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Clinical Trial Protocol

PHASE 1 TRIAL: T4 IMMUNOTHERAPY OF HEAD AND NECK CANCER

Version 10
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Clinical Trial Protocol Version History

Version No.	Issue Date	Reason for Update
1.0	4-8-2012	N/A
1.1	4-9-2012	Version 1 under revision following preliminary feedback from MHRA
2.0	24-11-2012	Several minor revisions following EAG/ MHRA and REC review
2.1	3-12-2012	Inclusion of tocilizumab in management of cytokine storm; add ferritin measurement
2.2	5-4-2013	Clarify the terms enrolment/ registration in Inclusion Criteria. Amend Table 3 to clarify screening period (top of table) and add 1 screening ECG that was missing. Clarify nature of AE (adverse events) in Figure 5
2.3	February 2015	Update immunophenotyping panel (p30); manufacturing process (p31); blood samples taken from inpatients (p33); management of toxicity (p34 and Appendix 2); add serological testing of blood to be used to manufacture T4 immunotherapy (p35); modify details of blood volumes/ bottles taken over the 6 weeks post treatment (Chapters 6/9). Update tumour biopsy storage details (p50). Sample handling in section 9.3.3 updated (p52). References updated.
2.4	March 2015	Provide further detail on manufacturing process including amended Figure 4 to comply with IMPD version 4.1
2.5	May 2015	Correction of errors in superscripted notes on page 40 Realignment of "Xs" in Table on p40 Replacement of the inaccurate term "metronomic" with "low dose", as pertains to cyclophosphamide (no effect on treatment). Correction of typographical error (creatine kinase rather than creatinine kinase) Clarification of wording regarding prior treatments that preclude participation in the trial (p24) Clarification that manufacture of T4 immunotherapy may take 14-16 days Update drugs that may be used for local anaesthesia
3.0	October 2015	- Substitute cytokine measurement for ELISPOT analysis to render monitoring of MAGE A3/ A4-reactive T-cells easier and more informative. - Clarify that a drop in ejection fraction of >10% is a DLT only if the resulting ejection fraction falls below the normal range (lower limit 50%). Add a comment to state that any suspected new cardiac symptom will be notified to the trial steering committee.

		- Page 23: change so that criterion 6 is assessed within 4 weeks of treatment (not enrolment), while criteria 9 and 10 are assessed within 14 (rather than 7) days of enrolment. - Page 26 Table 1 and Section 5.1.3: Update volume of injection to 1 - 4mL (instead of 1mL as previously stated). - Page 27: Remove definitive reference to the fact that the maximum tolerated dose will necessarily be the recommended dose for Phase 2 testing, since this will be determined by the data monitoring/ trial steering committee upon completion of the trial. - Page 31: manufacturing flow chart updated. - Page 32: Correction of errors as follows. Insert "10% AB serum" (not 20%). Add text as follows: Use of ultrasound, where necessary; interim sterility tests performed on day 7 or 8 …and on day of release (usually day 15). In all cases, the italicized text has been added. - Page 36: increase flexibility of day -3 visit to "within 4 days of treatment", to allow for bank holidays etc.
3.1	December 2015	Page 32: Broaden description of those able to undertake intra-tumoural injection of T4 immunotherapy to read "The autologous T4 immunotherapy cell product will be administered to the patient by a head and neck cancer surgeon or a clinician who has experience of intra-tumoural injection, using ultrasound guidance where necessary to identify the viable tissue within the tumour."
4.0	December 2016	1. Secondary trial objectives have been updated to include the investigation of (i) persistence of T4+ T-cells at the site of intra-tumoural administration and their dose-dependent migration from that site into the peripheral circulation; (ii) effect of T4 immunotherapy upon global gene expression within the tumour microenvironment 2. Immune monitoring for MAGE A3/ A4-dependent T-cell cytokine release had previously been planned using two different technologies (ELISPOT/ Luminex). A combined cytokine release assay has been developed which allows both approaches to be combined when these cryopreserved samples are analysed. 3. Additional information is presented about the administration of a T4 radiotracer to a subset of up to 3-6 patients. This had previously been discussed in the protocol at multiple points but had not previously been acknowledged as a secondary objective and trial endpoint. Furthermore, the number of T4+ T-cells required to generate this radiotracer had been over-estimated previously. 4. Inclusion criteria have been re-worded to state "Regarding previous treatment, patients may have received prior systemic

		therapy, including platinum chemotherapy, at least one week earlier than the planned administration of T4 immunotherapy." This is to resolve an inconsistency with exclusion criterion 6". 5. Eligibility criteria. Timing of cardiac and blood tests pre-treatment have been specified as within 4 weeks of treatment, to eliminate an inconsistency with statements set out in section 6.1 and Table 3. 5. Tumour biopsies will be gathered from some patients for RNA seq analysis (instead of other assays). This recently developed technique can provide more information on global gene expression changes that occur within the tumour microenvironment following administration of T4 immunotherapy. 6. Appendix 2 (algorithm for management of cytokine release syndrome) has been updated to incorporate a number of changes described in recent publications.
5.0	January 2018	1. Modification of Research Team (page 8) 2. The maximum dose for this study is 1 x 109 T4+ transduced T-cells (page 26) which experience indicates can be formulated in a volume of 4mL. The limit on a total of 1 x 109 cells has therefore been removed. 3. Modification of definition of end of trial (section 4.11, page 29) 4. Pages 53-54. Clarification of assays to be performed on tumour biopsies and that up to three core biopsies may be taken, as indicated in the patient information leaflet.
6.0	April 2018	Protocol 5.0 was not used since Regeneron (who had originally agreed to undertake biopsy RNASeq and RNAScope analysis) withdrew support for the study. Consequently all changes to protocol 4.0 have been tracked in the submitted draft of protocol 6.0 Incorporate use of celecoxib to mitigate local inflammatory response to T4 immunotherapy. Clarify that tumour biopsies undertaken post T4 immunotherapy will not be collected after 4 weeks.
6.1	September 2018	Clarify SPECT CT imaging approach. Clarify that biopsies may be analysed for immune cells and/ or immune cell markers Clarify that celecoxib may be administered in apple sauce to patients with swallowing difficulties
7.0	January 2019	Update NICE guidance re treatment with cetuximab and platinum chemotherapy Non-clinical toxicology information updated

		Clinical experience with T4 immunotherapy during dose escalation phase of study (e.g. patients 1-15) added Modification of cohort 6 so that patients receive fludarabine/ cyclophosphamide conditioning therapy rather than low dose cyclophosphamide. This will be followed by 108 T4+ CAR T-cells. Revision of inclusion/exclusion criteria and requirements for contraception in light of addition of a fludarabine/ cyclophosphamide lymphodepletion step in cohort 6. Revision of dose-limiting toxicities definitions in light of addition of a fludarabine/ cyclophosphamide lymphodepletion step in cohort 6. Specification of need for more intensive monitoring of patients with moderate renal dysfunction who are treated with fludarabine/ cyclophosphamide lymphodepletion. Section added to detail management of toxicities expected due to lymphodepletion. Discretionary use of antimicrobial prophylaxis allowed and suggested agents listed. Alludes to potential shortening of T4 manufacturing process to 11 days (IMPD) with interim sterility testing also performed on day 4. Updated monitoring table for cohort 6. Serial monitoring of regulatory T-cells and myeloid derived suppressor cells will be undertaken. Mage A3/ A4 reactive T-cells will not be monitored in this cohort. Addition of an interim analysis step in the protocol, with publication of this analysis.
7.1	April 2019	Version 7.0 rejected by MHRA. Therefore modified as follows: Clarification that Fludarabine and Cyclophosphamide are considered as IMPs when used in cohort 6 Dose of fludarabine may be reduced by up to 50% if creatinine clearance is between 30-70mL/minute A justification has been added for the anticipated occurrence of additional toxicity in cohort 6. Dose-limiting toxicities are defined for patients treated in cohort 6. Contraceptive requirements have been updated for patients in cohort 6 Live vaccines are contraindicated in patients in cohort 6 Skin cancer is listed as a contraindication for patients considered for cohort 6
8.0	April 2021	The primary change in protocol 8.0 entails the incorporation of a seventh patient cohort in which low dose Flu/Cy lymphodepleting chemotherapy (as per cohort 6) is followed by T4 immunotherapy, administered in conjunction with

		nivolumab. Three doses of nivolumab will be administered, commencing one day prior to T4 immunotherapy and followed by doses after 4 and 8 weeks. For this reason, patients will undergo extended monitoring for 12 weeks, including two assessments (at 6 and 12 weeks) of tumour status post CAR T-cell treatment. Some additional tests will be performed to monitor for nivolumab induced immune-related adverse events. Section 1.2.6 provides a justification for the inclusion of PD1 inhibition in the therapeutic regimen administered to patients in cohort 7. Since nivolumab may influence trial secondary objectives, it is considered to be an IMP. Contraindications to recruitment have been broadened to include interstitial lung disease and nivolumab allergy. If no DLT has occurred within the four-week period following CAR T-cell treatment of the first patient in cohort 7, recruitment will open for the next two patients in that cohort. Disparities have been corrected that pertain to timing of lymphodepleting chemotherapy (from 2-11 days before T4 immunotherapy) and number of patients who may undergo tumour biopsy, together with timing of these biopsies.
8.1	July 2021	No changes compared to 8.0
9.0	October 2022	T4 dose increased to 1 x 10^9 cells (3 patients; cohort 8), in combination with fludarabine, cyclophosphamide and nivolumab. Expected sample size increased Current version of CTCAE used to grade adverse events
9.1	November 2023	Updated personnel and blood volumes taken for exploratory assays. Correction of minor errors.

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SIGNATURES

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CONFIDENTIALITY STATEMENT

This Clinical Trial Protocol is a confidential document for the sole information and use of the investigating team and regulatory and other reviewing bodies. However, this document may be made available to other parties upon request to the Chief Investigator.

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List of Abbreviations

4αβ	-	A chimeric cytokine receptor in which the human IL-4 receptor α ectodomain is fused to the human IL-2 receptor β transmembrane and endomain
ACT	-	Adoptive Cell Therapy
AE	-	Adverse Event
ALT	-	Alanine Aminotransferase
API	-	Active Pharmaceutical Ingredients
AR	-	Adverse Reaction
ATMP	-	Advanced Therapeutic Medicinal Product
CAR	-	Chimeric Antigen Receptor
CD	-	Cluster of Designation
CI	-	Chief Investigator
CK	-	Creatine Kinase
CR	-	Complete Response
CRF	-	Case Report Form
CRP	-	C-Reactive Protein
CT	-	Computed Tomography
CTCAE	-	Common Terminology Criteria for Adverse Events
CXR	-	Chest X-Ray
DLT	-	Dose Limiting Toxicity
DSUR	-	Development Safety Update Report
ECG	-	Electrocardiogram
ECHO	-	Echocardiogram
EGF(r)	-	Epidermal Growth Factor (receptor)
ENT	-	Ear, Nose and Throat
EOP	-	End of Production
FACS	-	Fluorescence Activated Cell Sorting
FBC	-	Full Blood Count
FDG	-	Fluorodeoxyglucose
Flu/Cy	-	Fludarabine and cyclophosphamide
GMP	-	Good Manufacturing Practice
H&E	-	Haematoxylin & Eosin Staining
HIV	-	Human Immunodeficiency Virus
HLA	-	Human Leukocyte Antigen
HTLV	-	Human T-lymphotropic virus
ICANS	-	Immune Effector Cell Neurotoxicity Syndrome
IFN	-	Interferon
IL	-	Interleukin
IPC	-	Internal Process Control
KHP CTO	-	King's Health Partners Clinical Trials Office
LD	-	Lymphodepleting
LFT	-	Liver Function Tests
MABEL	-	Minimum Anticipated Biological Effect Level

MDM	-	Multi-disciplinary Team Meeting
MHRA	-	Medicines and Healthcare products Regulatory Agency
MRI	-	Magnetic Resonance Imaging
MSKCC	-	Memorial Sloan Kettering Cancer Center
MTD	-	Maximum Tolerated Dose
MUGA	-	Multi-Gated Acquisition Scan
NCI	-	National Cancer Institute
NE	-	Not Evaluable
PBMC	-	Peripheral Blood Mononuclear Cells
PBS	-	Phosphate Buffered Saline
PD	-	Progressive Disease
PR	-	Partial Response
QC	-	Quality Control
QP	-	Qualified Person
(q)PCR	-	(Quantitative) Polymerase Chain Reaction
RDPT	-	Recommended Dose for Phase 2 Testing
REC	-	Research Ethics Committee
RECIST	-	Response Evaluation Criteria In Solid Tumours
RTK	-	Receptor Tyrosine Kinase
RT-PCT	-	Reverse Transcriptase Polymerase Chain Reaction
SAE	-	Serious Adverse Event
SCCHN	-	Squamous Cell Carcinoma of Head and Neck
SCID	-	Severe Combined Immunodeficiency
SD	-	Stable Disease
SPECT	-	Single Photon Emission Computed Tomography
SmPC	-	Summary of Product Characteristics
SOP	-	Standard Operating Procedure
(S)SAR	-	(Suspected) Serious Adverse Reaction
SUSAR	-	Suspected Unexpected Serious Adverse Reaction
T2A	-	*Thosea Asigna* 2A Peptide
T1E	-	A chimeric peptide comprising transforming growth factor-α (upstream of cysteine 1) fused to epidermal growth factor (downstream of cysteine 1)
T1E28z	-	A chimeric antigen receptor in which the T1E peptide is fused to CD28 (hinge transmembrane and endodomain) followed by CD3ζ
TBI	-	Total Body Irradiation
TIL	-	Tumour-Infiltrating Lymphocytes
(free) T4	-	Thyroxine
T4	-	The combination of 4αβ co-expressed with T1E28z
TGF-α	-	Transforming Growth Factor-α
TMF	-	Trial Master File
TNF	-	Tumour Necrosis Factor
Treg	-	Regulatory T-Cells
TUNEL	-	Terminal Deoxynucleotidyl Transferase dUTP Nick End Labelling
UAR	-	Unexpected Adverse Reaction
U&E	-	Urea and Electrolytes
WOCB	-	Women of Childbearing Potential

1. INTRODUCTION

1.1 Background Disease Information

1.1.1 The Unmet Need: Improved Loco-regional Control of Head and Neck Cancer

Squamous cell carcinoma of the head and neck (SCCHN) is the sixth most common cancer worldwide, with 600,000 cases diagnosed annually (1, 2). Despite state of the art multimodal and multidisciplinary therapy incorporating surgery, radiotherapy, chemotherapy and targeted agents, five-year survival remains at only 50%. Indeed there has been little improvement in patient survival over the past 30 years (1, 3). In patients with recurrent or metastatic disease the median survival time is a mere six months (4, 5).

In designing this clinical trial, we have identified two key areas of unmet need.

(i) Locally recurrent SCCHN
Loco-regional disease accounts for the majority of deaths in patients with SCCHN (4, 5). This contrasts with most other solid tumour types in which metastatic spread constitutes the primary cause of death. Loco-regional treatment failure occurs in 60% - 70% of patients after conventional surgery and radiation (6-8). Recurrent tumours can be painful and may invade into vital tissues, resulting in considerable morbidity and mortality. In that setting, younger and fitter patients may be suitable for treatment with (re)-irradiation (9) or salvage surgery (9). Surgery is the treatment of choice for resectable lesions, although salvage rates tend to vary, based on the site of primary tumour. Unfortunately however, co-morbidity and/ or advanced disease stage commonly precludes the selection of either of these options for patients. Furthermore, re-irradiation may not be possible if tumours recur in a previously irradiated location (10). If surgery or radiation are not suitable, recurrent head and neck cancer is often managed with chemotherapy - either alone (11-13) - or together with the anti-EGF receptor antibody, cetuximab (14). In support of this approach, the combined use of cetuximab together with platinum-based chemotherapy has led to a three-month prolongation in median survival for patients with recurrent or metastatic SCCHN (15) and this combination is now recommended under some circumstances as an option for treating recurrent or metastatic SCCHN (https://www.nice.org.uk/guidance/ta473/chapter/1-Recommendations, accessed 07/11/2018). Furthermore, over 50% of patients with recurrent SCCHN died as a direct consequence of loco-regionally recurrent disease even though salvage treatment was performed (16). These considerations highlight a clear need for additional therapeutic options for patients with locally recurrent SCCHN.

(ii) Newly diagnosed patients with locally advanced SCCHN
About 1 in 10 patients with newly diagnosed SCCHN are not suitable for any form of active therapy whatsoever (17). In a two-year period, forty-four such patients were reviewed by the multidisciplinary team meeting (MDM) at Guy's and St Thomas' NHS Foundation Trust, which covers the South-East London Cancer Network. Patients are unsuitable for conventional therapies owing to co-morbidity, locally advanced disease, metastatic disease and patient refusal. For those patients, the 30 week mortality rate is

100% (17). Currently, management of such individuals involves counselling, symptom control and support of both airway (tracheostomy) and nutritional status (eg enteral feeding via PEG gastrostomy). A more effective therapeutic approach that may achieve some improvement in local disease control could provide meaningful additional benefit for some of these patients.

(iii) Patients with SCCHN commonly also develop metastatic disease. Even at the time of diagnosis, regional nodal involvement is found in 43% and distant metastasis in 10% of patients (18).

These considerations emphasise the unmet need for more effective treatment approaches for patients with SCCHN that act both locally and systemically. This study aims to investigate intra-tumoural administration of a novel cellular immunotherapy in patients with at least one measurable and accessible site of loco-regional progressive disease with or without concurrent distant metastases.

1.1.2 Target and Rationale

In SCCHN, the ErbB family of receptor tyrosine kinases (RTK) represents a highly attractive target for novel therapies. The ErbB family comprises four members, namely epidermal growth factor receptor (EGFr or ErbB-1), ErbB-2 (HER2/neu), ErbB-3 and ErbB-4 (19-21). These molecules provide a molecular network that plays a fundamental role in many biological systems. Individual ErbB molecules bind 2-8 distinct ligands with the exception of the orphan receptor, ErbB2. Signal complexity is diversified by the ability of ErbB RTK to undergo ligand-driven homo- or hetero-dimerization. Although all possible binary ErbB combinations have been detected, ErbB2 is the preferred dimerization partner for all other family members, owing to its constitutively "open" ectodomain (22). In the adult, ErbB receptors are expressed at low levels in several non-haemopoietic tissues. However, increased synthesis of ErbB family members correlates strongly with the development of several solid tumors, particularly squamous cell carcinomas.

Overwhelming evidence implicates dysregulated ErbB signalling in the pathogenesis of SCCHN (23-30). This tumour represents a classical model of EGF-driven oncogenesis since it strongly over-expresses ErbB1 in >90% of cases. Over-expression of ErbB1 is implicated in resistance to radiotherapy and is a strong prognostic marker for poor survival and metastasis (28-30). Furthermore, since the level of ErbB1 expression increases with tumour progression, this molecule represents an increasingly appropriate target with disease evolution (31, 32). Disappointingly however, clinical data indicate that only a minority of patients with SCCHN benefit from ErbB1 targeted therapies (33). In part, this may result from the frequency with which other ErbB family members are co-expressed, conferring worsened prognosis (23, 24, 26). In many tumours, therapeutic resistance to ErbB-directed therapies is mediated by upregulated activity of non-targeted family members (34-38). In agreement with this, resistance of SCCHN cell lines to ErbB1-targeted antibody or small molecule agents has been associated with increased ErbB2/ErbB3 signalling (33, 39).

1.2 Background Therapeutic Information

1.2.1 Chimeric Antigen Receptor-Engrafted T-cells in the Treatment of Relapsed Head and Neck Cancer

The above considerations provide a strong rationale for targeting of the extended ErbB network in SCCHN. This study aims to achieve this goal using a novel immunotherapeutic strategy based upon chimeric antigen receptor (CAR) technology. Chimeric antigen receptors are fusion molecules in which a targeting moiety (eg an antibody fragment or ligand) is coupled in series to hinge, transmembrane and T-cell activating domains (40, 41). When expressed in T-lymphocytes, CARs engage native target on the tumour cell surface, obviating the need for either HLA expression or antigen processing. Use of this technology is particularly suited to SCCHN in light of the frequent down regulation by tumour cells of HLA antigens, co-stimulatory ligands or other elements of the antigen processing machinery (42-45). The development of CAR-based immunotherapy has attracted increasing interest in recent years, largely owing to the development of efficient systems for genetic modification of primary human T-cells. Consequently, it is now feasible to engineer large numbers of patient-derived T-cells with specificity for a chosen tumour-associated target molecule.

A number of investigators have examined the efficacy and safety of adoptively transferred T-cells to treat SCCHN (46). Occasional striking and durable clinical responses have been described in patients with advanced disease, both following loco-regional and systemic delivery (47, 48). However, logistic difficulties involved in the generation and expansion of tumour-specific T-cells has compromised further study. With the advent of efficient genetic targeting and *ex-vivo* T-cell culture systems, we now wish to re-investigate this therapeutic modality for patients with SCCHN.

1.2.2 T4 Immunotherapy

The ErbB family of receptor tyrosine kinases will be targeted with a second generation CAR named T1E28z. In this fusion receptor, targeting is achieved with a novel chimeric peptide named T1E. To create the T1E peptide, the five most N-terminal amino acids (amino acids 971-975 of pro-epidermal growth factor precursor (NP_001954.2)) were replaced by sequences encoding the seven most N-terminal amino acids of the mature human TGF-α protein (amino acids 40-46 of pro-transforming growth factor α isoform 1 (NP_003227.1)). The T1E peptide retains the ability of its parent ligands to bind ErbB1 but can also bind with high affinity to the ErbB2/ErbB3 heterodimer combination (49-51).

To engineer the T1E28z fusion receptor (52), cDNA encoding for T1E (amino acids 1-55) was placed downstream of a colony-stimulating factor-1 leader (bases 1-75) and upstream of a human CD28-derived hinge, transmembrane and endodomain (amino acids 114-220), followed by the cytoplasmic domain of the T-cell receptor CD3ζ chain (amino acids 52-164). The structure of the T1E28z CAR is shown in cartoon form in **Figure 1A**. Expression of T1E28z in transduced human T-cells has been demonstrated by flow cytometry and western blotting.

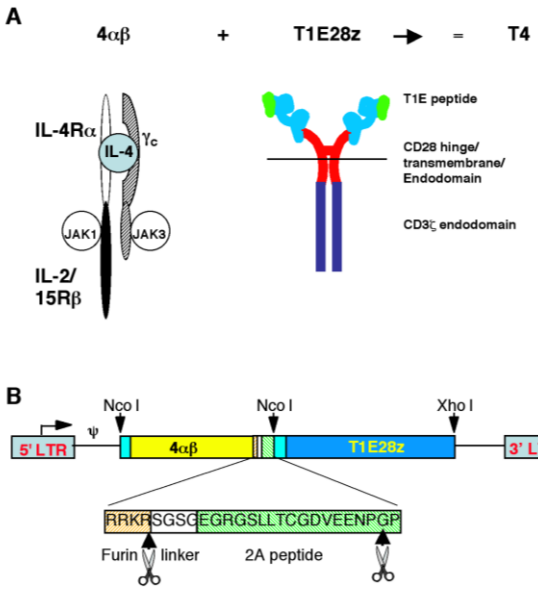


FIGURE 1. Transgene products. **A.** Cartoon structure of the transgenes to be co-expressed in patient T-cells. **B.** The SFG T4 retroviral vector. Stoichiometric co-expression of 4αβ and T1E28z is achieved using a *Thosea Asigna* (T2A) peptide. Since this leaves a short peptide overhang on the C-terminus of the N-terminal protein (4αβ), a Furin cleavage site is placed upstream as indicated.

In this clinical trial, the T1E28z CAR will be co-expressed in human T-cells together with a chimeric cytokine receptor named 4αβ. The 4αβ chimeric cytokine receptor consists of a fusion in which the human IL-4 receptor (IL-4R) α ectodomain (amino acids 1-233) is fused to the transmembrane and endodomain of the common β receptor subunit (β_c; amino acids 241-551) used by IL-2 and IL-15 (53). The structure of 4αβ is shown in cartoon form in **Figure 1A**. Binding of IL-4 leads to approximation of the 4αβ chimeric cytokine receptor with the common γ chimeric cytokine receptor. By this means, a potent IL-2-like growth signal is delivered selectively to the transduced T-cells by IL-4, a cytokine that is normally a much weaker growth factor for T-cells. Use of the 4αβ fusion receptor enables rapid, robust and selective expansion of T-cells *ex-vivo* in response to the cytokine interleukin-4 (IL-4) (53).

Expression of T1E28z and 4αβ will be achieved in patient T-cells using the SFG onco-retroviral expression vector (**Figure 1B**) (54). Stoichiometric co-expression of both transgenes will be obtained obtained by insertion of an intervening optimized Furin cleavage site (RRKR; (55)) followed by a linker (SGSG) and finally a *Thosea Asigna* 2A (T2A) peptide sequence (EGRGSLLTCGDVEENPGP; **Figure 1B**) (56). Such peptide sequences cause ribosomal skipping during protein translation, meaning that two polypeptides are produced from a single open reading frame (ie sequences encoded on either side of the "skip" motif). Since the upstream protein will contain a T2A-derived peptide on its C-terminus (EGRGSLLTCGDVEENPG), the incorporation of an upstream Furin cleavage site is a useful device to remove this unwanted sequence.

The combination of T1E28z and 4αβ is referred to as "T4". Consequently, we use the term "T4 immunotherapy" to describe the therapeutic application of autologous T-cells derived from patients that have been engineered to co-express 4αβ and T1E28z. This study will determine the safety and maximum tolerated dose of intra-tumoural T4 immunotherapy (alone, following lymphodepletion with fludarabine and cyclophosphamide, or following combined lymphodepletion and PD-1 immune checkpoint blockade) in patients with relapsed SCCHN. We will not administer exogenous IL-4 to patients enrolled in this study.

1.2.3 Pre-Clinical Efficacy

Pre-clinical efficacy data are presented in greater detail in the **Investigator Brochure** and the **Investigational Medical Product Dossier.** Findings are summarized briefly below.

First, we set out to characterize precisely which ErbB homo- and heterodimer combinations can be targeted using the T1E28z fusion receptor. To achieve this, T1E28z T-cells were co-cultivated with a panel of (ErbB ^{neg}) 32D haemopoietic cells (57) that had been engineered to express ErbB receptors in all possible single or dual combinations. T-cell activation was indicated by production of the pro-inflammatory cytokines, interferon-γ (IFN-γ) and IL-2. Comparison was made with a variety of control CARs. T1E28z but not control T-cells were activated by targets that express ErbB1 or ErbB2/3 heterodimers. Weaker activation was also observed in response to ErbB4 homo- and heterodimers. To assess anti-tumour activity, T1E28z T-cells were co-cultivated with a panel of SCCHN cell lines, representing a broad diversity of ErbB receptor expression. T1E28z, but not control T-cells, underwent activation when cultured with several SCCHN cell lines. This was accompanied by the selective destruction of tumour cell monolayers by T1E28z, but not control T-cells. Together, these findings confirm that human T1E28z-engrafted T-cells can recognize a broad range of human SCCHN cell types as a consequence of engagement of ErbB receptors on target cells.

To examine anti-tumour activity of T1E28z and T4-cells *in-vivo*, a model was established in SCID Beige (immunodeficient) mice engrafted with a firefly luciferase-expressing SCCHN xenograft. When compared to control animals, both populations of ErbB re-targeted T-cells achieved significant anti-tumour activity, without clinical evidence of toxicity. Owing to ease of generation/ expansion, the T4 vector has been chosen for further clinical translation.

1.2.4 Pre-Clinical Safety Testing

Pre-clinical safety testing data are presented in greater detail in the **Investigator Brochure** and the **Investigational Medical Product Dossier**. Findings are summarized briefly below.

We reasoned that it would be appropriate to test toxicity of the therapeutic product (eg human $T4^+$ T-cells) in mice for the following reasons.

- Human T-cells can elicit profound toxicity in mice, as illustrated by their ability to induce xenogeneic graft versus host disease following appropriate conditioning treatment (58).

- All human ErbB ligands (including EGF, TGF-α and heregulins) are active on murine target cells (59, 60). This means that human $T1E28z^+$ T-cells would be expected to engage murine cells that express relevant ErbB receptors. In agreement with this, we have shown that human T-cells engrafted with the human T1E28z fusion receptor can recognize and destroy a murine $ErbB^+$ SCCHN tumour cell line, B7E3 (61).

- Consequently, we examined the toxicity of human $T1E28z^+$ T-cells following adoptive transfer into SCID Beige recipient mice. T-cells were transferred either by the intra-tumoural or intravenous routes, directly following gene transfer or after *ex-vivo* activation on $ErbB^+$ tumour cells. In these studies, we observed no alteration in clinical status, weight or histological appearance of harvested organs in animals treated in this manner. Histology was assessed by an independent veterinary histopathologist, soon after *in-vivo* T-cell disappearance.

- In several studies that addressed the efficacy of T1E28z$^+$ or T4$^+$ T-cell immunotherapy, we observed no clinical evidence of T-cell mediated toxicity in mice, following intraperitoneal administration of T-cells. Animals were followed up for up to 128 days in these studies.

- Despite the above, dose-dependent toxicity has been demonstrated following intraperitoneal administration of larger doses of T4$^+$ T-cells. Toxicity appears to be aggravated by high tumour burden, and high T-cell transduction efficiency. The minimum dose at which toxicity has been observed in mice is 10 million transduced cells in SCID Beige mice and 4.5 million cells in NSG mice. By contrast, efficacy has been observed at lower doses indicating that even using this route of administration, there is a therapeutic window. Moreover, persistence of CAR T-cells has been demonstrated in NSG mice without any toxicity of pathology. Dose-dependent toxicity is observed in tumour-free mice at even higher T-cell doses. Evidence indicates that cytokine storm accounts for this finding.

Extrapolation to man of these findings is supported by the ability of the CAR to cross the species barrier and by the fact that dose-dependent toxicity can be elicited. However, it should be borne in mind that SCID Beige mice (in which these pre-clinical studies were undertaken) are highly immunodeficient. This may enhance the toxic potential of this approach since animals lack "cytokine sinks" that would be present in man in the absence of lymphodepletion.

We concluded that cautious dose escalation should be undertaken during clinical testing. Target cell doses reflected the efficiency of T-cell transduction. Use of the intra-tumoural route was deemed to be safest since pre-clinical imaging studies suggest that the cells remain at this site. We initiated dosing in man at 10^7 T4$^+$ T-cells - a level that, proportionately for weight, equates to 3000-fold below the toxic threshold as determined by IP injection in mice. This initial dose level has resulted in reproducible tumour regression in mice following regional (eg intraperitoneal) administration. Consequently, we set 10^7 T4$^+$ T-cells as the "minimum anticipated biological effect level" (MABEL) from which dose escalation will proceed cautiously, monitoring for dose-limiting toxicity as specified elsewhere in this Clinical Trial Protocol. In man however, efficacy at a starting dose of 10^7 cells is a remote possibility, considering that a typical 1cm^3 tumour mass contains approximately 10^9 cells (representing an effector to target ratio of 1:100).

At the time of drafting the protocol amendment of September 2022, we had treated a total of 19 patients with T4 immunotherapy. These comprised 5 groups (cohorts 1, 2, 3, 4 and 5) of 3 patients each that received 10, 30, 100, 300 and 1000 million CAR T-cells during what is referred to as the dose escalation phase of the study. Subsequently, a sixth cohort of 3 patients received lymphodepleting chemotherapy with fludarabine and cyclophosphamide followed by an intermediate T4 dose of 1 x 10^8 cells. In cohort 7, a single patient received fludarabine and cyclophosphamide followed by 1 x 10^8 T4$^+$ CAR T-cells together with nivolumab. However, this patient exhibited progressive disease at 6 weeks and was withdrawn from the study. Thus, after completion of 19 patients, no dose-limiting toxicities or CAR T-cell leakage into the circulation has been observed in any patient at any time in the six week period post treatment. Ten of 19 patients have achieved stable disease by RECIST criteria. However, no clinical responses have been observed, arguing that potency of this approach is still not adequate.

Following treatment of the first patient in cohort 7, the trial steering committee met to discuss the future of the trial. It was decided to suspend the current cohort 7 owing to lack of efficacy (and lack of toxicity).

Instead, it was proposed by the committee to open a new cohort (cohort 8) in which patients are treated with fludarabine and cyclophosphamide followed by 10^9 CAR T-cells, together with three doses of nivolumab. By this means, the dose of T4 will be re-escalated to the maximum deliverable dose. The trial steering committee will meet after completion of treatment of the first patient in this cohort to discuss whether to continue to treat further patients in the same cohort. If they approve the continuation of the cohort 8, the trial steering committee will meet again after the third patient has been treated. Should any of these 3 patients achieve a clinical response, cohort 8 will be expanded until all remaining retroviral vector units have been used. At the time of writing (September 2022) there are 8 units of vector remaining, allowing treatment of a maximum of 8 more patients in this clinical trial.

1.2.5 Lymphodepletion using Fludarabine and Cyclophosphamide

Several factors are believed to limit the success of adoptive T-cell immunotherapy (ACT) in patients with malignant disease. These include the presence of expanded populations of immunosuppressive cells in patients, including regulatory T-cells (Treg) and myeloid suppressor cells. Furthermore, access to supportive cytokines *in-vivo* is also believed to limit the survival of infused T-cells. To address these limitations, investigators are increasingly using combinations of cytotoxic agents in order to "pre-condition" patients prior to ACT. This approach, commonly known as lymphodepletion, was pioneered by Rosenberg to improve the efficacy of immunotherapy of metastatic melanoma using *ex-vivo* expanded tumour-infiltrating lymphocytes (TIL) (62). **Figure 2** shows that ACT achieves an increased response rate when TIL cells are administered after lymphodepletion with fludarabine and cyclophosphamide. Furthermore, when total body irradiation is added to intensify conditioning, the resultant myeloablation leads to a further improvement in clinical response rate. While these data do not derive from randomized controlled studies, they do nonetheless raise the possibility that appropriate lymphodepletion may enhance the efficacy of ACT using CAR-engineered T-cells. While this intervention will also increase the expected toxicity of the therapy, this is justified on the basis that lymphodepletion removes a key immunosuppressive obstacle to the effectiveness this therapy. In turn, this may allow this experimental CAR T-cell immunotherapy to effect tumour shrinkage in some patients, instead of disease stabilisation as previously noted. The incorporation of a lymphodepletion step converted an otherwise ineffective CAR T-cell immunotherapy for B-cell malignancy (63) or sarcoma (64) into one that achieved meaningful tumour regression for patients, albeit with the induction of inevitable chemotherapy-related toxicity. Moreover, a recent presentation of a CAR T-cell study for mesothelioma has reported

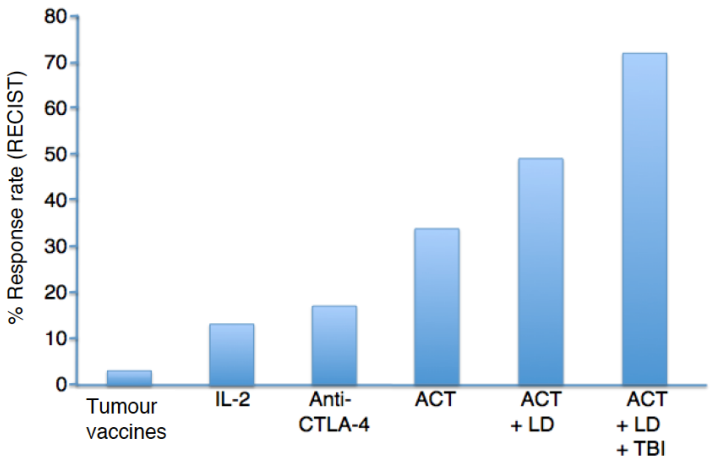


FIGURE 2. Preparatory lymphodepletion enhances response rate in patients with metastatic melanoma treated with adoptive cell therapy (ACT) using *ex-vivo* expanded tumour infiltrating lymphocytes. Results are compared to those achieved with tumour vaccines, IL-2 or anti-CTLA-4 alone. LD – non-myeloablative lymphodepletion using fludarabine and cyclophosphamide; TBI – total body irradiation. Data from the Surgery Branch, National Cancer Institute (adapted from reference 61).

unprecedented efficacy in patients who received lymphodepletion followed by CAR T-cells followed in turn by PD1 immune checkpoint inhibition. Responses were seen in 8 of 11 patients (6 partial responses and 2 complete responses) (https://www.cancernetwork.com/article/regional-delivery-mesothelin-targeted-car-t-cell-therapy-creates-win-solid-tumors, accessed 16/04/2019). A full risk benefit analysis in support of this proposed approach is presented in section 2.3.4 of the IMPD.

In keeping with this, early clinical studies using CAR-based immunotherapy which did not incorporate a lymphodepletion step achieved very limited clinical efficacy in a spectrum of malignancies (65-68). By contrast, lymphodepletion has been included in several more recent clinical studies in which regression of malignant disease has been repeatedly observed (69-73). Interpretation of these data is complicated by several factors. For example, CAR design has also improved over this period. Most current studies involve so-called second or third generation CARs in which signalling domains comprise two or three elements respectively, leading to enhanced potency of T-cell activation. However, in an ongoing study in which a second generation CAR is being used without lymphodepletion to treat patients with lymphoma, no clinical responses have been seen as yet beyond disease stabilisation (74). Taken together, while evidence is clearly limited, these observations raise the possibility that lymphodepletion has been an important contributory factor to the improved efficacy of ACT using CAR-engineered T-cells.

Currently, three mechanisms are believed to account for the beneficial effect of lymphodepletion, namely the depletion of Treg, the removal of competing cytokine sinks and the creation of "space" within which adoptively infused T-cells may expand, particularly in response to homeostatic cytokines such as IL-7 and IL-15.

Adoptive T-cell transfer in the absence of pre-conditioning generally results in poor T-cell engraftment in the patient and subsequent poor clinical responses. In this trial, we had aimed to address this issue in the first instance by intra-tumoural injection of T-cells. By this means, we hoped that T-cells would locate at the site of the disease for a sufficient period to elicit a meaningful therapeutic effect. While this approach has proven non-toxic, it has also demonstrated inadequate efficacy, even at the highest dose tested. Stable disease has proven to be the best clinical response seen in patients, whereas no examples of partial or complete tumour regression were seen. Against this background, it is now logical to explore immunomodulatory approaches that may improve T-cell survival and function *in-vivo*. The gold standard approach used to achieve this aim in the context of adoptive T-cell immunotherapy entails the pre-conditioning of patients with lymphodepleting chemotherapy, comprising the combination of fludarabine and cyclophosphamide (Flu/Cy).

The primary concern associated with the use of lymphodepletion relates to its potential toxicity, particularly in heavily pre-treated patients with malignant disease. Lymphodepletion has been a co-factor in two fatal Suspected Unexpected Serious Adverse Reactions (SUSAR), both of which occurred in patients treated with CAR-engineered T-cells. Both fatal reactions were characterized by uncontrolled immune cell activation and resulting cytokine storm (75, 76). In one case, toxicity may have been a manifestation of undetected sepsis (76). However, death of the second patient was clearly attributed to the uncontrolled activation of the T-cells, which were targeted against ErbB2 (75). A full description of that event and its implications for the design of our trial is provided in the Risk Benefit analysis section of the IMPD. Furthermore, a UK trial in which lymphodepletion has been used prior to infusion of CAR-engineered T-cells was terminated owing to toxicity, which was most apparent in the group that received Flu/Cy chemotherapy and high dose IL-2 cytokine support (77). That study entailed the use of a first

generation (eg lowest potency) CAR targeted against carcinoembryonic antigen which was administered to a patient population with a similar performance status and life expectancy to ours.

In all three cases, toxicity is likely to have been multi-factorial. Nonetheless, the ability of lymphodepletion to remove several cell populations and cytokine sinks is likely to have been a co-factor in the uncontrolled progression of these toxic events. Given the risk benefit analysis presented in section 2.3.4 of the IMPD, we did not feel that it would be appropriate to include a lymphodepletion step in this clinical trial until we had de-risked T4 immunotherapy by dose escalation to the maximum deliverable dose of 1 billion CAR T-cells.

Given the safety but inadequate efficacy of T4 immunotherapy observed to date, coupled with the lack of detectable emigration of CAR T-cells from the injected tumour, patients enrolled in cohorts 6-9 will receive conditioning with lymphodepleting chemotherapy prior to intra-tumoural T4 CAR T-cells. The gold standard regimen used in this context is the combination of fludarabine and cyclophosphamide (Flu/Cy). In this study, we have elected to employ a low dose Flu/Cy regimen, given the potential for toxicity of this treatment. On the one hand, evidence from studies of TIL cell immunotherapy and CD19 CAR T-cell immunotherapy has linked intensity of lymphodepletion with improved clinical response rate. Nonetheless, the price paid for enhanced anti-tumour activity is greater risk of toxicity due to cytopenias and enhanced activation of therapeutically infused T-cells in patients. To balance risk, we have selected a low dose Flu/Cy regimen for this study as is practiced currently in the conditioning of lymphoma patients for treatment with Kymriah, a licensed CD19-specific CAR T-cell product (https://www.fda.gov/downloads/UCM573941.pdf, accessed 05/11/2018). While Flu/Cy lymphodepletion regimens are used in conjunction with two NICE approved CAR T-cell products, both drugs are considered to be IMPs, given that their inclusion impacts upon the secondary objectives of this clinical trial.

We did consider whether it would be appropriate to initially reduce the dose of T4 immunotherapy in patients who receive Flu/Cy conditioning. Given the perceived increase in risk involved, we reduced the dose of T4 immunotherapy to 10^8 cells for cohort 6. In cohort 7 (single patient), we added three doses of nivolumab to this regimen in an effort to alleviate PD1-mediated suppression of the CAR T-cells in the tumour microenvironment. However, this cohort was suspended on the recommendation of the trial steering committee owing to lack of efficacy. In an attempt to boost efficacy of this approach, a new cohort (cohort 8) will be initiated in which the dose of T4 immunotherapy is re-escalated to the maximum deliverable dose of 1 billion cells. This is administered after lymphodepletion and combined with PD1 blockade using 3 doses of nivolumab.

1.2.6 The rationale for immunomodulation using PD1 immune checkpoint blockade

While fludarabine/ cyclophosphamide chemotherapy transiently removes inhibitory lymphoid cell populations, it does not impact on several other immunosuppressive factors that operate within SCCHN tumours. Several pharmaceuticals have been evaluated for their ability to counteract the immunosuppressive nature of the solid tumour-associated microenvironment. Unquestionably, greatest success has been achieved with monoclonal antibodies directed against “immune checkpoints” (ICP) –

molecules that serve to dampen adaptive immune responses. The prototypic example of an ICP is CTLA-4, a receptor that is upregulated on the surface of T-cells when they are activated by cognate antigen. Pioneering studies in malignant melanoma first demonstrated that CTLA-4 blockade can allow otherwise suppressed anti-tumour immune responses to flourish, promoting tumour remission and sustained response in a sizeable minority of patients (78).

More recently, even more impressive clinical data have been achieved with a second class of ICP inhibitor directed against the programmed cell death-1 (PD1) receptor or its ligand, PD-L1. PD1 is upregulated on chronically activated T-cells in addition to other leukocyte populations. This receptor delivers a SHP-2-dependent negative signal to these cells upon engagement by either of its natural ligands, PD-L1 and PD-L2 (79). Both PD1 and its primary ligand, PD-L1, are increased in the majority of SCCHN tumours, when compared to normal or dysplastic epithelium (80, 81). In addition, PD-L2 is commonly co-expressed in SCCHN tumours (82). Immunosuppression mediated by this axis is accentuated by T-cell activation and EGFr signalling (prevalent in SCCHN), both of which further upregulate PD-L1 and PD-L2 expression by these tumours (81, 83). PD1-expressing intra-tumoural lymphocytes found in head and neck tumours tend to co-localise with foci of PD-L1 expression. Unsurprisingly, when these T-cells are extracted from tumours and tested *in vitro*, they are defective in their ability to undergo activation (84).

Analysis of non-small cell lung carcinomas has demonstrated that tumours with the largest neo-antigen loads tend to respond best to treatment with ICP blockade (85). In keeping with this, cutaneous melanoma has the highest mutation frequency among human cancers (86, 87) and is the most responsive tumour type to ICP inhibition (88). Importantly, SCCHN tumours also have a high mutational burden (87, 89, 90), providing a rationale for the study of PD1 blockade in this disease. The first study to report was the KEYNOTE-012 open-label multicentre Phase Ib trial (91) which evaluated the anti-PD1 monoclonal antibody, pembrolizumab. A 16% response rate was achieved in this refractory patient population (n=174), accompanied by acceptable safety. Median overall survival of patients was 13 months and 51% of those treated survived for at least 1 year. Over 80% of the responses were durable at 6 months (some beyond 2 years). Responses were seen in patients with human papilloma virus (HPV) positive and negative tumours, both of which carry similarly high mutational loads (90). These are impressive data given that the median survival of patients with platinum chemotherapy-resistant SCCHN is 6 months or less (92). Based on these findings, the US Food and Drug Administration (FDA) granted accelerated approval to pembrolizumab in 2016 for the treatment of patients with refractory metastatic/ recurrent SCCHN, adding to its previous approvals in melanoma and lung cancer. Subsequently, another PD1 inhibitor (nivolumab; BMS-936558) was approved based on the Checkmate 141 randomised Phase III study of 361 patients (93). Both drugs have subsequently been incorporated into US clinical guidelines for SCCHN treatment (94). Nivolumab is approved in the UK for treatment of patients with head and neck cancer that is progressive within 6 months of chemotherapy treatment (https://www.nice.org.uk/guidance/ta490head and neck cancer, accessed 27-12-2019). The activity of pembrolizumab in platinum-refractory recurrent/ metastatic SCCHN has subsequently been confirmed in the phase II KEYNOTE-055 (95) and the phase III KEYNOTE-040 trials (96). Long-term follow up data for both nivolumab and pembrolizumab suggest that a tail to the survival curve is emerging, indicating that durable survival benefit may be achieved in a minority of patients. Moreover, recent data demonstrate that long-term benefit of nivolumab in this regard is independent of PD-L1 expression (97).

The PD-1:PD-L1 interaction is known to occur in the tumour microenvironment, where immune cells that express PD-1 are inactivated by expression of PD-L1 on tumour cells and a number of tumour-infiltrating

immune cells (98). Consequently, it is assumed that monoclonal antibodies that disrupt the interaction between PD1 and its ligands operate primarily within tumour deposits. However, IgG4 monoclonal antibodies such as nivolumab and pembrolizumab are characterized by a relatively high molecular mass, leading to a slow distribution in tissues. Pre-clinical studies indicate that uptake of antibody within tumours following intravenous administration is initially heterogeneous, with a predominantly peripheral distribution. Maximal tumour uptake and tumour to blood ratios may be found after 2-7 days (98). Given that CAR T-cells would be expected to upregulate PD1 in the days following anti-tumoural injection (owing to activation at that site), we have elected to administer the first dose of nivolumab 24 hours prior to CAR T cell administration.

Manufacturers of pembrolizumab recommend that treatment of patients with SCCHN is continued until disease progression, occurrence of unacceptable toxicity, or for up to 24 months in the absence of disease progression (https://www.merck.com/product/usa/pi_circulars/k/keytruda/keytruda_pi.pdf, accessed 27.12.2019). A similar recommendation applies to nivolumab, when prescribed under terms specified by the UK National Health Service (https://www.nice.org.uk/guidance/ta490head and neck cancer, accessed 27-12-2019). However, the optimum duration of treatment with PD1 antagonists remains unknown (99). In this study, a total of 3 doses of nivolumab will be delivered, enabling the assessment of this combination for safety and for efficacy over a 12 week period post CAR T-cell infusion. Importantly, patients enrolled into this study would not be eligible for (or will have already failed) immune checkpoint blockade with a PD1 or PD-L1 inhibitory antibody. When used in this manner, nivolumab is considered to be IMP since it may impact on the secondary objectives of this clinical trial.

Justification for experimental immunotherapy of patients with refractory SCCHN with intra-tumoural T4 CAR T-cells and systemic PD1 blockade is based on the following considerations.

- Analysis of T4 immunotherapy prepared from SCCHN patients using our GMP manufacturing process indicates that infused cells already express significant levels of PD1 (19.4 $\pm$ 26.2%).

- PD1 expression by T-cells is activation-dependent (100). Consequently, PD1 would be expected to increase further following activation of $T4^+$ CAR T-cells within the tumour microenvironment.

- Pre-existing stable adaptive immune responses (101) and T-cell "inflamed" gene expression profiles (83) are both predictive of positive response to anti-PD1 targeting in patients with SCCHN and other cancers. Exposure of tumour-infiltrating T-cells isolated from human SCCHN cancers to PD1 inhibitory antibodies promotes the activation of these cells, associated with an enhanced type 1 cytokine response (84). By injecting CAR T-cells directly within a SCCHN tumour, a bolus of tumour-specific T-cells is provided that produce type 1 cytokines and which would be expected to benefit similarly from PD1 blockade.

- Interferon (IFN)-γ is a key mediator of the protective effect of tumour-specific T-cells (including CAR T-cells), acting through several mechanisms. These include direct anti-proliferative, anti-angiogenic and pro-apoptotic effects, promotion of Treg fragility, enhanced macrophage activation, increased chemokine release and upregulated HLA (and therefore tumour antigen) expression (102, 103). Indeed, this cytokine is required for responsiveness to anti-PD1 therapies (103), while mice that are deficient in IFN-γ are significantly more prone to cancer development

(104). However, the effects of this pleiotropic cytokine are double-edged (102) in the sense that IFN-γ also upregulates PD-L1 and PD-L2 expression, both on tumour cells (105) and on mesenchymal stromal cells (which are also found in solid tumours) (106). This may account for the fact that expression of PD-L1 on SCCHN tumour cells is predictive of beneficial therapeutic response to PD1 blockade, since it may provide an indirect indicator that IFN-γ secreting (tumour-specific) T-cells are present at that site (91, 93, 107). Given the potent ability of $T4^+$ CAR T-cells to release IFN-γ (108-111) (and thereby upregulate tumour-associated PD-L1 expression), it is anticipated that this effect of IFN-γ would be counteracted by PD1 blockade.

- Unlike CTLA-4 (T-cell restricted), PD-1 is expressed on several other suppressive myeloid and lymphoid cell types (112). Neutralisation of PD1 has been reported to reverse the inhibitory effects of myeloid-derived suppressor cells, tumour-associated macrophages (80, 113) and regulatory B-cells (114) found in SCCHN tumours. Consequently, it would appear that PD1 inhibition can "re-educate" many elements found within the tumour microenvironment, favouring the activation of both endogenous tumour-specific T-cells and CAR T-cells that have been introduced at that site.

- Intra-tumoural injection of T4 immunotherapy is likely to promote the release of tumour antigen, leading to priming of endogenous tumour-specific T-cells within the draining lymph node(s). In turn, this would be expected to promote epitope spreading. Evidence in support of epitope spreading induced by CAR T-cell immunotherapy has been presented recently (115) and is a key mechanism in many autoimmune disorders (116). Consequently, it is considered desirable in fostering autoimmune responses against the self-antigens that are also found in tumours (117). Blockade of PD1 would be expected to amplify the propagation of such immune responses.

- Several pre-clinical studies have demonstrated enhanced anti-tumour activity of CAR T-cells when co-administered with a blocking PD1 or PD-L1 antibody (113, 118-120), or following the implementation of genetic approaches to disable PD1 (most notable for CD28+CD3ζ CAR, as is found in T4 immunotherapy) (119).

- PD1 is also expressed on some dysfunctional regulatory T-cell subsets which secrete IFN-γ, with further augmentation in the presence of anti-PD1 treatment (121). Consequently, PD1 blockade may hinder the effector activity of some Treg populations.

- PD1 inhibition enhances T-cell migration to tumours, via an IFN-γ dependent increase in chemokine release (122). Increased T-cell proliferation of T-cells within tumours was also apparent in this study (122).

- Recent clinical data from Memorial Sloan Kettering Cancer Center (MSKCC) provide strong clinical justification for the proposed approach. Twenty patients (18 malignant pleural mesothelioma, 1 lung cancer, 1 breast cancer) were treated with intra-pleural CAR T-cells targeted against mesothelin and containing a CD28 + CD3ζ endodomain. In 17 cases, CAR T-cells were administered following lymphodepletion with intravenous cyclophosphamide. Importantly, no

CAR T-cell–related toxicities higher than grade 1 were observed. Fourteen of the patients with mesothelioma received subsequent anti-PD-1 therapy. One of these patients developed grade 3 pneumonitis that responded to steroid treatment. CAR T cells were detected in the peripheral blood of 13 of 14 patients (1-39 weeks). Among the subgroup that received combination therapy, two patients achieved complete metabolic response, as determined by PET scanning (durable to 62 and 39 weeks at the time of publication). In addition, there were 5 partial responses and 4 patients who achieved stable disease in this subgroup (https://ascopubs.org/doi/abs/10.1200/JCO.2019.37.15_suppl.2511, accessed 27.12.2019). These data provide clinical affirmation of a raft of pre-clinical studies that demonstrate superior anti-tumour activity when CAR T-cells are combined with PD1 blockade (113, 118, 119, 123-126).

- While ground-breaking, the MSKCC study entailed the ad hoc (e.g. off protocol) administration of anti-PD1 immunotherapy, commencing at variable intervals after infusion of CAR T-cells. Given the considerations summarised above, it would be expected that this combinatorial approach would be most effective if PD1 blockade were initiated prior to infusion of CAR T-cells. Clinical proof of concept in support of the safety of combinatorial immunotherapy using CAR T-cells (third generation design) and pembrolizumab following fludarabine/ cyclophosphamide chemotherapy has recently been demonstrated (127). In that study, patients with neuroblastoma received more intensive lymphodepletion (e.g. cyclophosphamide 500mg/m^2 and fludarabine 30mg/m^2 x 3 days each) than is proposed here. Moreover, the final three patients also received two doses of pembrolizumab (2mg/kg) one day prior to CAR T-cells and 21 days post-infusion. Grade 3 and 4 toxicities were all cytopaenias attributable to fludarabine/ cyclophosphamide chemotherapy. No significant CAR T-cell mediated toxicity was observed. Only 1 case of grade 2 cytokine release syndrome and no cases of neurotoxicity were reported. Efficacy in this study was modest (2/3 stable disease), most likely due to the sub-optimal mature of the CAR, which is prone to tonic signalling (128). Nonetheless, both of these refractory patients subsequently achieved complete remissions with chemotherapy or surgery following participation in the trial.

- Further clinical evidence of efficacy of PD1 blockade in conjunction with CAR T-cell immunotherapy is provided by a case report of a patient with progressive diffuse large B-cell lymphoma in whom rapid disease progression was observed following CD19-specific CAR T-cell immunotherapy (129). Commencing 26 days after CAR T-cell infusion, he received pembrolizumab every 3 weeks and achieved a response that was durable to 12 months at the time of publication. The only pembrolizumab-induced toxicity noted was fever.

Taken together, these points provide a strong rationale for the clinical evaluation of the combination of T4 immunotherapy and PD1 inhibition in a challenging ErbB-expressing solid tumour setting.

1.2.7 Use of fludarabine, cyclophosphamide and nivolumab as IMPs

Fludarabine, cyclophosphamide and nivolumab are being used as IMPs in this study. However, all three are commercially available and consequently do not require manufacture on site. In this clinical trial, these pharmaceuticals will be purchased from the Pharmacy at Guy's Hospital. The pharmacy will maintain their own supplies and record dose, quantity dispensed, batch number and expiry for IMPs dispensed to individual subjects for monitoring purposes. There is no requirement to segregate stock of these medicines as long as sites are able to maintain adequate supplies for this study.

For details on storage conditions for these drugs, please refer to the latest version of the Summary of Product Characteristics (SPC) document. Temperature records are held in the department in a central folder. The sponsor may inspect these central records and make copies thereof for their own information. OHCT Pharmacy can print a trend graph for the sponsor to place in the individual trial file at the end of the study for the time that the IMP has been stored on site. For any temperature deviations on site, the Chief Investigator (CI) and Clinical Research Associate (CRA) must be informed and the local hospital policy must be followed. The affected stock will be placed in quarantine until confirmation is received from the manufacturers that the product is fit for use.

These drugs will be dispensed by the pharmacy upon receipt of a valid trial-specific prescription proforma. Drugs will be reconstituted aseptically, labelled (using the trial-specific IMP label) and packaged according to pharmacy standard operating procedures, ensuring that dispensing logs and patient records are completed. Once these drugs have been prepared, a staff member from the Clinical Research Facility (CRF) will collect the doses from the aseptics pharmacy and deliver them in a designated chemotherapy transfer box direct to the CRF for patient administration. Accurate records of all IMP dispensing by the study site must be recorded on individual patient accountability logs (local templates are permitted). Chemotherapy doses (unused/used) must be destroyed after reconciliation as per local Trust Waste Policy.

1.2.8 Attenuating cytokine-mediated local inflammatory effects of T4 immunotherapy using celecoxib

As the T4 trial has progressed, a dose-dependent increase in local inflammatory effects of the injected cells has become apparent. This is indicated by painful swelling of the injected lesion, accompanied initially by intermittent pyrexia, chills and/or rigors. These local reactions are likely due to CAR T-cell activation, accompanied by cytokine release within the tumour. Local inflammatory responses have persisted for several weeks in some cases, and generally require the prescription of potent corticosteroids to alleviate these reactions. Conceptually, this is undesirable since clinical experience indicates that sustained persistence of functional CAR T-cells is required for therapeutic efficacy in patients with haematological malignancies (130).

In an attempt to pre-empt and alleviate this toxicity, patients will be treated wherever possible (unless contraindicated) with the cyclooxygenase (COX-)2 inhibitor, celecoxib. Administration of celecoxib will not influence the data collection and analysis of the trial and it is being used for medical reasons to make the patient more comfortable. However, patients who cannot tolerate celecoxib can still enter the trial. Celecoxib is contraindicated in patients with active gastro-intestinal bleeding; active gastro-intestinal

ulceration, cerebrovascular disease, inflammatory bowel disease, ischaemic heart disease, mild to severe heart failure and peripheral arterial disease. In this context, administration of celecoxib to the patient is a clinical decision, which will be made by the treating clinician delegated on the study. This agent will be prescribed in combination with omeprazole to provide gastric protection, as undertaken successfully in the PRECISION clinical trial (131). Celecoxib has been selected for this purpose based on the following considerations:

- COX-2 is upregulated during severe inflammatory reactions, such as cytokine release syndrome (132, 133). Moreover, inhibition of COX-2 using celecoxib has been shown to mitigate cytokine release syndrome when it occurs in the context of selected disease processes, such as overwhelming influenza (133), or therapeutic interventions, such as intra-pleural oncolytic virus treatment of mesothelioma (132, 134). No adverse events were attributed to celecoxib in these studies (132, 134).

- Celecoxib acts in a discriminatory manner in curtailing inflammatory responses. This agent reduces deleterious forms of inflammation that are characterised by IL-17 and prostaglandin E2 production, but it does not impede potentially beneficial responses that are mediated via IFN-γ (135).

- COX-2 is upregulated not only in inflammatory processes, but also in SCCHN tumours (primarily in malignant cells) (136), in which it promotes angiogenesis and metastasis (137, 138). Consequently, SCCHN and nasopharyngeal (139) tumours that express high levels of COX-2 have poorer prognosis (137, 138).

- Celecoxib has been administered as a component of combination therapy in patients with diverse solid tumours, including SCCHN (140). Treatment has been well tolerated. Xue *et al* (139) safely administered this agent at doses of up to 800mg/day in patients with SCCHN who underwent radiotherapy for nasopharyngeal carcinoma. Stomach pain occurred in 2 patients and was alleviated by treatment to protect the gastric mucosa. No patients withdrew from the study because of toxicity. With the objective of enhancing response to EGFr inhibitors, a Phase 1 trial from the Dana-Farber Cancer Institute demonstrated a 22% response rate to concurrent gefitinib and celecoxib with minimal toxicity (141). A study from the Mount Sinai School of Medicine tested the combination of erlotinib, celecoxib, and re-irradiation in patients with recurrent SCCHN (142). No dose-limiting toxicities were observed in the patients at doses of celecoxib of up to 200mg BD. Safe administration of celecoxib at daily doses of 200-400mg was also reported in patients with SCCHN by Lalla (143), when combined with radiation.

- The PRECISION Phase 4 randomized controlled clinical trial (131) indicated that low dose celecoxib (average dose of 209mg/day) is associated with approximately the same cardiovascular risk as two non-selective nonsteroidal anti-inflammatory drugs with less COX-2 inhibitory activity, namely ibuprofen and naproxen. Moreover, risk of gastrointestinal events was significantly lower for celecoxib than naproxen or ibuprofen (all patients received omeprazole), while risk of renal events was lower for celecoxib than ibuprofen.

- Adverse cardiovascular events were noted previously when celecoxib was used in the dose range of 400-800mg/day (144).

2. OBJECTIVES OF THE TRIAL

2.1 Trial Objectives

The overall goal of this study is to investigate the safety of T4 immunotherapy when administered to treat loco-regional disease in SCCHN that is not suitable for conventional active therapy.

2.1.1 Primary Objectives

- To define dose limiting toxicities for T4 immunotherapy in SCCHN.
- To determine a safe and feasible recommended dose for phase II testing of intra-tumoural T4 immunotherapy.

2.1.2 Secondary Objectives

- To investigate serum cytokine levels after administration of T4 immunotherapy.
- To investigate persistence of $T4^+$ T-cells at the site of intra-tumoural administration and their dose-dependent migration from that site into the peripheral circulation.
- To achieve preliminary assessment of anti-tumour activity, using cross-sectional imaging to quantify objective responses.
- To investigate tumour ErbB receptor phenotype, before and after administration of T4 immunotherapy.
- To investigate immunomodulatory effects of lymphodepletion using fludarabine and cyclophosphamide on T4 immunotherapy.
- To investigate immunomodulatory effects of the combination T4 immunotherapy (administered post lymphodepletion) and PD1 immune checkpoint blockade.
- To investigate effect of T4 immunotherapy upon immune reactivity against endogenous tumour antigens.
- To investigate effect of T4 immunotherapy upon global gene expression within the tumour microenvironment.
- To investigate safety of T4 immunotherapy, when administered in combination with lymphodepleting chemotherapy (fludarabine and cyclophosphamide), alone or in combination with nivolumab.

2.2 Trial Endpoints

2.2.1 Primary Endpoint

Dose limiting toxicity of T4 immunotherapy graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE), Current Version.

2.2.2 Secondary Endpoints

- Cytokine levels present in serum taken pre-injection, at 30 min after injection, and at 1, 4, 24, 48-96 and 120-168 hours post T-cell infusion (flexible time points, to allow for weekends). Analysis will be performed using a multiplex cytokine bead array platform.
- Persistence of $T4^+$ T-cells in tumour biopsies (measured by quantitative PCR and RNAScope analysis) at two weeks post therapy.
- Presence of $T4^+$ T-cells in the circulation measured by quantitative PCR and flow cytometry analysis for $T1E28z^+$ T-cells at 4, 24, 48-96 and 120-168 hours (flexible time points, to allow for weekends), and days 8, 15, 22, 29 and day 43 post injection. In the case of patients who receive lymphodepleting chemotherapy and nivolumab (cohorts 7-8), this analysis will also be performed on day 28 (instead of 29), 56, 71 and 85 (where day of T4 injection is day 1).
- Evidence of response evaluated by appropriate cross-sectional imaging and, in the case of patients in cohorts 7-8, 12 weeks post therapy. Clinical response will be assessed according to RECIST 1.1 criteria.
- Effect of T4 immunotherapy upon endogenous T-cell reactivity against MAGE-A3 and MAGE-A4 cancer/testis antigens. Analysis will be performed 3 days before and 29 days after T4 immunotherapy is administered on day +1. Responses will be quantified as cytokine release, measured using a combined ELISPOT and multiplex cytokine bead array platform and/or CyTOF analysis after stimulation with overlapping peptides derived from each antigen.
- Evidence of immunomodulation by cyclophosphamide and fludarabine, as measured by circulating numbers of $CD4^+$ $CD25^{HIGH}$ $CD127^{DIM/NEG}$ regulatory T-cells and myeloid-derived suppressor cells. Cells will be quantified by serial flow cytometry of peripheral blood samples.
- Effect of T4 immunotherapy on gene expression in the tumour microenvironment will be assessed in serial tumour biopsies undertaken before, one week after, and two weeks after administration of T4 immunotherapy.
- Trafficking of T4 immunotherapy will be assessed in a subset of patients by SPECT-CT imaging, following administration of an aliquot of T4 immunotherapy that has been radiolabelled with Indium-111.

3. PATIENT SELECTION CRITERIA

3.1 Inclusion Criteria

1) Histologically and/ or cytologically confirmed SCCHN.

2) 18 years or older.

3) Locally advanced and/ or recurrent head and neck cancer with or without metastatic disease (excluding brain metastases) for whom no standard therapy remains or is suitable.

4) Regarding previous treatment, patients may have received prior systemic therapy, including platinum chemotherapy, up to one week prior to T4 immunotherapy. This one week limit does not apply to the use of lymphodepleting chemotherapy in cohorts 6-8 or PD1 immune checkpoint blockade in cohorts 7-8, as specified in this protocol. In the presence of metastatic disease, recent short-course palliative radiotherapy to non-target site(s) is allowed.

5) Those who refuse palliative treatment may be eligible for participation. However, their reasons for not opting for palliative treatment must be explored thoroughly.

6) At least one loco-regional target lesion measurable by RECIST v1.1 criteria on CT or MRI scanning within four weeks of treatment and amenable to intra-tumoural injection.

7) Eastern Co-operative Oncology Performance Status of 0-2 (0-1 for cohorts 6-8).

8) Normal cardiac function as assessed by electrocardiography and either echocardiography (ECHO), or multi-gated acquisition (MUGA) scanning. Left ventricular ejection fraction must be $\geq$ 50%. Assessment must take place within 28 days of treatment.

9) Haematology results within 28 days of treatment: neutrophils $\geq 1.5 \times 10^9$/L, platelets $\geq 100 \times 10^9$/L, haemoglobin $\geq$90g/L, INR <1.5.

10) Biochemistry results within 28 days of treatment:
- serum creatinine <1.5 upper limit of normal (ULN)
- bilirubin <1.25 times ULN;
- ALT/ AST <2.5 times ULN (<5 times ULN if liver metastases present)

11) Female patients must be postmenopausal (12 months of amenorrhea), surgically sterile or they must agree to use a physical method of contraception. Oral or injectable contraceptive agents cannot be the sole method of contraception. Women of childbearing potential (WOCB) who receive cyclophosphamide must adhere to these contraceptive requirements during the trial and until 6 months after the last dose of cyclophosphamide and fludarabine. Male patients, even if

sterilized, must agree to use a barrier method of contraception. Male subjects must also commit to use a barrier method of contraception until at least 3 months after the end of study treatment and this is extended to 6 months in the event that they have received cyclophosphamide and fludarabine.

12) Written informed consent prior to any trial procedure and registration.[1]

3.2 Exclusion Criteria

1) The presence of or imminent occurrence of airway obstruction, unless tracheostomy in place.

2) The presence of or imminent occurrence of tumour-mediated infiltration of major blood vessels.

3) Positive history of HIV-1, HIV-2, HTLV-1, HTLV-2, Hepatitis B, Hepatitis C or syphilis infection.

4) Prior splenectomy.

5) Clinically active autoimmune disease or interstitial lung disease. Sub-clinical or quiescent autoimmune disease does not exclude from participation.

6) Treatment in the week preceding the administration of T4 immunotherapy (or in cohorts 6-8, fludarabine/ cyclophosphamide/ nivolumab followed by T4 immunotherapy) with any of the following additional therapies: (i) systemic corticosteroids ($\geq$ 20mg prednisolone/ day); (ii) any systemic immunomodulatory agent; (iii) radiotherapy; (iv) chemotherapy or (v) any investigational medicinal product.

7) Concurrent use of anticoagulant therapy is not permissible.

8) The presence of major co-morbidity likely to impair ability to undergo trial therapy, such as recent myocardial infarction, congestive cardiac failure, active gastrointestinal bleeding, active gastrointestinal ulceration, inflammatory bowel disease, ischaemic heart disease, peripheral arterial disease or uncontrolled hypertension.

9) The presence of any psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule.

10) Cyclophosphamide or fludarabine allergy or contraindication (Cohorts 6-8 only).

11) Nivolumab allergy (Cohorts 7-8 only).

[1] enrolment/ registration occurs on the day that blood is collected to manufacture T4 immunotherapy

12) Pregnancy.

13) Breastfeeding.

14) Prior T4 immunotherapy. However, prior immune checkpoint blockade does not preclude participation.

15) With respect to cohorts 6-8 (fludarabine and cyclophosphamide pre-treatment), patients who have received a live vaccine four weeks or fewer before enrolment are ineligible for recruitment to the study. During treatment and for three months after treatment with fludarabine, administration of live vaccines is prohibited.

16) With respect to cohorts 6-8 (fludarabine and cyclophosphamide pre-treatment), patients with a history of skin cancer are ineligible for recruitment to the study.

4. TRIAL DESIGN

This is a phase 1 dose-finding open label study of T4 immunotherapy in ErbB1-expressing SCCHN, following loco-regional relapse.

4.1 Justification for a 3 + 3 trial design

A key guiding principle for dose escalation in phase I trials is to avoid exposing too many patients to sub-therapeutic doses while preserving safety. Rule-based designs (including the traditional 3+3 design) have two advantages in this respect. First, they do not stipulate any prior assumption of the dose–toxicity curve, which is appropriate for a first in man study involving T-cell immunotherapy. Second, the traditional 3+3 design is simple to implement and is generally safe. However, a potential disadvantage of this design is that it may involve an excessive number of dose escalation steps, which results in a large proportion of patients who are treated at low (ie, potentially sub-therapeutic) doses. To counterbalance this risk, only five dose escalation steps have been included in our study.

Using a 3+3 trial design, the occurrence of two dose-limiting toxicities (at a given dosing level) would indicate that the maximum tolerated dose has been exceeded. In light of this, measures are required to minimise the risk that three or more trial participants within a single dosing cohort suffer a dose-limiting toxicity. To achieve this, participants will be enrolled and treated in a staged manner (**Figure 3**). This approach is described in **section 4.5** below, which deals with methods to be used in this study. By this means, we aim to minimize the risk that two or more patients will suffer dose-limiting toxicities within a short time window so that appropriate actions are taken promptly throughout the trial.

In the event that dose-limiting toxicities occur, the trial steering committee will be notified in all cases. The trial steering committee will also review data pertaining to each cohort of treated patients prior to dose escalation.

4.2 Starting Dose of T4 Immunotherapy

The starting dose was 1 x 10^7 $T4^+$ transduced T-cells. Since this is a first in man study, the optimal dose of T-cells for intra-tumoural CAR therapy is unknown. Due to the widespread low-level distribution of the ErbB receptor family in normal tissues, there is the potential for toxicity with this approach. The chosen dose of 1 x 10^7 cells for intra-tumoural injection represents a low starting point relative to other studies of intra-tumoural T-cell administration (145) or intravenous phase 1 CAR studies (63, 65, 66, 68-72, 74, 75, 146-149). Allowing for a typical tumour volume for injection of $1cm^3$, this corresponds to approximately 10^9 tumour cells (150). Consequently, the initial effector to target cell ratio is 1:100, which is unlikely to be associated with either substantial efficacy or toxicity.

4.3 Dose Escalation of T4 Immunotherapy

T4 immunotherapy T-cell doses were first escalated in fixed increments according to the dose escalation scheme outlined in **Table 1**. Because cell products do not expand in a standardized manner, we defined a target dose accompanied by an acceptable range (to be used in the event that expansion to the target cell number is not attained at end of production). Three patients were enrolled at each dose level during dose escalation. If a dose-limiting toxicity (DLT) had occurred in any cohort, that cohort would have been expanded to six patients by enrolment of further patients. Since the highest dose level of 10^9 cells was reached (cohort 5) without achieving the maximum tolerated dose of T4 immunotherapy (**section 4.8**), a cohort of three patients (cohort 6) was next enrolled at a lower dose level of 10^8 CAR^+ T-cells, administered after lymphodepletion with fludarabine and cyclophosphamide. Since this combination was well tolerated, a further cohort (cohort 7 – one patient) was recruited in which nivolumab (three doses) was added to the regimen administered to patients in cohort 6. Once again, this combination was well tolerated but the patient was withdrawn from the study after his 6 week post T4 immunotherapy CT scan showed progressive disease. This event prompted an ad hoc meeting of the trial steering committee which recommended that cohort 7 should be suspended. The trial steering committee further recommended that a new cohort (cohort 8) should be opened in which the CAR T-cell dose is re-escalated to 10^9 cells (the maximum deliverable dose), administered post fludarabine/ cyclophosphamide and in combination with 3 doses of nivolumab. This proposal is based on the fact that no leakage of CAR T-cells into the systemic circulation was seen at the maximum deliverable dose during the dose escalation phase of the study. The trial steering committee will meet after completion of treatment of patient one and patient three in the new cohort (cohort 8) to review outcomes and make recommendations for further progression of the trial. If any responses are seen in the first 3 treated patients, cohort 8 will be expanded until all remaining retroviral vector units have been used (currently 8 vector units remain). Cohorts to be recruited are summarised in **Table 1**. The expected sample size in this study ranges from 22 to 29 patients. Twenty two patients corresponds to the number of patients already enrolled in the trial from cohort 1 to 7 (i.e. 21) plus the first patient to be recruited in cohort 8. After treatment of the 22nd patient is completed, seven vector units will remain which means that the maximum number of patients that could be treated in this trial would reach 29.

Table 1. Dose Escalation Protocol ($T4^+$ T-cells).

Notes	Dose Level (Cohort number)	Target dose	Acceptable dose range of $T4^+$ cells	Volume for injection (mL)	Number of patients
	-1*	3 x 10^6 cells	3 x 10^6 cells	1 ± 0.2	3
Starting T4 Dose level	1	1 x 10^7 cells	3 x 10^6 - 10^7 cells	1 ± 0.2	3
↓	2	3 x 10^7 cells	1.1 – 3 x10^7 cells	1 ± 0.2	3
	3	1 x 10^8 cells	3.1 – 10 x10^7 cells	2 ± 0.4	3
	4	3 x 10^8 cells	1.1 – 3 x10^8 cells	3 ± 0.6	3
Maximum deliverable dose	5	1 x 10^9 cells**	3.1 – 10 x10^8 cells	4 ± 0.8	3
Intravenous fludarabine 25mg/m² and cyclophosphamide 250mg/m² once daily for 3 days, administered 2-11 days prior to T4	6	1 x 10^8 cells	3.1 – 10 x10^7 cells	4 ± 0.8	3

immunotherapy					
Fludarabine and cyclophosphamide as above plus nivolumab 480mg IV x 3 doses q 4 weekly, commencing one day prior to T4 immunotherapy	7 (suspended)	1 x 10^8 cells	3.1 – 10 x10^7 cells	4 ± 0.8	1
Fludarabine, cyclophosphamide and nivolumab as above	8	1 x 10^9 cells**	3.1 – 10 x10^8 cells	4 ± 0.8	3***

* a "–1" dose level has been included in case dose de-escalation is required from dose level 1. Please note that doses relate to the number of $T4^+$ transduced cells (not total cell number).
** Maximum 1 x 10^9 cells in total.
*** There will be a mandatory trial steering committee meeting after completion of treatment of the first patient and the first three patients in this cohort. The cohort will be expanded to a maximum of 8 patients if any clinical responses are seen in first 3 patients treated.

4.4 Lymphodepletion with Fludarabine and Cyclophosphamide

Since the MTD was not defined following administration of T4 immunotherapy at the highest possible dose level (cohort number 5; Target dose of 1 x 10^9 cells **Table 1**), patients enrolled in the following cohorts undergo conditioning with a low dose fludarabine and cyclophosphamide (Flu/Cy) lymphodepleting regimen, administered alone (cohort 6) or in combination with the PD1 inhibitor, nivolumab (cohorts 7-8).

4.5 Mitigation of Inflammatory Reactions with Celecoxib

Unless prohibited by contra-indications or drug interactions, patients will receive a fixed oral dose of celecoxib 100mg BD, commencing 1 week prior to T4 immunotherapy and co-prescribed with oral omeprazole 20mg once daily. This is justified on the basis that steady state conditions are reached on or before day 5 with multiple dosing.[2] Treatment will continue for 6 weeks, unless adverse effects attributable to celecoxib occur. The capsules may be opened and the contents added to cold or room temperature apple sauce for administration to patients with swallowing difficulties.

In the event that celecoxib is contra-indicated or cannot be prescribed owing to an unacceptable drug interaction, patients will receive T4 immunotherapy as per protocol, without dosing with celecoxib.

In the event that tumour-associated inflammation causes painful swelling despite celecoxib 100mg BD, dosing of this agent may be increased to 200mg BD. Dosing at the higher level may continue as long as this proves beneficial and is well tolerated. This approach is preferred to administration of oral corticosteroids since it will not compromise the function of the CAR T-cells *in situ*. However, if celecoxib

[2] (https://www.accessdata.fda.gov/drugsatfda_docs/label/1998/20998lbl.pdf, accessed April 6th, 2018).

200mg BD fails to control toxicity due to local inflammation, oral corticosteroids may be prescribed instead, at the discretion of the treating physician.

4.6 Co-administration of T4 immunotherapy with Nivolumab

Patients recruited to cohorts 7 and 8 of this clinical trial receive three infusions of nivolumab, each comprising a flat dose of 480mg. Nivolumab is administered intravenously at four weekly intervals, commencing one day prior to intra-tumoural injection of T4 CAR T-cells. A dose of 10^8 CAR T-cells was administered to the first patient recruited to cohort 7. However, this patient was withdrawn from the study following his 6 week post CAR T-cell CT scan which revealed progressive disease. The trial steering committee convened an ad hoc meeting to discuss this patient outcome. Given the excellent safety record of T4 immunotherapy at doses of up to 1 x 10^9 T-cells combined with this lack of efficacy, the committee recommended the suspension of cohort 7 and the initiation of a new cohort (cohort 8) in which the dose of T4 immunotherapy is re-escalated to the maximum deliverable dose of 10^9 cells (Table 1). These patients continue to receive conditioning with fludarabine and cyclophosphamide together with 3 doses of nivolumab as before.

4.7 Methods

The rate of subject entry and escalation to the next dose level will depend upon safety profile assessment of patients entered at the previous dose level. Toxicity will be evaluated according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), Current Version, as described below.

Enrolment within each cohort will be consecutive and is summarized in **Figure 3**. After treatment, patients in cohorts 1-5 will be evaluated for 24 hours as an inpatient and then again after 72 hours as an outpatient. Patients in cohort 6 and cohort 7 (lymphodepletion cohorts) will be hospitalised for a mandatory 7 day minimum period after intra-tumoural administration of T4 immunotherapy. If no DLT has occurred within the two-week period following CAR T-cell treatment of the first patient in any cohort, recruitment will open for the next two patients in that cohort (meaning that they will not receive T4 immunotherapy for at least a further 2 weeks). This interval has been extended to 4 weeks in cohort 7 and cohort 8 to account for the addition of nivolumab to the therapeutic regimen. However, since only one patient cell product can be manufactured at a time, this means that patients 2 and 3 within each cohort will be treated with a minimum gap of 2 weeks. Once three patients are enrolled in a cohort, all will be evaluated for DLT for 28 days before escalation to the next dose level. In the case of cohort 7 and cohort 8, this 28 day monitoring period commences on the day that patient 3 receives the final (third) dose of nivolumab.

When originally planned, the expected sample size in this study was 30. By substantial amendment 13, the expected sample size is now between 22 to 29 patients, as indicated in section 4.3.

All participants enrolled in the study will be provided with a patient alert card. This provides contact details of clinical staff who can be contacted at any time by patients on the trial. Patients in cohorts 6

onwards will receive a card that indicates the fact that they have received lymphodepleting chemotherapy, either alone (cohort 6) or with nivolumab (cohorts 7-8).

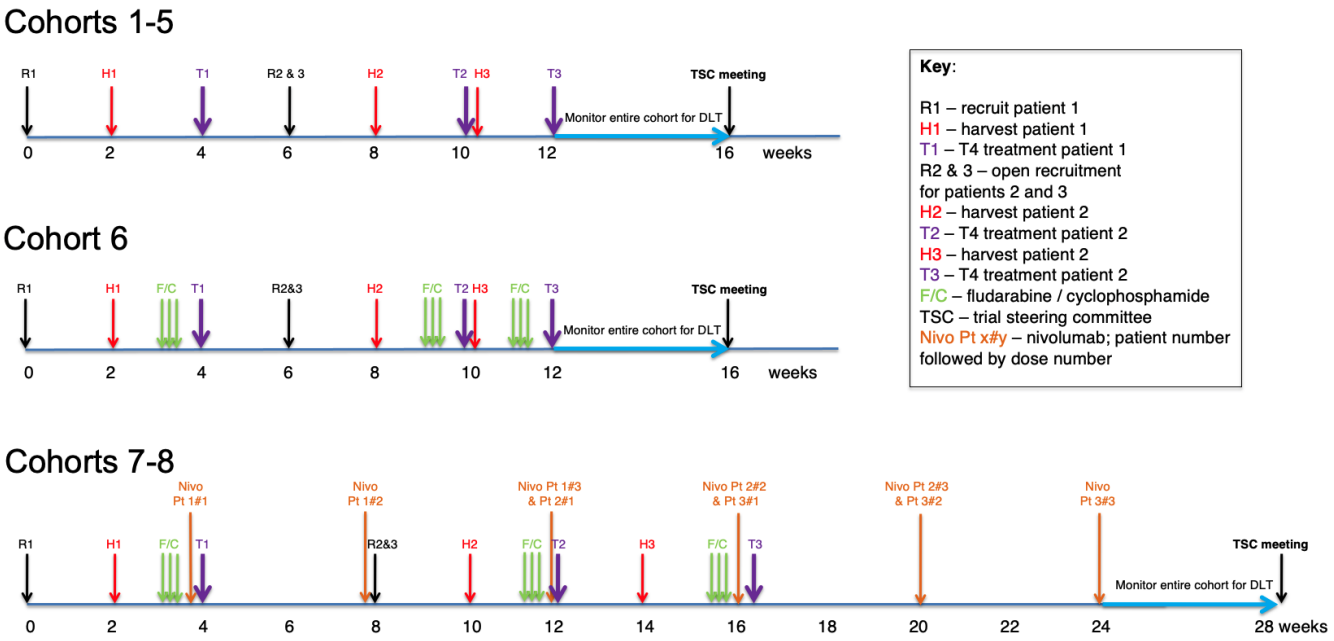


FIGURE 3. Timelines for consecutive patient treatment with T4 immunotherapy. Treatment with T4 immunotherapy is indicated by the vertical purple arrows. Administration of lymphodepleting chemotherapy with fludarabine and cyclophosphamide on 3 sequential days is indicated by the vertical green arrows (cohorts 6-7). Treatment with nivolumab is indicated by the extended vertical orange arrows (3 doses per patient). The indicated timeline indicates that there would be a minimum 6 week gap between the treatment of patients 1 and 2 within a cohort. This interval has been extended to 8 weeks in cohort 7 and cohort 8. The minimum interval would be 2 weeks between patients 2 and 3 (extended to 4 weeks in cohort 7 and cohort 8 to ensure that there is no possibility of 3 DLTs in these cohorts). In the event of dose-limiting toxicities (DLT) in patients 1 and 2, the trial steering committee would consider whether patient 3 could be enrolled at the lower dose level. Note also that in cohort 8, there will be a TSC meeting after the first patient has completed treatment and monitoring.

4.8 Maximum Tolerated Dose

A 3+3 dose escalation design will be followed in order to determine the maximum tolerated dose (MTD) and aid in the definition of the recommended dose for phase 2 testing (RDPT). Trial design has been modelled on dose escalation methods used in cancer drug trials (151).

The maximum administered dose occurs when 2 out of 3 patients (or 2 out of 6 with cohort expansion) experience a dose-limiting toxicity (DLT) at a given dose level. In this event, the MTD will have been exceeded. The MTD will then be taken as the dose level administered to the cohort below the maximum administered dose (151).

If one DLT occurs in a cohort of 3 patients, the cohort will be expanded with at least three further patients. If a second DLT occurs in the cohort, the MTD will have been exceeded and the next lowest dose level

will be expanded to establish the MTD. Thus, the MTD is the highest dose at which <33% of subjects experience a DLT.

If two DLT occur in the first three patients enrolled in any cohort, the MTD will also have been exceeded. If this occurs in cohort 1 (**Table 1**), de-escalation to a –1 dosing regimen will proceed.

If MTD is not reached, the trial will be completed when the last patient in cohort 8 has completed final evaluation at their last visit, 12 weeks after receiving T4 immunotherapy.

Before opening the next dose level, all adverse events recorded at the previous dose level (within 28 days of dosing) will be reviewed and discussed by the investigators.

4.9 Dose Limiting Toxicity

Dose limiting toxicities will be graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE), Current Version. Any DLT must be a toxic event that is considered at least possibly related to T4 immunotherapy. The period of evaluation for DLT after administration of T4 immunotherapy will be 6 weeks.

4.9.1 Dose Limiting Toxicities

Dose limiting toxicity (DLT) is defined as follows:

1. Cytokine storm and severe hypotension: There is a theoretical risk that if $T4^+$ T-cells activate, expand and pass in large numbers into the circulation that a rapid rise in serum cytokine levels could occur. To monitor for this eventuality, serum cytokine levels will be measured at multiple time points after injection of $T4^+$ T-cells. Although investigators will be vigilant for this toxic event, cytokine storm is considered unlikely in patients who do not receive lymphodepleting chemotherapy in view of the low dose of cells and use of the intra-tumoural route. For these reasons, the occurrence of cytokine release syndrome (as distinct from uncomplicated pyrexia) is considered to represent a DLT in cohorts 1-5. By contrast, risk of cytokine release syndrome is considered to be greater in cohorts 6-8 since regulatory T-cells will be depleted in these patients. For this reason, grade 3 cytokine release syndrome that lasts for 3 days or more will be considered a DLT in cohort 6-8 patients who receive Flu/Cy lymphodepleting chemotherapy prior to T4 immunotherapy.

2. Haematological: It is not anticipated that significant haematological toxicity will occur due to the effect of the CAR T-cells alone. This is because ErbB receptors are not expressed at significant levels by haemopoietic cells. The following events would constitute dose-limiting toxicities in cohorts 1-5:

- Febrile neutropenia (Absolute neutrophil count < 1.0×10^9/L with fever $\geq$ 38.5^0C; absolute neutrophil count < 1.0×10^9/L for more than one week).

- Platelet count < 25 x 10^9/L or thrombocytopenia associated with bleeding.

Given that cytopaenia-related toxicity is expected following Flu/Cy lymphodepletion (rather than an expected toxic effect of T4 immunotherapy), the haematological toxicities listed above will not be considered DLTs in cohorts 6-8. Instead, the following events will constitute DLTs in cohorts 6-8 (as well as in cohorts 1-5).

- Grade 4 neutropenia lasting longer than 21 days from the day of cell transfer.
- Grade 4 thrombocytopenia lasting longer than 35 days from the day of cell transfer.

3. Cardiac: ECG will be performed repeatedly during the monitoring phase, as detailed in section 6. Echocardiography will be performed on days 15, 29 and 43 (see section 6). Any suspected new cardiac symptom will be assessed urgently with the appropriate investigations and will be notified to the trial steering committee. A decline in ejection fraction of ≥10% between ECHO investigations will be considered a DLT if the resulting ejection fraction falls below the normal lower limit of 50%. This is because measurement of ejection fraction is subject to inter-observer variability of up to ± 25% (152). In keeping with this, it is well recognized that significant variation can be seen in ejection fraction when serially assessed in clinical trials, even in placebo-treated subjects.

4. Any other grade ≥3 non-haematological toxicity except incompletely treated nausea, vomiting or diarrhoea. Grade 3 fatigue will not be a DLT unless patients were grade 0 or 1 at baseline.

5. Any other toxicity agreed by the investigators to be dose-limiting.

Given increasing experience of the administration of CD19 CAR T-cells after Flu/Cy lymphodepletion (153), the following conditions are not considered DLTs:

- Aphasia/dysphasia or confusion/cognitive disturbance which resolves to grade 1 or less within 2 weeks of onset and to baseline within 4 weeks of onset.
- Expected chemotherapy-induced cytopaenias that occur in patients recruited to cohorts 6-8 will not be classified as DLTs *unless neutropenia and/ or thrombocytopenia meet the DLT definitions described above for cohorts 6-8*. Examples of expected chemotherapy-induced toxicities include lymphopenia, anaemia, anaemia that requires transfusion, neutropenia, bacterial infection in the setting of neutropenia, thrombocytopenia and bleeding in the setting of platelet count less than 50 x10^9/L.
- Immediate hypersensitivity reactions occurring within 2 hours of cell infusion (related to cell infusion) that are reversible to a grade 2 or less within 24 hours of cell administration with standard therapy.

In all cohorts, if a DLT occurs, recruitment will pause until all toxic events have been reviewed by the investigators and the trial steering committee.

4.10 Recommended Dose for Phase II Testing

If a MTD is identified, this defines the upper limit for the RDPT of T4 immunotherapy. If the MTD is not reached in the trial, the RDPT may be defined as the highest dose level tested, subject to discussion by the Trial Steering Committee.

4.11 Feasibility

Guy's and St Thomas' NHS Trust is a large tertiary referral centre for SCCHN. Although the percentage of patients with disease able to meet the eligibility criteria will be small (approximately 2 per month), study recruitment is feasible.

4.12 Patient Replacement

If after T-cell expansion, a sample fails end of production quality control (EOP QC), the patient will not be treated with T4 immunotherapy. In that event, the patient will be offered a second opportunity for treatment. Should they agree to this, the patient will be re-consented for treatment and a new blood sample taken. Alternatively, another patient will be enrolled at the same dose level as a replacement. The frequency of failure of EOP QC will be recorded.

4.13 End of the Trial

The end of the trial will be deemed to occur after database lock (following completion of monitoring of the last patient undergoing the trial) and completion of analysis of laboratory samples collected from patients.

5. THERAPEUTIC REGIMENS, EXPECTED TOXICITY, DOSE MODIFICATIONS

5.1 Treatment Plan

5.1.1 Preparation of T4 Immunotherapy

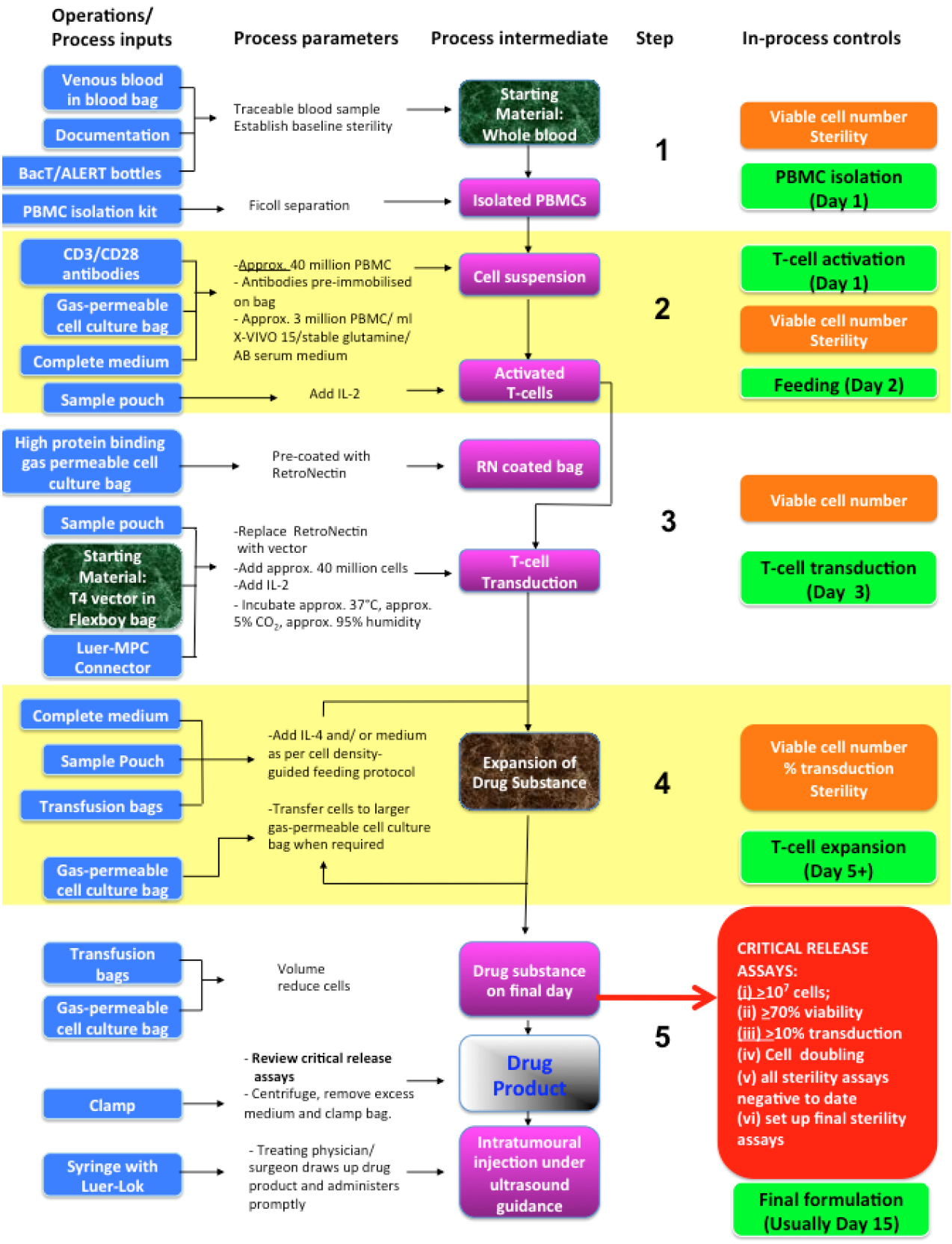
T4 immunotherapy consists of an autologous patient-derived cell product in which T-cells are genetically engineered and expanded *ex-vivo* thereafter (**section 1.2**). **Figure 4** presents an outline of the two-week closed manufacturing process whereby T4 immunotherapy is generated. A detailed description of this process is provided in the Investigational Medicinal Product Dossier.

5.1.2 Immunophenotypic analysis

Cells will be analyzed by flow cytometry for %T1E28z expression. Expression of T1E28z is detected using biotinylated anti-human EGF antibody (R&D systems, code BAF236) followed by PE-conjugated streptavidin (Invitrogen, code S866). This analysis will be performed 2-4 days after retroviral transduction and also at end of production.

Additional polychromatic flow and time of flight mass cytometry will be performed <u>for information only</u> in order to characterise the immunophenotype of the product, detecting expression of markers that include CD3, CD4, CD8, CD27, CD28, CD45RA, CD45RO, CD57, CD62L, CCR7, NKG2D, CD25, CD124 (to detect 4αβ), CD19 (B-cells), CD16+56 (NK cells), CD14 (monocytes) and PD1. CAR T-cells will also be characterised for polyfunctionality using a multiplex cytokine assay <u>for information only.</u>

Figure 4: Outline of Manufacturing Process.
Cell products are manufactured using the indicated process over a period of 11-17 days from phlebotomy to release of the final product. The process is closed meaning that cells are contained in gas-permeable bags throughout the manufacturing process. In step 1, peripheral blood mononuclear cells (PBMC) are isolated from patient blood. T-cells within the PBMC fraction are activated using immobilized antibodies, causing them to reproduce and rendering them suitable for retrovirus-mediated gene transfer (step 2). Gene-transfer is facilitated by the use of RetroNectin (RN)-coated bags. In step 3, gene-modified T-cells are selectively expanded by addition of IL-4, a drug that causes only the genetically engineered cells to grow well. This expansion phase continues through to the final day of manufacture (step 4) on which cells are washed and formulated for injection. In process control tests are shown in orange boxes and assist in maintaining the quality of the manufacturing process. Critical release assays are shown in the red box and indicate that the cell product is safe to release for patient administration.
Cell product manufacture will take place in the Good Manufacturing Process (GMP) Cell Therapy Suite of the Clinical Research Facility at Guy's Hospital.


Operations/ Process inputs
Process parameters
Process intermediate
Step
In-process controls
Venous blood in blood bag
Documentation
BacT/ALERT bottles
PBMC isolation kit
Traceable blood sample
Establish baseline sterility
Ficoll separation
Starting Material: Whole blood
Isolated PBMCs
1
Viable cell number
Sterility
PBMC isolation (Day 1)
CD3/CD28 antibodies
Gas-permeable cell culture bag
Complete medium
Sample pouch
-Approx. 40 million PBMC
- Antibodies pre-immobilised on bag
- Approx. 3 million PBMC/ ml X-VIVO 15/stable glutamine/ AB serum medium
Add IL-2
Cell suspension
Activated T-cells
2
T-cell activation (Day 1)
Viable cell number
Sterility
Feeding (Day 2)
High protein binding gas permeable cell culture bag
Pre-coated with RetroNectin
RN coated bag
Sample pouch
Starting Material: T4 vector in Flexboy bag
Luer-MPC Connector
-Replace RetroNectin with vector
-Add approx. 40 million cells
-Add IL-2
- Incubate approx. 37°C, approx. 5% CO_2, approx. 95% humidity
T-cell Transduction
3
Viable cell number
T-cell transduction (Day 3)
Complete medium
Sample Pouch
Transfusion bags
Gas-permeable cell culture bag
-Add IL-4 and/ or medium as per cell density-guided feeding protocol
-Transfer cells to larger gas-permeable cell culture bag when required
Expansion of Drug Substance
4
Viable cell number
% transduction
Sterility
T-cell expansion (Day 5+)
Transfusion bags
Gas-permeable cell culture bag
Volume reduce cells
Drug substance on final day
Clamp
- Review critical release assays
- Centrifuge, remove excess medium and clamp bag.
Drug Product
Syringe with Luer-Lok
- Treating physician/ surgeon draws up drug product and administers promptly
Intratumoural injection under ultrasound guidance
5
CRITICAL RELEASE ASSAYS:
(i) ≥10^7 cells;
(ii) ≥70% viability
(iii) ≥10% transduction
(iv) Cell doubling
(v) all sterility assays negative to date
(vi) set up final sterility assays
Final formulation (Usually Day 15)

5.1.3 T4 Immunotherapy Release and Administration

End of production (EOP) quality control (QC) assays will be performed on therapeutic cell products as summarized in **Table 2**. T4 immunotherapy products that pass QC will be volume reduced, centrifuged and re-suspended at the appropriate cell number in a final volume of 1-4mL X-VIVO 15 plus 10% AB serum. All cell handling will be undertaken in the GMP Cell Therapy Suite of the Clinical Research Facility at Guy's Hospital. Final cell products will be administered to patients in an adjacent treatment suite, immediately upon formulation.

In up to 3-6 selected patients, up to 30 million $T4^+$ T-cells will be passively labelled with ^{111}Indium, to generate a diagnostic radiotracer that can be used to infer the migration of the drug product for up to 72 hours by single photon emission computed tomography (SPECT)/CT imaging. This labelling procedure will be undertaken in the Guy's hospital Radiopharmacy unit. Both T4 immunotherapy and (where this is generated), the derived T4 radiotracer will be released by a qualified person (QP).

Table 2. End of Production Quality Control.

End Point	**Method**
>70% cell viability	DAPI staining and Flow cytometry analysis on day of release.
$>10^7$ T4-transduced cells	Flow cytometry analysis on day of release
>2 fold-increase in cell number, compared to the number of cells transduced on day 3.	Cell count
Cell surface phenotype >10% $T1E28z^+$	Flow cytometry analysis on day of release
Bacterial, fungal and mycoplasma sterility	Initiate sterility tests on day 1 (BacT/ ALERT), day 4 and 8 (BacT/ ALERT), day 8 (mycoplasma PCR) and on day of release (usually day 15; BacT/ ALERT, fungal & mycoplasma PCR). Final and interim culture results should be negative.
>30% radiolabel incorporation*	Clinical dose calibrator

*where a radiotracer is administered in which T4 engineered T-cells are labelled with Indium-111.

The autologous T4 immunotherapy cell product will be administered to the patient by a head and neck cancer surgeon or a clinician who has experience of intra-tumoural injection, using ultrasound guidance where necessary to identify the viable tissue within the tumour. Infiltration of the maximum possible volume of the target lesion will be achieved by injection along multiple radial paths. Cells will be delivered via a minimum number of puncture sites required to achieve as even a distribution of T4 immunotherapy throughout the viable area of the tumour. T4 immunotherapy will be delivered using a syringe with a Luer connection, via a 21-gauge needle. The total volume to be administered is 1-4mL and this volume will be distributed between the injection sites in accordance with the area of viable tumour seen at ultrasound. This means that the maximum volume that would be administered to a single site within the tumour would be 4mL.

In a subset of up to 3-6 patients, a T4 radiotracer will be administered to a single site in the tumour by a nuclear medicine physician or appointed deputy, within 2 hours of radiolabelling (preferably within 1 hour) and only if the drug product has been released for administration to the patient.

5.1.4 Pre-medication

Skin overlying the areas to be injected with T4 immunotherapy will be anaesthetised with lidocaine or bupivacaine, administered prior to treatment injection.

In cohorts 6-8, patients will receive pre-conditioning with lymphodepleting chemotherapy. This consists of fludarabine 25 mg/m^2 intravenously daily for 3 days and cyclophosphamide at 250 mg/m^2 intravenously daily for 3 days, starting with the first dose of fludarabine. Details of the administration of lymphodepleting chemotherapy are described in section 5.1.5 of this protocol.

5.1.5 Administration of Lymphodepleting Chemotherapy

These agents will be administered on 3 consecutive days, between 2-11 days prior to injection of CAR T-cells in cohorts 6-8. Patients enrolled into cohort 6 and cohort 7 will proceed to receive T4 immunotherapy at a lowered dose of 10^8 CAR$^+$ T-cells. Patients enrolled into cohort 8 will receive 10^9 T-cells, which is the maximum deliverable dose.

On each day that chemotherapy is administered, the following procedure will be followed.

- Patients will first receive intravenous hydration with 1L of 0.9% NaCl.
- This will be followed by Fludarabine 25mg/m^2 administered IV over 30 minutes.
- Next, cyclophosphamide 250mg/m^2 will be administered IV over 60 minutes
- Finally, patients will receive an additional 1L of 0.9% NaCl following the completion of the cyclophosphamide infusion

Patients should be kept well-hydrated but closely monitored to prevent fluid overload. Consequently, participants will be instructed to drink plenty of liquids during and for 24 hours following the chemotherapy (approximately 2 litres/24 hours). Since fludarabine is primarily cleared by renal excretion, the dose of this agent will be reduced by up to 50% if creatinine clearance is 30-70mL/minute. These patients must be monitored particularly closely in light of increased risk of fludarabine-related toxicity. Given that the terminal half-life of fludarabine is approximately 20 hours, investigators will also ensure that, in patients with a creatinine clearance of 30-70mL/minute, there will be a minimum 4 day interval between the final dose of fludarabine and the intra-tumoural administration of CAR T-cells.

Fludarabine is contraindicated if creatinine clearance is <30mL/minute.

5.1.6 Administration of Nivolumab

Patients enrolled into cohort 7 and cohort 8 will receive three doses of nivolumab. Nivolumab 480mg is administered as an intravenous infusion over approximately 60 minutes, as recommended by the manufacturer. In brief, the total dose can be infused directly as a 10 mg/mL solution or can be diluted with sodium chloride 9 mg/mL (0.9%) solution for injection or glucose 50 mg/mL (5%) solution for injection. The final infusion concentration should range between 1 and 10 mg/mL while the total volume of infusion must not exceed 160 mL.

During the infusion, patients should be monitored for signs and symptoms of infusion-related reactions including rigors, chills, wheezing pruritus, flushing, rash, hypotension, hypoxaemia and fever. Patients should be asked to immediately report chills, shaking, shortness of breath, wheezing, itching or rash, flushing, dizziness, fever or faintness.

Once trial participation has been completed, patients should be monitored continuously (at least up to 5 months after the last dose) by their referring oncologist, as an adverse reaction with nivolumab may occur at any time during or after discontinuation of therapy.

5.1.7 Patient Monitoring

Patient monitoring immediately prior to and over the 24-hour period following injection of T4 immunotherapy is summarized in **Figure** 5. Blood samples at T-30 minutes are for retrospective analysis. Baseline safety blood tests will be performed within a seven-day period prior to treatment as summarized in **Table 4**. All patients will remain in hospital for the first 24 hours post administration of T4 immunotherapy. This is to enable close monitoring in case of acute toxicity and to facilitate ease of regular sample collection for safety monitoring. In most cases, patients will be accommodated in an Oncology ward overnight. Patients in cohorts 6 to 8 will be monitored as hospital inpatients for a minimum period of 7 days after administration of T4 immunotherapy.

5.1.8 Dose Adjustments

Dose adjustments are not applicable. Once administered in a single dose, T4 immunotherapy cannot be adjusted.

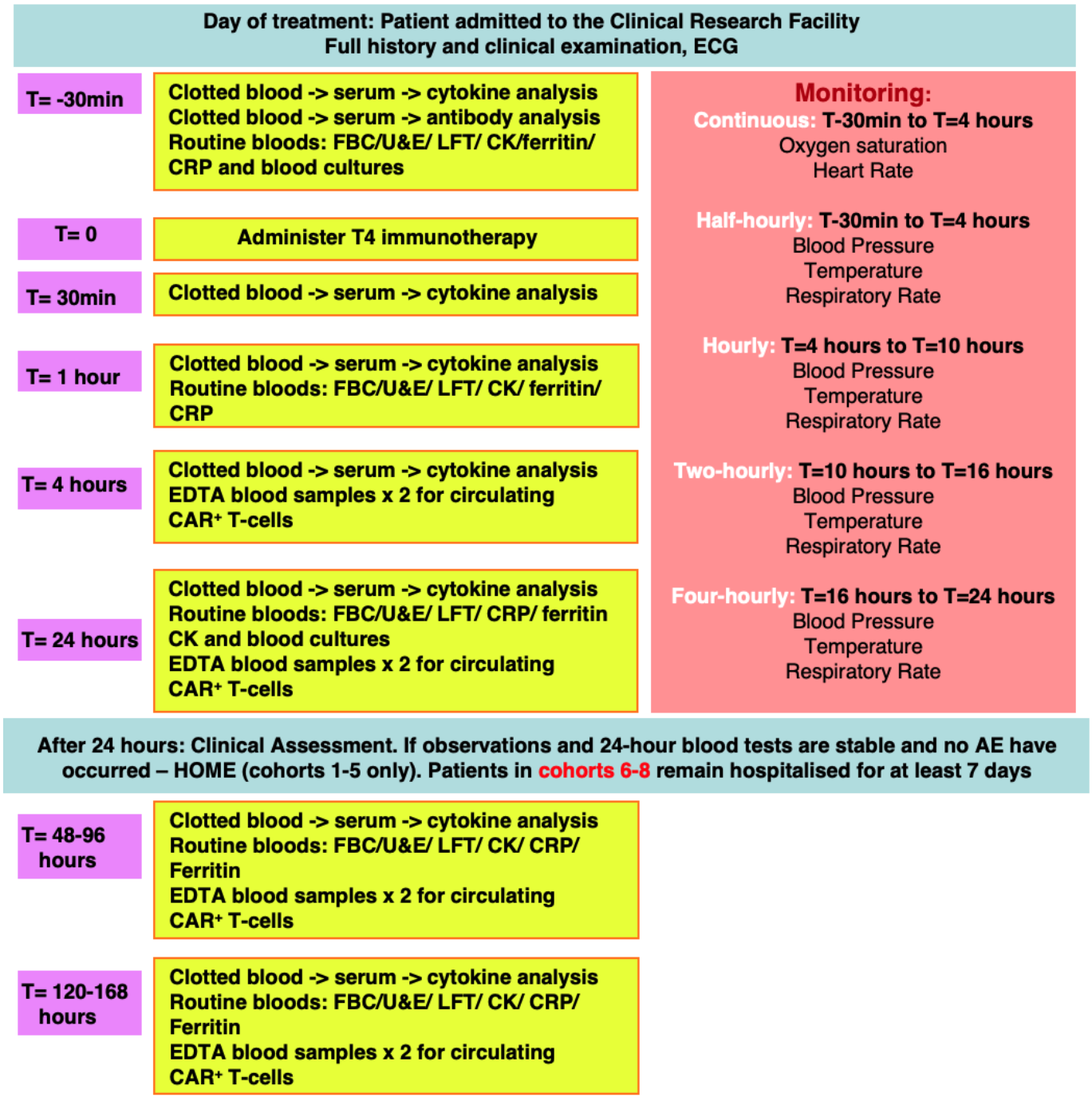


Figure 5: Patient Monitoring: Hours 0 – 168. The term "AE" refers to adverse events that are deemed clinically significant.

5.1.9 Duration of Therapy

T4 immunotherapy will be administered once to each patient.
Patients will be followed up until six weeks post T4 immunotherapy administration when the final response evaluations will be performed. In cohorts 7-8, the monitoring period will be extended to 12 weeks post T4 immunotherapy. At that time, patients will have completed protocol-related therapy and associated monitoring. Follow up thereafter will be as per referring clinician's normal practice.

5.1.10 Concomitant therapy

- Systemic corticosteroids and other immunomodulatory agents are not permitted, unless specified in this protocol. Such exceptions include nivolumab, cyclophosphamide, fludarabine and/or drugs prescribed by the investigators for the management of T4 immunotherapy-related toxicity.
- Chemotherapy or other anti-cancer agents are not permitted in conjunction with T4 immunotherapy (other than the use of lymphodepleting chemotherapy in cohorts 6 - 8).
- Radiotherapy is permitted for palliation of painful metastatic disease excluding the site of administration of T4 immunotherapy and target lesions used for RECIST evaluation.
- Otherwise there are no restrictions on concomitant medications. It is acceptable that patients are vaccinated against covid-19. Palliative care interventions for symptom management are permitted.
- Once the final response evaluation has been completed at six weeks post administration of T4 immunotherapy (or 12 weeks in the case of cohorts 7-8), these restrictions no longer apply.

5.2 Prevention and Management of Toxicities

Subjects will be hospitalised for at least 24 hours (cohorts 1-5) or 7 days (cohorts 6-8, in which lymphodepletion is undertaken) following CAR T-cell treatment. Subjects will remain in hospital until all non-haematological toxicities return to grade 1 or less. Subjects may be discharged with non-critical and clinically stable or slowly improving toxicities (e.g. renal insufficiency) even if >grade 1, if deemed appropriate by the clinical team. Subjects should remain hospitalised for ongoing fever, hypotension, hypoxia or ongoing neurological toxicity that is greater than grade 1. Wherever relevant guidelines have been issued by Guy's and St Thomas' NHS Foundation Trust ("Trust guidelines"), these should be followed.

Reference safety information (RSI) for T4 immunotherapy is located in section 5.3 of the Investigator Brochure. In the case of lymphodepleting agents (fludarabine and cyclophosphamide) and nivolumab, section 4.8 of the SmPCs are used as the RSI.

5.2.1 Infection prophylaxis

Chemotherapy with fludarabine and cyclophosphamide will be administered to patients in cohorts 6-8 and predisposes to neutropenia-associated and opportunistic infection (154). Consequently, patients in cohorts 6-8 may receive anti-microbial prophylaxis at physician's discretion, commencing 1 week prior to CAR T-cell treatment. It should be noted that this is not routinely recommended for patients with acute lymphoblastic leukaemia who receive CD19 CAR T-cell products following lymphodepleting chemotherapy (e.g. please see protocol published with (155)). Moreover, antibiotic use has recently been linked to poorer clinical outcome following CAR T-cell immunotherapy (156). Nonetheless, it is understood that some patients enrolled into this trial may be considered to be particularly susceptible to infectious complications. Hence, prophylactic anti-microbials such as the following may be considered: Cotrimoxazole 960mg BD on Monday, Wednesday and Fridays; Posaconazole 300mg BD on day 1 followed by 100mg daily; Aciclovir 400mg BD. Treatment may be stopped if CD4 count exceeds 200/µL.

5.2.2. Local inflammatory reactions

Management of local inflammatory reactions due to T4 immunotherapy (all cohorts) is discussed in section **4.5** above.

5.2.3 Cytokine release syndrome

Grading of cytokine release syndrome (CRS) will be performed according to the recently revised criteria described by Lee et al (157) (**Table 3**).

Table 3. American Society for Blood and Marrow Transplantation Consensus Grading for Immune Effector Cell Toxicities.≠

CRS parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever	Temperature≥38°C	Temperature≥38°C	Temperature≥38°C	Temperature≥38°C
With either				
Hypotension	None	Not requiring vasopressors	Requiring one vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
And/or				
Hypoxia	None	Requiring low-flow nasal cannula or blow-by	Requiring high-flow nasal cannula, facemask, non-rebreather mask or Venturi mask	Requiring positive pressure (e.g. CPAP, BiPAP, intubation and mechanical ventilation)

CPAP: Continuous positive airway pressure; BiPAP: Bilevel positive airway pressure
† Fever is defined as temperature ≥38°C not attributable to any other cause. In patients who have CRS then receive antipyretics or anti-cytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.

‡ CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a patient with temperature of 39.5°C, hypotension requiring one vasopressor and hypoxia requiring low flow nasal cannula is classified as having Grade 3 CRS.

≠Organ toxicities associated with CRS may be graded according to CTCAE Current Version but they do not influence CRS grading.

^ Low-flow nasal cannula is defined as oxygen delivered at ≤ 6 litres/minute. Low flow also includes blow-by oxygen delivery, sometimes used in paediatrics. High-flow nasal cannula is defined as oxygen delivered at > 6 litres/ minute.

If cytokine storm is suspected, the treatment algorithm described in the current Guy's and St Thomas CRS clinical management guideline will be followed. Agents will be stored on the ward. The proposed approach is justified as follows.

- Increasing evidence indicates that IL-6 is an important mediator of cytokine storm in patients treated with CD19 CAR-specific T-cells and that the anti-IL-6 receptor antibody, tocilizumab, can ameliorate toxicity (158, 159).
- With ongoing pyrexia, give consideration to administration of levetiracetam since seizures may occur in these patients.

5.2.4 Fever and neutropenia

Evaluation for a source of infection should be performed per Trust guidelines. Fevers should be treated with paracetamol and comfort measures. Subjects who are neutropenic and febrile should receive broad-spectrum antibiotics according to Trust guidelines. Maintenance IV fluids (normal saline) should be started on most subjects with high fevers, especially if oral intake is poor or if the subject has tachycardia. Even daily fluid balance should be achieved in subjects who are not hypotensive. Consideration should be given to the administration of Filgrastim for chemotherapy-induced neutropenia.

5.2.5 Blood product support

Haematological support measures should be implemented as per Trust guidelines. In brief, all blood products should be irradiated. Attempts should be made to keep haemoglobin >80 gm/L and platelets >20,000/mm^3 using packed red blood cell and platelet transfusions. Leukocyte filters should be utilised for all blood and platelet transfusions to decrease sensitization to transfused leukocytes and decrease the risk of infection with cytomegalovirus.

5.2.6 Neurotoxicity

Immune effector cell neurotoxicity syndrome (ICANS, e.g. encephalopathy, somnolence, aphasia) have been observed following immunotherapy with anti-CD19 CAR T cell immunotherapy. Evaluation of any new-onset neurotoxicity should include a neurological examination (including mini-mental state examination), brain MRI, neurological consultation and consideration of the need for examination of the cerebrospinal fluid. Endotracheal intubation may be needed for airway protection in severe cases. Corticosteroids may be considered for any severe or life-threatening neurotoxicity and anti-convulsant

and sedative medications may be considered as clinically indicated. Neurotoxicity has been linked with targeting of the CD19 antigen on occult tumour or non-tumour elements in the central nervous system (160). Consequently, it is not an expected toxicity in this trial.

5.2.7 Hypotension and Renal insufficiency

Subjects should generally be kept well-hydrated but closely monitored to prevent fluid overload. The management suggestions indicated below may need to be modified based on the clinical characteristics of individual subjects such as pulmonary status, cardiac function, and other factors.

The baseline systolic blood pressure is defined for this guideline as the average of all systolic blood pressure readings obtained during the 24 hours prior to the CAR T-cell infusion. The first treatment for hypotension is administration of IV normal saline boluses.

- Subjects with a systolic blood pressure that is 80% or less of their baseline blood pressure and less than 100 mm Hg should receive a 1L normal saline bolus.
- Subjects with a systolic blood pressure that is 80% or less of their baseline blood pressure and greater than 100 mm Hg on two consecutive blood pressure checks separated by at least 2 hours should receive a 1L normal saline bolus.
- Subjects with a systolic blood pressure less than 85 mm Hg should receive a 1L normal saline bolus regardless of baseline blood pressure.

5.2.8 Other toxicities attributable to cyclophosphamide and fludarabine

Gastrointestinal side effects such as nausea, vomiting, mucositis, and gastritis are commonly seen with this regimen. Although cyclophosphamide may cause haemorrhagic cystitis and haematuria, this is rare with doses of <1g/m^2, rendering the concomitant prescription of mesna unnecessary.[3] Fludarabine may also cause autoimmune haemolytic anaemia. Chemotherapy with fludarabine and cyclophosphamide can rarely cause major organ dysfunction. A comprehensive list of toxicities of these agents is presented in the Investigator Brochure.

[3] London Cancer North and East guidelines for the administration of mesna with ifosfamide and cyclophosphamide. UCLH guideline authored by Emma Morris. Version 1.0 (9/1/2014).

5.2.9 Toxicities and Recommended Treatment Modifications for Nivolumab

Nivolumab can induce several types of immune-related adverse reactions. Most of these will improve or resolved with appropriate management, including the initiation of corticosteroids and treatment modifications.

If immunosuppression with corticosteroids is used to treat an adverse reaction, a taper of at least 1 month duration should be initiated upon improvement. Rapid tapering may lead to worsening or recurrence of the adverse reaction. Non-corticosteroid immunosuppressive therapy should be added if there is worsening or no improvement despite corticosteroid use.

Nivolumab should not be resumed while the patient is receiving immunosuppressive doses of corticosteroids or other immunosuppressive therapy. Prophylactic antibiotics should be used to prevent opportunistic infections in patients receiving immunosuppressive therapy.

Nivolumab must be permanently discontinued for any severe immune-related adverse reaction that recurs and for any life-threatening immune-related adverse reaction.

Nivolumab-induced immune-related adverse reactions can exhibit a delayed onset. Consequently, patients should be monitored continuously for at least 5 months after their last dose by the referring oncologist.

A guide to the management of toxicity induced by nivolumab is provided in the Summary of Product Characteristics (SmPC) (Opdivo SmPC, https://www.medicines.org.uk/emc/product/6888/smpc, accessed 28.12.2019) and is summarised in **Table 4**.

Table 4. Recommended treatment modifications for nivolumab[†]

Immune-related adverse reaction	Severity	Treatment modification
Immune-related pneumonitis	Grade 2 pneumonitis	Withhold dose(s) until symptoms resolve, radiographic abnormalities improve, and management with corticosteroids is complete
	Grade 3 or 4 pneumonitis	Permanently discontinue treatment
Immune-related colitis	Grade 2 diarrhoea or colitis	Withhold dose(s) until symptoms resolve and management with corticosteroids, if needed, is complete
	Grade 3 diarrhoea or colitis	Withhold dose(s) until symptoms resolve and management with corticosteroids is complete
	Grade 4 diarrhoea or colitis	Permanently discontinue treatment
Immune-related hepatitis	Grade 2 elevation in aspartate aminotransferase (AST), alanine aminotransferase (ALT), or total bilirubin	Withhold dose(s) until laboratory values return to baseline and management with corticosteroids, if needed, is complete

	Grade 3 or 4 elevation in AST, ALT, or total bilirubin	Permanently discontinue treatment
Immune-related nephritis and renal dysfunction	Grade 2 or 3 creatinine elevation	Withhold dose(s) until creatinine returns to baseline and management with corticosteroids is complete
	Grade 4 creatinine elevation	Permanently discontinue treatment
Immune-related endocrinopathies	Symptomatic Grade 2 or 3 hypothyroidism, hyperthyroidism, hypophysitis, Grade 2 adrenal insufficiency Grade 3 diabetes	Withhold dose(s) until symptoms resolve and management with corticosteroids (if needed for symptoms of acute inflammation) is complete. Treatment should be continued in the presence of hormone replacement therapy[1] as long as no symptoms are present
	Grade 4 hypothyroidism Grade 4 hyperthyroidism Grade 4 hypophysitis Grade 3 or 4 adrenal insufficiency Grade 4 diabetes	Permanently discontinue treatment Permanently discontinue treatment Permanently discontinue treatment Permanently discontinue treatment Permanently discontinue treatment
Immune-related skin adverse reactions	Grade 3 rash	Withhold dose(s) until symptoms resolve and management with corticosteroids is complete
	Grade 4 rash	Permanently discontinue treatment
	Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN)	Permanently discontinue treatment Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN) Permanently discontinue treatment
Other immune-related adverse reactions	Grade 3 (first occurrence)	Withhold dose(s)
	Grade 3 myocarditis	Permanently discontinue treatment
	Grade 4 or recurrent Grade 3; persistent Grade 2 or 3 despite treatment modification; inability to reduce corticosteroid dose to 10 mg prednisone or equivalent per day	Permanently discontinue treatment Permanently discontinue treatment Permanently discontinue treatment

† From the Summary of Product Characteristics (SmPC) (Opdivo SmPC, https://www.medicines.org.uk/emc/product/6888/smpc, accessed 28.12.2019)

[1] Recommendations for the use of hormone replacement therapy are provided in section 4.4. of the SmPC.

5.2.9.1 Immune-related pneumonitis

Severe pneumonitis or interstitial lung disease, including fatal cases, has been observed with nivolumab monotherapy. Patients should be monitored for signs and symptoms of pneumonitis such as radiographic changes (e.g., focal ground glass opacities, patchy filtrates), dyspnoea, and hypoxia. Infectious and disease-related aetiologies should be ruled out.

For Grade 3 or 4 pneumonitis, nivolumab must be permanently discontinued, and corticosteroids should be initiated at a dose of 2 to 4 mg/kg/day methylprednisolone equivalents.

For Grade 2 (symptomatic) pneumonitis, nivolumab should be withheld and corticosteroids initiated at a dose of 1 mg/kg/day methylprednisolone equivalents. Upon improvement, nivolumab may be resumed after corticosteroid taper. If worsening or no improvement occurs despite initiation of corticosteroids, corticosteroid dose should be increased to 2 to 4 mg/kg/day methylprednisolone equivalents and nivolumab must be permanently discontinued.

5.2.9.2 Immune-related colitis

Severe diarrhoea or colitis has been observed with nivolumab monotherapy. Patients should be monitored for diarrhoea and additional symptoms of colitis, such as abdominal pain and mucus or blood in stool. Cytomegalovirus (CMV) infection/reactivation has been reported in patients with corticosteroid-refractory immune related colitis. Infectious and other aetiologies of diarrhoea should be ruled out, therefore appropriate laboratory tests and additional examinations must be performed. If diagnosis of corticosteroid refractory immune-related colitis is confirmed addition of an alternative immunosuppressive agent to the corticosteroid therapy, or replacement of the corticosteroid therapy, should be considered.

For Grade 4 diarrhoea or colitis, nivolumab or nivolumab in combination with ipilimumab must be permanently discontinued, and corticosteroids should be initiated at a dose of 1 to 2 mg/kg/day methylprednisolone equivalents.

Nivolumab monotherapy should be withheld for Grade 3 diarrhoea or colitis, and corticosteroids initiated at a dose of 1 to 2 mg/kg/day methylprednisolone equivalents. Upon improvement, nivolumab monotherapy may be resumed after corticosteroid taper. If worsening or no improvement occurs despite initiation of corticosteroids, nivolumab monotherapy must be permanently discontinued.

For Grade 2 diarrhoea or colitis, nivolumab should be withheld. Persistent diarrhoea or colitis should be managed with corticosteroids at a dose of 0.5 to 1 mg/kg/day methylprednisolone equivalents. Upon improvement, nivolumab may be resumed after corticosteroid taper, if needed. If worsening or no improvement occurs despite initiation of corticosteroids, corticosteroid dose should be increased to 1 to 2 mg/kg/day methylprednisolone equivalents and nivolumab must be permanently discontinued.

5.2.9.3 Immune-related hepatitis

Severe hepatitis has been observed with nivolumab monotherapy. Patients should be monitored for signs and symptoms of hepatitis such as transaminase and total bilirubin elevations. Infectious and disease-related aetiologies should be ruled out.

For Grade 3 or 4 transaminase or total bilirubin elevation, nivolumab must be permanently discontinued, and corticosteroids should be initiated at a dose of 1 to 2 mg/kg/day methylprednisolone equivalents.

For Grade 2 transaminase or total bilirubin elevation, nivolumab should be withheld. Persistent elevations in these laboratory values should be managed with corticosteroids at a dose of 0.5 to 1 mg/kg/day methylprednisolone equivalents. Upon improvement, nivolumab may be resumed after corticosteroid taper, if needed. If worsening or no improvement occurs despite initiation of corticosteroids, corticosteroid dose should be increased to 1 to 2 mg/kg/day methylprednisolone equivalents and nivolumab must be permanently discontinued.

5.2.9.4 Immune-related nephritis and renal dysfunction

Severe nephritis and renal dysfunction have been observed with nivolumab. Patients should be monitored for signs and symptoms of nephritis or renal dysfunction. Most patients present with asymptomatic increases in serum creatinine. Disease-related aetiologies should be ruled out.

For Grade 4 serum creatinine elevation, nivolumab must be permanently discontinued, and corticosteroids should be initiated at a dose of 1 to 2 mg/kg/day methylprednisolone equivalents.

For Grade 2 or 3 serum creatinine elevation, nivolumab should be withheld, and corticosteroids should be initiated at a dose of 0.5 to 1 mg/kg/day methylprednisolone equivalents. Upon improvement, nivolumab may be resumed after corticosteroid taper. If worsening or no improvement occurs despite initiation of corticosteroids, corticosteroid dose should be increased to 1 to 2 mg/kg/day methylprednisolone equivalents, and nivolumab must be permanently discontinued.

5.2.9.5 Immune-related endocrinopathies

Severe endocrinopathies, including hypothyroidism, hyperthyroidism, adrenal insufficiency (including secondary adrenocortical insufficiency), hypophysitis (including hypopituitarism), diabetes mellitus, and diabetic ketoacidosis have been attributed to nivolumab therapy.

Patients should be monitored for clinical signs and symptoms of endocrinopathies and for hyperglycaemia and changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation). Patients may present with fatigue, headache, mental status changes, abdominal pain, unusual bowel habits, and hypotension, or nonspecific symptoms which may resemble other causes such as brain metastasis or underlying disease. Unless an alternate aetiology has been identified, signs or symptoms of endocrinopathies should be considered immune-related.

For symptomatic hypothyroidism, nivolumab should be withheld, and thyroid hormone replacement should be initiated as needed. For symptomatic hyperthyroidism, nivolumab should be withheld and antithyroid medication should be initiated as needed. Corticosteroids at a dose of 1 to 2 mg/kg/day methylprednisolone equivalents should also be considered if acute inflammation of the thyroid is suspected. Upon improvement, nivolumab may be resumed after corticosteroid taper, if needed. Monitoring of thyroid function should continue to ensure appropriate hormone replacement is utilised. Nivolumab must be permanently discontinued for life-threatening hyperthyroidism or hypothyroidism.

For symptomatic Grade 2 adrenal insufficiency, nivolumab should be withheld, and physiologic corticosteroid replacement should be initiated as needed. Nivolumab must be permanently discontinued

for severe (Grade 3) or life-threatening (Grade 4) adrenal insufficiency. Monitoring of adrenal function and hormone levels should continue to ensure appropriate corticosteroid replacement is utilised.

For symptomatic Grade 2 or 3 hypophysitis, nivolumab should be withheld, and hormone replacement should be initiated as needed. Corticosteroids at a dose of 1 to 2 mg/kg/day methylprednisolone equivalents should also be considered if acute inflammation of the pituitary gland is suspected. Upon improvement, nivolumab may be resumed after corticosteroid taper, if needed. Nivolumab must be permanently discontinued for life-threatening (Grade 4) hypophysitis. Monitoring of pituitary function and hormone levels should continue to ensure appropriate hormone replacement is utilised.

For symptomatic diabetes, nivolumab should be withheld, and insulin replacement should be initiated as needed. Monitoring of blood sugar should continue to ensure appropriate insulin replacement is utilised. Nivolumab must be permanently discontinued for life-threatening diabetes.

5.2.9.6 Immune-related skin adverse reactions

Severe rash has been observed with nivolumab. Nivolumab should be withheld for Grade 3 rash and discontinued for Grade 4 rash. Severe rash should be managed with high-dose corticosteroid at a dose of 1 to 2 mg/kg/day methylprednisolone equivalents.

Rare cases of Stevens-Johnson syndrome and toxic epidermal necrolysis some of them with fatal outcome have been observed. If symptoms or signs of Stevens-Johnson syndrome or toxic epidermal necrolysis appear, treatment with nivolumab should be discontinued and the patient referred to a specialised unit for assessment and treatment. If the patient has developed Stevens-Johnson syndrome or toxic epidermal necrolysis with the use of nivolumab, permanent discontinuation of treatment is recommended. Caution should be used when considering the use of nivolumab in a patient who has previously experienced a severe or life-threatening skin adverse reaction on prior treatment with other immune stimulatory anticancer agents.

5.2.9.7 Other immune-related adverse reactions

The following immune-related adverse reactions were reported in less than 1% of patients treated with nivolumab monotherapy in clinical trials across doses and tumour types: pancreatitis, uveitis, demyelination, autoimmune neuropathy (including facial and abducens nerve paresis), Guillain-Barré syndrome, myasthenia gravis, myasthenic syndrome, aseptic meningitis, encephalitis, gastritis, sarcoidosis, duodenitis, myositis, myocarditis, and rhabdomyolysis. Cases of Vogt-Koyanagi-Harada syndrome and hypoparathyroidism have been reported post marketing.

For suspected immune-related adverse reactions, adequate evaluation should be performed to confirm aetiology or exclude other causes. Based on the severity of the adverse reaction, nivolumab should be withheld and corticosteroids administered. Upon improvement, nivolumab may be resumed after corticosteroid taper. Nivolumab must be permanently discontinued for any severe immune-related adverse reaction that recurs and for any life-threatening immune-related adverse reaction.

Rare cases of myotoxicity (myositis, myocarditis, and rhabdomyolysis), some with fatal outcome, have been reported with nivolumab. If a patient develops signs and symptoms of myotoxicity, close monitoring should be implemented, and the patient referred to a specialist for assessment and treatment without delay. Based on the severity of myotoxicity, nivolumab should be withheld or discontinued, and appropriate treatment instituted.

Solid organ transplant rejection has been reported in the post-marketing setting in patients treated with PD-1 inhibitors. Treatment with nivolumab may increase the risk of rejection in solid organ transplant recipients. The benefit of treatment with nivolumab versus the risk of possible organ rejection should be considered in these patients.

6. CLINICAL EVALUATION, LABORATORY TESTS, FOLLOW-UP

6.1 Prior to Commencement of Therapy

- Within four weeks before treatment:

  - Check against Inclusion criteria
  - Medical history/ Concomitant medication
  - Physical examination
  - ECHO and ECG
  - Screening blood tests (Full blood count, biochemical profile, HIV-1, HIV-2, HTLV-1, HTLV-2, HBV (HBsAg and anti-HB core (HBc) antibody), HCV, syphilis serology. Patients in cohort 6 and 7 will also undergo testing for creatinine clearance (Cockcroft-Gault equation, requires measurement of patient weight), CRP, creatine kinase and ferritin at this time.
  - Contrast-enhanced CT: head, neck, thorax, abdomen (Where deemed more useful, MRI imaging will be used to evaluate local tumour status).
  - Whole blood in EDTA (1 x 4mL) for circulating T1E28z^{+} cell analysis (qPCR), to assist in setting negative control for this assay.
  - **Selected patients only**: ^{18}Fluorodeoxyglucose (FDG) positron emission tomography (PET)-CT head, neck and half-body body scanning. The scan will be repeated on the same patients after treatment.
  - **Selected patients only:** In up to nine patients, up to three core biopsies will be taken from the target lesion selected as amenable to immunotherapy. Biopsies may be fixed in formalin and paraffin embedded. The necessary number of sections will be cut for any or all of the following analyses: (i) analysis of ErbB1 expression; (ii) dual staining of Cytokeratin A1/A3 and cleaved caspase 3 (apoptosis); (iii) detection of immune cells and/ or markers; (iv) H&E staining; (v) detection of CAR T-cells (RNAScope). Details of the assays are described in the Oral Pathology (Guy's Hospital) and CAR Research Group manual of standard operating procedures. One or more core biopsies may also be subjected to RNA extraction and RNA sequencing (RNASeq) as described in the manual of standard operating procedures produced by the company that undertakes this analysis. Subject to satisfactory quality control of RNA, transcriptomic profiling may also be analysed for differential gene expression analysis.

- **Cohorts 6-8 only**: Baseline blood sample to measure circulating Tregs (4mL whole blood in EDTA anticoagulant) and myeloid derived suppressor cells (31.5mL in citrate anticoagulant). Plasma and PBMC will be banked from excess material in the citrate sample and serum will be banked from a clotted sample for future comparative studies.

- **Cohort 7 and 8 only**: Random serum cortisol, random blood glucose, thyroid function tests (T4 and TSH).

☐ On the day of blood harvest (Day -14):

- HIV-1, HIV-2, HTLV-1, HTLV-2, HBV (HBsAg and anti-HBc antibody), HCV, syphilis serology on the harvested blood.

☐ **Cohorts 1-5 only**: Within four days before treatment (baseline safety screen/ testing):

- Clinical history
- Physical examination
- Bloods: FBC, U&E, LFT, CRP, creatine kinase, ferritin, MAGE-reactive T-cells (22.5mL whole blood in citrate anticoagulant).
- ECG

6.2 During Treatment

☐ **Cohorts 6-8 only**: Day -11 to Day -2 (three sequential days on which lymphodepleting chemotherapy is administered and which fall between day -11 and day -2 of CAR T-cell treatment)

- Clinical history
- Physical examination
- Bloods: FBC, U&E, LFT
- ECG (day -9 to day -2)

- Day 1:

  - The patient is admitted to hospital
  - Clinical history
  - Physical examination
  - ECG.
  - For monitoring and blood sampling over the first 24 hours please refer to the patient monitoring flow chart, **section 5.1.5** (**Figure 5**).
  - **Selected patients only**: SPECT-CT T-cell imaging following *ex-vivo* ^{111}In labelling of T4 engineered T-cells (first of three scans to be performed on these patients). The radiotracer (containing up to 30 million labelled T4-engineered T-cells) will be administered at a single site within the tumour.

- Day 2:

  - Bloods: FBC, U&E, LFT, CRP, creatine kinase, ferritin.
  - Whole blood in EDTA (1 x 4mL) for circulating T1E28z^{+} cell analysis (FACS, qPCR).
  - Clotted sample for cytokine analysis on serum.
  - **Selected patients only**: SPECT-CT T-cell imaging following *ex-vivo* ^{111}In labelling (second of three scans). Alternatively, at the clinician's discretion, SPECT alone may be conducted if it is deemed that this will not affect the quality of the data.

- Day 3-4:

  - Bloods: FBC, U&E, LFT, CRP, creatine kinase, ferritin.
  - Whole blood in EDTA (1 x 4mL) for circulating T1E28z^{+} cell analysis (FACS, qPCR).
  - Clotted sample for cytokine analysis on serum.
  - **Selected patients only**: On day 3, SPECT-CT T-cell imaging will be repeated following *ex-vivo* ^{111}In labelling (third of three scans). Alternatively, at the clinician's discretion, SPECT alone may be conducted if it is deemed that this will not affect the quality of the data.

- **Cohort 7 and 8 only**: Random serum cortisol, random blood glucose, thyroid function tests (T4 and TSH)

Day 5-7:

- Bloods: FBC, U&E, LFT, CRP, creatine kinase, ferritin.
- Whole blood in EDTA (1 x 4mL) for circulating T1E28z^{+} cell analysis (FACS, qPCR).
- Clotted sample for cytokine analysis on serum.

Day 8:

- Clinical history.
- Physical examination.
- Bloods: FBC, U&E, LFT, CRP, creatine kinase, ferritin.
- Whole blood in EDTA (1 x 4mL) for circulating T1E28z^{+} cell analysis (FACS, qPCR).
- ECG.
- **Selected patients only:** In up to nine patients, up to three core biopsies will be taken from the target lesion selected as amenable to immunotherapy. Biopsies may be fixed in formalin and paraffin embedded. The necessary number of sections will be cut for any or all of the following analyses: (i) analysis of ErbB1 expression; (ii) dual staining of Cytokeratin A1/A3 and cleaved caspase 3 (apoptosis); (iii) detection of immune cells and/ or markers; (iv) H&E staining; (v) detection of CAR T-cells (RNAScope). Details of the assays are described in the Oral Pathology (Guy's Hospital) and CAR Research Group manual of standard operating procedures. One or more core biopsies may also be subjected to RNA extraction and RNA sequencing (RNASeq) as described in the manual of standard operating procedures produced by the company that undertakes this analysis. Subject to satisfactory quality control of RNA, transcriptomic profiling may also be analysed for differential gene expression analysis.
- **Cohorts 6-8 only**: Circulating Tregs (4mL whole blood in EDTA anticoagulant) and myeloid derived suppressor cells (31.5mL in citrate anticoagulant). Plasma and PBMC will be banked from excess material in the citrate sample and serum will be banked from a clotted sample for future comparative studies.
- **Cohort 7 and 8 only**: Random serum cortisol, random blood glucose, thyroid function tests (T4 and TSH).

- Day 11:

  - **Cohorts 6-8 only**: Clinical history.

- Day 15:

  - Clinical history.
  - Physical examination.
  - Bloods: FBC, U&E, LFT, CRP, creatine kinase, ferritin.
  - Whole blood in EDTA (1 x 4mL) for circulating T1E28z^{+} cell analysis (FACS, qPCR).
  - **Cohorts 1-5 only**: Clotted sample for separation and storage of serum at –80°C. This will be used to examine for emerging anti-CAR antibodies.
  - ECG.
  - ECHO.
  - CXR.
  - **Selected patients only:** In up to nine patients, up to three core biopsies will be taken from the target lesion selected as amenable to immunotherapy. Biopsies may be fixed in formalin and paraffin embedded. The necessary number of sections will be cut for any or all of the following analyses: (i) analysis of ErbB1 expression; (ii) dual staining of Cytokeratin A1/A3 and cleaved caspase 3 (apoptosis); (iii) detection of immune cells and/ or markers; (iv) H&E staining; (v) detection of CAR T-cells (RNAScope). Details of the assays are described in the Oral Pathology (Guy's Hospital) and CAR Research Group manual of standard operating procedures. One or more core biopsies may also be subjected to RNA extraction and RNA sequencing (RNASeq) as described in the manual of standard operating procedures produced by the company that undertakes this analysis. Subject to satisfactory quality control of RNA, transcriptomic profiling may also be analysed for differential gene expression analysis.
  - **Cohorts 6-8 only**: Circulating Tregs (4mL whole blood in EDTA anticoagulant) and myeloid derived suppressor cells (31.5mL in citrate anticoagulant). Plasma and PBMC will be banked from excess material in the citrate sample and serum will be banked from a clotted sample for future comparative studies.
  - **Cohort 7 and 8 only**: Random serum cortisol, random blood glucose, thyroid function tests (T4 and TSH).

- Day 22:

  - **Cohorts 6-8 only**: Clinical history.
  - **Cohorts 6-8 only**: Physical examination.
  - **Cohorts 6-8 only**: Bloods: FBC, U&E, LFT, CRP, creatine kinase, ferritin.
  - **Cohorts 6-8 only**: Whole blood in EDTA (1 x 4mL) for circulating T1E28z^{+} cell analysis (FACS, qPCR).
  - **Cohorts 6-8 only**: Circulating Tregs (4mL whole blood in EDTA anticoagulant) and myeloid derived suppressor cells (31.5mL in citrate anticoagulant). Plasma and PBMC will be banked from excess material in the citrate sample and serum will be banked from a clotted sample for future comparative studies.
  - **Cohort 7 and 8 only**: Random serum cortisol, random blood glucose, thyroid function tests (T4 and TSH).

- Day 28 (cohort 7 and 8 only; no visit on day 28 for patients in cohorts 1-6):

  - **Cohort 7 and 8 only**: Clinical history.
  - **Cohort 7 and 8 only**: Physical examination.
  - **Cohort 7 and 8 only**: ECG.
  - **Cohort 7 and 8 only**: Bloods: FBC, U&E, LFT, CRP, creatine kinase, ferritin.
  - **Cohort 7 and 8 only**: Whole blood in EDTA (1 x 4mL) for circulating T1E28z^{+} cell analysis (FACS, qPCR).
  - **Cohort 7 and 8 only**: Circulating Tregs (4mL whole blood in EDTA anticoagulant) and myeloid derived suppressor cells (31.5mL in citrate anticoagulant). Plasma and PBMC will be banked from excess material in the citrate sample and serum will be banked from a clotted sample for future comparative studies.
  - **Cohort 7 and 8 only**: Random serum cortisol, random blood glucose, thyroid function tests (T4 and TSH).

- Day 29 (cohorts 1-6 only; no visit on day 29 for patients in cohort 7-8):

  - **Cohorts 1-6 only**: Clinical history.
  - **Cohorts 1-6 only**: Physical examination.
  - **Cohorts 1-6 only**: Bloods: FBC, U&E, LFT, CRP, creatine kinase, ferritin.
  - **Cohorts 1-5 only**: Whole blood in citrate for circulating T1E28z^{+} cell analysis (1 x 4mL in EDTA) and MAGE-reactive T-cells (22.5mL in citrate anticoagulant)
  - **Cohorts 1-6 only**: ECG.
  - **Cohorts 1-6 only**: ECHO.
  - **Cohorts 1-6 only**: CXR.
  - **Cohort 6-8 only**: Circulating Tregs (4mL whole blood in EDTA anticoagulant) and myeloid derived suppressor cells (31.5mL in citrate anticoagulant). Plasma and PBMC will be banked from excess material in the citrate sample and serum will be banked from a clotted sample for future comparative studies.

- Day 36 (cohorts 6-8 only; no visit on day 36 for patients in cohorts 1-5):

  - **Cohorts 6-8 only**: Clinical history.
  - **Cohorts 6-8 only**: Physical examination.
  - **Cohorts 6-8 only**: Bloods: FBC, U&E, LFT, CRP, creatine kinase, ferritin.
  - **Cohort 6-8 only**: Whole blood in EDTA (1 x 4mL) for circulating T1E28z^{+} cell analysis (FACS, qPCR).
  - **Cohorts 6-8 only**: Circulating Tregs (4mL whole blood in EDTA anticoagulant) and myeloid derived suppressor cells (31.5mL in citrate anticoagulant). Plasma and PBMC will be banked from excess material in the citrate sample and serum will be banked from a clotted sample for future comparative studies.
  - **Cohort 7-8 only**: ECHO.
  - **Cohort 7-8 only**: CXR.

- Day 43:

  - Clinical history.
  - Physical Examination.
  - Contrast-enhanced CT: head, neck, thorax, abdomen (Where deemed more useful at enrolment, MRI imaging will be used to evaluate local tumour status).
  - Bloods: FBC, U&E, LFT, CRP, creatine kinase, ferritin.
  - Whole blood in EDTA (1 x 4mL) for circulating T1E28z^{+} cell analysis (FACS, qPCR).
  - Clotted sample for separation and storage of serum at –80°C. This will be used to examine for emerging anti-CAR antibodies.
  - ECG.
  - **Cohorts 1-6 only**: ECHO.
  - **Selected patients only**: FDG PET-CT head, neck and half-body body scanning (performed on the same day as contrast-enhanced CT scan).
  - **Cohorts 6-8 only**: Circulating Tregs (4mL whole blood in EDTA anticoagulant) and myeloid derived suppressor cells (31.5mL in citrate anticoagulant). Plasma and PBMC will be banked from excess material in the citrate sample and serum will be banked from a clotted sample for future comparative studies.
  - **Cohort 7-8 only**: Random serum cortisol, random blood glucose, thyroid function tests (T4 and TSH).

- Day 56 (cohort 7-8 only; no visit on day 56 for patients in cohorts 1-6):

  - **Cohort 7-8 only**: Clinical history.
  - **Cohort 7-8 only**: Physical examination.
  - **Cohort 7-8 only**: Bloods: FBC, U&E, LFT, CRP, creatine kinase, ferritin.
  - **Cohort 7-8 only**: Whole blood in EDTA (1 x 4mL) for circulating T1E28z^{+} cell analysis (FACS, qPCR).
  - **Cohort 7-8 only**: Random serum cortisol, random blood glucose, thyroid function tests (T4 and TSH).

- Day 71 (cohort 7-8 only; no visit on day 71 for patients in cohorts 1-6):

  - **Cohort 7-8 only**: Clinical history.
  - **Cohort 7-8 only**: Physical examination.
  - **Cohort 7-8 only**: Bloods: FBC, U&E, LFT, CRP, creatine kinase, ferritin.
  - **Cohort 7-8 only**: Whole blood in EDTA (1 x 4mL) for circulating T1E28z^{+} cell analysis (FACS, qPCR).
  - **Cohort 7-8 only**: ECG.
  - **Cohort 7-8 only**: ECHO.
  - **Cohort 7-8 only**: CXR.
  - **Cohort 7-8 only**: Circulating Tregs (4mL whole blood in EDTA anticoagulant) and myeloid derived suppressor cells (31.5mL in citrate anticoagulant). Plasma and PBMC will be banked from excess material in the citrate sample and serum will be banked from a clotted sample for future comparative studies.
  - **Cohort 7-8 only**: Random serum cortisol, random blood glucose, thyroid function tests (T4 and TSH).

- Day 85 (cohort 7-8 only; no visit on day 85 for patients in cohorts 1-6):

  - **Cohort 7-8 only**: Clinical history.
  - **Cohort 7-8 only**: Physical examination.
  - **Cohort 7-8 only**: Contrast-enhanced CT: head, neck, thorax, abdomen (Where deemed more useful at enrolment, MRI imaging will be used to evaluate local tumour status).
  - **Cohort 7-8 only**: Bloods: FBC, U&E, LFT, CRP, creatine kinase, ferritin.
  - **Cohort 7-8 only**: Whole blood in EDTA (1 x 4mL) for circulating T1E28z^{+} cell analysis (FACS, qPCR).
  - **Cohort 7-8 only**: Clotted sample for separation and storage of serum at –80°C. This will be used to examine for emerging anti-CAR antibodies.

- **Selected patients cohort 7-8 only**: FDG PET-CT head, neck and half-body body scanning (performed on the same day as contrast-enhanced CT scan).

- **Selected patients only (cohort 7-8 only)**: Random serum cortisol, random blood glucose, thyroid function tests (T4 and TSH).

A 48-hour window will operate for visits at Days 8, 15, 28, 29, 43, 56, 71 and 85 to allow for weekend breaks.

Should circulating T1E28z$^+$ T-cells be detectable at the end of the study period, we would request that clinicians who follow up the patient after the study period has been completed would request that patients provide blood samples at each outpatient visit to monitor the persistence and proportion of these cells that remain.

6.3 Following Completion of Treatment (Follow Up)

After completion of the six weeks (or twelve weeks post T4 treatment in cohorts 7-8) on protocol:

- The patients will be followed up as per the referring clinician's practice.

- Appropriate cross-sectional imaging will be performed when clinically indicated.

- The Sponsor will continue safety follow-up for least two years and report safety data as per pharmacovigilance regulations. Patients will be followed up periodically after treatment for general health and survival. This will be achieved either by direct patient contact or telephoning of general practitioner.

- Time to progressive disease defined clinically or radiologically will be documented.

6.4 Summary Table

Table 5. Summary of Patient Monitoring Studies

a. COHORT 1-5

	Screen_T4 Manufacture			Treat	Post- Treatment Period[9]							Follow Up
Days	-3 to -28	-14	<-4	1[5]	2	3-4	5-7	8	15	29	43	
Clinical History	X		X	X				X	X	X	X	
Examination	X		X	X				X	X	X	X	
Entry criteria assessment/ bloods, including serology	X											
Blood harvest & serology		X										
CT (+/- MRI)[1]	X										X	As per referring clinician's practice
Biopsy cohort [2]	X							X	X			
^{18}FDG PET-CT[3]	X										X	
^{111}In SPECT-CT[4]				X	X[7]	X[7]						
FBC/U&E/LFT CK/CRP/ferritin	X		X	X	X	X	X	X	X	X	X	
Intra-tumoural T4 immunotherapy				X								
Serum for cytokines				X	X	X	X					
Serum for emerging antibodies				X					X		X	
Blood for CAR analysis				X	X	X	X	X	X	X	X	
Blood for MAGE-reactive T-cells			X							X		
CXR									X	X		
ECG	X		X	X				X	X	X	X	
ECHO	X								X	X	X	

b. COHORT 6

LYMPHODEPLETION COHORT
COHORT 6

			Lymphodepletion Manufacture			T4										
	Screen															
Days	-3 to -28	-14[7]	-4 to -11	-3 to -10	-2 to -9	1[5]	2	3-4	5-7	8[6]	11[6]	15[6]	22[6]	29[6]	36[6]	43[6]
Clinical History	X		X	X	X	X				X[8]	X[9]	X	X[10]	X	X	X
Examination	X		X	X	X	X				X		X	X	X	X	X
Entry criteria assessment/ bloods, including serology	X															
Flu/Cy lymphodepleting chemotherapy			X	X	X											
Blood harvest & serology		X[4]														
Intra-tumoural T4 immunotherapy						X										
CT[1]	X															X
Tumour biopsy[2]	X									X	or	X				
^{18}FDG PET-CT[3]	X															X
^{111}In SPECT-CT[4]						X	X[7]	X[7]								
FBC/U&E/LFT	X		X	X	X	X	X	X	X	X		X	X	X	X	X
CK/CRP/ferritin	X					X	X	X	X	X		X	X	X	X	X
Treg number	X									X		X	X	X	X	X
MDSC number	X									X		X	X	X	X	X
Serum for cytokines						X	X	X	X							
Serum for emerging antibodies						X										X
Blood for CAR analysis	X					X	X	X	X	X		X	X		X	X
Banking of PBMC/ plasma	X									X		X	X	X	X	X
Banking of serum	X									X		X	X	X	X	X
CXR												X		X		
ECG	X				X	X				X		X		X		X
ECHO	X											X		X		X

c. COHORTS 7 and 8

LYMPHODEPLETION + NIVOLUMAB
COHORTS 7-8

			Lymphodepletion				Nivolumab													
	Screen	Manufacture					T4													
Days	-3 to -28	-14[7]	-11 to-4	-10 to-3	-9 to -2	-1	1[5]	2	3-4	5-7	8[6]	11[6]	15[6]	22[6]	28[6]	36[6]	43[6]	56[6]	71[6]	85[6]
Clinical History	X		X	X	X		X				X[8]	X[9]	X	X[10]	X	X	X	X	X	X
Examination	X		X	X	X		X				X		X	X	X	X	X	X	X	X
Entry criteria assessment/ bloods, including serology	X																			
Flu/Cy lymphodepleting chemotherapy			X	X	X															
Nivolumab (480mg)						X									X			X		
Blood harvest & serology		X																		
Intra-tumoral T4 immunotherapy							X													
CT[1]	X																X			X
Tumour biopsy[2]	X										X	or	X							
^{18}FDG PET-CT[3]	X																X			X
^{111}In SPECT-CT[4]							X	X[7]	X[7]											
FBC/U&E/LFT	X		X	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X
CK/CRP/ferritin	X						X	X	X	X	X		X	X	X	X	X	X	X	X
Treg number	X										X		X	X	X	X	X		X	
MDSC number	X										X		X	X	X	X	X		X	
TFTs/ cortisol/ glucose	X								X		X		X	X	X		X	X	X	X
Serum for cytokines							X	X	X	X										
Serum for emerging antibodies							X										X			X
Blood for CAR analysis	X						X	X	X	X	X		X	X	X		X	X	X	X
Banking of PBMC/ plasma	X										X		X	X	X	X	X		X	
Banking of serum	X										X		X	X	X	X	X		X	
CXR													X			X			X	
ECG	X				X		X				X		X		X		X		X	
ECHO	X												X			X			X	

MDSC – myeloid-derived suppressor cells; Tregs – regulatory T-cell number

1. CT scanning of head, neck, thorax and abdomen. MRI scanning (tumour site only) will only be conducted where considered to be more informative than CT.
2. Selected patients: Biopsies for any or all of: a). analysis of ErbB1 expression; (ii) dual staining of Cytokeratin A1/A3 and cleaved caspase 3 (apoptosis); (iii) detection of immune cells and/ or markers; (iv) H&E staining; (v) detection of CAR T-cells (RNAScope); (vi) RNA extraction and RNA sequencing (RNASeq).
3. Head, neck and half-body PET-CT scan will be performed on selected patients.
4. Selected patients will receive up to 30 million T4-engineered T-cells that have been labelled with ^{111}Indium. This will permit tracking of the migration of $T4^{+}$ T-cells over the ensuing 72 hours.
5. Tests performed more than once on this day.
6. A 48-hour window will operate for visits at Days 8, 15, 28, 29, 43, 56, 71 and 85 to allow for weekend breaks.
7. At the clinician's discretion, SPECT alone may be conducted if it is deemed that this will not affect the quality of the data.
8. Patients may be discharged if well and apyrexial for 24 hours.
9. Patients should attend or make contact with the treating team daily until day 10 post CAR T-cell treatment.
10. Patients will be instructed to monitor temperature twice daily for the first 14 days after CAR T-cell administration and to remain within 2 hours travelling time from the treatment centre until 30 days after CAR T-cell treatment. Patients will be instructed to make immediate contact with the Phase I oncology team if they feel unwell or develop pyrexia.

7. CRITERIA OF EVALUATION

7.1 Evaluation of Efficacy

7.1.1 Primary End Point

The primary end point of this study is determination of the dose limiting toxicity induced by T4 immunotherapy, up to 6 weeks post administration. In cohorts 7-8, this observation period will be extended to 12 weeks post administration. Dose limiting toxicity will be graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE), Current Version.

All patients will be evaluable for toxicity from the time of their injection of T4 immunotherapy.

7.1.2 Secondary End Points

The secondary end points in this study and methods of evaluation are listed below.

Cytokine levels will be analyzed in serum taken pre-injection, at 30 minutes after injection, and at 1, 4, 24, 48-96 and 120-168 hours post T-cell injection. Please consult the CAR Research Group manual of standard operating procedures for current methodologies.

Presence of persistent $T4^+$ T-cells in tumour biopsies will be measured at one or two weeks post therapy in up to 9 patients. Please consult the CAR Research Group and Oral Pathology (Guy's Hospital) manual of standard operating procedures for current methodologies used (qPCR for $T1E28z^+$ T-cells and RNAScope).

Presence of $T4^+$ T-cells in the circulation will be analyzed at 4, 24, 48-96 and 120-168 hours, and on days 8, 15, 29 and 43 days post injection in cohorts 1-6. When T4 immunotherapy is combined with nivolumab (cohorts 7-8), analysis will be performed at 4, 24, 48-96 and 120-168 hours, and on days 8, 15, 22, 28, 43, 56, 71 and 85 days post T4 immunotherapy. Please consult the CAR Research Group manual of standard operating procedures for current methodologies used (FACS analysis and qPCR for $T1E28z^+$ T-cells).

Objective tumour response and time of progression will be measured according to RECIST criteria (161).

All eligible patients will be included in the response rate calculation. The subset that will be assigned a response category (CR, PR, SD or PD; see definitions below) are all patients who have received a single treatment with T4 immunotherapy and have had their disease re-evaluated.

Effects of lymphodepletion with fludarabine and cyclophosphamide or the combination of lymphodepletion and nivolumab on T4 immunotherapy will be evaluated by measurement of dose-limiting toxicity as described in **section 7.1.1** and objective tumour response rate and time of progression. In the case of cohort 7 and 8 (where lymphodepletion and nivolumab are included), assessment will continue for 12 weeks post administration of T4 immunotherapy.

The effect of T4 immunotherapy upon immune reactivity against endogenous tumour antigens will be assessed by measurement of T-cell reactivity against overlapping peptides derived from MAGE-A3 and MAGE-A4 (cohorts 1-5 only). Assessment will be performed using a multiplex cytokine bead array platform and/ or CyTOF combined with ELISPOT. Tumours will be assessed for MAGE-A3/ A-4 status by RT-PCR and immunohistochemistry. Please consult the CAR Research Group manual of standard operating procedures for current methodologies used.

Effect of T4 immunotherapy on gene expression in the tumour microenvironment will be assessed in serial tumour biopsies, to be undertaken in a subset of patients before, one week after and two weeks after administration of T4 immunotherapy. RNA sequencing analysis will be performed to assess the transcriptome in these samples. There will be up to three biological replicates derived from up to three core biopsies taken at each time point. Please consult the CAR Research Group manual of standard operating procedures for current methodologies used.

Trafficking of T4 immunotherapy will be assessed in a subset of patients by SPECT-CT imaging, following administration of an aliquot of T4 immunotherapy that has been radiolabelled with Indium-111.

7.1.3 Measurability of Tumour Lesions at Baseline

7.1.3.1 Definitions

Measurable disease - the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Measurable lesions - tumour lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with chest x-ray, and as ≥ 10 mm with CT scan or clinical examination [using calipers]. Bone lesions are considered measurable only if assessed by CT scan and have an identifiable soft tissue component that meets these requirements (soft tissue component ≥ 10 mm by CT scan). Malignant lymph nodes must be ≥ 15 mm in the short axis to be considered measurable; only the short axis will be measured and followed. All tumour measurements must be recorded in millimeters (or decimal fractions of centimeters) by use of a ruler or calipers. Tumour lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not

considered measurable unless there has been demonstrated progression in the lesion. If the only loco-regional site of disease is in a radiation field, which is likely in locally recurrent SCCHN, lesions will be considered measurable if there has been documented radiological evidence of disease progression at that site.

Non-measurable lesions - All other lesions (or sites of disease), including small lesions are considered non-measurable disease. Bone lesions without a measurable soft tissue component, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, lymphangitic involvement of lung or skin and abdominal masses followed by clinical examination are all non-measurable. Nodes that have a short axis <10 mm at baseline are considered non-pathological and should not be recorded or followed.

Target Lesions - When more than one measurable tumour lesion or malignant lymph node is present at baseline, all lesions up to a maximum of 5 lesions total (and a maximum of 2 lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. Note that pathological nodes must meet the criterion of a short axis of ≥ 15 mm by CT scan and only the short axis of these nodes will contribute to the baseline sum. At baseline, the sum of the target lesions (longest diameter of tumour lesions plus short axis of lymph nodes: overall maximum of 5) is to be calculated and recorded.

Non-target Lesions - All non-measurable lesions (or sites of disease) including pathological nodes (those with short axis ≥ 10 mm but < 15 mm), plus any measurable lesions over and above those listed as target lesions are considered non-target lesions. Measurements are not required but these lesions should be noted at baseline and should be followed as "present" or "absent".

All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

7.1.3.2 Methods of Measurements

In general, contrast-enhanced CT scanning will be used for response assessment. CT is the most reproducible method currently available to measure lesions selected for response assessment. "Measurability" of lesions on CT scan is based on the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.

In some cases, MRI imaging is preferable to CT, particularly for tumours that are located close to the skull base. The decision to use MRI in preference to CT will be taken in conjunction with an appropriately qualified radiologist.

The same method of assessment and the same technique should and will be used to characterize each identified and reported lesion at baseline and during follow-up. Assessments should be identified on a calendar schedule. While on study, all target lesions recorded at baseline should have their actual measurements recorded on the CRF at each subsequent evaluation, even when very small (e.g. 2 mm). If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned. For lesions which fragment/split, add together the longest diameters of the fragmented portions; for lesions which coalesce, measure the maximal longest diameter for the "merged lesion".

In selected patients ^{18}FDG PET-CT scanning will be performed to examine tumour status. Scans will be performed on patients who are willing to undergo this procedure at any stage of the trial, but preferably in those who receive at least 10^8 $T4^+$ cells.

In selected patients, up to 30 million T4-engineered T-cells will be passively labelled with ^{111}Indium, prior to intra-tumoural injection at a single site. This will permit tracking of T-cell migration for up to 72 hours by SPECT-CT scanning. It is envisioned that this will be performed once the highest tolerated dose has been determined.

7.1.4 Tumour Response Evaluation

All patients will have their best response from the start of study treatment until the end of study (i.e. six weeks after treatment; also assessed on day 85 in cohorts 7-8). This is classified as outlined below:

Complete or partial responses may be claimed only if the criteria for each are met at a subsequent time point at least 4 weeks later (please refer to **Table 6**).

Complete Response (CR): disappearance of all target and non-target lesions and normalization of tumour markers. Pathological lymph nodes must have short axis measures < 10 mm (Note: continue to record the measurement even if < 10 mm and considered CR). Tumour markers must have normalized. Residual lesions (other than nodes < 10 mm) thought to be non-malignant should be further investigated (by cytology or PET scans) before CR can be accepted.

Partial Response (PR): at least a 30% decrease in the sum of measures (longest diameter for tumour lesions and short axis measure for nodes) of target lesions, taking as reference the baseline sum of diameters. Non-target lesions must be non-PD.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as reference the smallest sum of diameters on study.

Progressive Disease (PD): at least a 20% increase in the sum of diameters of measured lesions taking as references the smallest sum of diameters recorded on study (including baseline) AND an absolute increase of ≥ 5 mm. Appearance of new lesions will also constitute PD (including lesions in previously un-assessed areas). In exceptional circumstances, unequivocal progression of non-target disease may be accepted as evidence of disease progression, where the overall tumour burden has increased sufficiently to merit discontinuation of treatment, for example where the tumour burden appears to have increased by at least 73% in volume (which is the increase in volume when all dimensions of a single lesion increase by 20%). Modest increases in the size of one or more non-target lesions are NOT considered unequivocal progression. If the evidence of PD is equivocal (target or non-target), treatment may continue until the next assessment, but on further documentation, the earlier date must be used.

Table 6. Integration of target, non-target and new lesions into response assessment.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Response for this category also requires
Patients with Target lesions ± non target lesions				
CR	CR	No	CR	Normalization tumour nodes < 10 mm
CR	Non-CR/Non-PD	No	PR	
CR	Not all evaluated	No	PR	
PR	Non-PD/ not all evaluated	No	PR	
SD	Non-PD/ not all evaluated	No	SD	Documented at least once ≥ 6 weeks from baseline
Not all evaluated	Non-PD	No	NE	
PD	Any	Any	PD	
Any	PD	Any	PD	
Any	Any	Yes	PD	

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression [or evidence of unequivocal disease progression] at that time should be reported as "symptomatic deterioration". This is a reason for stopping therapy, but is NOT objective PD. Every effort should be made to document the objective progression even after discontinuation of treatment.

NE: not evaluable.

Complete or partial responses may be claimed only if the criteria for each are met at a subsequent time point at least 4 weeks later. The best overall response can be interpreted as per **Table 7**.

Table 7. Best Overall Response.

Response: First time point	Subsequent time point	BEST overall response	Also requires
CR	CR	CR	Normalization tumour nodes < 10 mm
CR	PR	SD, PD or PR (see comment*)	
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD	
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD	
CR	NE	SD provided minimum criteria for SD duration met, otherwise NE	
PR	CR	PR	
PR	PR	PR	
PR	SD	SD	
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD	
PR	NE	SD provided minimum criteria for SD duration met, otherwise NE	
NE	NE	NE	
* may consider PR providing initial "CR" likely PR on subsequent review – then original CR should be corrected. Recurrence of lesion after true CR is PD. NE: not evaluable.			

7.1.4.1 Frequency of Tumour Re-evaluation

In the present study, tumours will be revaluated at six weeks after administration of T4 immunotherapy (cohorts 1-6) or at six and twelve weeks (cohorts 7-8) following administration of T4 immunotherapy. After discontinuation of protocol treatment, patients who have not progressed will still be re-evaluated according to the referring surgeons standard practice.

7.1.4.2 Date of Progression

This is defined as the first day when the RECIST (version 1.1) criteria for PD are met.

7.1.5 Reporting of Tumour Response

All patients included in the study must be assessed for response to treatment, even if there is a major protocol treatment deviation or if they are ineligible, or not followed/re-evaluated. Each patient will be assigned one of the following categories: complete response, partial response, stable disease, progressive

disease, early death from malignant disease, early death from toxicity, early death from other cause or unknown (not assessable, insufficient data).
Early death is defined as any death occurring before six weeks from administration of T4 immunotherapy. The responsible investigator will decide if the cause of death is malignant disease, toxicity or other cause.

Patients for whom response is not confirmed will be classified as "unknown", unless they meet the criteria for stable disease (or the criteria for partial response in case of an unconfirmed complete response). Patients' response will also be classified as "unknown" if insufficient data were collected to allow evaluation per these criteria.

7.1.6 Response Duration

Response duration will be measured from the time measurement criteria for CR/PR (whichever is first recorded) are first met until the first date that recurrent or progressive disease is objectively documented.

7.1.7 Stable Disease Duration

Stable disease duration will be measured from the time of start of treatment (or randomization for randomized studies) until the criteria for progression are met.

8. STATISTICAL CONSIDERATIONS

8.1 Statistical Design

This is a phase I, dose finding, open label study. A classic 3+3 design will be used (**section 4.1**).

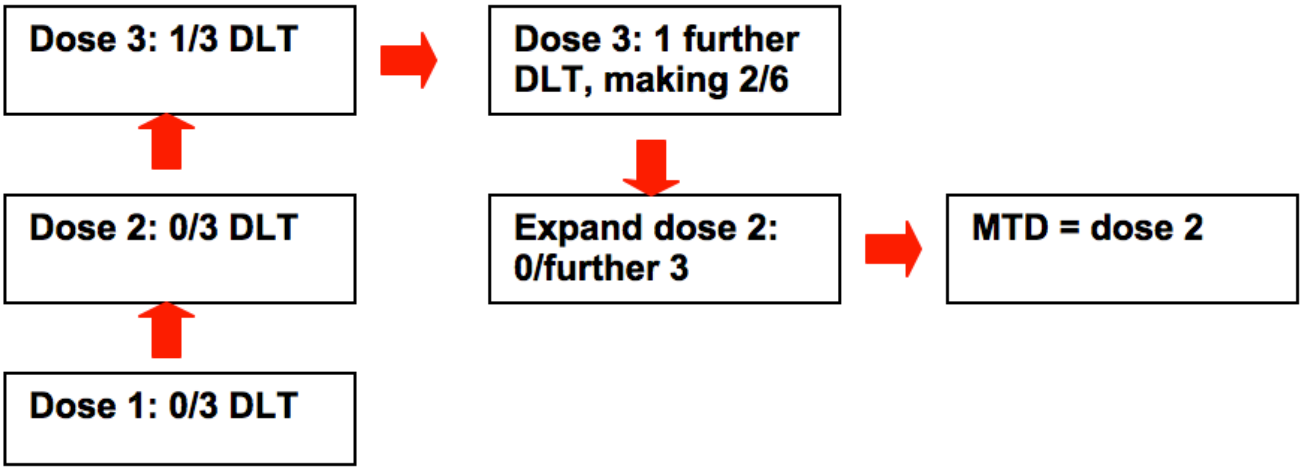


Figure 6: Statistical Design of the Study

Primary End Point:

- Dose limiting toxicity of T4 immunotherapy graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE), Current Version.

Secondary End Points:

- Cytokine levels present in serum taken pre-injection, at 30 min after injection, and at 1, 4, 24, 48-96 and 120-168 hours post T-cell infusion (later time points flexible, to allow for weekends). Analysis will be performed using a multiplex platform as per the CAR Research Group manual of standard operating procedures.

- /Persistence of $T4^+$ T-cells in tumour biopsies (measured by qPCR and RNAScope analysis) at one or two weeks post therapy. Analysis will be conducted as per the CAR Research Group manual of standard operating procedures.

- Presence of $T4^+$ T-cells in the circulation measured by qPCR and flow cytometry analysis for $T1E28z^+$ T-cells at 4, 24, 48-96 and 120-168 hours (flexible time points, to allow for weekends), and days 8, 15, 29 and 43 days post injection in cohorts 1-6. When T4 immunotherapy is combined with lymphodepletion and nivolumab (cohorts 7-8), analysis will be performed at 4, 24, 48-96 and 120-168 hours, and on days 8, 15, 22, 28, 43, 56, 71 and 85 days post T4 immunotherapy. Analysis will be conducted as per the CAR Research Group manual of standard operating procedures.

- Evidence of response evaluated by appropriate cross-sectional imaging 6 weeks post therapy in cohorts 1-6. Response will be assessed at 6 and 12 weeks in the case of the cohorts 7-8. Clinical response will be assessed according to RECIST 1.1 criteria.

- Effect of T4 immunotherapy upon endogenous T-cell reactivity against Cancer Tests antigens. Cytokine production will be measured in cohorts 1-5 using a multiplex cytokine bead array platform and/ or CyTOF combined with ELISPOT analysis, upon stimulation by overlapping peptides derived from MAGE-A3 and MAGE-A4. Assays will be conducted as per the CAR Research Group manual of standard operating procedures.

- Evidence of immunomodulation by lymphodepleting chemotherapy with fludarabine and cyclophosphamide. This will be assessed by quantification of circulating Treg cells and myeloid derived suppressor cells. Analysis will be conducted as per the CAR Research Group manual of standard operating procedures.

- Immunomodulatory effects of the combination T4 immunotherapy (administered post lymphodepletion) and PD1 immune checkpoint blockade will be examined by cross-sectional imaging at 6 and 12 weeks post CAR T-cell immunotherapy.

- Effect of T4 immunotherapy on gene expression in the tumour microenvironment with be assessed in serial tumour biopsies, to be undertaken before, one week after and two weeks after administration of T4 immunotherapy. Analysis will be by RNA-seq as per the CAR Research Group manual of standard operating procedures.

- Trafficking of T4 immunotherapy will be assessed in a subset of patients by SPECT-CT imaging, following administration of an aliquot of T4 immunotherapy that has been radiolabelled with Indium-111.

Entry to the next dose level: The primary end point of DLT will define entry to the next dose level. The period of evaluation for DLT is 28 days during the dose escalation phase of the study and cohort 6. After 3 patients have been enrolled in a cohort the next dose level cohort cannot be opened until there have been no DLT detected for 28 days for the last patient enrolled.

Estimate of patient numbers: The expected sample size in this study ranges from 22 to 29 patients. Twenty two patients corresponds to the number of patients already enrolled in the trial from cohort 1 to 7 (i.e. 21) plus the first patient to be recruited in cohort 8. After treatment of the 22nd patient is completed, seven vector units will remain which means that the maximum number of patients that could be treated in this trial would reach 29.

8.2 Statistical Analysis

All patients who started the treatment will be described. Patients without DLT who withdraw within 28 days from administration of T4 immunotherapy (cohorts 1-6) or within 28 days following the last dose of nivolumab (cohorts 7-8) will not be included in the toxicity analysis.

Statistical tests will be non-parametric due to the limited sample size. All analysis will be exploratory.

Analysis of the primary end point: The presence of DLT will be analyzed by tables of frequency compared with dose level. All other documented toxicity of any grade according to NCI Common Terminology Criteria for Adverse Events (CTCAE), Current Version will be similarly analyzed. Toxic side effects of all grades will be given by grade and dose level.

Serum cytokine levels: These data will be presented using median and ranges. Evolution in time will be given.

Presence of persistent $T4^+$ T-cells in tumour biopsies: Analysis will be performed by generating tables of frequency compared with dose level.

Presence of $T4^+$ T-cells in the circulation: Analysis will be performed by generating tables of frequency compared with dose level.

Evidence of response: disease status at six weeks after administration of T4 immunotherapy (cohorts 1-6) and six and twelve weeks after T4 immunotherapy (cohorts 7-8) will be analyzed by generating tables of frequency compared with dose level. Patients will be followed up for survival after leaving the study.

Endogenous T-cell reactivity against MAGE antigens: These data will be presented using median and ranges. Evolution in time will be given.

Lymphodepletion with fludarabine and cyclophosphamide: Frequency of circulating Treg cells and myeloid-derived suppressor cells will be presented prior to and after completion of lymphodepleting chemotherapy. Evolution in time will be given.

Toxic effects of nivolumab and/ or lymphodepleting chemotherapy, in conjunction with T4 immunotherapy: The presence of DLT will be analyzed by tables of frequency compared with dose level. All other documented toxicity of any grade according to NCI Common Terminology Criteria for Adverse Events (CTCAE), Current Version will be similarly analyzed. Toxic side effects of all grades will be given by grade and dose level.

RNA-seq analysis: Baseline and post-treatment gene expression will be analysed in biopsy samples by 100PE riboZero (ribosomal RNA-depleted) RNAseq of 150 million reads per sample. With a known sample size of 3, an α=0.05, power=0.9, and allowing for a coefficient of variation (CV) of counts within each of the groups of 0.4, we should be able to detect a relative expression δ of 2.946 (162). Tables of relative gene expression over time will be generated.

Trafficking of T4 immunotherapy: Signal intensity within the tumour and at other sites will be presented in a descriptive manner, based upon the report of the nuclear medicine physician/ radiologist for each patient scan. In addition, counts within individual regions of interest will be quantified and expressed as a % of whole body count.

9. TRANSLATIONAL RESEARCH/ PHARMACODYNAMIC STUDIES

9.1 Cytokine Analysis

9.1.1 Objectives

To analyse the level of cytokines in the peripheral circulation of patients who undergo T4 immunotherapy.

9.1.2 Materials Collected

Five mL of whole blood will be collected into a clotted sample collection tube by peripheral venous puncture. Blood samples will be taken at time points specified in **Table 8**.

Table 8. Timing of blood sampling for serum cytokine analysis.

1	2	3	4	5	6	7
-30 min	30 min	1 hour	4 hours	24 hours	48-96 hours	120-168 hours

9.1.3 Sample Handling

Full details of the current assay technologies in use to characterize clinical samples are described in the CAR Research Group manual of standard operating procedures.

The blood sample will stand at room temperature for 20min to permit clot retraction and is then centrifuged at 1500g for 15min. The serum layer will be gently aspirated and transferred to a cryovial for storage in a designated locked -80^0C freezer.

Cytokine analysis may be performed using a variety of platform technologies. Please see the CAR Research Group manual of standard operating procedures for currently used techniques. Using this type of approach, samples can be stored in the freezer for analysis either at the end of the study or when sufficient patients have had all blood samples collected to allow the use of 1 complete set of reagents.

9.1.4 Statistical Analysis

The evolution in time of cytokine levels will be monitored. Where relevant, the trend by dose level of particular cytokines will be analyzed.

9.2 Tumour Biopsies

9.2.1 Objectives

Biopsies may be analysed for some or all of the following, depending on the quality of the three core biopsies obtained:
Evaluation of the target lesion designated for intra-tumoural injection of T4 immunotherapy for levels of cell surface expression of ErbB1 on malignant cells, for apoptosis within the tumour and for cellular architecture.

Evaluation of the persistence of T1E28z^{+} T-cells within the tumour target lesion following injection with T4 immunotherapy.

Evaluation of global changes in gene expression following intra-tumoural injection of T4 immunotherapy.

9.2.2 Materials Collected

Within four weeks prior to administration of T4 immunotherapy, up to three core biopsies will be taken by the head and neck cancer surgeon, using ultrasound guidance if necessary.

One or two weeks after administration of T4 immunotherapy, further core biopsies will be taken from the lesion into which T4 immunotherapy was administered.

9.2.3 Sample Handling

Full details of the current assay technologies in use to characterize clinical samples are described in the CAR Research Group manual of standard operating procedures.

Biopsies taken pre-enrolment and post treatment may be fixed in formalin and paraffin embedded. The necessary number of sections will be cut for any or all of the following analyses: (i) analysis of ErbB1 expression; (ii) dual staining of Cytokeratin A1/A3 and cleaved caspase 3 (apoptosis); (iii) detection of immune cells and/ or markers; (iv) H&E staining; (v) detection of CAR T-cells (RNAScope). Details of the assays are described in the Oral Pathology (Guy's Hospital), Craniofacial and Regenerative Biology and CAR Research Group manual of standard operating procedures. The remainder of the block will be

stored in the Head and Neck and Oral Pathology Tissue Bank Archives of Guy's Hospital for future evaluation.

Core biopsies taken pre- and post-treatment may also be subjected to RNA extraction and RNA sequencing as described in the manual of standard operating procedures of the CAR Research Group and of commercial companies that are commissioned to undertake RNASeq analysis.

9.2.4 Statistical Analysis

ErbB1 expression will be recorded as % positive tumour cells. Expression of ErbB1 following treatment will be compared across dose levels.

Levels of apoptosis will be compared between the pre and post therapy biopsies for each individual patient.

The presence of T1E28z$^+$ T-cells will be analyzed by tables of frequency compared with dose level.

Subject to satisfactory quality control of RNA, transcriptomic profiling will be analysed for differential gene expression analysis. This will include analysis of CAR T-cell-derived transcripts and target ErbB receptor transcripts. Thresholds to define significant differences in gene expression will be decided upon based on a preliminary overview of the data. Differentially expressed genes will be annotated thoroughly with regards to their gene ontology (i.e secreted or transmembrane proteins) and involvement in relevant pathways.

9.3 Circulating T4$^+$ T-cells

9.3.1 Objectives

To evaluate the presence and (if present) levels of circulating T4$^+$ T-cells in the circulation of patients after treatment with intra-tumoural T4 immunotherapy.

9.3.2 Materials Collected

One sample containing 4mL of EDTA anti-coagulated whole blood will be collected by peripheral venous puncture. Depending on cohort number (please consult **Table 4**), blood samples will be taken at the following intervals post T4 immunotherapy:

- 4 hours
- 24 hours

- 48-96 hours
- 120-168 hours
- 8 days
- 15 days
- 28 or 29 days
- 36 days
- 43 days
- 56 days
- 71 days
- 85 days

A 48-hour window will be allowed for visits at Days 8, 15, 29, 43, 56, 71 and 85 at which point these samples will be collected.

9.3.3 Sample Handling

Full details of the current assay technologies in use to characterize clinical samples are described in the CAR Research Group manual of standard operating procedures.

Using the first sample, genomic DNA will be extracted from whole blood and will be stored at -20^{0}C until PCR analysis.

Using the second sample, T-cells will be immunostained in whole blood (using anti-CD3 and anti-EGF) to detect expression of the T1E28z CAR.

9.3.4 Statistical Analysis

The presence of T1E28z$^+$ cells detectable by FACS and/or PCR will be analyzed by tables of frequency compared with dose level.

9.4 Antibody Analysis

9.4.1 Objectives

To store serum for analysis of antibody generation as a response to T4 immunotherapy.

9.4.2 Materials Collected

Five mL of whole blood will be collected by peripheral venous puncture.
Depending on cohort number (please see **Table 4**), blood samples will be taken at the following intervals before or after T4 immunotherapy:

- -30 minutes
- 15 days
- 43 days
- 85 days

9.4.3 Sample Handling

A clotted blood sample will be obtained. After centrifugation, the serum layer will be gently aspirated, transferred to a cryovial and immediately transferred to a designated locked minus 80°C freezer.

9.4.4 Statistical Analysis

This is a stored serum sample for potential future analysis.

9.5 Circulating Regulatory T-cells

9.5.1 Objectives

To evaluate the level of circulating regulatory T-cells in the circulation of patients before and after treatment with fludarabine and cyclophosphamide. Lymphodepleting conditioning therapy will be

administered to the 6th, 7th and 8th cohorts of patients enrolled in the study. By this means, it is hoped to deplete circulating regulatory T-cells, thereby promoting enhanced efficacy of T4 immunotherapy.

9.5.2 Materials Collected

Four mL of EDTA anti-coagulated whole blood will be collected by peripheral venous puncture. Blood samples will be taken prior to and at several time points after the administration of T4 immunotherapy as indicated in **Table 5**. This analysis will be performed in cohorts 6-8 only.

9.5.3 Sample Handling

Full details of the current assay technologies in use to characterize clinical samples are described in the CAR Research Group manual of standard operating procedures.

In brief, anti-coagulated blood will be stained using an in-house assay. Following lysis of red blood cells, regulatory T-cells will be enumerated by flow cytometry as $CD4^{+}$ $CD25^{HIGH}$ $CD127^{DIM/NEG}$ events in tubes containing a defined volume of blood and number of counting beads.

9.5.4 Statistical Analysis

Pre- and post-treatment absolute number of circulating lymphocytes and $CD4^{+}$ $CD25^{HIGH}$ $CD127^{DIM/NEG}$ cells will be enumerated.

9.6 Circulating MAGE-reactive T-cells

9.6.1 Objectives

To evaluate the number of circulating T-cells capable of producing a panel of cytokines upon stimulation with overlapping peptides derived from MAGE-A3 and MAGE-A4. Comparison will be made before and after treatment with T4 immunotherapy.

9.6.2 Materials Collected

Citrate anti-coagulated whole blood (22.5mL) will be collected by peripheral venous puncture. Blood samples will be taken within 3 days prior to T4 immunotherapy and 29 days after administration of T4 immunotherapy. This analysis will be performed in cohorts 1-5 only.

9.6.3 Sample Handling

Full details of the current assay technologies in use to characterize clinical samples are described in the CAR Research Group manual of standard operating procedures.

Anti-coagulated blood will be separated by density gradient centrifugation over ficoll leading to isolation of peripheral blood mononuclear cells. Isolated cells will be transferred to Interferon-γ ELISPOT plates and will be stimulated with overlapping peptides from MAGE A3 and MAGE A4. Cytokine producing cells will be quantified in plates while cytokines present in supernatants will be measured using a multiplex cytokine bead array platform and/ or CyTOF.

9.6.4 Statistical Analysis

Pre- and post-treatment cytokine levels and cytokine production by stimulated cells will be enumerated.

9.7 Circulating myeloid-derived suppressor cells

9.7.1 Objectives

To evaluate the number of circulating myeloid-derived suppressor cells (MDSCs) before and after treatment with T4 immunotherapy.

9.7.2 Materials Collected

Whole blood will be collected by peripheral venous puncture (31.5mL in citrate anticoagulant) at intervals before and after T4 immunotherapy is administered. This analysis will be performed in cohorts 6 - 8 only.

9.7.3 Sample Handling

Full details of the current assay technologies in use to characterize clinical samples are described in the CAR Research Group manual of standard operating procedures.

In brief, cells will be stained with antibodies reactive against markers that include CD14, CD66, CD33 and HLA-DR (163).

9.7.4 Statistical Analysis

Cells are assigned to monocytic (M), polymorphonuclear (PMN) and early (e) MDSC subtypes. Pre- and post-treatment numbers of each subset will be enumerated.

10. INVESTIGATOR AUTHORISATION PROCEDURE

This is a single centre Phase I trial.

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments.

This protocol and related documents will be submitted for review to a Research Ethics Committee (REC), to the Medicines and Healthcare products Regulatory Agency (MHRA) for Clinical Trial Authorisation and to the Gene Therapy Advisory Committee.

The Chief Investigator will submit a final report at conclusion of the trial to the KHP CTO (on behalf of the Sponsor), the REC and the MHRA within the timelines defined in the Regulations.

11. PATIENT REGISTRATION / RANDOMIZATION PROCEDURE

This is a single centre Phase 1 trial.

Patients will be recruited via the head and neck cancer clinic and multidisciplinary team meeting or from external institution referral to the Phase I clinical trials unit at Guy's Hospital. Patients will be registered, and eligibility criteria checked against the inclusion and exclusion criteria. Subject numbers will be allocated sequentially beginning with 001.

12. FORMS AND PROCEDURES FOR COLLECTING DATA

All data collection will be captured in the medical notes on designated visit specific forms. Data will then be transcribed from the medical notes into an electronic case report form (CRF).

Data will be compiled on an Excel spreadsheet. All statistical analysis will be performed using SPSS and/ or GraphPad Prism software.

12.1 Data Flow

Personalised study data will be maintained at the study site in paper and/or electronic format. Paper records will be kept in a locked room or cabinet at all times. Access to the records is restricted to researchers working on the study and to representatives of regulatory authorities required to audit the conduct of the research study.

Electronic data containing personalised information will be saved on local study site computers only in password protected files and backed up regularly to hard copy (CD, flash disk), which will be kept in a secure cabinet or backed up to a remote server.

All data collection will be the responsibility of the Chief Investigator. All data will be collected by the research team members and investigators. After the final patient in a cohort has been recruited and observed for 28 days for DLT, all data from the cohort will be reviewed by the Chief Investigator and Co-Investigators. By this means, any missing or inconsistent data will be obtained and/or corrected, if possible.

13. REPORTING ADVERSE EVENTS

13.1 Definitions

Definitions used will be those under the Medicines for Human Use (Clinical Trials) Regulations 2004 and Amended Regulations 2006 as follows:

Adverse Event (AE):
This is defined as any untoward medical occurrence in the patient administered a medicinal product that does not necessarily have a causal relationship with this treatment. An AE is therefore described as any unfavourable and unintended sign (including abnormal laboratory results), symptoms or disease temporally (timely) associated with the use of a medicinal product whether or not related to the product.

Adverse Reaction (AR):
This is defined as any untoward and unintended response in a subject to an investigational medicinal product, which is related to any dose administered to that subject. This means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

Unexpected Adverse Reaction (UAR):
An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in:

- The summary of product characteristics (SmPC) for that product (for products with a marketing authorisation)
- The Investigator's Brochure (IB) relating to the trial in question (for any other investigational product)

Serious adverse Event (SAE), Serious Adverse Reaction (SAR) or Unexpected Serious Adverse Reaction (USAR):
Any adverse event, adverse reaction or unexpected adverse reaction, respectively, that

- Results in death;
- Is life-threatening;
- Required hospitalisation or prolongation of existing hospitalisation;
- Results in persistent or significant disability or incapacity;

- Consists of a congenital anomaly or birth defect.

Although not a serious adverse event, any unplanned pregnancy should be reported via the SAE reporting system.

Suspected Serious Adverse Reaction (SSAR):
This is defined as an adverse reaction that is classified in nature as serious and which is consistent with the information about the medicinal product in question – in the case of a licensed product in the Investigator Brochure for that product.

Suspected Unexpected Serious Adverse Reaction (SUSAR):
The definition of a SUSAR is a serious adverse drug reaction, the nature or severity of which is not consistent with the applicable product information, e.g. summary of product characteristics (SmPC) or Investigator Brochure.

A serious event or drug reaction is not defined as a SUSAR when:

- It is serious but expected
- It does not fit the definition of a SAE, whether expected or not

13.2 Severity of Adverse Events

Severity for each adverse event, including any laboratory test abnormality, will be determined by using the National Cancer Institute Common Toxicity Criteria (NCI CTCAE, current version) as a guideline, wherever possible. The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html and are appended to this Clinical Trial Protocol. In those cases where NCI CTCAE criteria do not apply, severity should be defined according to the following criteria:

- Mild — Awareness of sign or symptom but easily tolerated
- Moderate — Discomfort enough to cause interference with normal daily activities
- Severe — Inability to perform normal daily activities
- Life Threatening — Immediate risk of death from the reaction as it occurred

13.3 Determining Causality

Relationship to study drug administration will be determined as follows:

- None No relationship between the experience and the administration of the study drug; related to other aetiologies such as concomitant medications or patient's clinical state.
- Unlikely The current state of knowledge indicates that the relationship is unlikely.
- Possible A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction might also have been produced by the patient's clinical state or other modes of therapy administered to the patient.
- Likely A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction cannot be reasonably explained by the known characteristics of the patient's clinical state or other modes of therapy administered to the patient.
- Definitely An adverse event, which is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation, e.g., a concomitant drug(s), concomitant disease(s).

13.4 Procedures for Adverse Event Reporting

Toxicity will be monitored from the first study-related procedure until 6 weeks post administration of T4-transduced T-cells (cohorts 1-6) and 12 weeks post T4 immunotherapy (cohorts 7-8). All adverse reactions and serious adverse reactions that occur during this period will be recorded by the Chief Investigator. All SAEs/ SAR designated as reportable (see below) and all SUSARs will be reported to the sponsor (the KHP CTO). Those meeting the definition of serious adverse events must be reported using the Serious Adverse Event Form.

Adverse events that are not considered serious should be included on the relevant case report forms (CRFs) as defined in the trial Protocol. This data will be included in the final trial report.

Investigators must record in the CRF and the patient notes their opinion concerning details of nature, onset, duration, severity, seriousness, expectedness and relationship to T4-transduced T-cells. In cases of doubt, Investigators will liaise in order to come to a final decision, which will be documented as above. Medical terminology should always be used to describe any event. Investigators should avoid vague terms such as "sick".

13.5 Investigator Reporting to the KHP CTO and Research Ethics Committee

The Chief Investigator (CI) will report all SAEs and SARs to the KHP CTO as soon as he is aware of and has assessed the event. It is the responsibility of the Chief Investigator to report all SUSARs to the Research Ethics Committee.

All SAEs, SARs & SUSARs (including any follow up information), must be reported using the SAE Report form no later than 24 hour hours of the investigators becoming aware. This form will be completed and faxed to the KHP CTO using the number quoted on the SAE Report Form. This form should also be e-mailed to the KHP CTO using the address quoted on the form.

The KHP CTO will acknowledge receipt of the SAE Report using the KHP CTO SAE Form Receipt Form. If the CI has not received receipt within 24 hours of sending the report (during office hours), the SAE Report Form should be re-sent to the KHP CTO by email or fax.

Additional information, as it becomes available, will also be reported on the SAE Report Form and returned to the KHP CTO by email or fax as above.

The original SAE Report Form will be filed in the Trial Master File (TMF), with copies filed in the patient's hospital notes, the case record form and the Sponsor file.

13.6 Sponsor Reporting Responsibilities

The co-sponsors have delegated the delivery of the Sponsor's responsibility for Pharmacovigilance (as defined in Regulation 5 of the Medicines for Human Use (Clinical Trials) Regulations 2004 to the King's Health Partners Clinical Trials Office (KHP CTO)).

All SAEs, SARs and SUSARs (excepting those specified in this protocol as not requiring reporting) will be reported immediately by the Chief Investigator to the KHP CTO in accordance with the current Pharmacovigilance Policy.

Death as a result of disease progression and other events that are primary or secondary outcome measures are not considered to be SAEs and should be reported in the normal way, on the appropriate CRF.

The KHP CTO will report SUSARs to the regulatory authorities (MHRA only in this case, since this is a single site trial).

The Chief Investigator will report to the relevant ethics committee. Reporting timelines are as follows:

- SUSARs which are fatal or life-threatening must be reported not later than 7 days after the sponsor is first aware of the reaction. Any additional relevant information must be reported within a further 8 days.
- SUSARs that are not fatal or life-threatening must be reported within 15 days of the sponsor first becoming aware of the reaction.
- The Chief Investigator and KHP CTO (on behalf of the co-sponsors), will submit a Development Safety Update Report (DSUR) relating to this trial IMP to the MHRA and REC annually.

13.7 Urgent Safety Measures

The Regulations allow the sponsor and investigator to take appropriate urgent safety measures to protect clinical trial subjects from any immediate hazard to their health and safety, these measures should be taken immediately but the sponsor must notify the MHRA and the Main REC in writing, of the measures taken and the reason for the measures within 3 days by submitting a substantial amendment. The **CI** must inform the KHP CTO **as soon as possible after the implementation of the urgent safety measures**. The CI should phone the Clinical Trials Unit at the MHRA and discuss the issue with a medical assessor immediately.

The substantial amendment should be faxed and emailed to the Clinical Trials Unit **marked 'Urgent Safety Measure'** (contact details on MHRA website) or sent as a PDF document on disk to:
Information Processing Unit,
Area 6,
Medicines and Healthcare products Regulatory Agency,
151 Buckingham Palace Road
Victoria
London
SW1W 9SZ

The decision to undertake appropriate safety measures may be taken by:

- The CI
- The KHP CTO - on behalf of the Sponsor and in consultation with the CI.

14. QUALITY ASSURANCE

14.1 Control of Data Consistency

After the final patient in a cohort has been recruited and observed for 28 days for DLT, all data from the cohort will be reviewed by the Chief Investigator, and Co-Investigators to ensure consistency.

15. ETHICAL CONSIDERATIONS

15.1 Patient Protection

The responsible investigator will ensure that this study is conducted in agreement with either the Declaration of Helsinki (Tokyo, Venice, Hong Kong, Somerset West and Edinburgh amendments) or the laws and regulations of the country, whichever provides the greatest protection of the patient.

The protocol has been written, and the study will be conducted according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice (ref: http://www.ifpma.org/pdfifpma/e6.pdf).

15.2 Subject Identification

Subject data will be anonymised by the use of study numbers. These will be assigned at the start of the study and all blood samples and results reports will be identified by study number. A copy of the study number code identifying subjects will be kept in a secure cabinet at study site accessible to the Chief Investigator and Co-Investigators at all times. It is not anticipated that any information will be sent outside the study site. However, in the event that it is, it will be fully anonymised.

Analysis will be conducted by the study team. Analysis will only be conducted on anonymised data.

The Chief Investigator will act as custodian of the data on behalf of co-sponsors.

Personal data will be stored for a minimum of 15 years. Access will be controlled by the Chief Investigator.

The following guidelines will be strictly adhered to:

- All anonymised data will be stored on a password-protected computer.
- All trial data will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the Data Protection Act and archived in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 as defined in the Joint KHP CTO Archiving SOP.

15.3 Informed Consent

All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he/she will be exposed. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorised individuals other than their treating physician.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are registered or randomised in the study. This will be done in accordance with the national and local regulatory requirements.

15.4 Quality Assurance

Monitoring of this trial will be to ensure compliance with Good Clinical Practice and scientific integrity will be managed and oversight retained, by the KHP CTO Quality Team.

The Investigator(s) will permit trial-related monitoring, audits, REC review, and regulatory inspections by providing the Sponsor(s), Regulators and REC direct access to source data and other documents (eg patients' case sheets, blood test reports, X-ray reports, histology reports etc).

16. ADMINISTRATIVE RESPONSIBILITIES

Ultimate responsibility for Trial Administration rests with the Chief Investigator.

17. TRIAL SPONSORSHIP AND FINANCING

The trial is co-sponsored by Guy's and St Thomas' NHS Foundation Trust and King's College London as the employer of the Chief Investigator. Full details are provided on the front cover of this Clinical Trial Protocol.

Funding to support this trial has been provided by the J.P. Moulton Charitable Foundation, the Wellcome Trust and from the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London.

18. INTERIM ANALYSIS

One interim analysis for safety and efficacy will be conducted after the dose escalation phase of the study has been completed (i.e. first fifteen patients). Analysis will consist of assessment of trial primary and secondary endpoints e.g.

- quantification of DLTs within the first 6 weeks post CAR T-cell therapy
- assessment of clinical response by RECIST 1.1 criteria at 6 weeks post treatment and relationship to CAR T-cell dose
- analysis of circulating CAR T-cells after CAR T-cell therapy.
- quantification of circulating cytokine levels before and after CAR T-cell therapy.
- quantification of MAGE A3/ A4 reactive T-cells before and after CAR T-cell therapy.
- assess intra-tumoural CAR T-cells in post-treatment biopsies
- alteration in gene expression induced by T4 immunotherapy within the tumour microenvironment

We will also undertake exploratory analysis to

- assess the presence and magnitude of anti-CAR antibodies before and after CAR T-cell therapy.
- assess of EGFr expression by tumours before and after CAR T-cell therapy.

19. PUBLICATION POLICY

The data generated from this trial will be submitted for publication in a peer-reviewed journal. It is envisioned that a publication will be submitted following the interim analysis described in the preceding section. Authorship will include all Investigators listed in the Clinical Trial Protocol in addition to other scientific and clinical staff who contribute to the study.

20. TRIAL MANAGEMENT AND OVERSIGHT

20.1 Trial Management Team

This group is led by the CI for this study. The group will consist of the CI, co-investigators and core study team members including statisticians, clinical trial manager, research nurses and representatives of the GM/P team. The trial management team will be responsible for the day to day management of the trial activities and will meet on a regular basis to discuss any trial related activities or issues.

20.2. Trial Steering and Data Monitoring Committee

Oversight of the trial is provided by a joint Trial Steering and Data Monitoring Committee (TSC/ DMC). The TSC/ DMC is responsible for maintaining the quality of the study in addition to ongoing monitoring of individual toxicities and adverse events on behalf of the Co-Sponsors and Funders. The TSC/ DMC also ensures that the study is conducted according to the guidelines for Good Clinical Practice (GCP), UK Clinical Trial Regulations, the UK Policy Framework for Health and Social Care and all relevant regulations and local policies.

The committee was constituted prior to the opening of the trial and consists of an independent Chair and Deputy Chair (both clinicians) and a Statistician, together with the trial chief investigator and co-investigators. Over the duration of funding from the Wellcome Trust, an external advisor to the charity is also a participant. In addition to opening and closing meetings (prior to study commencement and after study completion respectively), the TSC/DMC meets at the end of each cohort of 3 patients and in the event of a dose-limiting toxicity event. However, for Cohort 8, there will be a mandatory trial steering committee meeting after completion of treatment of the first patient and the third patient. Following committee approval, Cohort 8 may be expanded to a maximum of 8 patients. At each meeting, the TSC/ DMC makes a recommendation regarding continuation of the trial and whether amendments, modifications or study termination is advised. Further details on current membership and terms of reference is provided in the TSC/ DMC charter.

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