CTC within 5 working days.

Sites that persistently do not return data within the required timelines may be suspended from recruiting further patients into the trial by UCL CTC and subjected to a 'for cause' monitoring visit. See section 14.3 ('For Cause' On-Site Monitoring) for details.

11.4. Data Queries

Data arriving at UCL CTC will be checked for legibility, completeness, accuracy and consistency, including checks for missing or unusual values. Data Clarification Requests will be sent to the data contact at site. Further guidance on how data contacts should respond to data queries can be found on the Data Clarification Request forms.

12. PHARMACOVIGILANCE

12.1. Definitions

The following definitions have been adapted from Directive 2001/20/EC, ICH E2A "Clinical Safety Data Management: Definitions and Standards for Expedited Reporting" and ICH GCP E6.

Adverse Event (AE)

Any untoward medical occurrence in a patient treated on a trial protocol, which does not necessarily have a causal relationship with an IMP. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of an IMP, whether or not related to that IMP. See section 12.2.1 for AE reporting procedures.

Adverse Reaction (AR)

All untoward and unintended responses to an IMP related to any dose administered. A causal relationship between an IMP and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)

An adverse event or adverse reaction that at any dose:

- Results in death
- Is life threatening (the term "life-threatening" refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe)
- Requires in-patient hospitalisation or prolongs existing hospitalisation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect
- Is otherwise medically significant (e.g. important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed above)

See section 12.2.2 for SAE reporting procedures.

Suspected Unexpected Serious Adverse Reaction (SUSAR)

An adverse event meeting the following criteria:

- Serious – meets one or more of the serious criteria above
- Related – assessed by the local investigator or sponsor as causally related to one or more elements of the trial treatment
- Unexpected – the event is not consistent with the applicable reference safety information (RSI).

See section 12.3 for reporting procedures for these events.

Adverse event of special interest

An AE that is of particular interest to the Trial Management Group, even if it occurs outside the standard AE reporting timeframes for the trial.

See section 12.3 for reporting procedures for these events.

Overdose, Trial treatment error

Refer to section 8.6 for details on reporting of these events.

12.2. Reporting Procedures

Adverse Event Term

An adverse event term must be provided for each adverse event. Wherever possible a valid term listed in the Common Terminology Criteria for Adverse Events (CTCAE) v5.0 should be used. This is available online at:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

Severity grade

Severity grade of each adverse event must be determined by using CTCAE v5.0.

Causality

The relationship between the treatment and an adverse event will be assessed.

For AEs (including SAEs), the local PI or designee will assess whether the event is causally related to trial treatment.

For SAEs, a review will also be carried out by the Sponsor's delegate.

As phase II is a placebo-controlled trial, the evaluation of causality must be performed as if the patient is on active treatment for the second phase of the study.

Causal relationship to each trial treatment must be determined as follows:

- Related (reasonable possibility) to a trial treatment
- Not related (no reasonable possibility) to a trial treatment

NB Events will be classified as related to trial treatment if evaluated as possibly, probably or definitely related by the investigator or sponsor.

UCL CTC will consider events evaluated as related to be adverse reactions.

12.2.1. Reporting of Adverse Events (AEs)

All adverse events that occur between day 2 of Cycle 1 (first infusion of MSCTRAIL/placebo) and 21 days post last MSCTRAIL/placebo administration (or after this date if the investigator feels that the event is related to the MSCTRAIL or trial procedures) must be recorded in the patient notes and the trial CRFs. Those meeting the definition of a Serious Adverse Event (SAE) and Adverse Event of Special Interest (AESI) must also be reported to UCL CTC using the trial specific SAE Report. Also refer to section 12.2.2 (Reporting of Serious Adverse Events (SAEs)).

Pre-existing conditions (i.e. present on Day 2 of cycle 1, prior to infusion of MSCTRAIL/placebo) do not qualify as adverse events unless they worsen or recur (i.e. improves/resolves and then worsens/reappears again).

E.g. an AE could be an exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition (worsening of the event). Another example of an AE is when a pre-existing condition improves during the trial (e.g. from grade 3 to grade 1) and then it worsens again (e.g. from grade 1 to grade 2), even if the event is of severity equal or lower to the original condition (improvement and recurrence of the event).
NB the disease(s) under study and its anticipated day-to-day fluctuations would not be an AE.

12.2.2. Reporting of Serious Adverse Events (SAEs)

All SAEs that occur between the signing of informed consent and 21 days post last MSCTRAIL administration **(or after this date if the site investigator feels the event is related to the ATIMP/placebo)** must be submitted to UCL CTC by fax within **24 hours** of observing or learning of the event, using the trial specific SAE Report. All sections on the SAE Report must be completed. If the event is **not being reported within 24 hours** to UCL CTC, the circumstances that led to this must be detailed in the SAE Report to avoid unnecessary queries.

Please note that disease progression (including disease related deaths) must be reported to UCL CTC using the trial specific SAE Report within **24 hours** of learning of the event.

Exemptions from SAE Report submission

For this trial, the following events are exempt from requiring submission on an SAE Report, but must be recorded in the relevant section of the trial CRFs:

- events that occur after 21 days post last MSCTRAIL/placebo administration that are not considered to be side-effects of the their infusion (unless they are a negative pregnancy outcome)

Please note that hospitalisation for elective treatment, for palliative care or for socio-economic/logistical reasons does not qualify as an SAE.

Completed SAE Reports must be faxed to UCL CTC within 24 hours of becoming aware of the event

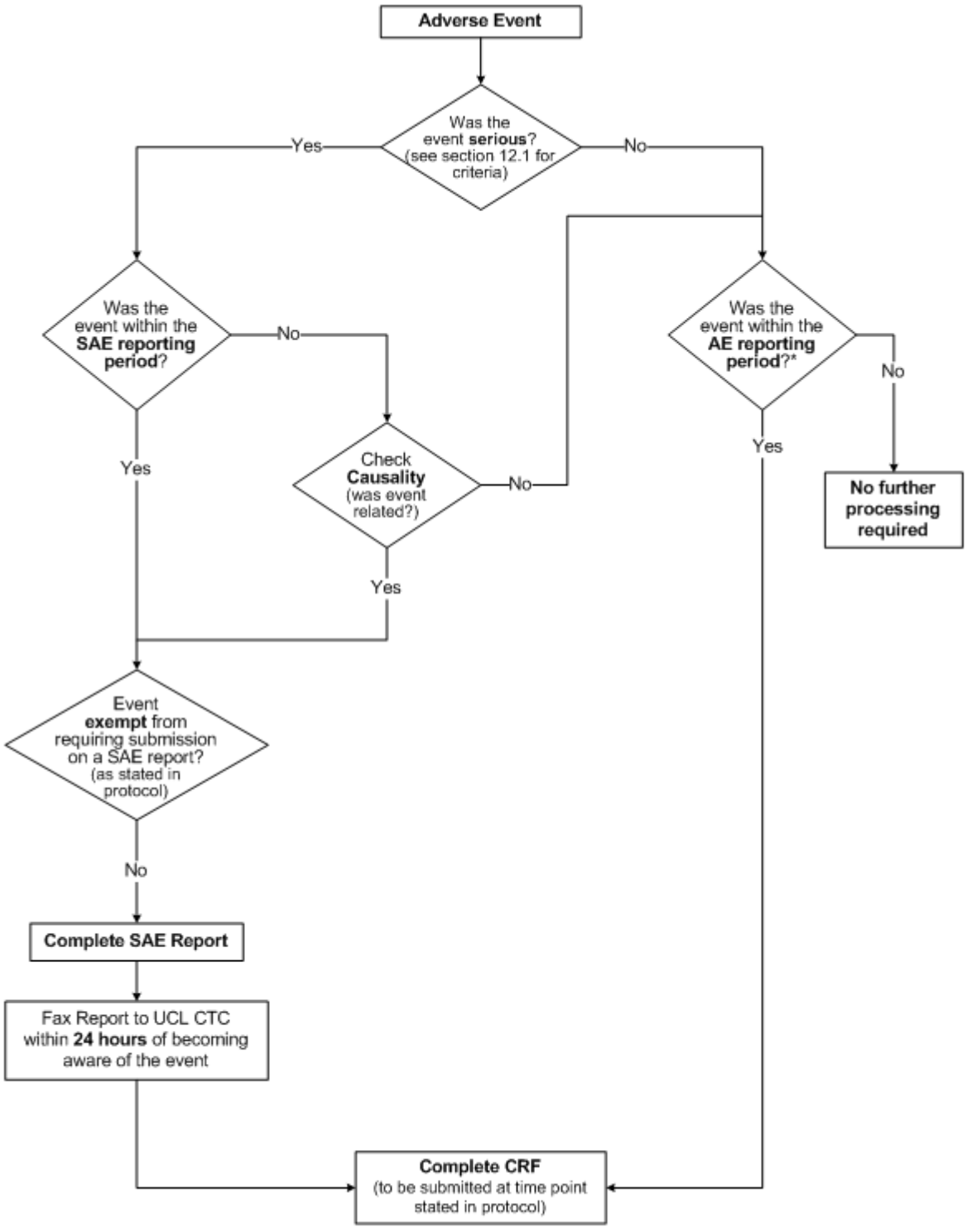
Fax:[redacted]

SAE Follow-Up Reports

All SAEs/SARs must be followed-up until resolution and until there are no further queries.

Sites must ensure any new and relevant information is provided promptly. If the event term changes or a new event is added, the causality must be re-assessed by an Investigator. If the event is not being reported within 24 hours to UCL CTC, the circumstances that led to this must be detailed in the SAE/SAR Report to avoid unnecessary queries.

Adverse Event Reporting Flowchart



*This applies if AE and SAE reporting periods differs.

SAE Processing at UCL CTC

On receipt of the SAE Report, UCL CTC will check for legibility, completeness, accuracy and consistency. Expectedness will be evaluated, to determine whether or not the case qualifies for expedited reporting, using the approved RSI (the list of expected adverse events in the approved IB for MSCTRAIL). While MSCs have been used in many clinical trials, there is no clinical experience with the MSCTRAIL used in this study. Therefore currently there are no expected adverse events related to this specific ATIMP, hence all SARs will be reported as SUSARs.

The CI, or their delegate (e.g. a clinical member of the TMG), may be contacted to review the SAE and to perform an evaluation of causality on behalf of UCL CTC.

SUSARs

If the event is evaluated as a Suspected Unexpected Serious Adverse Reaction (SUSAR), UCL CTC will submit a report to the MHRA and REC within the required timelines.

UCL CTC will unblind any SUSARs before expedited reporting, if not already unblinded. Refer to section 8.12 for details.

Wherever possible, evaluations of causal relationship by both the site and the Sponsor's clinical reviewer will be reported.

Informing Sites of SUSARs

Phase I is a single site study. During phase II UCL CTC will inform all PIs of any SUSARs that occur on the trial at the time of submission to MHRA and GTAC. PIs will receive blinded expedited SUSAR reports that must be processed according to local requirements.

12.3. Adverse events of special interest

The following adverse events of special interest for MSCTRAIL must be reported on an SAE report within **24 hours of becoming aware of the event**

- Thromboembolic event ≥ CTCAE grade 4 within 48 hours of MSCTRAIL infusion

All AEs of special interest must be reported by faxing a completed SAE report to UCL CTC within 24 hours of becoming aware of the event
Fax: [redacted]

12.4. EU Tissue & Cells Directive

12.4.1. SAEs

The EU Tissue and Cells Directive (2004/23/EC) defines an SAE as follows:

"'Serious Adverse Event' means any untoward occurrence associated with the procurement, testing, processing, storage and distribution of cells that might lead to the transmission of a communicable disease, to death or life-threatening, disabling or incapacitating conditions for patients or which might result in, or prolong, hospitalisation or morbidity."

12.4.2. SARs

The EU Tissue and Cells Directive (2004/23/EC) defines a SAR as follows:

"'Serious Adverse Reaction' means an unintended response, including a communicable disease, in the donor or in the recipient associated with the procurement or human application of tissues and cells that is fatal, life threatening, disabling, incapacitating or which results in, or prolongs, hospitalisation or morbidity."

SARs as defined in the Tissue and Cell Directive may occur a considerable time after administration. This is particularly the case with communicable diseases.

Event	**Reporting requirements**
SAE event of MSCTRAIL during distribution to study site	The study site should notify the manufacturer **within one business day** of learning of the event for their DI to report to the HTA/donation site as per regulatory requirements. The study site should notify the CTC **within one business day** of learning of the event if MSCTRAIL can't be administered to the study patient.
SAE/SAR in recipient *causally related* to MSCTRAIL	The trial site reports the SAE/SAR to the CTC for assessment and reporting as per protocol section 12.2.2. Where the trial treatment is an ATIMP/placebo the CTC will forward the report for the SAR to the QP/manufacturer of MSCTRAIL. If the SAE is a transmission of a communicable disease possibly, probably or definitely related to the donated tissues or cells, the CTC should report the event **within one business day** of learning of the event to the manufacturer for reporting to the HTA–DI at the donation site as per regulatory requirements.

12.5. Safety Monitoring

UCL CTC will provide safety information to the Trial Management Group (TMG) and the Independent Data Monitoring Committee (IDMC) on a periodic basis (detailed in 14.4 Oversight Committees) for review.

Trial safety data will be monitored to identify:

- new adverse reactions to the IMP
- trial related events that are not considered related to the IMP
- AEs of Special Interest as per section 12.3

For phase I, the TMG and IDMC will review safety and efficacy data as detailed in section 14.4 (Oversight Committees) and will provide recommendations on dose assignment for future patients until the RP2D has been determined, or the trial terminated for safety reasons.

Recommendations for dose de-escalation and trial termination will be aided by the model-based design, which uses a dose-toxicity model to estimate the probability of DLT at each dose level of MSCTRAIL, given all past and current DLT data in the trial (see section 17.1). If DLTs are observed in the first cohort of 3 patients, then dose de-escalation decisions can be made using the table provided in subsection 8.3.1. Clinical opinion and secondary efficacy data may also be used to determine the best course of action for future patients.

If either of the following two occurs, the IDMC may suspend the trial, or have more frequent safety assessments:

1. Death of a patient after MSCTRAIL administration that is considered to be related (reasonable possibility) to MSCTRAIL

2. Occurrence of a secondary malignancy after MSCTRAIL administration

The trial may only be restarted following acceptance of a substantial amendment by the MHRA/GTAC.

If UCL CTC identifies or suspects any issues concerning patient safety at any point during the trial, the CI or TMG will be consulted for their opinion, and if necessary the issue will be referred to the IDMC.

12.6. Safety communication

UCL CTC will inform PIs of participating sites of any SUSARs, DLTs and serious safety-related protocol deviations that occur on the trial. PIs will receive a copy of the expedited SUSAR report at the time of submission to MHRA and GTAC (see also 12.2). Any DLTs and/or safety-related protocol deviations will be reviewed by the TMG (which includes PIs of participating sites). UCL CTC will notify site investigators about any DLTs/safety-related protocol deviations and actions to be taken as decided by TMG (if applicable).

MSCTRAIL treatment of patients in the 1st cohort is ‘staggered’ as described in section 14.4.1. UCL CTC will inform site Investigators whether the next patient can be treated (and dose de-escalated, if applicable) once the TMG has reviewed trial safety data.

TACTICAL is a single site trial during phase I when the recommended dose of MSCTRAIL for phase II will be determined. Between 2 to 4 sites may take part in the second randomised phase of the trial depending on rate of patient recruitment. PIs of all participating sites will be members of the TMG and will regularly review trial data as detailed in section 14.4.1. Additional teleconferences between PIs/TMG members and CTC to discuss the trial will be arranged where necessary.

12.7. Pregnancy

Reporting Period

If a female patient or the female partner of a male trial patient becomes pregnant between the start of trial treatment and 6 months after last MSCTRAIL administration, the site must submit a trial specific Pregnancy Report to UCL CTC by fax **within 24 hours** of learning of its occurrence.

The site must request consent from the pregnant trial patient or female partner of a male patient to report information regarding a pregnancy using:

- For female patients: the trial-specific Pregnancy Monitoring Information Sheet and Informed Consent Form for trial patients
- For female partners of male patients: the trial specific Pregnancy Monitoring Information Sheet and Informed Consent Form for partners of study patients

If consent is not given, the notification that a pregnancy has occurred will be retained by UCL CTC, however no further action will be taken on the information detailed in the report.

All pregnancies must be reported by faxing a completed Pregnancy Report to UCL CTC within 24 hours of becoming aware of the pregnancy
Fax: +[redacted]

Pregnancy Follow-Up Reports

For pregnant patients or partners who consent, their pregnancies must be followed -up for up to 6 weeks after the end of the pregnancy (or later if there are ongoing issues) to collect information on any ante- and post-natal problems for both mother and child. If significant new information is received, follow-up Pregnancy Reports must be submitted to UCL CTC by fax within **24 hours** of learning of the outcome. Reports must include an evaluation of the possible relationship of each trial treatment to the pregnancy outcome.

SAEs during pregnancy

Any SAE occurring in a pregnant patient must be reported using the trial specific SAE Report, according to SAE reporting procedures. Refer to section 12.2.2 (Reporting of Serious Adverse Events (SAEs)) for details.

Pregnancy Report processing at UCL CTC

UCL CTC will submit a report to the MHRA and the REC if the pregnancy outcome meets the definition of a SUSAR. Refer to section 12.2.2 (Reporting of Serious Adverse Events (SAEs)) for details.

12.8. Development Safety Update Reports (DSURs)

Safety data obtained from the trial will be included in DSURs that UCL CTC will submit to the MHRA and the REC.

13. INCIDENT REPORTING AND SERIOUS BREACHES

13.1. Incident Reporting

Organisations must notify UCL CTC of all deviations from the protocol or GCP immediately. An incident report may be requested and will be provided, but an equivalent document (e.g. Trust Incident form) is acceptable where already completed.

If site staff are unsure whether a certain occurrence constitutes a deviation from the protocol or GCP, the UCL CTC trial team can be contacted immediately to discuss.

UCL CTC will use an organisation's history of non-compliance to make decisions on future collaborations.

UCL CTC will assess all incidents to see if they meet the definition of a serious breach.

13.2. Serious Breaches

A "serious breach" is defined as a breach of the protocol or of the conditions or principles of Good Clinical Practice (or equivalent standards for conduct of non-CTIMPs) which is likely to affect to a significant degree the safety or physical or mental integrity of the trial subjects, or the scientific value of the research.

Systematic or persistent non-compliance by a site with GCP and/or the protocol, including failure to report SAEs occurring on trial within the specified timeframe, may be deemed a serious breach.

In cases where a serious breach has been identified, UCL CTC will inform the MHRA and REC within 7 calendar days of becoming aware of the breach.

Sites must have written procedures for notifying the sponsor of serious breaches (MHRA Guidance on the Notification of Serious Breaches).

14. TRIAL MONITORING AND OVERSIGHT

Participating sites and PIs must agree to allow trial-related on-site monitoring, Sponsor audits and regulatory inspections by providing direct access to source data/documents as required. Patients are informed of this in the patient information sheet and are asked to consent to their medical notes being reviewed by appropriate individuals on the consent form.

UCL CTC will determine the appropriate level and nature of monitoring required for the trial. Risk will be assessed on an ongoing basis and adjustments made accordingly.

14.1. On-Site Monitoring

The degree of on-site monitoring will be proportionate to the objective, purpose, phase, design, size, complexity, blinding, endpoints and risks associated with the trial.

Details of monitoring activities will be included in the trial monitoring plan which will be provided to Sites. The Monitoring Plan will be under review throughout the trial and updates provided as necessary.

Sites will be sent a letter in advance, confirming when a routine monitoring visit is scheduled to take place. The letter will include a list of the documents to be reviewed, interviews that will be conducted, planned inspections of the facilities and who will be performing the visit.

Monitoring Follow Up

Following a monitoring visit, the Trial Monitor/Trial Coordinator will provide a follow up email to the site, which will summarise the documents reviewed and a statement of findings, incidents, deficiencies, conclusions, actions taken and/or actions required. The PI at each site will be responsible for ensuring that monitoring findings are addressed in a timely manner, and by the deadline specified.

14.2. Central Monitoring

Sites will be requested to submit screening logs and staff delegation logs to UCL CTC at the frequency detailed in the trial monitoring plan, or on request, and these will be checked for consistency and completeness. Also refer to sections 4.2.2 (Required documentation) and 6.1 (Screening Log).

Ensuring patient eligibility is the responsibility of the PI or other delegated Investigator(s). Checks of the criteria listed on the registration form (phase I) or randomisation form (phase II) will be undertaken by an appropriately trained UCL CTC staff member prior to registration/randomisation. Also refer to section 7.1).

Details relating to the informed consent process will be collected on the registration form (phase I) or randomisation form (phase II) and are subject to review by UCL CTC as part of patient eligibility.

Copies of completed ATIMP/placebo drug accountability logs must be returned to UCL CTC for all trial patients. Sites will be required to submit logs in accordance with the trial

monitoring plan.Data received at UCL CTC will be subject to review in accordance with section 11.4 (Data Queries).

Sites will be requested to conduct quality control checks of documentation held within the Investigator Site File and ATIMP Management File at the frequency detailed in the trial monitoring plan. Checklists detailing the current version/date of version controlled documents will be provided for this purpose.

Where central monitoring of data and/or documentation submitted by sites indicates that a patient may have been placed at risk ***(e.g. evidence of an overdose having been administered)***, the matter will be raised urgently with site staff and escalated as appropriate (refer to section 13 (Incident Reporting and Serious Breaches) and 14.3 ('For Cause' On-Site Monitoring) for further details).

14.3. 'For Cause' On-Site Monitoring

Additional on-site monitoring visits may be scheduled where there is evidence or suspicion of non-compliance at a site with important aspect(s) of the trial protocol/GCP requirements. Sites will be sent a letter in advance outlining the reason(s) for the visit and confirming when it will take place. The letter will include a list of the documents that are to be reviewed, interviews that will be conducted, planned inspections of the facilities and who will be performing the visit.

UCL CTC will assess whether it is appropriate for the site to continue participation in the trial and whether the incident(s) constitute a serious breach. Refer to section 13 (Incident Reporting and Serious Breaches) for details.

14.4. Oversight Committees

14.4.1. Trial Management Group (TMG)

The TMG will include the Chief Investigator, PIs of all participating sites, clinicians and experts from relevant specialities and TACTICAL trial staff from UCL CTC (see page 4). The TMG will be responsible for overseeing the trial.

Phase I

Treatment start for the first 3 patients in dose cohort 1a will be staggered by at least 21 days to allow safety data from the 1st cycle of treatment to be reviewed by the TMG before the next patient is treated with MSCTRAIL. The clinical members of the TMG and the trial statistician will review toxicity data after each patient has reached Day 21 following first MSCTRAIL infusion (end of cycle 1). A decision on whether the next patient can be treated with MSCTRAIL (and any dose de-escalation guidelines followed if needed, as defined in section 8.3) will be taken based on this review. This decision may be made by minimum 3 clinical members of the TMG and the statistician (or in the absence of the statistician, the Trial Group Lead) in a face-to-face meeting (minuted), via teleconference (minuted) or in writing via email.

Once the 3rd patient in the 1st dose cohort (1a) has reached day 7 after the last MSCTRAIL infusion, the TMG will review the safety data and will decide whether the cohort should be expanded (1b) and the next patient treated on the same dose or

whether the dose should be de-escalated based on the 'Guidelines for dose de-escalation in first cohort' listed in section 8.3 Trial Treatment Details. This decision may be made in a face-to-face meeting (minuted), via teleconference (minuted) or in writing via email by minimum 3 clinical members of the TMG and the statistician agreeing to what dose level to continue with. The advice of the IDMC will also be sought, as described in 14.4.2, to confirm they agree with decisions on cohort expansion or dose de-escalation for the next cohort. Where there are no concerns about safety during the treatment of the first 3 patients in cohort 1a, the treatment interval between each patient in cohort 1b can be dropped if this is confirmed by the IDMC.

The TMG will review toxicity data when the last patient in the dose cohort 1b have reached day 21 following first MSCTRAIL infusion (end of cycle 1) to decide if the dose can be used as RP2D in phase II of the protocol or dose should be de-escalated (this decision will need to be confirmed by the IDMC as described in 14.4.2).

The TMG will continue to review the trial data for patients in the lower dose cohorts (if needed) as described above until the RP2D is determined.

Once RP2D has been determined the TMG will review the phase I data and decide if the trial can proceed to phase II with all subsequent patients receiving the RP2D (this decision will need to be confirmed by the IDMC as described in 14.4.2). The TMG will advise at this point whether any changes to the trial conduct are needed before the start of phase II in which case a substantial amendment addressing their suggestions will be submitted for approval.

In addition, the TMG will meet regularly (approximately twice a year) to review the trial, or as necessary to address any issues.

Phase II

During the second, randomised phase of the trial the TMG will meet regularly, approximately twice a year, unless concerns about safety have been raised (in which case IDMC will be consulted for advice), and will send updates to the NCRI Lung Clinical Studies Group.

A TMG charter summarising the roles and responsibilities of the TMG will be signed off by each member of the TMG*.*

14.4.2. Independent Data Monitoring Committee (IDMC)

The role of the IDMC is to provide independent advice on data and safety aspects of the trial.

Phase I protocol:

The role of the IDMC is to provide independent advice on data and safety aspects of the trial. Unless there are concerns regarding safety during the treatment of patients in the first cohort of phase I, the IDMC will review toxicity data when the 3rd patient in the first dose cohort (1a) have reached day 7 after the last MSCTRAIL infusion. A report on these patients will be provided to the IDMC, who will advise the TMG whether the cohort should be expanded (1b) and the next patient treated on the same dose or whether the dose should be de-escalated based on the safety and efficacy data assessed.

Unless there are concerns regarding safety during the treatment of patients in cohort 1b of phase I, the IDMC will review toxicity data when the last patient in the dose cohort 1b have reached day 21 following first MSCTRAIL infusion (end of cycle 1). A report on these patients will be provided to the IDMC, who will advise the TMG of the dose can be used as RP2D in phase II of the protocol or dose should be de-escalated.

The IDMC will continue to review the trial data for patients in the lower dose cohorts (if needed) as described above until the RP2D is determined.

When RP2D is determined the IDMC will review the phase I data and decide if the trial can proceed to phase II with all subsequent patients receiving the RP2D. The IDMC will advise at this point whether any changes to the trial conduct are needed before the start of phase II in which case a substantial amendment addressing their suggestions will be submitted for approval.

In addition, meetings of the Committee will be held approximately twice annually to review the trial. Other meetings may be organized as necessary to address any issues. The IDMC is advisory to the TMG and can recommend premature closure of the trial to the TMG.

Phase II protocol:

The IDMC will review trial data approximately twice annually (unless concerns about safety have been raised) during the second, randomised phase of the study. Other meetings may be organised as necessary to address any issues regarding the study. The IDMC is advisory to the TMG and can recommend premature closure of the trial to the TMG.

An IDMC charter summarising the roles and responsibilities of the IDMC will be signed off by each member of the IDMC prior to the first meeting.

14.4.3. Role of UCL CTC

UCL CTC will be responsible for the day to day coordination and management of the trial and will act as custodian of the data generated in the trial (on behalf of UCL). UCL CTC is responsible for all duties relating to pharmacovigilance which are conducted in accordance with section 12 (Pharmacovigilance).

15. WITHDRAWAL OF PATIENTS

In consenting to the trial, patients are consenting to trial treatment, assessments, follow-up and data collection.

15.1. Discontinuation of Trial Treatment

A patient may be withdrawn from trial treatment whenever such treatment is no longer in the patient's best interests, but the reasons for doing so must be recorded in the patient's notes and on the relevant Case Report Form(s). Reasons for discontinuing treatment may include:

- Unacceptable toxicity whether a DLT or not
- Intercurrent illness which prevents further treatment
- Patient decision not to continue with trial treatment
- Any alterations in the patient's condition which justifies the discontinuation of treatment in the site investigator's opinion
- Treatment delay of >21 days

In these cases patients will remain within the trial for the purposes of follow-up and data analysis according to the treatment option to which they have been allocated (in phase II) unless they explicitly withdraw consent.

If a patient expresses their wish to withdraw from trial treatment, sites should explain the importance of remaining on trial follow-up, or failing this of allowing routine follow-up data to be used for trial purposes and for allowing existing collected data to be used. If the patient gives a reason for their withdrawal, this should be recorded.

15.2. Future Data Collection

If a patient explicitly states they do not wish to contribute further data to the trial their decision must be respected, with the exception of essential safety data, and recorded on the relevant CRF. In this event data due up to the date of withdrawal must be submitted but no further data, other than essential safety data, sent to UCL CTC.

15.3. Losses to Follow-Up

If a patient moves from the area, every effort should be made for the patient to be followed up at another participating trial site and for this new site to take over the responsibility for the patient, or for follow-up via GP. Details of participating trial sites can be obtained from the UCL CTC trial team, who must be informed of the transfer of care and follow up arrangements. If it is not possible to transfer to a participating site, the registering site remains responsible for submission of forms.

If a patient is lost to follow-up at a site every effort should be made to contact the patient's GP to obtain information on the patient's status.

16. TRIAL CLOSURE

16.1. End of Trial

For regulatory purposes the end of the trial will be 24 months after last patient in phase II has reached the end of treatment (section 9.4) at which point the 'declaration of end of trial' form will be submitted to the MHRA and Ethics Committee, as required.

Following this, UCL CTC will advise sites on the procedure for closing the trial at the site.

Once the end of trial has been declared, no more prospective patient data will be collected but sites must co-operate with any data queries regarding existing data to allow for analysis and publication of results.

16.2. Archiving of Trial Documentation

At the end of the trial, UCL CTC will archive securely all centrally held trial related documentation for a minimum of 25 years. Arrangements for confidential destruction will then be made. It is the responsibility of PIs to ensure data and all essential documents relating to the trial held at site are retained securely for a minimum of 25 years after the end of the trial, and in accordance with national legislation.

Essential documents are those which enable both the conduct of the trial and the quality of the data produced to be evaluated and show whether the site complied with the principles of GCP and all applicable regulatory requirements.

UCL CTC will notify sites when trial documentation held at sites may be archived. All archived documents must continue to be available for inspection by appropriate authorities upon request.

16.2.1. Archiving of essential trial documentation relating to traceability

In accordance with the Advanced Therapy Regulations (1394/2007/EC), all parties (the sponsor of the trial, the manufacturer and the investigator/institution where the ATIMP is used) should keep their parts of the traceability records for a minimum of 30 years after the expiry date of the ATIMP. These requirements will be set out in contractual agreements between the parties and the sponsor.

To comply with the regulatory requirements, each responsible party must ensure that the information relating to the traceability and accountability, from the production of ATIMPs to the recipient (patient) receiving the ATIMPs, are archived for a minimum 30 years after the expiry date of the ATIMP.

The following essential documents/traceability data must be retained by the investigator and institution responsible for the human application of the ATIMP:

- Shipping Records for ATIMP
- Certificate of analysis of the ATIMP
- Treatment allocation and decoding documentation
- Patient identification code list

- ATIMP accountability at the site including final disposition of both used and unused product.

These records contain relevant information for traceability purposes and at least the following minimum data set from these records should be kept for 30 years after the expiry date of the product, or longer if required by the terms of the clinical trial authorisation or by the agreement with the sponsor:

- Identification of the investigator/institution
- Identification of the sponsor
- Identification of the manufacturing site
- Product name/code
- Pharmaceutical form, route of administration, quantity of dosage units and strength
- Batch and/or code number
- Trial reference code
- Patient trial code/number
- Patient identification code list (links name of patient to the Patient trial code/number)
- Product expiry/retest date
- Date of administration
- Records of any product that was unused or destroyed at site and its final status

The patient medical records must contain the product name/code, the trial reference code, trial subject code and administration dates and dose in order to ensure that a link can be made back to the identity of the product and the further traceability records of the investigator and sponsor.

16.3. Early Discontinuation of Trial

The trial may be stopped before completion as an Urgent Safety Measure on the recommendation of the IDMC (see section 14.4.2 Independent Data Monitoring Committee (IDMC)). Sites will be informed in writing by UCL CTC of reasons for early closure and the actions to be taken with regards the treatment and follow up of patients.

16.4. Withdrawal from Trial Participation by a Site

Should a site choose to close to recruitment the PI must inform UCL CTC in writing. Follow up as per protocol must continue for any patients recruited into the trial at that site and other responsibilities continue as per the CTSA.

17. STATISTICS

17.1. Sample Size Calculation

Phase I:

The phase I part of this study will be conducted as a dose de-escalation study, using the modified Continual Reassessment Method (mCRM) design [39]. The aim is to identify the Maximum Tolerated Dose (MTD) and thus the RP2D of MSCTRAIL when given with SOC treatment. The MTD is the largest dose of MSCTRAIL that has an estimated risk of causing DLT equal to or closest to 35% (the target toxicity level). Assuming a working model for the relationship between dose and the risk of DLT, data are used to estimate each dose's risk of causing DLT. These estimates inform which doses are likely to be tolerable, and which should be given to the next cohort.

Patients will be assigned to dose levels in groups of 3. The first 3 patients will be treated at the highest MSCTRAIL dose of $4x10^8$. Based on the DLT outcomes of these patients, estimates of the probability of DLT will be calculated and the next 3 patients will receive the dose of MSCTRAIL with a probability of DLT less than but closest to 35%. A minimum sample size of n=6 patients will be required, assuming that no DLTs are observed in any of these six patients. Otherwise, we will require a maximum of 12 patients. For comparison, conducting this trial as a dose escalation study under a 3+3 design, if there were zero DLTs, a minimum of 12 patients might be required (3 per dose to reach the MTD, plus a further 3 to test the MTD); if any DLTs were observed during the dose-escalation part of the study, at least another three patients would be required. Hence in this trial we plan for a minimum of 6 patients to confirm the RP2D (assuming zero DLTs occur), or a maximum of n=12 if one or more DLTs occur.

Table 1 shows the chance of recommending each dose as the MTD under six different scenarios, as well as the percentage of patients who will receive each dose over 1000 simulated trials. In scenarios where $4x10^8$ is the MTD (has true risk of DLT of at most 35%), there is at least an 89% chance that $4x10^8$ is recommended as the MTD, with a 8%-73% chance that the MTD will be declared after 6 patients rather than the maximum sample size of 12.

Table 1: Recommendation (Experimentation) percentages for phase I trial using mCRM across six scenarios (10,000 simulations per scenario). Start dose = $4x10^8$; mean prior belief of DLT = (5%, 10%, 35%); max. sample size of 12 patients; MTD declared as $4x10^8$ if no DLTs observed in first 6 patients (with first 6 patients dosed at $4x10^8$).

Scenario (Risks of DLT for doses $8x10^7$, $2x10^8$ and $4x10^8$)		**Dose**			**Percentage of trials stopping at 6 patients**
	No MTD (all doses unsafe or no patients given final estimated MTD)	**$8x10^7$**	**$2x10^8$**	**$4x10^8$**	
1 (5%, 10%, *35%*)	1%	0% (2%)	10% (12%)	89% (87%)	7.8%
2 (5%, *25%*, 45%)	2%	2% (5%)	32% (22%)	63% (73%)	2.5%
3 (5%, 10%, *15%*)	0%	0% (0%)	1% (3%)	99% (97%)	37.3%
4 (15%, *25%*, 45%)	2%	6% (7%)	29% (21%)	63% (73%)	2.5%

5 (1%, 5%, *10%*)	0%	0% (0%)	0% (1%)	100% (99%)	52.4%
6 (2%, 3%, *5%*)	0%	0% (0%)	0% (0.3%)	100% (99.7%)	73.0%

Italics is MTD/RP2D. Underlined scenario = true DLT probabilities are same as prior mean belief.

Phase II:

Standard response rates (Complete or Partial response) in this population on chemotherapy alone are in the region of about 25% [40, 41] The target response rate of at least 45% is considered reasonable based on a review of recent phase I/II studies in NSCLC and protocols published on the ISCRT website. Seto et al (2013) [42] powered for a more than doubling in response rates (from 25% up to 70%); Komiyama et al (2012) [43] a 15% improvement (10% vs 25%); Kurata et al (2012) [44] a difference of 15% (20% vs 35%) ; and Bral et al (2010) [45] reported response rates of 52%. Recently, Reck et al (2016) [1] observed a 44.8% response rate in PD-L1 positive NSCLC patients receiving pembrolizumab (updated to 45.5%) and Gandhi et al (2018) [4] observed a 47.6% response rate in NSCLC patients receiving pembrolizumab plus pemetrexed and platinum-based chemotherapy. Moreover, since MSCTRAIL is expensive to manufacture at this point in time, it would be important to demonstrate that any observed effect is likely to show cost-effectiveness in future trials. Therefore, in this trial detecting at least a 15% improvement from 45% to >70% is consistent with the type of effects we anticipate in early phase II NSCLC trials and possibly more cautious.

In total, 44 patients will be randomized 1:1 between the MSCTRAIL treatment arm and the placebo arm. Under a one-sided exact test at the 20% significance level we can detect a difference in response rate of at least 25% between treatment arms (i.e. 45% on control arm vs 70% on the investigational arm), with 80% power. To account for potential dropouts/non-evaluable patients, we will recruit 46 patients in total (assumes ~5% dropout). Accrual will be about 18-24 months, assuming approximately 2 patients per month are recruited.

Further to the primary test of efficacy compared to the placebo arm, we will also test whether the observed response rate in the MSCTRAIL arm is significantly different to a historical control rate of 45%, but under stricter type I and type II error criteria. To detect 25% improvement with MSCTRAIL and chemotherapy (i.e. to at least 70% response rate), at least 13 out of 22 patients are required to respond to MSCTRAIL. This assumes a power of about 90% and type I error (one sided) of 13%. The sample size method is based on using exact Binomial methods with approximate alpha [46].

17.2. Statistical analysis

17.2.1. Analysis of main endpoint

Phase I

Population for analysis.

For DLT analysis, the primary population for analysis will be the safety population (all patients who receive at least one dose of MSCTRAIL and one dose of SOC treatment).

For the efficacy assessments, patients included will be those who are included in the safety population and also have evaluable tumour response (i.e. non missing baseline tumour assessments).

Primary endpoint:

- The dose limiting toxicity (DLT) rate after first cycle of MSCTRAIL and SOC treatment per dose level
- Recommended phase II dose of MSCTRAIL in combination with SOC as first line treatment for lung adenocarcinoma

Model based analysis for the mCRM: Analysis of DLTs

The mCRM model will be used to model the risk of DLT after each patient (after the accelerated phase) to estimate the dose for subsequent patients using a one parameter power model with a vague log-Normal prior distribution (mean = 0, variance = 1.34 on the log scale). The DLT outcomes and dose assigned to each patient will be used in the statistical model. The model will be updated after each patient's outcome is known and the recommended dose for the next patient will be based on the smallest difference between the target toxicity level of 35% and the estimated probability of toxicity at each dose level. Uncertainty in the probability of DLT at each dose level will be given by Bayesian credible intervals. In addition, clinical judgment will be used to assist with dose assignment for patient cohorts, since the CRM is meant to be a guide to clinical decision for dose escalation.

At the end of phase I, empirical DLT rates per dose and those estimated from the model (with credible intervals to show uncertainty) will be presented. The dose level with estimated DLT rate closest to the Target Toxicity Level (35%) will be the proposed Recommended phase II Dose, subject to approval from the trial Independent Data Monitoring Committee.

Phase II:

Population for analysis.

The primary population for analysis will be the efficacy/intent to treat (ITT) population defined as all patients randomized who receive at least one dose of protocol (randomized) study medication (patients on the investigational arm must receive one dose of MSCTRAIL). For analysis of tumour response patients should also have evaluable tumour response (i.e. non missing baseline tumour assessments). The safety population will include all patients who receive protocol (randomised) study medication.

Primary endpoint:

- Tumour response rate by RECIST(v1.1) criteria after 12 weeks (iRECIST if patient received pembrolizumab)

At each visit patients will be programmatically assigned a RECIST (iRECIST if patient received pembrolizumab) visit response of CR, PR, SD or PD depending on the status of their disease compared to baseline and previous assessments. Progression of target

lesions (TL) will be calculated in comparison to when the tumour burden was at a minimum (i.e. smallest sum of diameters previously recorded on study). In the absence of progression, tumour response (CR, PR, SD) will be calculated in comparison to the baseline tumour measurements obtained before starting treatment. If a patient has had a tumour assessment, which cannot be evaluated, then the patient will be assigned a visit response of not evaluable (NE) unless there is evidence of progression in which case the response will be assigned as PD. If > 1/3 of lesions recorded at baseline are missing then the TL response will be NE. However, if the sum of non-missing TL diameters would result in PD (i.e. if using a value of 0 for missing lesions the sum of diameters has still increased by > 20% or more compared to the smallest sum of diameters on study), PD takes precedence over NE. A visit response of CR will not be allowed if any of the TL data is missing

Objective response rate is defined as the percentage of patients who have a confirmed visit response of CR or PR prior to any evidence of progression (as defined by RECIST 1.1 or iRECIST if patient received pembrolizumab). A visit response of CR is defined when all TLs and non-target lesions (NTLs) present at baseline have disappeared (with the exception of lymph nodes which must be <10mm to be considered nonpathological) and no new lesions have developed since baseline. A visit response of PR is defined when the sum of diameters of the TLs has decreased by 30% or more compared to baseline (with no evidence of progression) and the NTLs are at least stable with no evidence of new lesions.

In the case of stable disease, measurements should have met the stable disease criteria at least once during the study, observed at least 6 weeks after the start of treatment. When the investigator is in doubt as to whether progression of disease has occurred and therefore reassesses the patient at a later date, the date of the initial scan should be declared as the date of progression if the repeat scans confirm progression. Best overall response will be calculated as the best response recorded from date study treatment started for each patient. Percentage change in tumour size will be determined for patients with measurable disease at baseline and is derived at each visit by the percentage change in the sum of the diameters of TLs compared to baseline.

17.2.2. Analysis of secondary endpoints and secondary analyses

Phase I:

Secondary endpoints:

- Frequency of adverse events after the first cycle
- Best overall response,
- Change from baseline in sum of target lesions at 6 and 12 weeks
- Duration of response
- Progression free survival

Analysis of Adverse events

Other safety data will be summarised. All patients who receive at least one dose of MSCTRAIL will be included in the assessment of the safety profile (safety analysis set). At the end of the study, appropriate summaries of all safety data will be produced, as defined below. Data from all cycles of initial treatment will be combined in the presentation of safety data. Adverse events (AEs) will be listed individually by patient and dose group (dose and schedule). For patients who have a dose modification, all AEs (due to drug or otherwise) will be assigned to the initial dose group. The number of patients experiencing each AE will be summarised by the CTCAE grade. The number and percentage of patients with adverse events in different categories (e.g. causally related, CTCAE grade ≥3) will be summarised by dose group, and events in each category will be further summarised. Serious AEs will be summarised separately if a sufficient number occur.

Analysis of Tumour response

Tumour response data will be listed and summarised by dose, if appropriate, using the following response categories: Complete Response (CR), Partial Response (PR), Stable Disease (SD), Progressive Disease (PD) and Non-Evaluable (NE). In addition, the percentage of patients who have a confirmed PR or CR or have a visit response of SD that is at least 12 weeks after the first dose of study therapy will be summarised. Waterfall plots (bar charts) indicating the percentage change from baseline in sum of the diameters of TLs may be produced by dose level depending on how much data is obtained in patients with measurable disease at baseline. These may be individual patient plots of changes in tumour size over time or dose level plots with the best percentage change per patient displayed. If there is only limited data then percentage change in tumour size will be listed only. Duration of response will be summarised.

Progression Free Survival

Progression Free Survival (PFS) is defined as the time from randomization to time of progression (as per RECIST v1.1 criteria or iRECIST if patient received pembrolizumab) or time of death from any cause. Patients with no confirmed time of progression/death will be censored at the time that they were last confirmed as non-progressive/alive. PFS will be analysed using KM plots and will be presented along with median PFS time per dose level. PFS rate (along with 95% confidence interval) will be presented at 3, 6 and 12 months.

Phase II:

Secondary endpoints:

- Frequency of adverse events
- Best overall response
- Change from baseline in sum of target lesions
- Tumour response at each time point

- Duration of response
- Progression free survival
- Time to progression
- Overall survival

Frequency of Adverse events

Other safety data will be summarised. All patients who receive at least one dose of MSCTRAIL will be included in the assessment of the safety profile (safety analysis set). At the end of the study, appropriate summaries of all safety data will be produced, as defined below. Data from all cycles of initial treatment will be combined in the presentation of safety data. Adverse events (AEs) will be listed individually by patient and dose group (dose and schedule). For patients who have a dose modification, all AEs (due to drug or otherwise) will be assigned to the initial dose group. The number of patients experiencing each AE will be summarised by the CTCAE grade. The number and percentage of patients with adverse events in different categories (e.g. causally related, CTCAE grade ≥3) will be summarised by dose group, and events in each category will be further summarised. Serious AEs will be summarised separately if a sufficient number occur.

Tumour Response Analysis

Exact confidence intervals will be generated for the response rates (CR + PR + SD over number of patients) in the investigational arm for the best response and also at each visit. A logistic regression model will also be used to compare best response rates between arms adjusting for any covariates (e.g. Performance status, baseline tumour measurements). This analysis will also be repeated at 12 weeks and other time points if data are available. In addition, a mixed effects repeated measures model will be used to model response rates over time using generalized linear models with a logit link. Absolute differences in response rates at each time point and for the best response will also be computed with confidence intervals.

Waterfall plots (bar charts) indicating the percentage change from baseline in sum of the diameters of TLs may be produced by dose level depending on how much data is obtained in patients with measurable disease at baseline. These may be individual patient plots of changes in tumour size over time or dose level plots with the best percentage change per patient displayed. If there is only limited data then percentage change in tumour size will be listed **only**. Duration of response will be computed from the time of first response to progression or death and will be estimated using Kaplan Meier (KM) methods.

Progression Free Survival, Time to Progression, and Overall Survival

Progression Free Survival (PFS) is defined as the time from randomization to time of progression (as per RECIST v1.1 criteria or iRECIST if patient received pembrolizumab)

or time of death from any cause. Time to Progresson (TTP) is defined as the time from randomization to time of progression. Overall Survival (OS) is defined as the time from randomization to time of death from any cause. Patients with no confirmed time of progression/death will be censored at the time that they were last confirmed as non-progressive/alive. PFS, TTP and OS will be analysed using KM plots and will be presented along with median PFS, TTP and OS times. If the assumption of proportional hazards between MSCTRAIL and placebo is deemed reasonable, a Cox proportional hazards model will be fitted to the data for each endpoint and hazard ratios will be calculated. PFS, TTP and OS rates (along with 95% confidence intervals) will be presented at 3, 6 and 12 months.

18. ETHICAL AND REGULATORY CONSIDERATIONS

In conducting the trial, the Sponsor, UCL CTC and sites shall comply with all relevant guidance, laws and statutes, as amended from time to time, applicable to the performance of clinical trials including, but not limited to:

- the principles of ICH Harmonised Tripartite Guideline for Good Clinical Practice as set out in Schedule 1 (Conditions and Principles of Good Clinical Practice and for the Protection of Clinical Trial Subjects) of the Medicines for Human Use (Clinical Trials) Regulations 2004 and the GCP Directive 2005/28/EC, as set out in SI 2006/1928
- Human Rights Act 1998
- Data Protection Act 2018 and General Data Protection Regulation (EU)2016/679 (GDPR)
- Freedom of Information Act 2000
- Human Tissue Act 2004
- Medicines Act 1968
- Medicines for Human Use (Clinical Trials) UK Regulations SI 2004/1031, and subsequent amendments
- Good Manufacturing Practice
- Detailed guidelines on good clinical practice specific to advanced therapy medicinal products (ENTR/F/2/SF/dn D(2009) 35810)
- Genetically Modified Organisms (Contained use) Regulations 2014
- the UK Policy Framework for Health and Social Care Research, issued by the Health Research Authority , issued by the UK Department of Health (Second Edition 2005) or the Scottish Health Department Research Governance Framework for Health and Community Care (Second Edition 2006)
- Where applicable, UCL CTC and sites will work towards implementation of the EU Clinical trials Regulation EU/536/2014.

18.1. Ethical Approval

The trial will be conducted in accordance with the World Medical Association Declaration of Helsinki entitled ‘Ethical Principles for Medical Research Involving Human Subjects’ (1996 version) and in accordance with the terms and conditions of the ethical approval given to the trial.

The trial has received a favourable opinion from the Gene Therapy Advisory Committee and Health Research Authority (HRA) approval for conduct in the UK.

UCL CTC will submit Annual Progress Reports to the REC, commencing one year from the date of ethical approval for the trial.

18.2. Regulatory Approval

A Clinical Trial Authorisation (CTA) has been granted for the trial.

The trial will be conducted at approved trial sites in accordance with the trial protocol and the terms of the CTA granted by the MHRA.

18.3. Site Approvals

Evidence of assessment of capability and capacity by the Trust/Health Board R&D for a trial site must be provided to UCL CTC. Sites will only be activated when all necessary local approvals for the trial have been obtained.

18.4. Protocol Amendments

UCL CTC will be responsible for gaining ethical and regulatory approvals, as appropriate, for amendments made to the protocol and other trial-related documents. Once approved, UCL CTC will ensure that all amended documents are distributed to sites as appropriate.

Site staff will be responsible for acknowledging receipt of documents and for implementing all amendments promptly.

18.5. Patient Confidentiality & Data Protection

Patient initials and year of birth will be required for the registration (phase I) /randomization (phase II) process and will be provided to UCL CTC. UCL CTC will preserve patient confidentiality and will not disclose or reproduce any information by which patients could be identified. Data will be stored in a secure manner and UCL CTC trials are registered in accordance with the Data Protection Act 2018 and General Data Protection Regulation (EU)2016/679 (GDPR) with the Data Protection Officer at UCL.

19. SPONSORSHIP AND INDEMNITY

19.1. Sponsor Details

Sponsor Name: University College London

Address: [redacted]Office
[redacted]

Contact: Director of Research Support

Tel: [redacted](unit admin)
Fax: [redacted]

19.2. Indemnity

University College London holds insurance against claims from participants for injury caused by their participation in the clinical trial. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, as this clinical trial is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical trial. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.

Participants may also be able to claim compensation for injury caused by participation in this clinical trial without the need to prove negligence on the part of University College London or another party. Participants who sustain injury and wish to make a claim for compensation should be advised to do so in writing in the first instance to the Chief Investigator, who will pass the claim to the Sponsor's Insurers, via the Sponsor's office.

Hospitals selected to participate in this clinical trial shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to University College London, upon request.

20. FUNDING

The MRC is supporting the central coordination of the trial through UCL CTC.

Sites will be provided with some funding to assist with the coordination of the trial locally.

21. PUBLICATION POLICY

The first publication of the trial results will be in the name of the Trial Management Group, if this does not conflict with the journal's policy. The TMG (or a subgroup of the TMG) will form the basis of the writing committee and advise on the nature of publications. If there are named authors, these should include the Chief Investigator(s), Trial Coordinator(s), and Statistician(s) involved in the trial. Contributing site investigators in this trial will also be acknowledged. Data from all sites will be analysed together and published as soon as possible. Participating sites may not publish trial results prior to the first publication by the TMG or without prior written consent from the TMG. The trial data are owned by UCL. The Clinicaltrials.gov number allocated to this trial will be quoted in any publications resulting from this trial.

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APPENDIX 1: ABBREVIATIONS

ABPI	Association of British Pharmaceutical Industry
ADL	Activities of Daily Living
AE	Adverse Event
ALP	Alkaline phosphatase
ALK	Anaplastic Lymphoma Kinase gene
ALT	Alanine transaminase
ANC	Absolute Neutrophil Count
AR	Adverse Reaction
AST	Aspartate aminotransferase
ATIMP	Advanced Therapy Investigational Medicinal Product
AUC	Area Under the Curve
CEA	Carcinoembryonic Antigen
CI	Chief Investigator
CLRN	Comprehensive Local Research Network
CR	Complete response
CRF	Case Report Form
CT	Computerised Tomography
CTA	Clinical Trial Authorisation
CTAAC	Clinical Trials Advisory & Awards Committee
CTCAE	Common Terminology Criteria for Adverse Events
CTSA	Clinical Trial Site Agreement
CXR	Chest X-Ray
DFS	Disease Free Survival
DPA	Data Protection Act
DSUR	Development Safety Update Report
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDTA	Ethylene Diamine Tetra Acetate
EGFR	Epidermal Growth Factor Receptor
EEA	European Economic Area
EudraCT	European Clinical Trials Database
FBC	Full Blood Count
FSH	Follicle Stimulating Hormone
G-CSF	Granulocyte Colony Stimulating Factor
GFR GMO	Glomerular Filtration Rate Genetically Modified Organisms
Hb	Haemoglobin
HSE	Health and Safety Executive
HRT	Hormon Replacement Therapy
IB	Investigator's Brochure
ICH GCP	International Conference of Harmonisation-Good Clinical Practice
IDMC	Independent Data Monitoring Committee
IMP	Investigational Medicinal Product
INR	International Normalised Ratio
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Trial Number
IV	Intravenous
LDH	Lactate Dehydrogenase

LFT	Liver Function Tests
LLN	Lower Limit of Normal
MRC	Medical Research Council
MRI	Magnetic Resonance Image
MHRA	Medicines and Healthcare products Regulatory Agency
NCRI	National Cancer Research Institute
NCRN	National Cancer Research Network
NICE	National Institute for Health and Care Excellence
NRES	National Research Ethics Service
NSCLC	Non-small Cell Lung Cancer
OS	Overall Survival
PA	Posteroanterior
PD	Progressive Disease
PFS	Progression Free Survival
PI	Principal Investigator
PO	By mouth
PR	Partial Response
REC	Research Ethics Committee
RECIST	Response Evaluation Criteria in Solid Tumours
RSI	Reference Safety Information
RTOG	Radiotherapy Oncology Group
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SD	Stable Disease
SOC	Standard of Care
SODA	Summary Of Drug Arrangements
SPC	Summary of Product Characteristics
SSA	Site Specific Assessment
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
TMG	Trial Management Group
UCL CTC	CR UK and UCL Cancer Trials Centre
U&E	Urea and Electrolytes
ULN	Upper Limit of Normal
WBC	White Blood Cells
WOCBP	Women Of Child Bearing Potential

APPENDIX 2: QUICK REFERENCE GUIDE TO PATIENT ASSESSMENTS DURING PHASE I TREATMENT

Assessment			Interventional phase of study															
	Pre Intervention		Cycle 1 Pemetrexed/Cisplatin/ Pembrolizumab & ATIMP administration					Cycle 2 Pemetrexed/Cisplatin /Pembrolizumab & ATIMP administration			Cycle 3 Pemetrexed/Cisplatin /Pembrolizumab & ATIMP administration			Cycle 4 Pemetrexed / Cisplatin / Pembrolizumab			Follow Up
	Prior to registration	Within 14 days prior to registration	Day 1	Day 2	Day 3	Day 8	Day 15	Day 1	Day 2	Days 3, 8 and 15	Day 1	Day 2	Days 3, 8 and 15	Day 1	Day 15	Day 21	Every 6 weeks until 24 months after end of treatment
Interventions																	
Pemetrexed/Cisplatin /Pembrolizumab Infusion			x					x			x			x			
MSCTRAIL Infusion				x					x			x					
Examination/Investigation																	
Clinical Review			x	x	x	x	x	x	x		x	x		x		x	x
Physical examination		x	x	x	x	x	x	x	x		x	x		x		x	x
Vital signs **(1)**		x	x	x	x	x	x	x	x		x	x		x		x	x
ECG		x		x[2]	x	x	x		x[2]			x[2]				x	
Weight		x						x			x			x			
ECOG status		x						x			x			x			
CT Scan	X[6]									X[6]						X[6]	X[6]
Laboratory tests																	
Haematology (FBC) **(3)**		x	x			x	x	x			x			x		x	
Oncological Profile **(4)**		x	x			x	x	x			x			x		x	
Urinalysis			x					x			x			x			
Transaltional research Sample **(5)**			x	x	x	x	x	x	x	x	x	x	x	x	x		
Thyroid Function tests(8)		x									x					x	
Pregnancy test (if needed)		x	x					x			x						
Adverse event and Con Med collection		x	x	x	x	x	x	x	x		x	x		x		x	

Assessment			Interventional phase of study														Follow Up
	Pre Intervention		Cycle 1 Pemetrexed/Cisplatin/ Pembrolizumab & ATIMP administration					Cycle 2 Pemetrexed/Cisplatin /Pembrolizumab & ATIMP administration			Cycle 3 Pemetrexed/Cisplatin /Pembrolizumab & ATIMP administration			Cycle 4 Pemetrexed / Cisplatin / Pembrolizumab			
	Prior to registration	Within 14 days prior to registration	Day 1	Day 2	Day 3	Day 8	Day 15	Day 1	Day 2	Days 3, 8 and 15	Day 1	Day 2	Days 3, 8 and 15	Day 1	Day 15	Day 21	Every 6 weeks until 24 months after end of treatment
Pre- Registration Assessments Only																	
Informed Consent		x															
Cancer signs & symptoms		x															
Height		x															
Procurement of FFPE block		x															
Renal Function (GFR)		x															
Coagulation Screening **(7)**		x															
Histological Confirmation	x																

(1) Vital signs include heart rate, oxygen saturation, blood pressure, and pulse (blood pressure and pulse to be measured after 2 minutes supine rest; heart rate and oxygen saturation monitored continuously throughout MSCTRAIL infusion)
(2) On the day of MSCTRAIL infusion ECG tests will be performed pre and 4 hours post infusion
(3) FBC including haemoglobin, white cell count, platelets, neutrophils
(4) Oncological profile – including sodium, potassium, urea, creatinine, bilirubin, ALP, AST or ALT, LDH, albumin, total protein, calcium, magnesium, glucose, CRP
(5) Up to 30ml blood sample will be collected during cycles 1-3 on Day 1, Day 2 (pre MSCTRAIL-infusion, 3 and 6hrs post MSCTRAIL), Day 3, 8 and 15; up to 30mls blood samples on days 1 and 15 during cycle 4. Samples sent to the the research laboratoryat UCL as detailed in section 10 and the Laboratory manual for the study.
(6) Screening CT scan performed within 28 days of registration; post treatment CT scans at weeks 6 and 12 (within 5 days of D21 of C2 or C4), 6 weekly during follow up until evidence of progression, then 3 monthly up to 1 year, 18 and 24 months after end of treatment
(7) Coagulation screen: PT, APTT and INR
(8) Patient's receiving pembrolizumab will undergo thyroid function tests (T4 and TSH) at the specified timepoints. Following the end of cycle 4 patient's will continue to have these tests every 6-8 weeks according to site local policy

APPENDIX 3: QUICK REFERENCE GUIDE TO PATIENT ASSESSMENTS DURING PHASE II TREATMENT

Assessment	Pre Intervention		Interventional phase of study											Follow Up
			Cycle 1 SOC & ATIMP / Placebo administration					Cycle 2 SOC & ATIMP / Placebo administration		Cycle 3 SOC & ATIMP / Placebo administration		Cycle 4 Standard of care treatment		
	Prior to registration	Within 14 days prior to registration	Day 1	Day 2	Day 3	Day 8	Day 15	Day 1	Day 2	Day 1	Day 2	Day 1	Day 21 of cycle 4	Every 6 weeks until 24 months post end of treatment
Interventions														
Pemetrexed/Cisplatin/ Pembrolizumab Infusion			x					x		x		x		
MSCTRAIL / Placebo Infusion				x					x		x			
Examination/Investigation														
Clinical Review			x	x	x	x	x	x	x	x	x	x	x	x
Physical examination		x	x	x	x	x	x	x	x	x	x	x	x	x
Vital signs **(1)**		x	x	x	x	x	x	x	x	x	x	x	x	x
ECG		x		x^2	x	x	x		x^2		x^2	x	x	
Weight		x						x		x		x		
ECOG status		x	x					x		x		x		
CT Scan	X(5)								X (D14-21)				X**(5)**	x**(5)**
<u>Laboratory tests</u>														
Haematology (FBC) **(3)**		x	x			x	x	x		x		x	x	
Oncological Profile **(4)**		x	x			x	x	x		x		x	x	
Urinalysis			x					x		x		x		
Thyroid Function tests(7)		x								x			x	

Assessment	Pre Intervention		Interventional phase of study											Follow Up
			Cycle 1 SOC & ATIMP / Placebo administration					Cycle 2 SOC & ATIMP / Placebo administration		Cycle 3 SOC & ATIMP / Placebo administration		Cycle 4 Standard of care treatment		
	Prior to registration	Within 14 days prior to registration	Day 1	Day 2	Day 3	Day 8	Day 15	Day 1	Day 2	Day 1	Day 2	Day 1	Day 21 of cycle 4	Every 6 weeks until 24 months post end of treatment
Pregnancy test (if applicable)		x	x					x		x				
Adverse event and Con Med collection		x	x	x	x	x	x	x	x	x	x	x	x	
Pre- Randomisation Assessments Only														
Informed Consent		x												
Cancer signs & symptoms		x												
Height		x												
Procurement of FFPE block		x												
Renal Function (GFR)		x												
Coagulation Screening **(6)**		x												
Histological Confirmation	x													

(1) Vital signs include heart rate, oxygen saturation, blood pressure, and pulse (blood pressure and pulse to be measured after 2 minutes supine rest; heart rate and oxygen saturation monitored continuously throughout MSCTRAIL/placebo infusion)

(2) On the day of MSCTRAIL infusion ECG tests will be performed pre and 4 hours post infusion

(3) FBC including haemoglobin, white cell count, platelets, neutrophils

(4) Oncological profile – including sodium, potassium, urea, creatinine, bilirubin, ALP, AST or ALT, LDH, albumin, total protein, calcium, magnesium, glucose, CRP

(5) Screening CT scan performed within 28 days of randomisation; post treatment CT scans at weeks 6 and 12 (within 5 days of D21 of C2 and C4), then 6 weekly during follow up until evidence of progression, then 3 monthly up to 12 months, then a further scan at 18 months

(6) Coagulation screen: PT, APTT and INR

(7) Patient's receiving pembrolizumab will undergo thyroid function tests (T4 and TSH) at the specified timepoints. Following the end of cycle 4 patient's will continue to have these tests every 6-8 weeks according to site local policy

APPENDIX 4: ECOG PERFOMANCE STATUS

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

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

APPENDIX 5: PROTOCOL VERSION HISTORY

Protocol:		**Amendments:**		
Version no.	**Date**	**Amendment no.**	**Protocol Section (no./title)**	**Summary of main changes from previous version.**
1	03/11/2017	N/A		
1.1	10/01/2018	N/A *changes in response to MHRA initial review prior to CTA*	12.2.2.Reporting of SAEs	Start of SAE reporting period changed from 'C1 D2 (1st MSCTRAIL/placebo infusion) to 'time of consent signed' 'Disease progression (including disease related deaths)' are no longer exempt from SAE reporting and must be submitted to CTC on SAE report within 24hrs of site learning about the event.
			3.Trial Design	Paragraph on 'Dose selection' has been added to justify to the selected MSCTRAIL starting dose and subsequent dose de-escalations.
			8.1.3.IMP/placebo Administration Details	Infusion changed from 'as quickly as practically possible' to 'over 20-30 minutes'
			12.6 Safety communication	New subsection detailing communication of SUSARs, DLTs and safety-related protocol deviations to participating sites added.
			Throughout protocol	Phase I of the trial has been changed from 'multi-site' to 'single-site'

2.0	23/02/2018	N/A Changes in reponse to GTAC initial review prior to trial approval	Throughout protocol	Clarification that the trial treatment in this study is defined as 3 cycles cisplatin/ pemetrexed and MSCTRAIL (or placebo in phase II) followed by a 4th cycle of cisplatin / pemetrexed (without MSCTRAIL/placebo). After completion of the four cycles of trial treatment, the patient will revert to local standard of care therapy as decided by their treating clinician.
			Throughout protocol	The follow up period for patients in phase I has been changed from 12 months to 24 months to be the same as for patients in phase II.
			Throughout protocol	Clarification that a decision by the TMG/IDMC will be needed before the trial can proceed with phase II and any changes to the trial conduct as advised by the TMG/IDMC will be submitted for approval as substantial amendment before the start of phase II.
			Throughout protocol	The 2nd randomised phase of the study changed from 'single blind' to 'double blind' trial.
			Throughout protocol	Removal of requirement for NHS number and full date of birth. Only patient initials and year of birth will be collected.
			6.3.Pregnancy and birth control	Contraception period changed from 12 months to 6 months after end of trial treatment
3.0	05/11/18	SA02 Update to clarify standard of care	6.2 and Trial Summary	Removal of PDL1 expression <50% as inclusion criteria Removal of *'any contraindication to the administration and use of cisplatin, pemetrexed, vitamin B12 or folic acid'* as an exclusion criteria as patients may not receive chemotherapy regimen
			6.3	Clarification to the definitions of WOCBP
			Throughout Protocol	Protocol has been updated to allow patients to receive pembrolizumab if clinically appropriate following the update of standard of care treatment for the patient population, Clarification that iRECIST will be used for disease assessments when patients are treated with pembrolizumab
			Section 10	Change in central laboratory from CRUK institute, Manchester to Lungs for Living Research Centre, UCL, London. Translational samples will initially be stored at lab then used to investigate biomarkers of apoptosis, Donor immune response to allogeneic MSCTRAIL therapy and future ethically approved research.
			Section 17	Statistics section updated due to emerging data in disease area and pembrolizumab treatement

4.0	14/02/19	SA03 Update to trial inclusion criteria and toxicity management of pembrolizumab	1.1 & 6.2	ALT or AST inclusion criteria changed from <3 x ULN to <2.5 x ULN in the absence of liver metastases Exclusion Criteria: - Severe intercurrent infection replaced by active infection requiring systemic therapy - Live vaccination exclusion updated to 'within 30 days prior to trial registration, during dose administration and 90 days after last dose' - Clarity that contraindications to cisplatin, pemetrexed, vitamin b12 or folic acid only relates to patients receiving chemotherapy - Additional exclusion criteria relating to pembrolizumab
			8.6.4	Addition of possible pembrolizumab toxicities and precautions/treatment
			9.4 & appendices	Addition of thyroid function tests for pembrolizumab patients
			12.2	Update to AE reporting timeframe to clarify that if an investigator feels that an event is related to the MSCTRAIL or trial procedures the AE should be reported, even if it occurs outside the stipulated 3 weeks post last MSCTRAIL infusion timeframe

(INSERT HOSPITAL/INSTITUTION LOGO HERE WITH CR UK LOGO INCLUDED)

PATIENT INFORMATION SHEET – PHASE 1

TACTICAL

Targeted stem cells expressing TRAIL as a therapy for lung cancer

IRAS No.: 228124

We are inviting you to take part in a research study called TACTICAL

- We would like to invite you to take part in a research study.
- You are free to decide if you want to take part. If you choose not to take part, this will not affect the care you receive in any way.
- Before you decide whether or not to take part, we will go through this Patient Information Sheet with you and answer any questions you may have, so that you fully understand why we are carrying out this research and what it would involve for you.
- Please take the time to read the information carefully and talk to others about the study if you wish. Ask us if there is anything you don't understand or if you would like more information. Take your time to decide whether or not you wish to take part.
- You can decide to stop taking part in the study at any time without giving a reason.
- If you decide to take part, we will ask you to sign a form to give your consent to take part in the study.

The first part of the Patient Information Sheet provides you with a summary and if you would like to find out more, goes on to tell you about the purpose of the study and what will happen if you take part. Then we give you more detailed information about the conduct of the study. A glossary is also provided at the end of the Patient Information Sheet to describe any acronyms or abbreviations used.

Summary of the research study

- We are carrying out this research to test a possible new therapy for lung cancer. The study will be looking to see if genetically modified stromal cells (called MSCTRAIL) are a safe therapy for certain lung cancers.
- We have altered these stromal cells with a specific protein called TRAIL that we hope will treat cancer.
- This study is defined as phase 1, which means the therapy has not previously been tested in humans. It is important to know you may not directly benefit from taking part.
- As well as testing whether the therapy is safe, we will also be looking for the best tolerated dose of the modified cells.
- You are being considered for this study because you have been diagnosed with inoperable adenocarcinoma of the lung and are healthy enough to have chemotherapy.
- If you enter the study, as well as the standard treatment for your condition (4-6 cycles of cisplatin and pemetrexed and/or pembrolizumab), you will also receive an additional intravenous therapy of the genetically modified cells (MSCTRAIL) for the first 3 cycles.
- It is important to know that by joining the study, you will attend the hospital more often than you would normally have to. This will be explained further in section 2.

Who is organising and funding the research?

The study is funded by the Medical Research Council, sponsored by University College London and run by Cancer Research UK & UCL Cancer Trials Centre (UCL CTC).
Your doctor will not be paid for including you in the study.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect the interests of any patients that may take part. This study has been reviewed and granted a favourable opinion by the London – West London & Gene Therapy Advisory Committee (GTAC) Research Ethics Committee and has also been approved by the Research and Development department at your hospital and the Medical Research Council.

How have patients been involved in the study?

In designing this study, we have taken into account patient opinions on the information provided in this Patient Information Sheet.

Members of the UCL/UCLH Cancer Research Patient Representative Group have been involved in reviewing the content of the Patient Information Sheet and have provided helpful comments.

Commercial exploitation

Data collected in this study may be included as part of an application to license this treatment. You will not be identified in this application and you will not benefit financially from this.

If you have questions

We hope you find this information sheet helpful. We appreciate it may not answer all of your questions, so please do not hesitate to contact us on the telephone numbers given at the end of this Patient Information Sheet if you would like to discuss any aspect of the study further.

Contents

1. Why are we doing this study?

Adenocarcinoma is the most common type of lung cancer. Treatment depends on how much the tumour has grown or spread, how abnormal the cells look and the patients general health and level of fitness. The main treatment options are surgery, chemotherapy, radiotherapy and biological therapies.

If patients cannot be cured by surgery they may be treated with one of the following treatments:

- Two chemotherapy drugs (usually cisplatin and pemetrexed)
- An immunotherapy drug called pembrolizumab
- A combination of chemotherapy and immunotherapy

Throughout this information sheet, when 'standard treatment' is mentioned, we are referring to one of the above regimens.

The chemotherapy treatments often control the cancer for a number of months but disease may come back and/or spread further. Immunotherapies are newer treatments which can help your body control and kill cancer cells but these do not work in all patients and again the disease may come back and/or spread further. This has led us to look for new more effective treatment options.

The aim of this study is to develop a new targeted therapy (MSCTRAIL) given alongside conventional treatments (such as cisplatin and pemetrexed or immunotherapy) to try to improve treatment for adenocarcinoma.

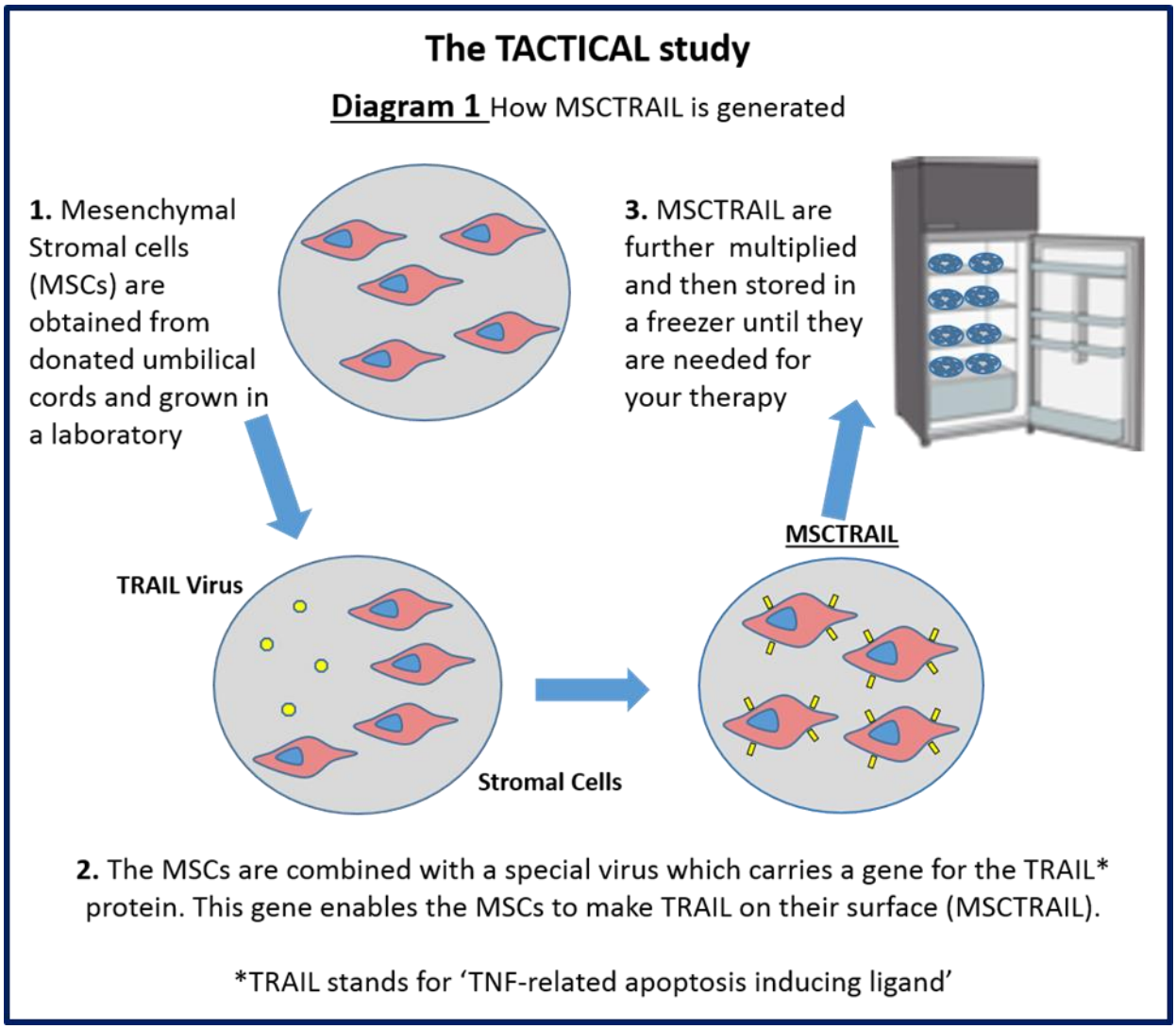
2. What is this study about?

Mesenchymal stromal cells (MSC) occur naturally in the body. They have the capability to divide and multiply easily and grow into different types of cells. Research suggests they are also able to recognise cancer cells and travel to them.

We have obtained MSCs from multiple umbilical cords donated through the Anthony Nolan (a blood cancer charity). We have genetically modified these MSCs using a special virus which carries a gene for a protein called TRAIL (TNF Related Apoptosis Inducing Ligand). The virus has been changed so it cannot grow and cause infection, but instead lets the MSCs make the TRAIL protein. TRAIL has been shown to cause death in different types of cancer cells when tested in a laboratory.

We will give these genetically modified MSCs (MSCTRAIL) to you along with the standard treatment for your cancer. We think these MSCs will act as a carrier to bring the anti-cancer therapy (TRAIL) directly to the site of cancer. We want to test if giving these modified MSCs (MSCTRAIL) to patients is safe and can be used as a therapy for lung adenocarcinoma.

MSCTRAIL are manufactured at the Centre for Cell, Gene & Tissue Therapeutics at the Royal Free Hospital (see details in diagram 1)



THE STUDY HAS TWO PHASES.

Clinical studies are divided into different stages, called phases. A phase I study is often the first time a new therapy is tested in humans. These studies look at whether the new therapy is safe to give, what the side effects are, how well the body copes and what is the right dose to give. They are usually small studies, recruiting only a few patients.

YOU ARE INVITED TO TAKE PART IN PHASE I

This phase I trial will assess the safety of MSCTRAIL in combination with the standard treatment for lung adenocarcinoma and the best dose to give. We will treat between 6 and 18 patients in this phase of the study.

Once phase I is complete, a formal review will take place to assess the side effects of the therapy and to determine the best tolerated dose of MSCTRAIL. The study will continue with the second phase if this review confirms that MSCTRAIL is tolerated and it is safe.

Phase II studies often involve more patients than phase I and may compare the new therapy with another treatment already in use, or with a dummy drug (placebo). They aim to find out more about side effects and how to manage them, and whether the new therapy works well enough to test in a large (phase III) trial.

The second phase of this study will assess whether MSCTRAIL alongside standard treatment may be better for treating patients with adenocarcinoma than standard treatment alone. Phase II will include a further 46 patients. Half of the patients will be given MSCTRAIL (at the dose established in phase I) with standard treatment, while the other half will have placebo (a 'dummy treatment') instead of MSCTRAIL as well as standard treatment. The allocation for each patient will be decided at random by a computer. Patients will not know whether they will receive MSCTRAIL or placebo (this is called a blinded trial). The purpose of allocating the therapy randomly and of the blinding is to ensure the results of the study are reliable.

3. Why have I been invited to take part?

- You have inoperable adenocarcinoma of the lung.
- You have stage 3b or stage 4 cancer. This means that your cancer has spread from its original site so cannot be controlled by surgery alone.
- Your health and fitness level mean you could receive chemotherapy or immunotherapy.
- You are therefore a potential candidate for MSCTRAIL research.

4. Do I have to take part?

No. It is up to you to decide whether or not to take part in the study. We will describe what would be involved and go through this Patient Information Sheet with you. You should take it away so that you can read it carefully and discuss with others if you wish. If you decide to take part, we will ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. If you decide not to take part, or later to withdraw, this will not affect the standard of care you receive.

If you decide to withdraw after you have received the modified stromal cells (MSCTRAIL), you will not need to attend any further clinic visits for the study. We may need to continue to collect safety data about you from your doctor because the study is looking at how safe MSCTRAIL is.

5. What will happen to me if I take part?

Consent

We will explain the study to you in detail and answer your questions. You can discuss participation with your family and friends. Take as much time as you need to consider taking part.

If you agree to take part, you will be asked to sign a consent form. We will give you a copy of the form and a copy of this information leaflet to take away.

If you chose not to take part or do take part and withdraw, your doctor will talk through alternative treatment options with you. The care you receive will not be affected in any way.

Screening

You will have or will already have had some initial tests to ensure you are suitable for the study - we call these screening tests.

These include:

- Clinical review including your medical history and medications
- Physical review including examination, height and weight
- Electrocardiogram (ECG) measuring of heart
- Blood tests to look at kidney, liver and blood function
- Computerised tomography (CT) scan of chest, abdomen and pelvis
- Pregnancy test (if applicable)

These tests, with the exception of the pregnancy test, form part of routine clinical care and you would need them whether you take part in the trial or not.

Once your assessments are complete we will tell you whether the study is suitable for you.

We would also like to collect blood samples to study how MSCTRAIL affects the cancer cells (see details in section 'What will happen to any samples I give'). We will take blood samples throughout the study for your regular assessments and would like to take an additional up to 30mls (about 6 teaspoons) at some of these visits for this analysis.

You would have previously had a biopsy where a small sample of your tumour tissue was taken for testing. With your permission, if some of this previously collected tissue sample is available, we would like to use it for research related to this study and to lung cancer.

Treatment (Phase 1)

All patients have MSCTRAIL with standard treatment (either Pemetrexed/Cisplatin chemotherapy, Pembrolizumab or both) (diagram 2).

Standard treatment will be given at the same dose and over the same time schedule as patients not in the study. The two chemotherapy agents are called Pemetrexed and Cisplatin (if you do not tolerate cisplatin, your doctor may decide to give you carboplatin instead). If you receive immunotherapy, this will be with an agent called Pembrolizumab.

You will receive 4 cycles of standard treatment for lung adenocarcinoma: given every 21 days. You will have MSCTRAIL for the first 3 cycles in addition to the chemotherapy/immunotherapy.

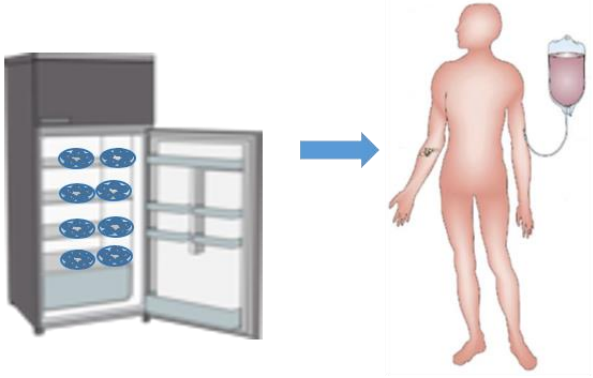
After 4 cycles you may have further treatment if your oncologist thinks that you need it. This further treatment will be according to the 'standard of care' at your hospital and will not be part of this study.

The TACTICAL study

	Cycle 1 (3 weeks)		Cycle 2 (3 weeks)		Cycle 3 (3 weeks)		Cycle 4 (3 weeks)	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Standard Treatment*	•		•		•		•	
MSCTRAIL		•		•		•		

*Standard treatment will be either Pemetrexed & Cisplatin only, Pembrolizub only or Pemetrexed & Cisplatin & Pembrolixumab

If you are eligible for the trial you will receive MSCTRAIL infusions 3 times alongside 4 cycles of standard treatment (Pemetrexed & Cisplatin and/or Pembrolizumab).
Standard treatment will be given on day 1 of each cycle. MSCTRAIL will be given on day 2 of the first 3 cycles



You will be infused with MSCTRAIL 3 times over the 4 treatment cycles. Studies in the lab have shown that MSCs can recognise the cancer cells and travel to them.
We hope that the TRAIL protein on the surface if the MSCs will then cause the cancer cells to die whilst leaving healthy cells unaffected

Diagram 2 What will happen to you if you take part in TACTICAL

What is included in a cycle of treatment?

Prior to any therapy, you will have a brief clinical review including observations and vital signs. You may also require blood tests, urinalysis and a tracing of your heart (ECG). This will ensure you are well enough to receive further therapy and to monitor for any side effects.

These tests will be carried out by a trained specialist who understands the procedure and will be able to talk you through everything they are doing.

You will receive standard treatment and MSCTRAIL through a drip into your arm, we call this intravenous. A nurse will put a small tube (a cannula) into one of your veins and connect the drip to it. This will be removed after you have had the treatment for the day.

Sometimes people need a more permanent longer plastic tube that gives drugs into a larger vein in their chest or arm. If you already have one of these tubes in place, we can use this instead of a cannula for chemotherapy, immunotherapy and MSCTRAIL .

You will receive therapy as follows:

Day 1

If you receive chemotherapy:

Pemetrexed - chemotherapy given intravenously over 10 mins; cycles 1 - 4

Cisplatin – chemotherapy given intravenously 30mins later and over 2 hours (you may require fluids to be given before and after to keep you well hydrated); cycles 1 – 4

If you receive immunotherapy:

Pembrolizumab – immunotherapy given intravenously over 30 mins: cycles 1-4

Day 2

MSCTRAIL - given intravenously over 30mins on day 2 of cycles 1-3

Each cycle is 21 days.

Following MSCTRAIL infusion (day 2 of cycles 1, 2 and 3) you will need to stay in the hospital for up to 6 hours. During this time you will have observations taken regularly, these will include temperature, heart rate, blood pressure and checking the oxygen level in your blood. This allows us to check you are well after MSCTRAIL infusion. You will also have blood samples taken.

We may ask you to stay overnight if we think we need to observe you longer.

If you subsequently need treatment for complications you may need to be re-admitted to hospital as normal.

Supportive medications

During the course of your therapy you will be given medications to help prevent some side effects. These may include anti-sickness medication that is given routinely to patients receiving chemotherapy or immunotherapy. It is also important to stay well hydrated and you may require some intravenous fluids to help with this.

If you require further medications while on the study these will be decided by your doctor.

If you are treated by a doctor that is not part of the study, we ask that they contact us on the 24 hour trial phone, before giving any non-emergency medications.

Visits during trial cycles

The main aims of the phase I part of the study are to find out whether this therapy has any significant side effects and what the best MSCTRAIL dose to give patients is. This is why it is very important you attend all visits as required so we can monitor your progress and health.

Visits during cycle 1

In addition to the infusion days during cycle 1 (Day 1 and Day 2), you will need to come for assessments on days 3, 8 and 15. During these visits you will have a clinical examination, ECG and may have some blood tests. These are to ensure you are fit for further infusions and assess any side effects you may have.

Visits during cycles 2-3

You will need to visit the hospital on the treatment days (day 1 for standard treatment and day 2 for MSCTRAIL) and will have a clinical review, ECG as well as blood and urine tests. You will also need to come on days 3, 8 and 15 to give a blood sample. At the end of cycle 2 you will have a CT scan.

Cycle 3 will include your final dose of MSCTRAIL.

Visits during cycle 4

You will need to visit the hospital on day 1 of cycle 4 for your standard treatment (you will not have further MSCTRAIL infusions). This visit will also include a clinical review and blood and urine tests. You will also need to come on day 15 to give a blood sample.

At the end of cycle 4 you will be reviewed by your clinician and have a full examination including vital signs (heart rate, blood pressure), ECG, bloods tests (full blood count, oncology profile, liver and kidney function), medication check and a CT scan (to assess your disease).

Based on the results after cycle 4, your doctor may advise you to continue standard treatment as they would if you were not in the trial. Pemetrexed/cisplatin may be given for another 1-2 cycles (up to maximum of 6), pembrolizumab may be given every 3 weeks for up to 2 years but these visits will no longer be because you take part in the study. If you continue with further cycles of standard treatment (after cycle 4), you will have these visits and assessments according to the local standard of care.

Follow up Visits after cycle 4

Following completion of the 4th cycle of standard treatment we will ask you to continue to attend the clinic for regular follow ups.

Visits will occur every 6 weeks until 2 years after the 4th cycle. At each visit you will have a clinical review, physical examination and a CT scan.

If you received pemetrexed and/or pembrolizumab, your doctor may also advise you to continue this treatment (or with another appropriate therapy) according to the local standard of care as you would if you were not in trial. This is called maintenance therapy. You may need to visit the hospital for this standard of care maintenance therapy but these visits will no longer be because you take part in the study.

Total Visits

From screening to the end of cycle 4 (visits while on study therapy) you will need to attend the hospital 19 times. Of these visits, 6 will be routine care (you will have these visits whether you decide to be in the study or not). 13 of the visits are study related and additional to routine care (you would not have to attend these visits if you are not taking part in the study)

After cycle 4 you may have further treatment but these visits will be part of routine care and not part of the study.

Routine visits are shown in black text in Diagram 3 whilst additional visits in green text.

Please see diagram 3 on the next page summarising the study visits and investigations required.

6. What will I have to do?

It is important that you attend all scheduled appointments for clinic visits and tests as described above.

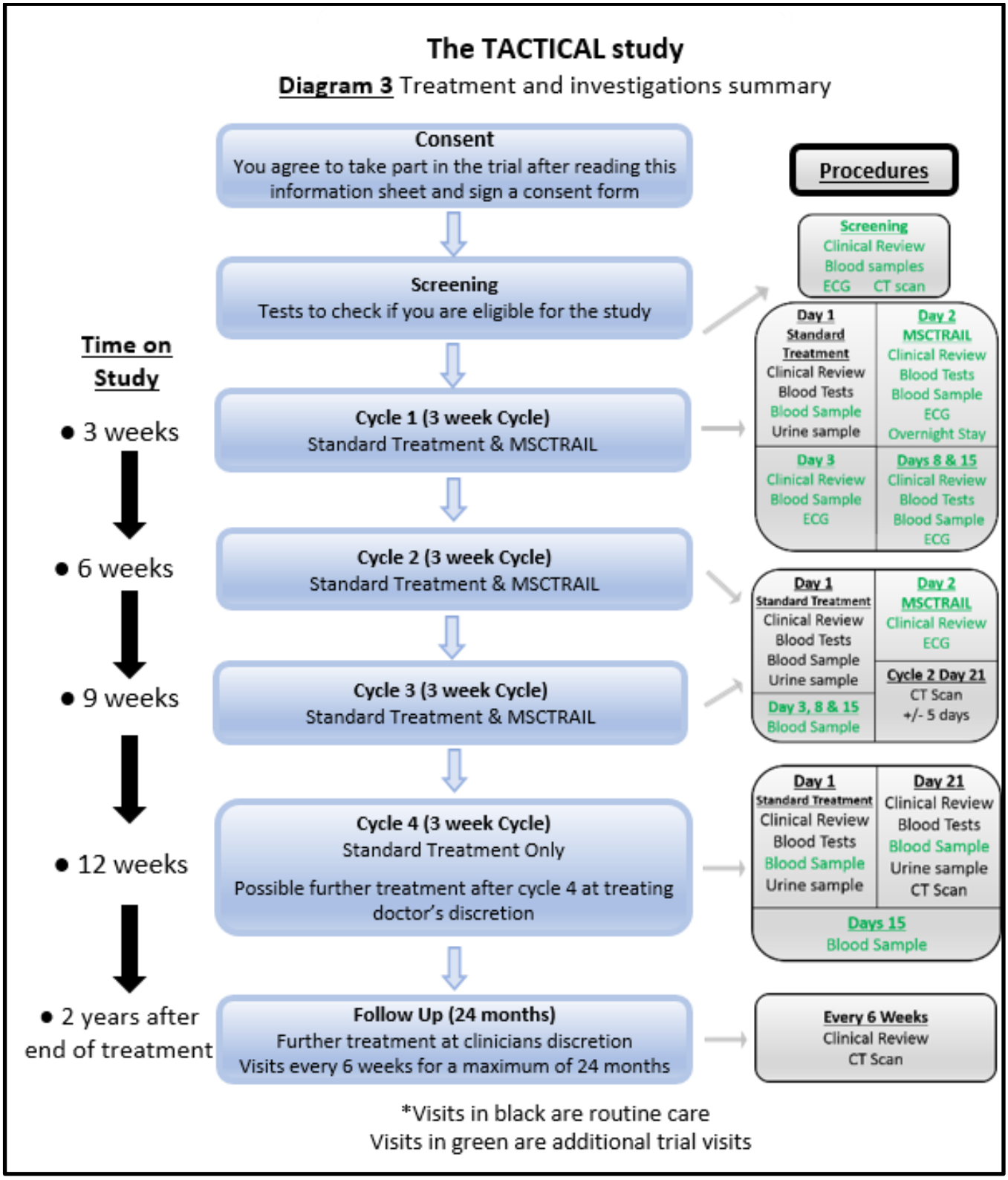
You should let us know about any side effects you may have experienced between the scheduled visits.

If you already take any regular medications you should let us know so we can check that they can be continued while you are on the study.

You should also let us know before taking any new medications while you are receiving the study therapy. This includes 'over-the-counter' treatments as some medications may interact with the study therapy. We would ask you not to take any over-the-counter, herbal or homeopathic medication except for paracetamol without discussing it with us first.

It is essential you inform us if you are prescribed new medication.

If you become unwell you should seek medical help as required. We are available to offer advice to you as well as your GP and other standard NHS services. We ask that if you do have contact with other doctors and healthcare professionals that you tell them of your involvement in the study. We will give you a contact card with information about the study that you can show them.


The TACTICAL study
Diagram 3 Treatment and investigations summary
Consent
You agree to take part in the trial after reading this information sheet and sign a consent form
Screening
Tests to check if you are eligible for the study
Cycle 1 (3 week Cycle)
Standard Treatment & MSCTRAIL
Cycle 2 (3 week Cycle)
Standard Treatment & MSCTRAIL
Cycle 3 (3 week Cycle)
Standard Treatment & MSCTRAIL
Cycle 4 (3 week Cycle)
Standard Treatment Only
Possible further treatment after cycle 4 at treating doctor's discretion
Follow Up (24 months)
Further treatment at clinicians discretion
Visits every 6 weeks for a maximum of 24 months
Time on Study
• 3 weeks
• 6 weeks
• 9 weeks
• 12 weeks
• 2 years after end of treatment
Procedures
Screening
Clinical Review
Blood samples
ECG CT scan
Day 1
Standard Treatment
Clinical Review
Blood Tests
Blood Sample
Urine sample
Day 2
MSCTRAIL
Clinical Review
Blood Tests
Blood Sample
ECG
Overnight Stay
Day 3
Clinical Review
Blood Sample
ECG
Days 8 & 15
Clinical Review
Blood Tests
Blood Sample
ECG
Day 1
Standard Treatment
Clinical Review
Blood Tests
Blood Sample
Urine sample
Day 2
MSCTRAIL
Clinical Review
ECG
Day 3, 8 & 15
Blood Sample
Cycle 2 Day 21
CT Scan
+/- 5 days
Day 1
Standard Treatment
Clinical Review
Blood Tests
Blood Sample
Urine sample
Day 21
Clinical Review
Blood Tests
Blood Sample
Urine sample
CT Scan
Days 15
Blood Sample
Every 6 Weeks
Clinical Review
CT Scan
*Visits in black are routine care
Visits in green are additional trial visits

7. What are the side effects of the therapy received during the research study?

All drugs and procedures can cause side effects. Side effects vary from person to person and can range from mild to severe and sometimes can be life threatening. We do not expect you will have all or even most of the side effects listed but we cannot predict which ones you may experience or how serious they will be.

General Chemotherapy/Immunotherapy Side Effects

Many of the side effects of Pemetrexed, Cisplatin and Pembrolizumab are well known. The side effects for the standard treatment you receive will be discussed with you separately as they are not specific to this trial. Your doctor will give you a leaflet and explain the side effects and general risk of chemotherapy and/or immunotherapy. A list of the most common chemotherapy/immunotherapy side effects can also be found in Appendix 1 of this information sheet**.** You can also ask your doctor for the Patient Information Leaflets on Pemetrexed, Cisplatin or Pembrolizumab if you want to see a detailed list of their individual side effects.

MSCTRAIL potential side effects

As MSCTRAIL is a new therapy and has never been given before we do not know the full extent of the side effects.

SIGNS AND SYMPTOMS TO BE AWARE OF:

- Fevers and sweats which may indicate you have an infection or a low immune system
- Worsening breathlessness
- Chest pain or palpitations
- Dizziness or episodes of collapse
- Local skin irritation at the site of infusion

Side Effects	Symptoms
Local site reactions	*Pain, swelling, redness, itching, bruising at the infusion site* These can be common side effects from any infusion and the symptoms don't usually last very long. In some cases the side effects may be more severe, therefore it is important to let your treating nurse or doctor know if you experience any of the above symptoms.
Infusion related embolism (blood clots) There is a risk that the MSCTRAIL infusion may increase the risk of the formation of blood clots, which may lead to complications.	*Chest pain, worsening breathlessness* Whilst there is a potential risk for thromboembolic events, they have not been observed in previous studies using MSCs infused in a similar way. Your doctors and nurses will monitor you closely during and after the infusion of the cells. If such a reaction does occur, you will be given appropriate medication. If you notice these symptoms please let your treating doctor or nurse know.
Infusion related cardiac arrhythmias	*Chest pain or palpitations, worsening breathlessness, light-headedness/ dizziness, sweats, fainting* Whilst there is a potential risk for cardiac arrhythmias, they have not been observed in previous studies using MSCs infused in a similar way. Your doctors and nurses will monitor you closely during and after the infusion of the cells. If such a reaction does occur, you will be given appropriate medication and you will be monitored until the symptoms resolve.

Graft-versus-Host Disease (GvHD) This is if the infused MSC cells see your body cells as foreign and start to attack them. The skin, liver, digestive system, eyes, joints, lungs can be affected.	*Rash, diarrhoea, sickness, loss of appetite, and yellowing of the skin (jaundice).* We cannot predict if you will experience this possible side effect, but the occurrence of GvHD has not been observed in previously reported studies using donor mesenchymal stem cells
Risks of developing new cancer due to the properties on mesenchymal stromal cells (MSCs) One of the properties of the mesenchymal stromal cells is their ability to divide many times. Because of this there is a potential risk that the MSCs can change and become themselves 'malignant' therefore leading to the appearance of a new tumour.	*Appearance of new cancer cells (different from your lung cancer)* While there are some studies suggesting that MSCs obtained from animals can lead to the formation of new cancer in animal models, this has not been seen when human MSCs are used. To date MSCs have been used in over 400 clinical studies in the last 10 years for treatment of a wide range of diseases and there have been no reported cases of new cancers related to MSCs.
Risks of developing new cancer due to the virus used to make MSCTRAIL (insertional mutagenesis) There is a theoretical risk that the special virus used to make MSCTRAIL may make some cells grow without control thus leading to developing a new cancer.	*Appearance of new cancer cells (different from your lung cancer)* Early versions of the virus were found to cause some changes to the cells and make them cancerous. The virus used to make MSCTRAIL has been changed to reduce the risk of this. It has been used in a number of clinical trials including one involving children and there have been no reports of appearance of new cancer in these studies. Even though we cannot exclude the possibility that the virus can make some cells grow without control, we believe that the chance of you developing a new cancer after MSCTRAIL infusion is very low.
Risks related to the virus *(replication competent virus)* There is a theoretical risk that the special virus used to make MSCTRAIL may start dividing and infecting other cells.	The virus used to make MSCTRAIL has been changed in a way that prevents it from becoming active once inside the cells. It has been also tested in a laboratory to confirm the absence of a virus that can divide inside the cells. Similar viruses have been previously used in other studies and have shown to be safe to administer to patients.

When you come for your hospital visits, we will ask you about any side-effects you may have experienced. It is important that you tell us about any problems as they arise, as it is often possible to deal with side effects by adjusting the study therapy or giving you some other medications. We will monitor you closely for any possible side effects and we may suggest additional investigations if we consider it appropriate.

We would like to be updated about any changes in your health. When you join the study, we will give you a contact card to let you know the correct number to call, you should carry this with you at all times.

If you are admitted to a hospital or have to see your GP in between hospital visits, please remember to show them the contact card in case they need to speak us. Please ask your doctor to get in contact if there are any questions or queries regarding treatment.

8. Are there any other risks?

CT scans are used to assess the extent of your disease and how you respond to treatment. As part of this study you will have up to 20 CT scans. These scans use x-rays and each scan, exposes you to radiation.

Using radiation in research is strictly governed. An independent expert has confirmed that the risk of this excess radiation to your future health is very small compared with the underlying cancer and its treatment.

The independent assessors have also confirmed that the investigations are a justifiable part of the study.

CT scans are part of the standard way in which we assess your disease and how effective treatment is. It is likely that you would have the same number of CT scans even if you were not taking part in this study.

9. Pregnancy and Contraception

The chemotherapy with pemetrexed and cisplatin may have damaging effects on unborn babies. Currently it is not known if MSCTRAIL or Pembrolizumab are harmful to unborn babies. For that reason it will be important that you use reliable methods of contraception. Where advisable, your partner may be recommended to use reliable contraception too.

If you or your partner are of child bearing potential please refer to Appendix 1 for further information on pregnancy & contraception.

10. What are the possible advantages and disadvantages of taking part?

Advantages

We cannot promise the study will help you but we hope the information we receive from you taking part in this study will help improve our knowledge of treating metastatic lung cancer, which will benefit the treatment of people with it in the future.

Disadvantages

The disadvantages of taking part in the study are mostly associated with the side effects mentioned in Section 7. As this is a new therapy we do not yet know all the side effects that you may experience

If you decide to take part in this study you will need to attend the hospital more often, this involves extra travel and inconvenience. We will be able to pay for reasonable travel costs for these additional visits (outlined in section 5) if requested.

You may also need to have extra tests at these visits including extra blood samples (see section 5).

Before participating in this study, you should consider if taking part will affect any insurance you have and seek advice if necessary.

11. What happens when the study stops?

Should you require any further treatment after the end of this study or should the study stop early, your oncologist will discuss alternative treatments with you.

As MSCTRAIL is an investigational drug it will not be available once the study has ended. If needed, you will have standard cancer treatment as determined by your oncologist.

12. What are the alternatives for treatment?

Should you choose not to take part in the study this will not affect your treatment options.

You will receive the standard treatment for your condition.

You can discuss other standard treatment or experimental therapy options with your oncologist.

13. More information about taking part

Will my taking part in this study be kept confidential?

Yes, all information collected during the study will be kept strictly confidential.

Details about you, your therapy, any side-effects you have, how the cancer responds and how you are during and following study therapy will be recorded in your medical notes.

Information relevant to your participation in the study will be passed to the Cancer Research UK & University College London Cancer Trials Centre (UCL CTC)**.** This information will include your initials and year of birth.

All information held at UCL CTC will be stored securely and handled according to the Data protection Act and the General Data Protection Regulation (GDPR) requirements. The research is conducted in the public interest as it may lead to improvements in future treatment. You will be assigned a study number by UCL CTC and your study data (including your initials and year of birth) held there will be linked by this number. This study number will also be used to link your study data to any tissue or blood samples you agree to being collected and sent to relevant central laboratories. Your name will never be used in any reports about the study.

UCL (as a sponsor) will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. Your hospital, the laboratory making MSCTRAIL and UCL will all keep information about you for at least 30 years after the trial has finished. This is required by law for this type of treatment. Your rights to access, change or move the information are limited, as we need to manage collected data in specific ways to ensure the study is reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained.

The study information collected will be used to help improve our knowledge of treating metastatic lung cancer and may also be shared anonymously with other researchers in the future to help answer other important questions (additional ethics approval would be obtained if appropriate)

Staff from UCL CTC, the sponsor (or its representatives), regulatory authorities and your NHS Trust/Health Board might look at information collected about you as part of the study. This is to ensure that the study is being carried out properly and that the information collected is accurate. These organisations will always keep information about you confidential.

We will tell your GP about your participation in the study.

You can find out more about how we use your information at: http://www.ctc.ucl.ac.uk/Privacy.aspx

If you wish to raise a complaint on how we have handled your information, you can contact our Data Protection Officer on:

data-protection@ucl.ac.uk

If you are not satisfied with our response, you can contact the Information Commissioner's Office (ICO) on:

https://ico.org.uk/

What if relevant new information becomes available during the research study?

Sometimes we get new information about treatments being studied. If this happens, we will tell you and discuss with you whether you should continue in this study. If you decide not to carry on, we will make arrangements for your care to continue outside the study. If you decide to continue in the study, you may be asked to sign an updated consent form. In some circumstances, we might

consider it best for you to withdraw from the study. If this is the case, we will explain the reasons and arrange for your care to continue outside the study.

If the study is stopped for any other reason, we will tell you and arrange your continuing care.

What will happen if I don't want to carry on with the study?

You can withdraw from the study at any time without giving a reason and without your rights being affected but we would like to continue to collect information about you, so that we know about your progress following study therapy. We will also need to use the information collected up to your withdrawal. Any stored blood or tissue samples that can be identified as yours can be destroyed if you wish.

What if the study therapy harms me or I have a complaint?

Every care will be taken in the course of this study. In the unlikely event that you are injured by taking part, compensation may be available. If you suspect that the injury is the result of the Sponsor's (University College London) or the hospital's negligence then you may be able to claim compensation.

Please talk to your study doctor first and then make the claim in writing to Professor Sam Janes who is the Chief Investigator for this study and is based at University College London Hospital. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

You may also be able to claim compensation for injury caused by taking part in this study without the need to prove negligence on the part of University College London or another party. You should also discuss this possibility with your study doctor in the same was as above.

In addition, the normal National Health Service complaints arrangements are available to you. Please ask your study doctor if you would like more information on this. Details can be obtained from the hospital or the Department of Health website: http://www.dh.gov.uk.

You can also contact the Patient Advice and Liaison Service (PALS) provided by the NHS for advice or to raise an issue or concern about the hospital where you are treated.

You can contact the PALS staff:

By phone: << Insert telephone number for PALS at the NHS Trust>>

By email: << Insert email for PALS at the NHS Trust>>

What will happen to the samples I give?

We would like to collect additional blood samples (up to 30mls or about 6 teaspoons) for research at the following study visits: Days 1, 2, 3, 8 and 15 during cycles 1, 2 and 3, and days 1 and 15 during cycle 4. Where possible, these will be taken at the same time as a routine blood sample.

We would like to use these blood samples and the tumour sample that was taken at the time of your diagnosis for research to look at the following:

- Whether we can tell if the cancer is responding to the MSCTRAIL by looking at microscopic tumour cell fragments circulating in your blood
- Whether there is any sign of an immune response to the donor cells (MSCTRAIL)
- Whether different tumours respond differently to the MSCTRAIL to see if we can predict which patients are more likely to benefit from this therapy

This analysis will be performed in Central laboratories at the 'Lungs for Living Research Centre' at University College London. Samples will be under the control of UCL who will handle your samples confidentially. The samples will be labelled with your study number and initials, and will be linked to the study data through this unique study number.

The consent form will include optional choices to allow us to take blood and tumour samples for research purposes. As these are not essential for involvement in the study, if you do not wish to have samples taken then this will be noted on the consent form.

With your permission, your samples may also be stored and used in other ethically approved studies.

The routine blood samples will be tested at your hospital and will be processed as per normal clinical practice.

Will any genetic tests be done?

Genetic tests will be performed to study the genetic influences on lung cancer and treatment. Specifically we will be looking at whether different tumours respond differently to the MSCTRAIL to see if we can predict which patients are more likely to benefit from this therapy, including testing for genetic mutations.
The results will not affect you directly. No clinical genetic tests will be done for specific known inherited diseases.

What will happen to the results of the study?

We will publish a summary of results on the UCL CTC study website. Results will also be presented at national/international meetings and published in medical journals so that other researchers can see them. Your doctor will be informed when the results are available and you can ask him/her about the progress of the study. A summary with the results will be also available on the Cancer Research UK website.

Your identity and any personal details will be kept confidential. No named information about you will be published in any report of the study.

Thank You

Thank you for considering taking part in this study and for taking the time to read this Patient Information Sheet, which is yours to keep. If you decide to take part in the study, you will also be given a copy of your signed consent form.

Further Information

You may wish to contact one of the following organisations that are independent of the hospital at which you are being treated:

- Macmillan Cancer Support provides practical, medical and financial support and work towards the improving cancer care. They can be contacted at:

  Tel: 0808 808 00 00 (Freephone)

  Or visit their website at:

  http://www.macmillan.org.uk/HowWeCanHelp/HowWeCanHelp.aspx

- CancerHelp (Cancer Research UK) who provide all aspects of information for people with cancer. Their contact details are:

  Tel: 0808 800 4040 (Freephone)

  Or visit their website at: http://cancerhelp.cancerresearchuk.org

If you decide you would like to take part then please read and sign the consent form. You will be given a copy of this information sheet and signed consent form to keep. You can have more time to think this over if you are unsure or have more questions.

Local Site Contacts

If you have any questions, please do not hesitate to discuss any questions with your study doctor or members of the study team:

Your study doctor is:

Name: Contact phone number:

Your study/specialist nurse/trial co-ordinator is:

Name: Contact phone number:

Glossary

Abbreviation	Full Name	What it means
CT scan	Computer Tomography	Using x-rays to create a 2D picture of inside the body. The image produced is very detailed.
ECG	Electrocardiogram	A test that measures the electrical impulses that make your heart beat.
GTAC	Gene Therapy Advisory Committee	GTAC is the UK national Research Ethics Committee for gene therapy clinical research
I.V.	Intravenous	Drugs administered into a vein are administered intravenously. Many chemotherapy drugs are given this way.
NSCLC	Non-small cell lung cancer	Type of lung cancer including: • squamous cell cancer – these develop from cells that line the airways and is often found near the centre of the lung in one of the main airways • adenocarcinoma cancer – these develop from the cells that produce mucus (phlegm). It is often found in the outer areas of the lungs • large cell lung cancer – this is so called because the cells look large and rounded under a microscope
RT	Radiotherapy	Treatment using x-rays to shrink the tumour.
UCL	University College London	This is the organisation that takes responsibility for the running of the study, known as the Sponsor.
UCL CTC	UCL Cancer Trials Centre	The organisation centrally carrying out the day to day work on the study.
	Gene therapy	The introduction of genes into cells in order to treat or prevent disease
	Genetically modified	Containing genetic material that has been added (or altered) in order to obtain a desired feature.
	Genetic mutations	Permanent alteration in the DNA sequence that makes up a gene, such that the sequence differs from what is found in most people.
	Genetic tests	Type of medical test that identifies changes in chromosomes, genes, or proteins. The results of a genetic test can confirm or rule out a suspected genetic condition or help determine a chance of developing or passing on a genetic disorder
	Immune response	The reaction of the cells and fluids of the body to the presence of a foreign substance
	Mucus	a slimy substance secreted by the cells lining the body cavities (such as nose, esophagus) and serving primarily to protect and lubricate surfaces
	Oncologist	A medical doctor qualified to treat cancer
	Protein	Large molecules composed of long chains of amino acids and are an essential part of cells. Each protein has a unique function.
	Sponsor	The organisation which takes responsibility for the management of a clinical trial
	Vital signs	Clinical measurements, such as pulse, temperature, respiration rate, and blood pressure that indicate the state of essential body functions.

Appendix 1

General chemotherapy side effects

Lowered resistance to infection

Chemotherapy can reduce white blood cells made by the bone marrow, making you more prone to infection. You may have headaches, aching muscles, a cough, sore throat, pain passing urine or feel cold and shivery. If your temperature goes up, or you suddenly feel unwell, even with a normal temperature, contact us or the hospital straight away. Usually a temperature above 38ºC (100.5ºF) is considered to be high.

Anaemia (low number of red blood cells)

While having treatment with chemotherapy, you may become anaemic. This may make you feel tired and breathless. Let us know if you have such problems.

Bruising or bleeding

Chemotherapy can reduce the production of platelets, which help the blood to clot. Let us know if you have any unexplained bruising or bleeding, such as nosebleeds, blood spots or rashes on the skin, and bleeding gums.

Nausea and vomiting

If you feel sick it may begin soon after the treatment is given and last for a few days. You may feel sick but it is unusual to actually vomit. We will give you anti-sickness (anti-emetic) drugs to prevent or greatly reduce nausea and vomiting. If you are sick and this continues, tell us so we prescribe anti-sickness drugs that may be more effective.

Diarrhoea

This can usually be controlled with medicine but let your doctor know if it is severe or if it continues. It is important to drink plenty of fluids if you do have diarrhoea.

Constipation

You may experience some constipation. It is important that you talk to your nurse or doctor before you take any over-the-counter laxatives or stool softeners.

Hair loss

This usually starts after the first or second cycle of chemotherapy. Hair is usually lost completely but may just thin. You may also have thinning and loss of eyelashes, eyebrows and other body hair. Hair loss is temporary and your hair will re-grow once the treatment is finished.

Kidneys

Usually this does not cause any symptoms, and the effect on the kidneys is mild, but if the effect is severe the kidneys can be permanently damaged unless the treatment is stopped. Your kidneys will be checked by a blood test before each cycle of chemotherapy. Fluid will be given into the vein before and after treatment to keep your kidneys working normally. You may be asked to drink extra fluid before and after treatment; it is important to do this.

Hearing

Your hearing can be affected. You may get ringing in your ears (tinnitus) and lose the ability to hear some high-pitched sounds. Tinnitus usually gets better after treatment. Some hearing changes can be permanent. Tell us if you notice any changes in your hearing.

Numb or tingling hands or feet

Peripheral neuropathy is the numb or tingling sensations you may experience and is caused by the effect of the treatment on the nerves. You may find it hard to fasten buttons or do other fiddly tasks. Tell us if you have these symptoms. The symptoms usually improve slowly after treatment finishes, but in some people they may never go away.

Allergic Reactions

Skin rashes (including severe blistering and peeling) can occur so your doctor will regularly ask about any rashes. Fever has also been associated with treatment. Please tell us if you experience any of these symptoms. We can give you treatment to ease discomfort from these side effects.

Sore Mouth

Your mouth may become sore, or you may notice small ulcers during the treatment. Drinking plenty of fluids and cleaning your teeth regularly and gently with a soft toothbrush can help reduce the risk of this happening. Tell us if you have any of these problems as we can prescribe special mouthwashes and medicine to prevent or clear any mouth infection.

General immunotherapy side effects

Nausea and vomiting

You might feel sick or be sick. Anti-sickness injections and tablets can control it. Tell your doctor or nurse if you feel sick. You might need to try different anti sickness medicines to find one that works.

Pain and swelling in your joints

Let your doctor or nurse know if you have pain in your joints during or after having treatment. There are lots of ways to treat pain, including relaxation and painkillers.

Diarrhoea

Contact your doctor or nurse straight away if you have any signs of diarrhoea or you open your bowels more often than usual.

Depending on how severe your symptoms are, you might need treatment such as anti diarrhoea medicine and fluids into your vein. You may stop treatment for a time or stop altogether.

Skin Changes

You might notice skin changes, such as dryness, itching and rashes similar to acne on your face, neck and trunk.

Tell your doctor if you have any rashes or itching. Don't go swimming if you have a rash because the chlorine in the water can make it worse.

Tiredness & weakness

You might feel very tired and as though you lack energy.

Various things can help you to reduce tiredness and cope with it, for example exercise. Some research has shown that taking gentle exercise can give you more energy. It is important to balance exercise with resting.

Appendix 2 – Pregnancy and contraception

Please read below the relevant information for you and, if appropriate, share it with your partner.

For female patients

- Pemetrexed and cisplatin are excreted in human milk, and harmful effects on the child cannot be excluded. It is not known if MSCTRAIL or pembrolizumab are secreted in human milk. Therefore if you are breast-feeding or pregnant you will not be able to take part in the study.
- If there is a chance that you could become pregnant, we will ask you to take a pregnancy test) before entering the study to ensure you are not pregnant. We will repeat pregnancy testing at the beginning of treatment.
- You must agree to use at least 1 form of highly effective contraception from registration, during and up to 6 months after your last treatment. These are hormonal contraceptives, intrauterine devices or systems (also called the hormonal coil), sterilisation where both fallopian tubes have been blocked/cut, a vasectomised male partner or absolute and continuous abstinence. This must continue for the time you are receiving treatment and for 6 months after you finish your treatment.
- If you do become unexpectedly pregnant during the study, you must inform us immediately. You will stop being treated on the study and we will discuss your treatment options. We will also refer you for specialist counselling on the possible risks to yourself and your unborn baby.

For male patients

- It is possible that the MSCTRAIL and standard treatment will affect sperm or semen and therefore you should not father a child during and up to 6 months after your last treatment.
- If there is a possibility that your partner might become pregnant you must use at least 1 highly effective form of contraception from registration, during and up to 6 months after your last treatment. Highly effective forms of contraception are vasectomy also called male sterilisation (with appropriate post-vasectomy documentation of the absence of sperm in the ejaculate) or absolute and continuous abstinence. It is also advisable for your female partner to start using contraception (see guidance for women)
- If your partner becomes pregnant during the study, or within 6 months of stopping treatment, you must tell us immediately. We would discuss referral for specialist counselling on the possible risks to your partner and your unborn baby.

For female and male patients

We would like to monitor pregnancies occurring during and up to 6 months after last treatment, provided consent is given for this. We will give you (or your partner) a pregnancy monitoring information sheet and consent form and we will ask for consent for us to monitor the pregnancy.

If consent is obtained, we will collect information about the pregnancy, details of any previous pregnancies and information about medications taken. We will want to continue to collect information for up to 6 weeks after the end of the pregnancy to collect information on ante- and post-natal problems, whether the pregnancy continued to term and if so, how the baby was after birth. This information is important because it helps us to better understand the effect of the study treatment on pregnancy and the unborn baby.

Agreeing to collect information about the pregnancy is entirely voluntary. If you decide that you do not want to allow us to collect this information, or if you agree and then later change your mind, this will not affect the care you receive in any way. If you (or your partner) change your mind we will stop collecting any more information. However information that has already been sent to the organisers of the study be kept by them.

Fertility Advice

Due to the possibility of treatment-related infertility, we can provide you with information on egg cryo-preservation or sperm banking. Please let us know if you would like to discuss this further and if you would like further information.

TACTICAL

Targeted stromal cells expressing TRAIL as a therapy for lung cancer

SAMPLE COLLECTION FORM

Please complete the following details for each sample and send to the lab along with the samples

Please remember to use patient trial number - DO NOT USE PATIENT'S FULL NAME

Patient and Sample Details

Patient trial number:		**Patient Initials:**	
Type of sample: *(Please tick all that apply)*	☐ Blood in EDTA ____ tubes X _____ ml	☐ Blood in EDTA ____ tubes X _____ ml	☐ Blood in EDTA ____ tubes X ____ ml

All blood samples are transported at **ambient temperature** as long as they are delivered to the Lungs for Living Research Centre **within 30mins of being taken**.

Date samples collected:		Time samples collected:	
Site name:		Trial Time-point	*See time-points listed on page 2 and mark as appropriate*
Samples sent by: *(Staff Member Name)*			
Contact tel:		Contact email:	
Deliver samples to Lungs for Living Research Centre Queries regarding delivery of samples should be addressed to: [redacted]		F.A.O [redacted]	

Sample details

Sample	Quantity	SAMPLE TIMEPOINT **Please tick all that apply** (days/months post MSCTRAIL infusion)		
Blood in EDTA *Biomarkers of apoptosis* *Donor immune response* *Future ethically approved research*	_____ mls Up too 30mls	**Cycle:**	**1** ____	Day 1 ☐ Day 2 pre MSCTRAIL ☐ Day 2 - 3 hours post MSCTRAIL ☐ Day 2 - 6 hours post MSCTRAIL ☐ Day 3 ☐ Day 8 ☐ Day 15 ☐ At Progression ☐
			2 ____	
			3 ____	
			4 ____	Day 1 ☐ Day 15 ☐ At Progression ☐

Any deviations from lab manual procedure to be documented below:

FOR LAB USE ONLY			
Samples received / sufficient quantity	☐ Yes ☐ No If No, please specify: _____ _____		
Date samples received:		Time samples received:	
Name:		Signature:	

Please see TACTICAL Lab Manual for details on obtaining/delivery of samples

SUMMARY OF DRUG ARRANGEMENTS

TACTICAL PHASE I

Full Title Clinical Trial:	**Targeted stromal cells expressing TRAIL as a therapy for lung cancer**
Short Title:	**TACTICAL**
Trial Drugs	**ATIMP: MSCTRAIL (supplied as clinical trial stock)**
EudraCT Number	**2015-005526-18**
Sponsor:	**University College London**
Sponsor's Project ID:	**UCL/14/0453**

The procedures described within this Summary of Drug Arrangements apply only to patients registered on TACTICAL to receive allogeneic MSCTRAIL

SUMMARY OF DRUG ARRANGEMENTS

TACTICAL PHASE I

CONTACT DETAILS

For further information on trial drugs, trial protocol, dosing, ATIMP supply and distribution, please contact:

Key Contacts	Contact Details	Main Responsibilities
TACTICAL Trial Coordinator [redacted]	[redacted]	Sponsor representative Central Trial coordination and management
Professor Sam Janes [redacted]	[redacted]	Chief Investigator Contact for questions related to ATIMP administration and patient care
Dr Alice Davies [redacted]	[redacted]	Trial Investigator Contact for questions related to ATIMP administration and patient care
[redacted]	[redacted]	MSTRAIL manufacture MSCTRAIL QP release MSCTRAIL storage until needed at Site MSCTRAIL transport to Site
[redacted]	Tel: [redacted]	
CitySprint	Tel: Same Day Delivery: 0207 880 1117 Overnight Delivery: 0207 880 1115 Email: LondonEastHealthCT@citysprint.co.uk	Trial courier for MSCTRAIL transport from manufacturer (CCGTT) to site Return of vapour shipper from site to CCGTT

SUMMARY OF DRUG ARRANGEMENTS

TACTICAL PHASE I

Contents

SUMMARY OF DRUG ARRANGEMENTS

TACTICAL PHASE I

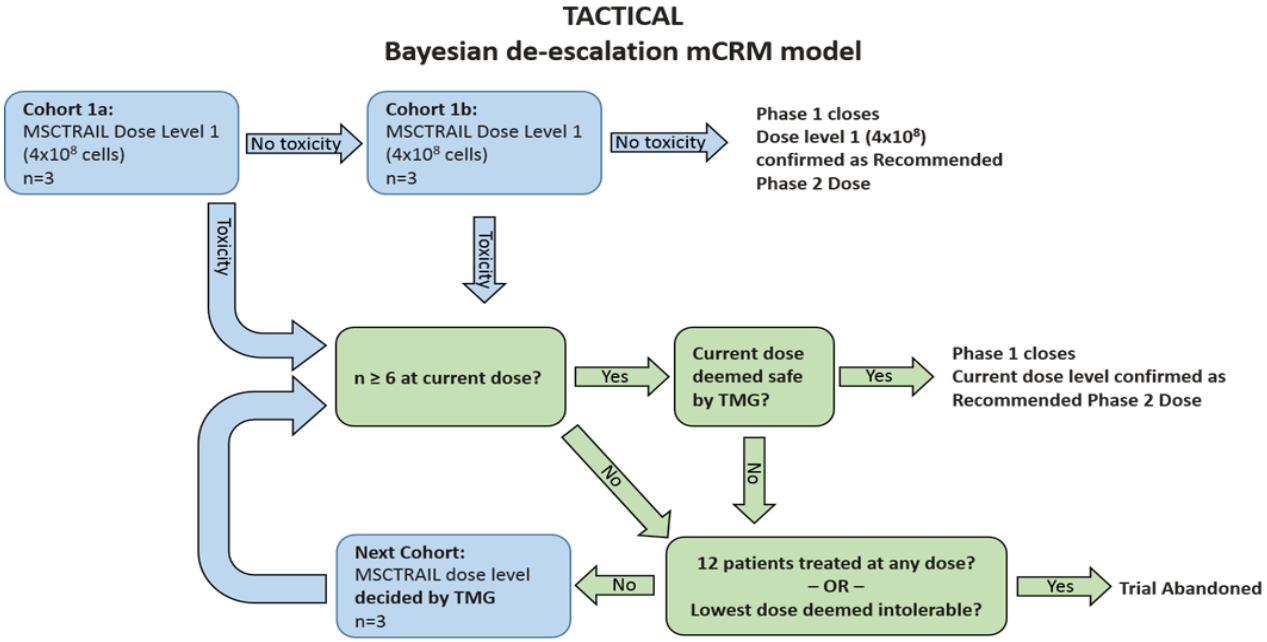
1. OVERVIEW

1.1 APPLICABILITY

This Summary of Drug Arrangements is applicable to the research and pharmacy members of site staff who are involved in receipt and administration of the trial drugs (MSCTRAIL, pemetrexed and cisplatin/carboplatin and/or pembrolizumab) used in the **phase I of TACTICAL**.

2. TRIAL INFORMATION

Phase I:



Phase I is a first-in-human, accelerated dose de-escalation study: 3 patients, deemed cohort 1a, will receive $4x10^8$ cells of MSCTRAIL in combination with standard therapy (pemetrexed and cisplatin and/or pembrolizumab) for 3 cycles, followed by a 4th cycle of standard therapy only (without MSCTRAIL). After the 4 cycles are completed patients will continue with a standard of care therapy as decided by their treating clinician.

If there are no dose limiting toxicities (DLT) a further 3 patients (cohort 1b) will receive the same dose of MSCTRAIL in combination with standard therapy as described above. If there are no toxicities in all 6 patients this will be the recommended Phase II dose (RP2D).

If patients in cohort 1 have DLTs then cohort 2 will receive a reduced dose of MSCTRAIL decided by the Trial Management Group (either $2x10^8$ or $8x10^7$ cells), if there are no DLTs after 3 patients, a further 3 patients will receive the same dose. If there are no toxicities in all 6 patients this will be the recommended Phase II dose (RP2D).

This pattern will continue until the RP2D is discovered.

For detailed information on TACTICAL please refer to the current version of the protocol.

3. TRIAL SITE AND PHARMACY SET-UP

The Principal Investigator (PI) must ensure site staff involved in receipt, thawing and administration of the ATIMP (MSCTRAIL) are trained according to local guidelines for infusion of cryopreserved cellular products. Staff performing MSCTRAIL related procedures must have read and understood the TACTICAL protocol and Summary of Drug Arrangements (SoDA) in order to be delegated this duty and be signed off by the PI on the Delegation log for the trial.

The PI must ensure pharmacy is informed about the trial and the Non-Investigational Medicinal Products (NIMPs): pemetrexed, cisplatin, carboplatin and pembrolizumab. A designated member of the pharmacy staff, who takes overall responsibility for all pharmacy aspects of the clinical trial (e.g. dispensing and accountability of pemetrexed, cisplatin, carboplatin and pembrolizumab), must be identified and listed on the Site Delegation Log for TACTICAL.

Prior to site initiation, documents for the Investigator Site File (ISF) and for the Pharmacy Site File (PSF) will be sent electronically to the Research and Pharmacy Leads (respectively) from UCL CTC. The contents of the ISF will include copies of forms for MSTRAIL ordering/reordering, transport and quality checks and MSCTRAIL accountability logs. All MSCTRAIL related documentation should be filed in the ISF.

4. PATIENT REGISTRATION (Phase I)

Phase I of the trial aims to establish the recommended MSCTRAIL dose when given in combination with standard therapy in metastatic adenocarcinoma patients.
Following site activation, patients may be recruited into the trial. Once an eligible patient has been identified and screening assessments performed, the site research team will complete the registration form and email/fax it to UCL CTC.

UCL CTC will use the completed form to confirm patient eligibility and register the patient into TACTICAL. UCL CTC will then email 'Confirmation of patient registration' to the PI, site research and pharmacy contacts, and the manufacturer (CCGTT-RFH). The 'Confirmation of patient registration' will contain the following information:

- patient trial number
- patient 'MSCTRAIL dose cohort' allocation:
    - Dose Level 1: 4 x 10^8 cells (per cycle)

If applicable, patients in further cohorts will receive either 1 bag of 2x10^8 MSCTRAIL or 1 bag of 0.8x10^8 MSCTRAIL as decided by the dose allocated by the TMG and/or IDMC

For details on how to order MSCTRAIL for the patient, please see section 8 'MSCTRAIL Prescribing and Ordering'.

5. TRIAL DRUGS

5.1 Trial IMP

In accordance with the Clinical Trial Authorisation (CTA) granted by the MHRA on the 19/02/2018, the following are classed as **Investigational Medicinal Products (IMPs):**

- **MSCTRAIL:** allogeneic mesenchymal stromal cells (from umbilical cord donors) genetically modified to express TRAIL (TNF-related apoptosis inducing ligand). The IMP in this trial is an advanced therapy investigational medicinal product (ATIMP) manufactured and QP released at the Centre for Cell, Gene & Tissue Therapeutics (CCGTT) at Royal Free Hospital (RFH). Brief details about MSCTRAIL manufacture are listed in the Investigator Brochure (IB) section 3.2 'MSCTRAIL' and protocol section 8.1 'Investigational Medicinal Product'. MSCTRAIL is suspension for infusion containing a minimum concentration of $2x10^6$ MSCTRAIL/ml in 50% DPBS (Dulbecco's Phosphate Buffered Saline), 4.5% HAS (Human Albumin Solution) and 10% DMSO (Dimethtyl sulfoxide)

5.2 Trial NIMPs

The following drugs have been classified as **Non Investigational Medicinal Products (NIMPs)** according to the protocol:

- Pemetrexed
- Cisplatin
- Carboplatin
- Pembrolizumab

Pemetrexed, cisplatin and pembrolizumab are standard treatment for patients with advanced/metastatic lung cancer and are given at standard doses in the trial. Carboplatin can be used instead of cisplatin if clinically appropriate at the discretion of treating clinician. Treatment details are listed in protocol section 8.3.2.

6. SUPPLY OF TRIAL DRUGS

6.1 Supplied drugs

For TACTICAL the following drugs will be provided free of charge to sites for the duration of the trial:

- **MSCTRAIL (ATIMP)** – dose as per protocol cohort, suspension for infusion, provided by CCGTT-RFH.

MSCTRAIL is manufactured and QP released at CCGTT. The manufacturing process is detailed in the ATIMP Dossier and has been approved by the Medicines and Healthcare products Regulatory Agency (MHRA). After manufacture, MSCTRAIL is labelled and cryopreserved in CryoMACS® freezing bags and stored in a temperature monitored vapour phase Dewars between -135°C to -196°C at the CCGTT's BioBank.

MSCTRAIL is NOT patient specific and the QP released ATIMP is ready to be shipped from CCGTT to the site following patient registration into the trial and prior to cycles 2 and 3 as needed.

6.2 Hospital commercial stock

The following drugs **will not** be provided for the trial and so hospital commercial stock should be used. Pharmacies must ensure they have adequate supplies for the trial.

- **Pemetrexed (NIMP**) – 500mg/m^2, solution for infusion
- **Cisplatin (NIMP)** - 75mg/m^2, solution for infusion
- **Carboplatin (NIMP)** – dose determined by renal function (AUC 5 or according to local protocol), solution for infusion
- **Pembrolizumab (NIMP)** – 200mg, solution for infusion

7. MSCTRAIL LABELLING

MSCTRAIL is supplied by CCGTT-RFH as labelled clinical trial stock. The ATIMP (at least 2x10^6 MSCTRAIL/ml) are cryopreserved in clear CryoMACS Freezing Bags containing a final volume of up to 100ml. Each bag is labelled, then placed inside a secondary bag and vacuum sealed.

Label wording for the CryoMACS Freezing Bags is provided in the ISF and example is shown below:

Clinical Trial: TACTICAL

EudraCT number: 2015-005526-18
Sponsor: University College London, WC1E 6BT
Chief Investigator: Prof. Sam Janes

MSCTRAIL

ISBT Code: _____

Batch UIN: _____

Patient Trial number: _____

__X__x10^8 MSCTRAIL in _____ml cell suspension for intravenous infusion

Date Frozen: _____/_____/_____ **Expiry Date:** _____/_____

Transport/Storage Condition: ≤ -135°C and ≥ -196°C

THAW AND THOROUGHLY RESUSPEND IMMEDIATELY BEFORE USE

INFUSE WITHIN 60 MINUTES OF THAWING

FOR CLINICAL TRIAL USE ONLY
CCGTT/LABEL/065/01

'X': the cell number per bag will be completed by the manufacturer where X is either '2' or '0.8'.

Patients in cohort 1 will receive 2 bags of 2x10^8 MSCTRAIL.
If applicable, patients in further cohorts will receive either 1 bag of 2x10^8 MSCTRAIL or 1 bag of 0.8x10^8 MSCTRAIL as decided by the dose allocated by the TMG and/or IDMC

SUMMARY OF DRUG ARRANGEMENTS

TACTICAL PHASE I

Patient trial number must be added to the label at the clinical site post administration by staff involved in MSCTRAIL administration (section 12.2.5).

MSCTRAIL is not patient specific and the patient trial number will not be listed on the primary and secondary labels as it will not be known at the time of labelling/MSCTRAIL freezing. Once the site orders MSCTRAIL for patient administration, the bags with the cryopreserved MSCTRAIL will be placed in a 3rd larger bag for transport from CCGTT to the site. This 3rd bag will contain a label with the patient trial number filled in.

8. MSCTRAIL PRESCRIBING AND ORDERING

Prior to MSCTRAIL treatment (Day 2 of cycles 1-3), the site investigator must ensure the required assessments (as detailed in protocol section 9.2 'Assessments during treatment') have been performed and the patient can be treated with MSCTRAIL. MSCTRAIL reduction to a 'lower dose cohort' may be required if patient has experienced a DLT (protocol section 8.7 DLTs). Decision on MSCTRAIL dose reduction for individual patients will be taken by the TMG after review of the patient's safety data. UCL CTC will inform the site about the TMG decision and the MSCTRAIL dose patient has been assigned to.

UCL CTC will provide the site with the following forms: '**TACTICAL Prescription for MSCTRAIL' and 'MSCTRAIL Request Form'.** Both forms need to be completed for each patient prior to each MSCTRAIL dose. The **'Prescription for MSCTRAIL'** will list the required MSCTRAIL dose for the patient and must be signed by the PI or appropriate member of staff (as identified on the site delegation log). The **'MSCTRAIL Request Form'** is then completed by site staff to order MSCTRAIL from the manufacturer, CCGTT-RFH. The '**MSCTRAIL Request form'** will specify the dose required and the date MSCTRAIL is needed at the site for patient administration.

8.1. Initial MSCTRAIL request for Day 2 of Cycle 1

Following patient registration into TACTICAL, the PI or delegated Site Investigator will prescribe MSCTRAIL using the **Tactical Trial Prescription for MSCTRAIL** provided in the ISF. Upon receipt of the completed MSCTRAIL prescription, site staff will complete **section A of the MSCTRAIL Request Form** and will email it to the CCGTT, copying UCL CTC. Site staff should specify on the **Request form** the MSCTRAIL dose required (corresponding to the 'dose cohort' patient was assigned to in the 'Confirmation of Patient Registration' email (section 4). Staff should also list on the form the date MSCTRAIL is required at the site and the planned infusion date of MSCTRAIL.

8.2. Subsequent MSCTRAIL request for Day 2 of Cycles 2 and 3

If patient has NOT experienced any Dose Limiting Toxicity *(protocol section 8.7 DLTs) AND does NOT require treatment delay due to adverse events related to standard chemo or MSCTRAIL (protocol section 8.5 Dose modifications)*

Once patient has reached Day 15 of cycle 1 or 2 (and no later than Day 18 to ensure MSCTRAIL delivery for the next cycle), the PI or delegated Site Investigator will prescribe MSCTRAIL using the **Tactical Trial Prescription for MSCTRAIL**. Upon receipt of the completed MSCTRAIL prescription

site staff will complete **section A of the MSCTRAIL Request Form**. Site staff should specify on the **Request form** the MSCTRAIL dose required (taking into account any dose reductions the patient may have had in the previous cycle). The Site should also list on the form the date MSCTRAIL is required at the site and the planned infusion date of MSCTRAIL.

If patient has had a Dose Limiting Toxicity *(protocol section 8.7 DLTs) AND is continuing with MSCTRAIL treatment at a reduced dose (protocol section 8.5 Dose modifications)*

- If a patient experiences MSCTRAIL related DLT, the TMG will review the patient safety data to decide if the patient can be treated with a reduced dose of MSCTRAIL. CTC will inform the site about the TMG decision and the 'lower dose cohort' that the patient has been assigned to.
- The PI or delegated Site Investigator will prescribe the reduced MSCTRAIL dose (as informed by CTC) using the **Tactical Trial Prescription for MSCTRAIL**. Upon receipt of the completed MSCTRAIL prescription site staff will complete **section A of the MSCTRAIL Request Form** specifying the reduced MSCTRAIL dose. This dose should be also used for the next cycle (if applicable). The Site should list on the form the date MSCTRAIL is required at the site and the planned infusion date of MSCTRAIL.

MSCTRAIL order acknowledgement by manufacturer, CCGTT-RFH

Following receipt of the **MSCTRAIL Request Form** from the site, CCGTT will complete **section B** of the form to acknowledge the MSCTRAIL order and to confirm that they will book MSCTRAIL shipment with the trial courier (City Sprint) for the required date/time (am or pm). CCGTT will then email the fully completed **MSCTRAIL Request Form** back to the PI, site staff and UCL CTC.

☆ **If the patient becomes unwell after the MSCTRAIL Request Form has been completed and sent, CCGTT and CTC should be informed as soon as site staff are aware.** The **MSCTRAIL Request Form** should be amended (if dose reduction is needed) or cancelled (if patient standard therapy/MSCTRAIL will be delayed) and the amended form re-sent to CCGTT (copying CTC).

The fully completed **MSCTRAIL Prescription and Request Forms** must be retained in the relevant section of the ISF.

9. PRESCRIBING OF TRIAL DRUGS

The PI is responsible for ensuring all trial drugs are prescribed appropriately for patients on the trial. During phase I of the trial all patients will have the following treatment:

	Cycle 1						Cycle 2						Cycle 3						Cycle 4					
Week	**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**		**12**	
Day	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Standard of care treatment Pemetrexed & Cisplatin	•						•						•						•					

or Pemetrexed, Cisplatin & Pembrolizumab or Pembrolizumab																							
MSCTRAIL $4x10^8$, $2x10^8$ or $8x10^7$ cells iv, depending on cohort		•						•						•									

Carboplatin (AUC 5 or according to local protocol) can be used instead of cisplatin if clinically appropriate at the discretion of treating clinician.

After completion of the four cycles of trial treatment, the patients will revert to local standard of care therapy as decided by their treating clinician.

Please refer to the trial protocol for dosing schedules and dose reductions of pemetrexed, cisplatin/carboplatin & pembrolizumab (trial NIMPs) and for MSCTRAIL (ATIMP).

9.1 PRESCRIBING OF TRIAL NIMPs

Prior to standard therapy (cycles 1-4), the site investigator must ensure required assessments (as detailed in protocol section 9.2 'Assessments during treatment') have been performed. Standard therapy dose adjustments may be required based on the patient's pre-cycle blood results and according to local policy (protocol section 8.5.2)

Sites should use their own prescriptions for standard therapy. A copy should be forwarded to UCL CTC prior to the first patient being registered.

Prescriptions must be signed by the PI or appropriate member of staff (as identified on the site delegation log) and a copy must be retained in the Pharmacy Site File.

9.2 PRESCRIBING OF TRIAL IMPs (MSCTRAIL)

Details regarding prescribing MSCTRAIL are listed in section 8 MSCTRAIL Prescribing and Ordering.

10. MSCTRAIL SHIPMENT FROM CCGTT TO TRIAL SITE

Following manufacture and QP release, MSCTRAIL is stored at the CCGTT until requested by the trial site for patient administration (section 8. MSCTRAIL Prescribing and Ordering). Shipment to the site is performed by the trial courier (City Sprint) using a temperature monitored vapour phase cryoshipper, which has been validated to hold temperature (-135°C to -190°C) for 10 days.

10.1 Booking courier for MSCTRAIL shipment from CCGTT to Site

CCGTT will contact City Sprint to arrange a shipment of the ATIMP for the required date as specified by the site on the **MSCTRAIL Request Form**. Contact details for City Sprint (if needed) are listed on page 2 of the SoDA.

CCGTT will provide the following information to the courier:

- Contact name at CCGTT and address for collection of the shipper with MSCTRAIL
- Date and collection time required at CCTGTT
- Contact name and delivery address at the site
- Type of service: direct same day delivery, ambient temperature
- Type of goods: ATIMP in a temperature monitored vapour shipper

CCGTT will confirm the MSCTRAIL shipment details with the PI, site staff and UCL CTC.

CCGTT will also email the site and UCL CTC the following documents:

- MSCTRAIL QP Batch Release Certificate
- MSCTRAIL Certificate of Analysis
- TSE statement

11. MSCTRAIL RECEIPT AT TRIAL SITE

11.1 Shipper arrival at site:

- Upon arrival at site, the courier will deliver the shipper to the ward where MSCTRAIL will be administered.
- The following documents will accompany the shipment and should be brought to the ward with the shipper:
  - **MSCTRAIL QP Batch Release Certificate**
  - **MSCTRAIL Certificate of Analysis**
  - **TSE statement**
  - '**MSCTRAIL Transport and Quality checklist'**:
    - Section 1 of this document lists MSCTRAIL dose, number of bags, batch number and expiry date and has been completed by CCGTT
    - Site staff completes Section 2 upon receipt of MSCTRAIL (once the quality checks described in 11.2 have been performed
    - Section 3 will be completed by CCGTT once the empty shipper has been returned to CCGTT and the temperature log during transport downloaded (section 11.3).

If MSCTRAIL delivery has not arrived by the expected time, CCGTT should be contacted in the first instance. If the delay is substantial (e.g. delivery not on anticipated date) the PI and UCL CTC must also be notified.

11.2 Quality checks upon shipper arrival at the ward

On delivery at the ward site staff must check the following before the vapour shipper can be returned to CCGTT:

- General condition of the liquid nitrogen vapour shipper
- Check the status of any external alarms (Normal/ Alert/ Alarm light flashing)
  If temperature alarm is Flashing/Alert – do NOT take MSCTRAIL out of the shipper
  Contact CCGTT and UCL CTC (section 11.4)

- If the temperature is reading 'Normal', record the time the shipper was inspected and opened on the **MSCTRAIL Transport and Quality Checklist**

Open the shipper fully and, wearing protective gloves, remove the MSCTRAIL bag(s) from the shipper and perform the checks listed below:

- Check that MSCTRAIL is frozen, the bag(s) are intact and that no leakage has occurred
- Check the **Batch Release Certificate** confirms MSCTRAIL has passed all product release criteria and is signed by the QP
- Check **MSCTRAIL Transport and Quality Checklist** section 1 has been completed by CCGTT

(If more than one bag of MSCTRAIL is to be infused, additional bags should be stored in the shipper until they are thawed for administration)

- Site staff must then complete Section 2 of the **MSCTRAIL Transport and Quality Checklist** (any problems with the vapour shipper or the ATIMP should be noted).
- The MSCTRAIL Transport and Quality Checklist with section 2 completed should be stored with the shipper for return to CCGTT*

**If the courier can wait until the above checks have been completed, the empty shipper can be returned immediately. Otherwise the shipper should be kept at the ward until CCGTT arrange the courier to pick it up later on the same day.*

N.B. If there is any concern regarding the condition of the shipper or the ATIMP, put the bag(s) back in the shipper and notify the PI, UCL CTC and CCGTT. Do NOT administer MSCTRAIL to the patient.

For details on MSCTRAIL thawing and infusion procedures see section 12.2.3 of SoDA**.**

11.3 Return of the empty shipper to CCGTT:

- When the empty shipper is returned, CCGTT staff will download and review the temperature data from the electronic logger to confirm that temperature remained within the specified range (between -135°C and -190^{0}C) during MSCTRAIL transport to the trial site.
- CCGTT will complete section 3 of the 'MSCTRAIL Transport and Quality Checklist' and will email the fully completed form with a copy of the downloaded Temperature Data Report to the site staff and to UCL CTC.

The MSCTRAIL QP Batch Release Certificate, MSCTRAIL Certificate of Analysis, TSE statement, Temperature log during transport, MSCTRAIL Transport and Quality Checklist need to be filed in the ISF together with any correspondence relating to the ATIMP.

11.4 MSCTRAIL TEMPERATURE EXCURSION during transport from CCGTT to trial site

Temperature excursions outside of the acceptable range -135°C to -190°C occurring during MSCTRAIL shipment from CCGTT to the trial site must be reported to UCL CTC and CCGTT as soon as possible.

MSCTRAIL should not be taken out from the shipper and should not be administered to the patient until notice from UCL CTC.

UCL CTC will contact CCGTT, and the Sponsor pharmacist, who will advise whether MSCTRAIL may be administered or not. Decisions regarding final product disposition are the responsibility of the releasing QP.

UCL CTC will notify the site and the PI whether MSCTRAIL can be used to treat the patient, returned with the shipper to CCGTT or destroyed at the site.

All correspondence relating to notification of temperature excursion must be filed in the ISF.

Procedures are outlined in the "UCL CTC Procedures for Reporting Temperature Excursions" document, which is held in the ISF and PSF. UCL CTC should be notified of temperature excursions using the Notification of Temperature Excursions document.

12. PROCEDURES RELATED TO TRIAL DRUGS ADMINISTRATION

12.1 Procedures related to the trial NIMPs

Pemetrexed, cisplatin and pembrolizumab are given as separate intravenous infusions according to the site's local policy and current SPC. Carboplatin can be used instead of cisplatin if clinically appropriate at the discretion of treating clinician. Details can be found in protocol section 8.3.2.

12.2 Procedures related to MSCTRAIL infusion

The PI should ensure that MSCTRAIL administration is performed by experienced staff trained according to local guidelines for infusion of cryopreserved cellular products. All staff performing procedures related to ATIMP infusion must have read and understood the TACTICAL protocol and Summary of Drug Arrangement in order for them to be delegated this duty and be signed off by the PI on the Delegation Log for the Trial.

Staff nurses administering cellular products in standard care are permitted to administer ATIMPs after receiving trial specific training i.e. reading relevant sections of the SoDA. Training should be recorded appropriately. A research nurse may be delegated oversight of staff nurses by the PI, in lieu of listing staff nurses on the Delegation Log.

12.2.1 Patient assessments prior to, during and after MSCTRAIL infusion

The Principal Investigator (or delegate) must ensure all required pre-infusion investigations have been performed as detailed in TACTICAL protocol sections 8.4 and 9.2. In addition patient should

be monitored immediately prior to MSCTRAIL infusion, every 15 minutes during the infusion, every 30 minutes for the 2 hours after the infusion and hourly thereafter until 4 hours post infusion. Monitoring should include:

- Temperature
- Pulse*
- Blood pressure
- Respiratory rate
- Oxygen saturation level*

*Heart rate and Oxygen saturation should be monitored continuously throughout infusion

An ECG is also required pre and 4 hours post MSCTRAIL infusion
All assessments performed should be recorded in the patient medical notes.

12.2.2 ATIMP Checks BEFORE MSCTRAIL thawing

The cryopreserved MSCTRAIL is delivered from CCGTT to the ward in a temperature monitored vapour phase cryoshipper as outlined in section 11 of the SoDA. Once the shipper quality checks described in section 11.2 have been performed and passed, the designated nurse/doctor responsible for MSCTRAIL infusion should complete the relevant details on the **MSCTRAIL Patient Accountability Log** (the same log is completed for each MSCTRAIL infusion for the trial patient). The following checks should be performed:

- MSCTRAIL dose listed on the paperwork received with the shipper matches the dose prescribed on the **TACTICAL Prescription for MSCTRAIL** for the patient.
- The number of MSCTRAIL cryobag(s) received matches the accompanying paperwork
- **MSCTRAIL Dose** listed on the label to be infused matches the dose prescribed on the **TACTICAL Prescription for MSCTRAIL** for the patient.
- **MSCTRAIL** is within the expiry date specified on the label

Examples of MSCTRAIL labels are shown in section 7 of the SoDA.

12.2.3 MSCTRAIL Thawing

MSCTRAIL is contained within clear and individually labelled 250 ml CryoMACS® freezing bags, placed inside a secondary 'overwrap' bag. There are likely to be 2 cryobags of cryopreserved MSCTRAIL to infuse.

Cryobags should be thawed individually and the 2nd bag only thawed once the 1st bag has been infused.

MSCTRAIL is thawed in a temperature monitored water bath at 37°C using the site standard procedure for thawing of frozen cellular products. The principles of aseptic non-touch technique (ANTT) should be observed throughout the procedure.

Upon thawing, the appearance of MSCTRAIL suspension for infusion is a turbid yellowish liquid which should be free from visible particulates upon mixing/shaking.

The MSCTRAIL suspension should be closely visually inspected for clumping of cells and bag should be gently mixed/agitated to break up clumps and re suspend product before beginning infusion.

MSCTRAIL MUST BE INFUSED WITHIN 60 MINUTES OF THAWING

The time of MSCTRAIL thawing completed and time infusion completed must be recorded on the **MSCTRAIL Patient Accountability Log**.

12.2.4 MSCTRAIL Infusion

MSCTRAIL is supplied in 250 ml CryoMACS® freezing bags, with a maximum of 100ml cell suspension per bag and a minimum concentration of $2x10^6$ MSCTRAIL per ml.

The target MSCTRAIL dose will be administered as an intravenous injection by a trained member of staff. Infusion of MSCTRAIL should be in accordance with local policies for infusion of cryopreserved cellular product.

Infusion must be done over 20-30 minutes and must be within 60 minutes of thawing.

MSCTRAIL suspension should be uniformly dispersed in the bag/infusion line ensuring there are NO CLUMPS or CELL AGGREGATES present.

The nurse should remain with the patient during the infusion taking observations as detailed in section 12.2.1 and protocol sections 8.4.

Infusion from CryoMACS bag

- These bags can be attached to a blood transfusion giving set by screwing the cap off one of the two ports on the cryobag and piercing this with a spike from the giving set.
- The bag should be infused over 20 - 30 minutes through a CVC/Hickman line or newly inserted peripheral IV access as standard local practice line. If observations are abnormal, the infusion should be slowed and the medical team should review. **The time from thawing to completing infusion of each bag should be no longer than 60 minutes.**
- The bag should be visually inspected every 10minutes for cell clumping and to ensure cells have not gravitated and collected at the bottom of the bag invert and gently mix the bag.
- Once the contents of the bag have been infused, refill the bag with approximately 20 ml sterile 0.9% sodium chloride (saline) and infuse the remainder of the product, thus rinsing the cryobag and minimising any loss of cells.
- If more than 1 bag is to be infused, **once the first bag has been infused without problems, the second bag can be thawed** and infused via the same giving set, remembering to rinse each bag with a further 20 ml of saline. This process should be repeated until the required volume of cells has been infused.

- After the final bag of the MSCTRAIL has been transfused and rinsed, the blood administration set should be flushed using 20-30 ml of saline, to ensure that no cells are left in the giving set.

The time of thawing completed, time infusion started and completed, and clinical observations (pre and post infusion) must be recorded on the **MSCTRAIL Patient Accountability Log**.

12.2.5 Post MSCTRAIL infusion procedures

Once all bags have been infused, the empty cryobags are disposed of in the appropriate containers in accordance with local hospital waste management policy.

Patients should continue to be monitored every 30 minutes for the 2 hours after MSCTRAIL infusion and hourly thereafter until 4 hours post infusion (details in protocol section 8.4.1).

All documentation relevant to the infusion, including MSCTRAIL prescription, MSCTRAIL Patient Accountability log and any pre-medication given must be completed in full and filed in the appropriate patient's medical/nursing notes.

The following is recorded in the medical notes:

- Date of infusion
- Patient Trial number
- Name of product (MSCTRAIL)
- Batch number
- Volume of cells infused
- Total dose of MSCTRAIL infused
- Number of cryobags infused
- Time intervals for infusion (time of thawing complete, start time and end time of infusion)

The completed **MSCTRAIL Transport and Quality Checklist, MSCTRAIL prescription and MSCTRAIL Patient Accountability Log** should be filed in the ISF.

Patient trial number must be added to the label at the clinical site post administration by staff involved in MSCTRAIL administration

If an error, accident or adverse event/reaction occurs during the infusion process it is essential to record this in the patient's medical/nursing notes and report to UCL CTC according to the Trial protocol.

13. MSCTRAIL SPILLAGE AND DESTRUCTION

13.1 Accidental spillage of MSCTRAIL

In the event of MSCTRAIL spillage no additional hazards above those encountered when administrating cellular blood products and handling blood samples are required, unless different requirements have been stipulated by the local GMO Safety Committee or Biological Safety Officer.

Gloves should be worn and the spillage covered with 2% Virkon solution or other suitable Sodium Hypochlorite disinfectant.

Contaminated materials (bags, tissues, swabs, needles, syringes etc.) should be disposed in the appropriate containers for incineration, according to local clinical waste management policy.
Traces of the disinfectant should be removed from the spill site by wiping the area thoroughly with 70% alcohol.

The TACTICAL Trial Coordinator at UCL CTC should be notified immediately if there is a considerable spillage of the product, for example loss of product.

13.2 MSCTRAIL Destruction

a. where MSCTRAIL is still frozen in the vapour shipper/ NOT thawed at the trial site

In the event that MSCTRAIL is not used for patient infusion (e.g. patient is withdrawn from trial treatment after the ATIMP has been shipped to the site) AND the ATIMP is still frozen in the vapour shipper, the PI/site investigator will inform UCL CTC and CCGTT. CTC and CCGTT will instruct the Site whether MSTRAIL should be returned to CCGTT (to be used for another patient or for use in future ethically approved research). CCGTT will arrange for the trial courier to pick up the vapour shipper with the frozen MSCTRAIL in such cases.

If MSCTRAIL does not need to be returned to CCGTT, CTC will authorise the Site (by email) to destroy the ATIMP according to the Site's local procedures for disposal of cellular therapy products. Destruction of MSCTRAIL should be recorded on the **MSCTRAIL Patient Accountability Log**. The log should be filed in the AMF and a copy sent to the Trial Coordinator at the CTC on request.

b. where MSCTRAIL has been thawed at the trial Site

In the event that MSCTRAIL was thawed but not used for patient infusion (e.g. patient became ineligible for MSCTRAIL infusion, not all thawed MSCTRAIL was infused within 60min), the Site staff should destroy the (remaining) ATIMP according to the local procedures for disposal of cellular therapy products. Destruction of MSCTRAIL should be recorded on the **MSCTRAIL Patient Accountability Log** and the log filed in the AMF. CTC should be informed and a copy of the log sent to the TACTICAL Trial Coordinator.

Details of the Site local drug destruction policy should be also filed in the ISF.

14. ACCOUNTABILITY DOCUMENTS FOR TRIAL DRUGS

14.1 MSCTRAIL Accountability logs (trial ATIMP)

In compliance with the ATMP regulations (120/2009/EC), the PI (or delegate) must ensure that there is a system in place allowing for full traceability of MSCTRAIL received from CCGTT and administered to patients. Information related to MSCTRAIL traceability has to be kept for a minimum of 30 years after the expiry date of the ATIMP.

During the course of the trial UCL CTC will request copies of the following documents related to MSCTRAIL accountability:

- **MSCTRAIL Request Form**
- **MSCTRAIL Transport and Quality Checklist**
- **MSCTRAIL Patient Accountability Log**

These forms should be completed and retained in the relevant section of the ISF for each patient treated with MSCTRAIL.

14.2 Pemetrexed, cisplatin/carboplatin and pembrolizumab Accountability logs (trial NIMPs)

Sites should ensure that appropriate systems are implemented that will allow adequate reconstruction of movement and administration of all NIMPs identified in the Protocol (pemetrexed, cisplatin, carboplatin and pembrolizumab).

15. MSCTRAIL SHELF LIFE EXTENSION

MSCTRAIL shelf life is 12 months when stored between -135 and -196^0. The expiry date is specified on the ATIMP label.

In addition, ongoing stability data will be acquired by the manufacturer, CCGTT, using the 'stability protocol' described in the ATMPD. This data may be used to extend MSCTRAIL shelf life if needed and the ATIMP will be relabelled with the new expiry date by CCGTT.

CCGTT will ship MSCTRAIL to the trial site only if QP released and within the specified expiry date. Site staff should confirm the expiry date on the label prior to patient MSCTRAIL infusion as detailed in section 12.2.2.

16. MSCTRAIL RECALL

MSCTRAIL is stored at CCGTT and is only shipped to the trial site if the release criteria specified in the ATMPD have been met and the product QP released. In the event of MSCTRAIL being recalled following shipment to the trial site, UCL CTC and CCGTT will instruct the PI and site staff whether to destroy the recalled MSCTRAIL (section 13.2) or to arrange transfer of the recalled MSCTRAIL back to CCGTT. CTC and CCGTT will liaise with the site to ensure replacement MSCTRAIL is supplied where necessary.

17 HEALTH AND SAFETY

Staff must follow local site guidelines on safety dealing with biological materials. When handling biological samples disposable gloves must be worn at all times.

Thermoprotective gloves must be used when retrieving cryobags from the liquid nitrogen cryoshipper. If liquid contacts the skin, frozen tissues should be flooded with tepid water. Cryogenic burns that result in blistering or deeper tissue freezing should be promptly seen by a physician.

Appendix 1 SUMMARY OF DOCUMENTS ASSOCIATED WITH SoDA

Document	Completed by	Timepoint
TACTICAL Prescription for MSCTRAIL	Site Investigator	Prior to cycle 1 Prior to cycle 2 Prior to cycle 3
MSCTRAIL Request Form	**Section A**: UCLH (to order MSCTRAIL) **Section B**: CCGTT (to acknowledge MSCTRAIL order)	Following registration Prior to cycle 2 Prior to cycle 3
MSCTRAIL product labels	N/A - MSCTRAIL labelled by CCGTT *(Site to add patient trial number to the label post ATIMP thawing for administration)*	Following manufacture, prior to MSCTRAIL cryopreservation
QP Batch Release Certificate **Certificate of Analysis** **TSE statement**	CCGTT (Documents provided with the shipped MSCTRAIL)	Cycle 1 shipment Cycle 2 shipment Cycle 3 shipment
MSCTRAIL Transport and Quality checklist	**Section 1:** CCGTT when packing MSCTRAIL for shipping **Section 2:** Site staff when MSCTRAIL received at site **Section 3**: CCGTT when shipper returned and Temperature log downloaded from site	At each MSCTRAIL shipment
Temperature data log	N/A – temperature log during MSCTRAIL transport from CCGTT to Site	Upon return of empty shipper to CCGTT
MSCTRAIL Patient Accountability log	Site	At each MSCTRAIL infusion

Appendix 2 ABBREVIATIONS

ATIMP	Advanced Therapy Investigational Medicinal Product
ATIMPD	Advanced Therapy Investigational Medicinal Product Dossier
CCGTT	Centre for Cell and Gene Tissue Therapeutics (ATIMP manufacturer)
CTA	Clinical Trial Authorisation
DLT	Dose Limiting Toxicity
DMSO	Dimethyl sulfoxide
DPBS	Dulbecco's Phosphate Buffered Saline
GMO	Genetically modified organism
HAS	Human Albumin Solution
IMP	Investigational Medicinal Product
IB	Investigator Brochure
ISF	Investigator Site File
MHRA	Medicines and Healthcare Regulatory Authority
MSCTRAIL	Mesenchymal Stromal Cells genetically modified to express TRAIL (TNF-related apoptosis inducing ligand
NIMPs	non Investigational Medicinal Products
PI	Principal Investigator
PSF	Pharmacy Site File
QP	Qualified Person
SoDA	Summary of Drug Arrangements
TMG	Trial Management Group
TMF	Trial Master File
UCL CTC	University College London Cancer Trials Centre

SUMMARY OF DRUG ARRANGEMENTS

TACTICAL PHASE I

VERSION HISTORY

Version number	Date	Summary of changes from previous version	Changes made by	Released to sites on
1.0	09/01/2019	N/A - initial version	Bilyana Popova and Alex Day	18/01/2019
2.0	02/04/2019	Addition of pembrolizumab as a nIMP Change in MSCTRAIL courier Section 12.2 update - Clarity over assessments pre and post MSCTRAIL infusion Section 4 & 7 update – clarity that patients will receive the maximum dose in cohort 1 and doses for further cohorts will be decided by the TMG/IDMC	Alex Day	22/05/2019

PATIENT MSCTRAIL ACCOUNTABILITY & MONITORING OBSERVATION LOG			
ATIMP Name:	**MSCTRAIL:** allogeneic mesenchymal stromal cells (from umbilical cord donors) genetically modified to express TRAIL (TNF-related apoptosis inducing ligand).		
Site Name:	**UCLH**		
Patient Trial No:	**TAC -**	**Initials:**	

MSCTRAIL Infusion Cycle ___	**MSCTRAIL Dose:** *Please select one only*	☐ **4 x 10^8**	☐ **2 x 10^8**	☐ **0.8 x 10^8**
Date MSCTRAIL received on ward:	*dd/mm/yyyy*	**Time MSCTRAIL received ward:**	*24 hour clock e.g. 14:10*	

PRIOR TO INFUSION PATIENT'S IDENTITY MUST BE CHECKED BY TWO (2) DELEGATED INDIVIDUALS

Date of infusion: (dd/mm/yyyy)

Cryobag 1	**Batch Number:**		
Time thawing started: (24h clock) cryobag 1		**Time thawing finished:** (24h clock) cryobag 1	
Time infusion started (24h clock) cryobag 1		**Time infusion completed:** (24h clock) cryobag 1	
Cryobag 2	**Batch Number:**		
Time thawing started: (24h clock) cryobag 2	N/A (1 bag only)	**Time thawing finished:** (24h clock) cryobag 2	
Time infusion started: (24h clock) cryobag 2	N/A (1 bag only)	**Time infusion completed:** (24h clock) cryobag 2	
Bag inspected for clumping throughout infusion?	☐	**Bag gently mixed and/or flushed when appropriate?**	☐

Volume infused: cryobag 1	_____ ml	**MSCTRAIL dose infused:**	_____ x10^8 MSCTRAIL	**Total MSCTRAIL dose infused:**
Volume infused: cryobag 2	_____ ml ☐ **N/A**	**MSCTRAIL dose infused:**	_____ x10^8 MSCTRAIL	_____x 10^8 MSCTRAIL

Completed by: (print name)		**Signature:**	
		Date:	
Checked by: (print name)		**Signature:**	
		Date:	

Comments

PATIENT MSCTRAIL ACCOUNTABILITY & MONITORING OBSERVATION LOG			
ATIMP Name:	**MSCTRAIL:** allogeneic mesenchymal stromal cells (from umbilical cord donors) genetically modified to express TRAIL (TNF-related apoptosis inducing ligand).		
Site Name:	**UCLH**		
Patient Trial No:	**TAC -**	**Initials:**	

Time	**Completed by** (initials)	**Blood Pressure** (mmHg)		**Pulse Rate** (b/min)	**Temperature** (^{0}C)	**Respiratory Rate**	**Oxygen Saturation** (%)
		Systolic	**Diastolic**				
Baseline Observations							
15 minute observations during infusion (CryoBag 1)							
Further 15 minute infusion observations (Cryobag 2)							

Time	**Completed by** (initials)	**Blood Pressure** (mmHg)		**Pulse Rate** (b/min)	**Temperature** (^{0}C)	**Respiratory Rate**	**Oxygen Saturation** (%)
		Systolic	**Diastolic**				
30 minute observations for 2 hours post infusion							
+30							
+60							
+90							
+120							
Hourly observations until 4 hours post infusion							
+180							
+240							

Comments

Completed by: (print name)		**Signature:**	
		Date:	

When requested, please email a copy to ctc.TACTICAL@ucl.ac.uk

For UCL CTC use only		Selected for in-depth checks?	Yes		No	
Date received: _____	Date checked:_____	Queries? Y / N	Date queries resolved: _____			
CTC Staff member Initials: _____	Date: _____	*File all correspondence relating to queries with this accountability log*				

STRATEGIC

A phaSe IIA randomised controlled TRiAl of firsT line chemotherapy with/without MSCTRAIL in BAP1 mutatEd maliGnant pleural mesothelIoma Cases (STRATEGIC)

Trial Sponsor:	University College London
Trial Sponsor reference:	UCL/124627
Trial funders:	Innovate UK
Funders reference:	Innovate UK Reference: 105197
ISRCTN/Clinicaltrials.gov no:	[delete as applicable]
EUDRACT no:	2019-003021-16
CTA no:	TBC
Protocol version no:	1.0
Protocol version date:	

The STRATEGIC protocol V1 DRAFT can be found at the following link:

https://drive.google.com/file/d/17xFp_0CkVP_hQ34x0hs3SBPO5GenvrzG/view?usp=sharing

TACTICAL PATIENT SCREENING LOG

Principal Investigator	Sam Janes	Site Name	UCLH

Date of Initial Screen (dd/mm/yy)	Patient Initials	Gender (M/F)	Age	Did patient consent to trial? (Y/N)	Main reason for exclusion (use code on reverse)	Comments	Patient recruited to trial? (Y/N)
08/04/2019	[redacted]	M	81	N/A	29	For Pembro monotherapy	N
08/05/2019	[redacted]	M	50	N/A	15		N
08/05/2019	[redacted]	M	63	Y			Y
20/05/2019	[redacted]	F	57	N/A	29	TAC-01 started and could not wait for staggering	N
03/06/2019	[redacted]	M	59	N/A	2	Did not want a trial	N
17/06/2019	[redacted]	M	75	N/A	12		N
24/06/2019	[redacted]	M	56	N/A	12		N
17/06/19	[redacted]	M	63	Y			Y
01/07/2019	[redacted]	F	65	N/A	29	TAC-02 consented first and could not wait for staggering	N
19/08/2019	[redacted]	F	59	N/A	24		N
19/08/2019	[redacted]	M	70	N/A	24		N
16/09/2019	[redacted]	F	76	N/A	24		N
21/10/2019	[redacted]	M	74	N/A	12		N
28/10/2019	[redacted]	M	67	Y			Y
11/11/2019	[redacted]	F	60	N/A	7		N
13/11/2019	[redacted]	M	74	N/A	29	Squamous cell	N
13/11/2019	[redacted]	M	83	N/A	15		
18/11/2019	[redacted]	F	85	N/A	12		N
20/11/2019	[redacted]	F	74	N/A	29	Pembro monotherapy	N
19/11/2019	[redacted]	M	76	Y			Y
09/12/2019	[redacted]	M	74	N/A	31	Trial halted	N

Please make all entries in BLACK ink. Any errors should be crossed out with a single pen stroke, initialled and dated. When starting a new page, please fill in page number in bottom right hand corner.

Screening Log Instructions

When do I start using the screening Log?
Please begin using the screening log as soon as possible after site activation.

Which patients should I include on the Screening Log?
All patients aged 18+ identified with inoperable stage IIIb/IV lung adenocarcinoma

Maintenance of the screening Log.
It is important that completion of this log is undertaken on an ongoing basis rather than retrospectively. It is suggested that potential patients are assessed from clinic lists and at MDT meetings.

What do I do with the logs?
Logs should be kept in the the Investigator Site File, and faxed (0207 679 9871) or sent to the UCL CTC when requested. CTC staff will review the log at monitoring visits and may collect copies. Logs can also be used at regular in-house meetings to discuss missed patients and methods to increase accrual.

How do I complete the logs?
Date of screen, patient initials, gender and age should be recorded on the log. The codes below should be u[illegible] expressing the main reason for exclusion. If the reason does not fit into one of the categories provided, pleas[illegible] 'Other' and specify the reason in the comments section. If the patient is going to participate in the stud[illegible] **Y** or **N** in the final box 'Patient recruited to trial?'.

PAGE:

Who should I call if I have any questions or problems?
For further information, please contact TACTICAL Trial Coordinator Tel: 02076799644 or ctc.tactical @ucl.ac.uk

CODES for 'Main reason for exclusion'

Patient Refusal

1. Potential side-effects
2. Other – specify reason in comments

Protocol Exclusions*:*

5. EGFR mutation positive
6. EML4-ALK translocation positive
7. ECOG performance status >1
8. Inadequate haematological status
9. Inadequate organ function
10. Prior chemotherapy
11. Prior hormonal therapy
12. Prior radiotherapy
13. Prior immunotherapy
14. Prior treatment with cellular therapy
15. Surgical procedure in the previous 6 weeks prior to registration
16. Respiratory failure with baseline resting SpO2 <88%
17. Long term oxygen therapy
18. WHO Class III or IV pulmonary hypertension
19. Severe intercurrent infection
20. Active or infected wounds
21. Vaccination with any live attenuated vaccine within 30 days prior to trial registration
22. Subject has known sensitivity to any of the trial drugs
23. Prior malignancy
24. Symptomatic brain metastases
25. Myocardial infarction, or unstable or uncontrolled heart condition
26. Venous thromboembolism within the last 6 monthsLife expectancy of <12 weeks
27. Unwilling to use adequate and effective contraception during, and for 6 months after trial treatment
28. Pregnant or lactating
29. Other protocol exclusion (record reason in comments)

Other Exclusions

30. Geographical or logistical difficulties
31. Other (record reason in comments)