KING'S College LONDON
University of London

Guy's and St Thomas' NHS
NHS Foundation Trust

STATISTICAL ANALYSIS PLAN

Phase 1 trial: T4 immunotherapy of head and neck cancer

TRIAL IDENTIFIERS

EudraCT Number: 2012-001654-25

REC Number: 12/LO/1834

ClinicalTrials.gov Identifier: NCT01818323

Document Version: 5.0

Date: 20th May 2025

Authors: Rachel Burman

School of Population Health Sciences | Faculty of Life Sciences and Medicine
King's College London,
4th Floor, Addison House, Guy's Campus,
London, SE1 1UL
Tel: 0207 848 6635
Email: rachel.c.burman@kcl.ac.uk

Chief Investigator

Dr John Maher
Division of Cancer Studies,
Third Floor Bermondsey Wing, Guy's Hospital,
Great Maze Pond, London SE1 9RT
Phone: (44) 020 7960 354517
Fax: (44) 020 7188 0919
Email: john.maher@kcl.ac.uk

Co-investigators
Prof James Spicer
Division of Cancer Studies,
Third Floor Bermondsey Wing, Guy's Hospital,
Great Maze Pond, London SE1 9RT
Phone: (44) 020 7188 4251
Fax: (44) 020 7188 0919
Email: james.spicer@kcl.ac.uk

Mr Jean-Pièrre Jeannon,
Department of Ear, Nose and Throat/ Head & Neck Surgery,
Guy's Hospital, Great Maze Pond, London SE1 9RT
Phone: (44) 020 7188 2213
Fax: (44) 020 7188 2206
Email: Jean-Pierre.Jeannon@gstt.nhs.uk
jp.jeannon@kcl.ac.uk

Dr Sophie Papa (née Burbridge)
Department of Medical Oncology
Fourth Floor Bermondsey Wing, Guy's Hospital,
Great Maze Pond, London SE1 9RT
Phone: (44) 020 7188 1468/9313
Fax: (44) 020 7188 0919
Email: sophie.burbridge@kcl.ac.uk

Dr Teresa Urbano-Guerrero
Department of Clinical Oncology
Oncology Offices, Ground Floor, Lambeth Wing
St Thomas' Hospital
Westminster Bridge Rd
London SE1 7EH
Phone: (44) 020 7188 4219
Fax: (44) 020 7928 9968
Email: Teresa.GuerreroUrbano@gstt.nhs.uk

RESEARCH TEAM MEMBERS

Ms Antonella Adami
Leucid Bio,
Third Floor Bermondsey Wing, Guy's Hospital,
Great Maze Pond, London SE1 9RT
Phone: (44) 020 7188 1482
Fax: (44) 020 7188 0919
Email: antonella.adami@kcl.ac.uk

APPROVAL

The undersigned hereby declare that they have prepared/examined the Statistical Analysis Plan and agree to its form and content.

Prepared by: Statistician

20/05/2025

Sign *Date*

RACHEL BURMAN

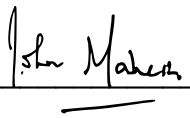
Reviewed by: Trial Statistician

20/05/2025

Sign *Date*

ABDEL DOUIRI

Approved by: Principal/ Chief Investigator



29.11.2025

Sign *Date*

DR JOHN MAHER

REVISION HISTORY

Version	Author	Description of change	Date of change
1.0	Rachel Phillips/ Fiona Reid	Original version of SAP	N/A
2.0	Nick Beckley-Hoelscher/ Fiona Reid	Addition of interim analysis, change of statistician, changes made in line with protocol 7.1	29/08/2020
2.1	Nick Beckley-Hoelscher/ Fiona Reid	Addition of table A14, the template table for biopsy data	22/04/2021
3.0	Marc Delord	Updated secondary objectives (section 1.1.2), inclusion/exclusion criteria (section 1.9) and outcome measures (section 1.10) in line with protocol. Updated sections for cohort 7 and 8 (sections 1.3, 1.5, 1.6, 2.1,2.5, 2.6) Modification of figure 1, table 1 and table 2 to include cohorts 7/8. The software section was modified Expected sample size increased in line with protocol (section 1.11) Appendices updated with addition of new table A1 : Summary of dosing schedule/ cohort structure) and information relevant to cohort 7/8.	19/02/2024
4.0	Rachel Burman	Administrative corrections to Inclusion/exclusion of criteria and secondary endpoints in line with the protocol version 10.1. Statistician update.	22/04/2025

5.0	Rachel Burman	Fixed titles of tables, data analysis figures and tables, emails, page numbering, appendix and referencing.	

TABLE OF CONTENTS

1 Description of the trial

See current protocol for full details.

1.1 Trial objectives

The overall goal of this study is to investigate the safety of T4 immunotherapy when administered to treat loco-regional disease in squamous cell carcinoma of the head and neck (SCCHN) that is not suitable for conventional active therapy.

1.1.1 Primary objectives

i. To define dose limiting toxicities for T4 immunotherapy in SCCHN.

ii. To determine a safe and feasible recommended dose for phase II testing of intra-tumoural T4 immunotherapy.

1.1.2 Secondary objectives

i. To investigate serum cytokine levels after administration of T4 immunotherapy.

ii. To investigate persistence of T4+ T-cells at the site of administration and in the peripheral circulation.

iii. To achieve preliminary assessment of anti-tumour activity, using cross-sectional imaging to quantify objective responses.

iv. To investigate tumour ErbB receptor phenotype, before and after administration of T4 immunotherapy.

v. To investigate immunomodulatory effects of lymphodepletion using fludarabine and cyclophosphamide on T4 immunotherapy.

vi. To investigate effect of T4 immunotherapy upon immune reactivity against endogenous tumour antigens.

vii. To investigate effect of T4 immunotherapy upon global gene expression within the tumour microenvironment.

viii. To investigate safety of T4 immunotherapy, when administered in combination with lymphodepleting chemotherapy (fludarabine and cyclophosphamide), alone or in combination with nivolumab.

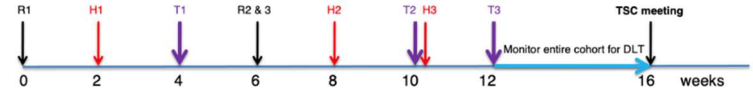
1.2 Trial design

This is a phase 1 dose-finding open label study of T4 immunotherapy in locally advanced or recurrent squamous cell carcinoma of the head and neck (SCCHN), with or without metastatic disease (excluding brain metastases) for whom no standard therapy remains or is suitable. A 3+3 dose escalation design will be followed in order to determine the maximum tolerated dose (MTD).

1.3 Dose escalation timeline

The rate of subject entry and escalation to the next dose level (see section 1.5 for details of dosing regimen) will depend upon safety profile assessment of patients entered at the previous dose level and is summarised in Figure 1. After treatment, patients in cohorts 1-5 will be evaluated for at least 24 hours as an inpatient and then again after 72 hours as an outpatient. Patients in cohorts 6 to 8 (lymphodepletion cohorts) will be hospitalised for a mandatory 7-day minimum period after intra-tumoural administration of T4 immunotherapy. If no dose limiting toxicity (DLT – see section 1.10.1) 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 cohorts 7 and 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 cohorts 7 and 8, this 28 day monitoring period commences on the day that patient 3 receives the final (third) dose of nivolumab.

Cohorts 1-5

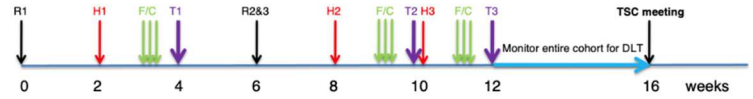


Key:

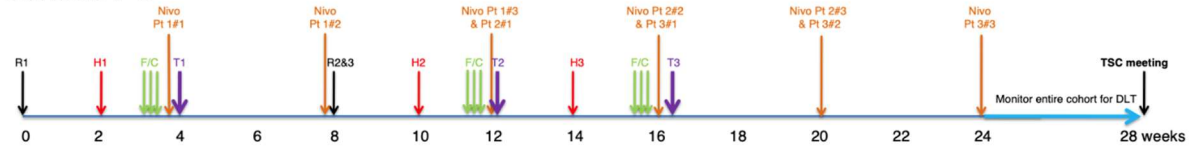
R1 – recruit patient 1
H1 – harvest patient 1
T1 – T4 treatment patient 1
R2 & 3 – open recruitment for patients 2 and 3
H2 – harvest patient 2
T2 – T4 treatment patient 2
H3 – harvest patient 2
T3 – T4 treatment patient 2
F/C – fludarabine / cyclophosphamide
TSC – trial steering committee
Nivo Pt x#y – nivolumab; patient number followed by dose number

1

Cohort 6



Cohorts 7-8



Day	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M
Harvest	X																	
Flu/Cy					X	X	X											
T4											X							
Mandatory Inpatient stay											X	X	X	X	X	X	X	X
Discharge if well																		X

Figure 1: Timelines for consecutive patient treatment with T4 immunotherapy. Treatment administration is indicated by the vertical arrows. The timeline indicates that patient 3 would be recruited only if less than 2 dose-limiting toxicities (DLT) has been observed in patients 1 and 2 within a cohort. In this event, the trial steering committee would consider whether patient 3 could be enrolled at the lower dose level (cohort 1-6). Note also that in cohort 8, there will be a joint TSC/DMC meeting after the first patient has completed treatment and monitoring.

1.4 Method of allocating groups / randomisation procedure

This is a phase 1, non-randomised, open-label, single group, proof-of-concept study. All participants will receive the IMP. Therefore, method of allocation and randomisation procedure are not applicable.

1.5 Dosing regimen and maximum tolerated dose (MTD)

T4 immunotherapy T-cell doses will be escalated in fixed increments according to the dose escalation scheme outlined in Table 1. A 3+3 dose escalation design will be followed in order to determine the maximum tolerated dose (MTD) and thus the recommended dose for phase 2 testing (RDPT).

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.

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 have been exceeded. If this had occurred in cohort 1 (Table 1), de-escalation to a –1 dosing regimen would have proceeded. The highest dose level (10^9 CAR+ T-cells) was reached without achieving the maximum tolerated dose of T4 immunotherapy by the end of cohort 5. Next, combination therapy was administrated to patients in cohort 6.

In that cohort, patients were enrolled at a lower dose level of 10^8 CAR+ T-cells, administered after lymphodepletion with fludarabine and cyclophosphamide. Thereafter a further cohort of three patients (cohort 7) was planned in which nivolumab (three doses) was added to the regimen administered to patients in cohort 6 (Table 1). However, this cohort was suspended after treatment of the first patient owing to lack of efficacy, on the recommendation of the trial steering committee. Instead the trial will now proceed to cohort 8, in which patients will be treated with lymphodepletion plus nivolumab (three doses), administered in combination with the highest dose level of T4 immunotherapy, 10^9 CAR+ T-cells.

Before opening the next dose level, all adverse events recorded at the previous dose level (within 28 days of T4 immunotherapy dosing in the case of cohorts 1-6 and up to 28 days after last dose of nivolumab for cohorts 7) will be reviewed and discussed by the investigators.

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)**
	-1*	3 x 10^6 cells	3 x 10^6 cells	1 ± 0.2
Starting T4 Dose level	1	1 x 10^7 cells	3 x 10^6 - 10^7 cells	1 ± 0.2
↓	2	3 x 10^7 cells	1.1 – 3 x10^7 cells	1 ± 0.2
↓	3	1 x 10^8 cells	3.1 – 10 x10^7 cells	2 ± 0.4
↓	4	3 x 10^8 cells	1.1 – 3 x10^8 cells	3 ± 0.6
Final T4 dose level	5	1 x 10^9 cells**	3.1 – 10 x10^8 cells	4 ± 0.8
Intravenous fludarabine 25mg/m² and cyclophosphamide 250mg/m² once daily for 3 days, 2-11 prior to T4 immunotherapy	6	1 x 10^8 cells	3.1 – 10 x10^7 cells	4 ± 0.8
Fludarabine and cyclophosphamide as above plus nivolumab 480mg IV x 3 doses q 4 weekly, commencing one day prior to T4 immunotherapy	7	1 x 10^8 cells	3.1 – 10 x10^7 cells	4 + 0.8
Fludarabine, cyclophosphamide and nivolumab as above	8	1 x 10^9 cells**	3.1 – 10 x10^8 cells	4 ± 0.8

Table 1* 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.

1.6 Visit schedule

Within the four weeks prior to T4 immunotherapy being administered patients will be screened for eligibility and undergo a series of tests. Blood will be harvested 14 days prior to therapy being administered. Baseline data will be collected within the three days prior to receiving therapy.

For cohorts 1-5, participants will be admitted overnight as inpatients and data collected at several time points during the first 24 hours following receipt of the T4 immunotherapy. They will undergo further examinations at study visits as outlined in Table 2.

In addition to the above, participants in cohort 6 will receive 3 days of lymphodepletion before T4 immunotherapy and remain as inpatients for 7 days following T4 immunotherapy.

Similarly, participants in cohorts 7 and 8 will remain as inpatients for 7 days following lymphodepletion and T4 immunotherapy, but will additionally receive 3 doses of Nivolumab. Nivolumab will be administered on the day prior to T4 immunotherapy and then at 4 weeks and 8 weeks after T4 immunotherapy.

Study visits and examinations for all cohorts are outlined in the Table 2.

1.7 Procedures by visit

Data collected at each time point for all cohorts are summarized in Table 2. During the 24 hours following T4 immunotherapy being received (day 1), data will be recorded at regular intervals, as summarized in Figure 2.

Table 2: Summary of Patient Monitoring Studies

a. COHORTS 1-5

	Screen T4 Manufacture			Treat	Post- Treatment Period[6]							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													
	Screen	Manufacture				T4										
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

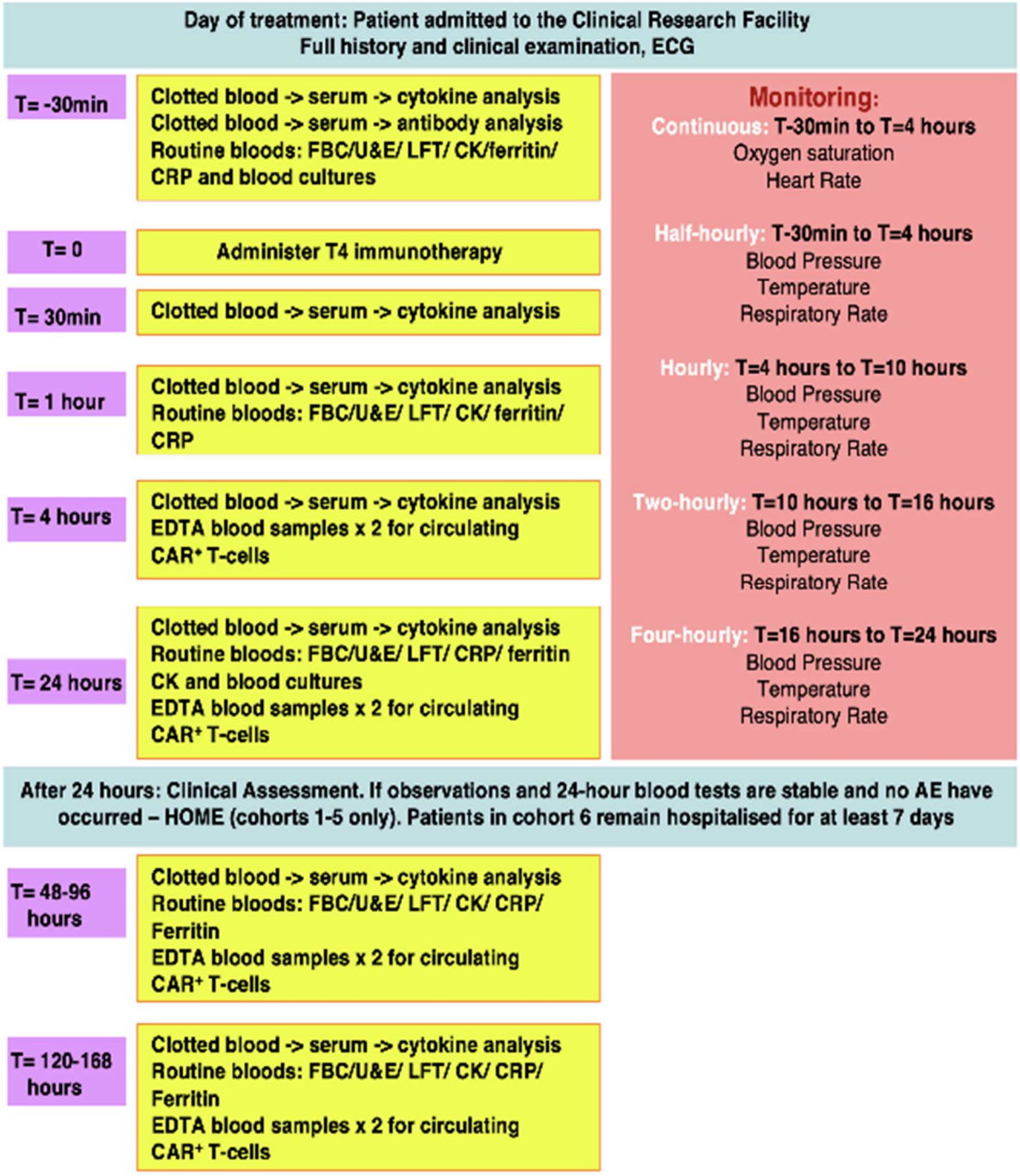
c. Cohort 7-8

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	

Manuf. – manufacture; 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 111In oxine. 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, 29 and 43, 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.



The term "AE" refers to adverse events that are deemed clinically significant.

Figure 2: Patient Monitoring: Hours 0 – 168.

1.8 Long-term follow-up

After completion of the six weeks 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.

1.9 Selection of participants

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.

1.9.1 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 cohort 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 ≥50%. Assessment must take place within 28 days of treatment.

9) Haematology results within 28 days of treatment neutrophils ≥1.5 x 109/L, platelets ≥100 $x10^9$/L, haemoglobin ≥90g/L, INR <1.5.

10) Biochemistry results within 28 days of treatment:
    - serum creatinine <1.5 upper limit of normal
    - bilirubin <1.25 times upper limit of normal;
    - LT/ AST <2.5 times upper limit of normal (<5 times upper limit of normal 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*.

* Enrolment/registration occurs on the day that blood is collected to manufacture T4 immunotherapy

1.9.2 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 (≥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 (Cohort 7-8 only).

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.

1.10 Outcome measures

1.10.1 Primary endpoint - Dose limiting toxicity

Dose limiting toxicities will be graded according to National Cancer Institute (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. Although patients are observed for DLTs for 6 weeks, the minimum period of evaluation for DLTs before recruitment can open to the next cohort is 28 days. Dose limiting toxicity 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 x 10^9/L with fever ≥38.50C; absolute neutrophil count < 1.0 x 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 at day 8, 15, 28 and 43. Echocardiography will be performed on days 15, 29 and 43. Any suspected new cardiac symptom will be assessed urgently with the appropriate investigations. 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%.

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, the following conditions are not considered DLTs:

•

Aphasia/dysphasia or confusion/cognitive disturbance which resolves to grade 1 or less within 2 weeks and to baseline within 4 weeks.

•

Expected chemotherapy-induced cytopaenias that occur in patients recruited to cohort 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 independent safety review committee.

1.10.2 Secondary endpoints

1. 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.

2. Persistence of $T4^+$ T-cells in tumour biopsies (measured by quantitative PCR) at two weeks post therapy.

3. 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, 29 and 43 days post injection. In the case of patients who receive lymphodepleting chemotherapy and nivolumab (cohort 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).

4. Evidence of response evaluated by appropriate cross-sectional imaging, and in the case of patients in cohorts 7 and 8, 12 weeks post therapy. Clinical response will be assessed according to RECIST criteria version 1.1, please see section 1.10.3 below for definitions of tumour response.

5. Effect of T4 immunotherapy upon endogenous T-cell reactivity against MAGE-A3 and MAGEA4 cancer/testis antigens (cohorts 1-5 only). 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.

6. Evidence of immunomodulation by cyclophosphamide and fludarabine (cohorts 6-8 only), 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.
7. 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.

8. 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.

1.10.3 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 later deemed ineligible. Each patient will be assigned one of the following categories: complete response, partial response, stable disease, progressive disease, or early death. See protocol for full definitions of complete response, partial response, stable disease and progressive disease. 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, other cause or unknown (not assessable, insufficient data).

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.

1.10.4 Adverse events

The protocol defines adverse events as follow:

Adverse Event (AE):
Any untoward medical occurrence in the patient administered a medicinal product that does not necessarily have a causal relationship with this treatment.

Adverse Reaction (AR):
Any untoward and unintended response in a subject to an investigational medicinal product, which is related to any dose administered to that subject.

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 either the summary of product characteristics (SmPC) for that product, or the Investigator's Brochure (IB) relating to the trial in question.

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:

i. Results in death
ii. Is life-threatening,
iii. Requires hospitalisation or prolongation of existing hospitalisation;
iv. Results in persistent or significant disability or incapacity;
v. Consists of a congenital anomaly or birth defect
vi. Important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

Suspected Unexpected Serious Adverse Reaction (SUSAR):
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.

1.11 Sample size

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.

2 Data analysis plan

2.1 Recruitment of patients

A flow chart in accordance with the requirements of the CONSORT statement will be presented (see Appendices, Figures 1, 2 and 3). This will include the number of patients consenting to enter the trial, the number of patients excluded during screening, the number of patients excluded due to unsuccessful blood harvest, and the number treated with T4 immunotherapy. For cohorts 1 to 6, this will include the number of patients who complete the minimum 29 day follow up post T4 immuno-therapy and complete the trial (43 days). However, for cohort 7 and 8, this will include 29 days follow up post last dose of Nivolumab and complete the trial (85 days). Information on the number of patients who withdraw or who are lost to follow-up will be included. This will be repeated for each individual cohort of patients.

The number screened and enrolled per month will be tabulated (Table 1).

The summary of dosing schedule and cohort structure will be tabulated (see Appendices, Table 2)

2.2 Baseline characteristics

The baseline characteristics of enrolled patients will be reported, including demographics and clinical characteristics (table 3-5).

2.3 Treatment delivery

The table of scheduled visits (table 6) and the number of treated patients will be reported (table 7). Treatment delivery will be described (table 8) and T4 dosing will be described (Table 9)

2.4 Analysis of primary outcome

The study's primary outcome is the assessment of dose limiting toxicity (DLT) of T4 immunotherapy (see section 1.10.1).

In cohorts 1 to 8 the minimum period for evaluation of DLTs is 28 days from last treatment administration (T4 treatment in cohorts 1 to 6, and nivolumab in cohorts 7/8) before recruitment can open to the next cohort. Patients without a DLT who withdraw within 28 days from last treatment administration will be withdrawn from its cohort and a new patient will be recruited to replace this patient. Patients with a DLT who

withdraw within 28 days from last treatment administration will be counted in the corresponding cohort.

In cohorts 8 the minimum period for evaluation of DLTs is 28 days from last dose of Nivolumab treatment, no dose escalation or de-escalation will be pursued after last patient in cohort 8 whatever the number of patient recruited in this cohort (from 2 to 6).

Individual listings of all DLTs will be presented (table 10). The presence of a DLT will also be analysed by tables of frequency by dose level (table 11).

2.5 Analysis of secondary outcomes

For descriptive analysis of secondary outcomes, means and standard deviations or medians and ranges will be reported for continuous variables, and frequencies and percentages will be reported for categorical variables.

All comparative analysis of secondary outcomes will be exploratory.

2.5.1 Adverse events

Adverse events (AE), adverse reactions (AR), serious adverse events (SAE) and serious adverse reactions (SAR) as specified in the protocol will be reported to the joint Trial Steering and Data Monitoring Committee (TSC/DMC) on an ongoing basis, and summarised at the end of the trial.

2.5.2 Total number of events (AE, SAE, SUSAR and SARs) according to CTCAE grading and number of patients experiencing at least one of each event will be tabulated (table 12). Individual listings of all adverse events will also be presented (table 13).Evidence of tumour response

Disease status at day 43 (visit 11), six weeks after administration of T4 immunotherapy will be analysed by generating tables of frequency compared with dose level (table 14). In cohort 7 and 8, disease status will be assessed at visit 14 (43 days after T4 immunotherapy) and visit 17 (85 days after T4 immunotherapy). Patients will be followed up for survival after leaving the study and censored after two years.

2.5.3 Serum cytokine levels

These data will be presented using median and ranges for each dose level and at all time points (-30 minutes pre-injection and 30 minutes, 1, 4, 24, 48-96 and 120-168 hours post injection) (table 15 and 16). Evolution over time will be illustrated graphically with individual patient values plotted over time, with an individual plot for each dose level (figure 4).

2.5.4 Presence of T4+ T-cells in the circulation

The number of CAR+ T-cells in circulation (number per mL blood and vector copy number per gram of genomic DNA) will be presented by patient at 4, 24, 48-96 and 120-168 hours and days 8, 15, 29 and 43 days post injection in cohorts 1-5 (table 17). In cohort 6, this analysis will be performed at 4, 24, 48-96 and 120-168 hours and days 8, 15, 22, 36 and 43 days post injection. In cohorts 7 and 8, this analysis will be performed at 4, 24, 48-96 and 120-168 hours and days 8, 15, 22, 28, 43, 56, 71 and 85 days post injection

2.5.5 Endogenous T-cell reactivity against MAGE antigens

These data will be presented using median and ranges for each dose level at 3 days prior to injection and day 29 post injection in cohorts 1-5 only (table 18). Evolution over time will be illustrated graphically with individual patient values plotted over time, with an individual plot for each dose level.

2.5.6 Biopsy test results

Biopsy test results will be available for a subset of patients. These results will be listed for each patient, at screening and at day 8 or 15 post-injection. (table 19).

2.6 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. Cohorts 1-5). Analysis will consist of assessment of trial primary and secondary endpoints as described in 2.4 and 2.5 above.

Please see appendix 1 for:

- outline TSC/DMC meeting report (Tables 1-10 and Figures 1-2)
- outline Statistical Analysis report (Tables 1-15 and Figures 13)

Software

Data management: The secure data management system MedScinet will be used.

Statistical analysis: R statistical environment for statistical computing version 4.1.3 (2022-03-10) or later will be used for data description and analysis.

Appendices

LIST OF FIGURES

LIST OF TABLES

Figure 1: Trial Flowchart (all cohorts)

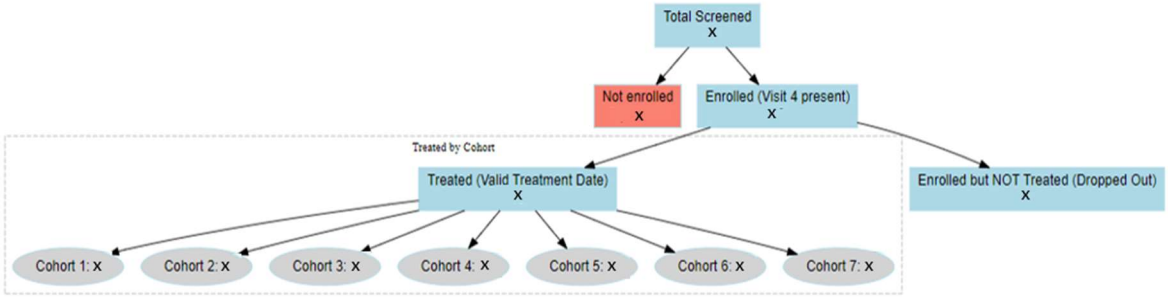


Figure 1: Recruitment CONSORT

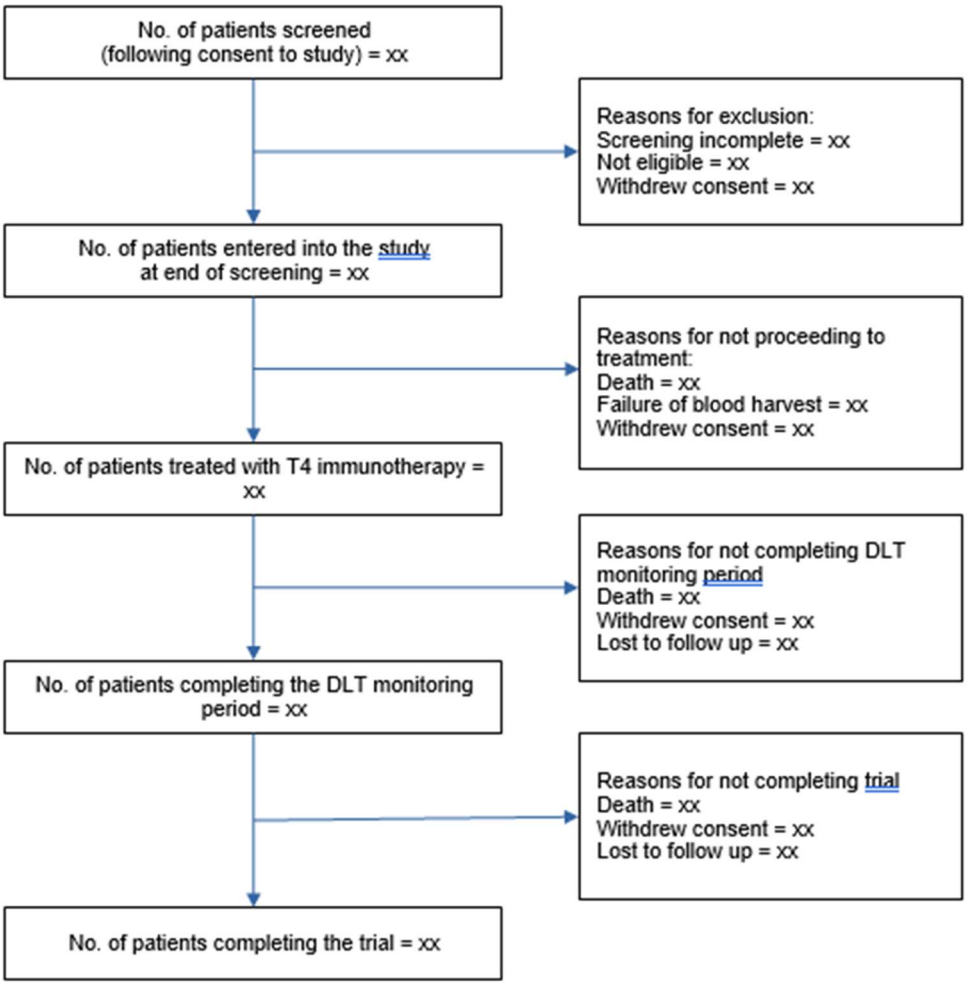


Figure 2: Trial Flowchart (all cohorts)

* Trial completion includes all patients followed up for at least 6 weeks after administration of T4 immunotherapy.

**DLT monitoring period = 29 days post T4 Immunotherapy for cohorts 1-6; 29 days post last dose of Nivolumab for cohorts 7/8.

Figure A2: Trial Flowchart, by cohort, from treatment onwards

Cohort 1

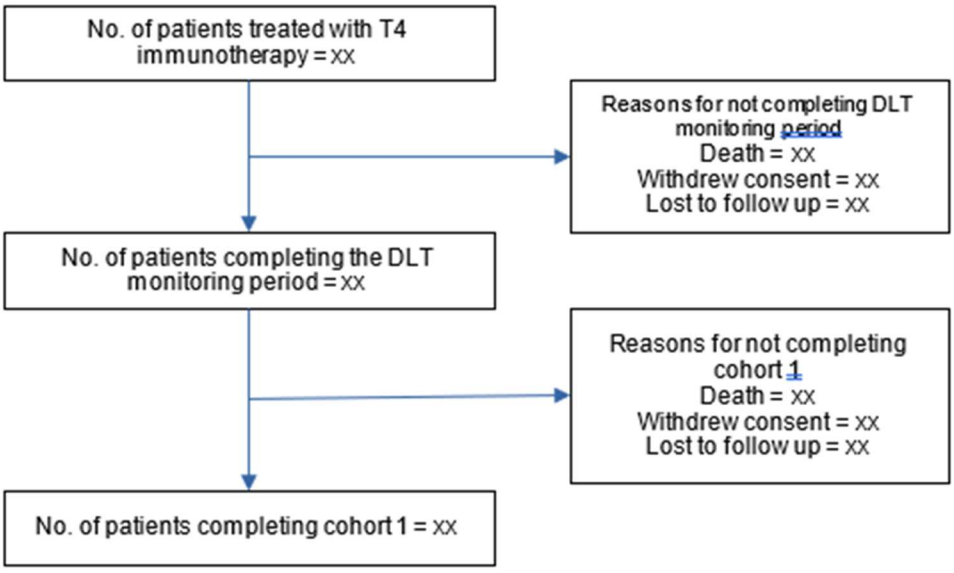


Figure 3: Trial flowchart, by cohort from treatment onwards

* Trial completion includes all patients followed up for at least 6 weeks after administration of T4 immunotherapy.

**DLT monitoring period = 29 days post T4 Immunotherapy for cohorts 1-6; 29 days post last dose of Nivolumab for cohorts 7/8.

Table 1: Summary of recruitment, by month

Month	Screened†	Enrolled††	Cohort (actual)
2015:			
June	xx	xx	xx
July	xx	xx	xx
August	xx	xx	xx
September	xx	xx	xx
October	xx	xx	xx
November	xx	xx	xx
December	xx	xx	xx
2016:			
January	xx	xx	xx
….			
….			

Total			

Table 1: Summary of recruitment, by month

† Screened: month of consent to screening

†† Enrolled: month confirmed eligible and entered into the study

Table 2: Summary of dosing schedule / cohort structure

Notes	Cohort	Target dose of $T4^+$ T-cells	Acceptable dose range for enrolment
Minus 1 dose level*	**-1**	**3×10^6**	**3×10^6**
Starting dose level	**1**	**1×10^7**	**$3 \times 10^6 – 1 \times 10^7$**
	2	**3×10^7**	**$1.1 - 3 \times 10^7$**
	3	**1×10^8**	**$3.1 - 10 \times 10^7$**
	4	**3×10^8**	**$1.1 - 3 \times 10^8$**
Final dose level	**5**	**1×10^9**	**$3.1 - 10 \times 10^8$**
	6	**1×10^8**	**$3.1 - 10 \times 10^7$**
	7	**1×10^8**	**$3.1 - 10 \times 10^7$**
	8	**1×10^9**	**$3.1 - 10 \times 10^8$**

Table 2: Summary of dosing schedule/cohort structure

*** To be used only if 2 DLTs seen in Cohort 1**

Table 3: Disease history of screened patients

Patient ID	Age	Sex (Male/Female)	Primary diagnosis*	
Patient 01	xx			Surgical pro Courses of r Discrete cou Courses of c
Patient 02	xx			Surgical pro Courses of r Discrete cou Courses of c
Patient 03	xx			Surgical pro Courses of r Discrete cou Courses of c

Table 1: Disease history of screened patients

***Primary diagnosis**: Oral cavity=No, Lips, Tongue, Palate, Other; Pharynx; Larynx; Nasal Cavity; Paranasal sinuses; Salivary glands.

Table 4: Baseline disease and past medical history of screened patients

Patient ID	Relevant medical history (summary)	Clinical abnormalities (summar
Patient 01		
Patient 02		
Patient 03		

Table 2: Baseline disease status and past medical history of screened patients

** **Clinical examination**: General appearance; Skin/scars; Head, Eye/ENT; Respiratory; Cardiovascular; Abdominal; Neurological; Lymph nodes; Musculoskeletal; Extremities; Other.

*** **Laboratory results**: Hep B=Hepatitis B surface antigen; Anti-HBV=Anti-HBV core antibody; Hep C=Hepatitis C, HIV 1 / 2=Human immunodeficiency virus, HTLV=*Human T-lymphotropic virus*; Syphilis.

Table 5: Baseline investigation results in screen patients

Patient ID	Full blood count†	CT Tumour imaging results (summary)	ECG abnormalities (or normal)	Echocard
Patient 01	Hb= xxx.x WCC= xxx.x Lymph= xxx.x Plts= xxx			
Patient 02	Hb= xxx.x WCC= xxx.x Lymph= xxx.x Plts= xxx			
Patient 03	Hb= xxx.x WCC= xxx.x Lymph= xxx.x Plts= xxx			

Table 3: Baseline investigation results in screened patients

†**Full blood count:** Hb=Haemoglobin (g/L), WCC=White cell count ($x10^9$/L), Lymph=Lymphocyte count ($x10^9$/L), Plts=Platelet count ($x10^9$/L)
††**PET-CT** only assessed on a sub-group of patients

Table 6: Schedule of Visits

Table 7: Completion Status of visits (x = visit completed)

Table 8: Treatment Delivery

Patient ID	Cohort (actual)	Date of treatment	Time from treatment of previous patient (days)
Patient 01	#	dd/mm/yyyy	-
Patient 02	#	dd/mm/yyyy	xx

Cohorts 1-5	Cohort 6	Cohorts 7-8
V1 – Screening	V1 – Screening	V1 – Screening
V2 – Day -14 Blood harvest	V2 – Day -14 Blood harvest Lymphodepletion Day 1 Lymphodepletion Day 2 Lymphodepletion Day 3	V2 – Day -14 Blood harvest Lymphodepletion Day 1 Lymphodepletion Day 2 Lymphodepletion Day 3
V3 – Pre-treatment final checks		Visit 3 – Day -1 Nivolumab
V4 – Day =1 Inpatient administration of T4 immunotherapy	V4 – Day =1 Inpatient administration of T4 immunotherapy	V4 – Day =1 Inpatient administration of T4 immunotherapy
V5 – 24 hours after T4 administration	V5 – 24 hours after T4 administration	V5 – 24 hours after T4 administration
V6 – 3-4 days after T4 administration	V6 – 3-4 days after T4 administration	V6 – 3-4 days after T4 administration
V7 – 5-7 days after T4 administration	V7 – 5-7 days after T4 administration	V7 – 5-7 days after T4 administration
V8 – 8 days after T4 administration	V8 – 8 days after T4 administration	V8 – 8 days after T4 administration

					Visits (see visits schedules definition)													
Cohort	Patient ID	Date consented (visit 1) Dd/mm/yyyy	Intended Cohort	Actual Cohort	V1	V2	V3	V4	V5	V6	V7	V8	V9	V9a	V10	V10a	V11	V
1-5	Patient #	dd/mm/yyyy	#	#					V13 – 36 days after T4 administration V14 – 43 days after T4 administration					x		X		
6	Patient #	dd/mm/yyyy	#	#			x		V15 - 56 days after T4 administration									

Table 5: Completion Status of Visits

Patient 03			

Table 6: Treatment Delivery

For reference - Minimum times between treatments (see section 4.7 of Protocol):

Patient 1 to Patient 2 (within cohort) = 42 days *Patient 2 to Patient 3 (within cohort) = 14 days* *Patient 3 to Next Cohort (dose escalation) OR Patient 4 in expanded cohort = 42 days* *Patient 4 to Patient 5 (within cohort) = 14 days* *Patient 5 to Patient 6 (within cohort) = 14 days* *Patient 6 to Next Cohort (dose escalation) = 42 days*

Table 9: T4 Dosing (treated patients only)

Patient ID	Cohort (actual)	CAR+ T-cell dose injected	Total T-cell dose injected	Radiolabelled cells administered (Yes/No)
Patient 01	#	xx.x	xx.x	
Patient 02				
Patient 03				
Patient 04				

Table 7: T4 Dosing (treated patients only

Table 10: Dose-limiting toxicities, per patient (treated patients only)

Patient ID	Cohort (actual)	Dose-limiting toxicity (Yes/No)	Event	Days from treatment to onset	Start date	End date	CTCAE grade (1-5)
Patient 01	#			xx	dd/mm/yyyy	dd/mm/yyyy	x
Patient 02							
Patient 03							

Table 8: Dose-limiting

Table 11: Dose-limiting toxicities and escalation, summary by cohort (treated patients only)

Cohort (actual)	Number of patients treated	Number of DLTs	Number withdrawn or lost to follow-up (reasons other than DLT)	Decision to escalate* (Yes/No)	Date of decision*
-1	x	x	x		dd/mm/yyyy
1					
2					
3					
4					
5					
6					
7				[redacted]	
8					

Table 9: Dose-limiting toxicities and follow-up summary per cohort

Table 12: Adverse events and reactions, including SAEs, summary (treated patients only)

	Number of events	Number of patients*
Serious adverse events (SAE)	xx	xx
Of which:		
Suspected Unexpected Serious Adverse Reactions (SUSAR)	*xx*	*xx*
Serious Adverse Reactions (SAR)	*xx*	*xx*
Adverse events (not serious) (AE)	xx	xx
CTCAE grade:		
1 – mild	xx	xx
2 – moderate	xx	xx
3 – severe	xx	xx
4 – life-threatening	xx	xx
5 – death	xx	xx

Table 10: Adverse events and reactions, including SAEs, summary (treated patients only)

* Number of patients experiencing at least one event

Table 13: Adverse events and reactions, including SAEs, per patient (treated patients only)

Patient ID	Cohort (actual)	Event	Dose-limiting toxicity (Yes/No)	Event Type: *	Days from treatment to onset	Start date	End date	CTCAE grade (1-5)
Patient 01	#				xx	dd/mm/yyyy	dd/mm/yyyy	
Patient 01								
Patient 01								
Patient 02								
Patient 02								
Patient 02								
…								

Table 11: Adverse events and reactions, including SAEs, per patient (treated patients only)

* **Event type:** SAE / AE
** **Related:** Unlikely / Possibly / Likely / Definitely
*** **Status:** Resolved / Resolved with sequelae / Ongoing (still in study) / Ongoing, at end of study

Table 14: Evidence of tumour response by dose cohort (Treated patients only)

Patient ID	Cohort (actual)	RECIST response at d43 – overall*	RECIST d43 - lesion 1*	RECIST d43 – lesion 2*	RECIST response at d85** - overall
Patient 01	#				
Patient 02					
Patient 03					

Table 12: Evidence of tumour response by cohort (Treated patients only)

* **RECIST response**: CR=Complete Response, PR=Partial Response, SD=Stable Disease, PD=Progressive Disease
** RECIST response is measured again at V17 for cohort 7 and 8 only

Table 15: Serum Cytokines (part I)

Cohort (actual)	Number of patients treated	Time (hours post-injection)	GM-CSF	INF-alpha	INF-gamma	IL-1 beta	IL-1RA	IL-2	IL-2R	IL-4	IL-5	IL-6
			Median (range)									
-1		-0.5										
		0.5										
		1										
		4										
		24										
		48-96										
		120-168										
1		-0.5										
		0.5										
		1										
		4										
		24										
		48-96										
		120-168										
2		-0.5										
		0.5										
		1										
		4										
		24										
		48-96										
		120-168										
3		-0.5										
		0.5										
		1										
		4										
		24										
		48-96										
		120-168										
4		-0.5										
		0.5										
		1										
		4										

		24										
		48-96										
		120-168										
5		-0.5										
		0.5										
		1										
		4										
		24										
		48-96										
		120-168										
6		-0.5										
		0.5										
		1										
		4										
		24										
		48-96										
		120-168										
7		-0.5										
		0.5										
		1										
		4										
		24										
		48-96										
		120-168										
8		-0.5										
		0.5										
		1										
		4										
		24										
		48-96										
		120-168										

Table 13: Serum Cytokines (part I)

Figure 3: Serum cytokine 'variable' over time, by cohort

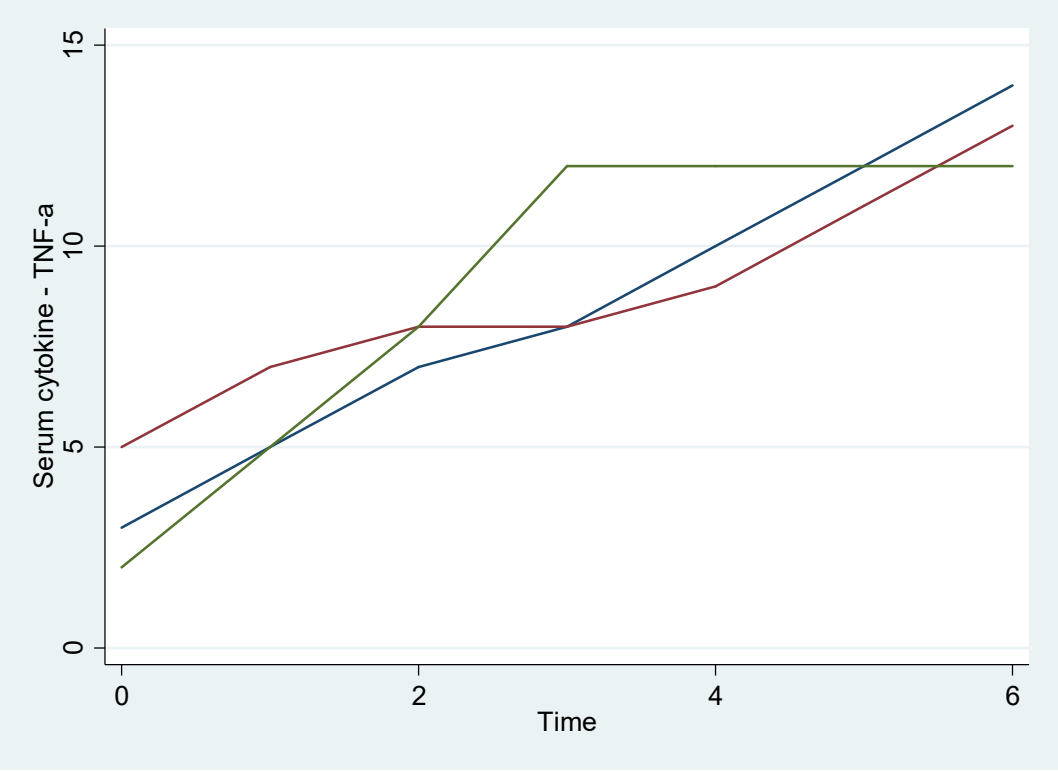


Figure 4: Serum cytokine 'variable' over time, by cohort

Table 15: Serum Cytokine (part II)

Cohort (actual)	Number of patients treated	Time (hours post-injection)	IL-17A	CCL5/ RANTES	EGF	Granzyme B	IL-1 alpha	VEGF - A	Fractalkine	IP-10	MC 1
			Median (range)								
-1		-0.5									
		0.5									
		1									
		4									
		24									
		48-96									
		120-168									
1		-0.5									
		0.5									
		1									

		4									
		24									
		48-96									
		120-168									
2		-0.5									
		0.5									
		1									
		4									
		24									
		48-96									
		120-168									
3		-0.5									
		0.5									
		1									
		4									
		24									
		48-96									
		120-168									
4		-0.5									
		0.5									
		1									
		4									
		24									
		48-96									
		120-168									
5		-0.5									
		0.5									
		1									
		4									
		24									
		48-96									
		120-168									
6		-0.5									
		0.5									
		1									
		4									
		24									

		48-96									
		120-168									
7		-0.5									
		0.5									
		1									
		4									
		24									
		48-96									
		120-168									
8		-0.5									
		0.5									
		1									
		4									
		24									
		48-96									
		120-168									

Table 14: Serum Cytokines (part ii)

Table 17: Circulating CAR T-cells*

Patient ID	Cohort (actual)	Time (post-injection)	Flow cytometry (no. CAR T-cells/ml blood)	qPCR (vector copy no. per gram DNA)
Patient 01	#	4 hours		
		24 hours		
		48-96 hours		
		120-168 hours		
		8 days		
		15 days		
		29 days		
		43 days		
Patient 02		4 hours		
		24 hours		
		48-96 hours		
		120-168 hours		
		8 days		
		15 days		
		29 days		
		43 days		
Patient 03		4 hours		

		24 hours		
		48-96 hours		
		120-168 hours		
		8 days		
		15 days		
		29 days		
		43 days		

Table 15: Circulating Car T-cells

***Analysis of circulating CAR-T cells is performed on different days dependent on the cohort.**
Cohort 1-5 : 4 hours, 24 hours, 48-96 hours, 120-168 hours, 8 days, 15 days, 29 days and 43 days post T4 immunotherapy.
Cohort 6: 4 hours, 24 hours, 48-96 hours, 120-168 hours, 8 days, 15 days, 22 days, 36 days, and 43 days post T4 immunotherapy.
Cohort 7 and 8: 4 hours, 24 hours, 48-96 hours, 120-168 hours, 8 days, 15 days, 22 days, 28 days, 43 days, 56 days, 71 days, and 85 days post T4 immunotherapy.

Table 18: ELISpot analysis: T-cell responses to MAGE A3 and A4 Antigens

Cohort (actual)	Number of patients treated	Time (days post-injection)	Spot forming cells per million PBMC, median (range)	
			MAGE A3	MAGE A4
-1		-3		
		29		
1		-3		
		29		
2		-3		
		29		
3		-3		
		29		
4		-3		
		29		
5		-3		
		29		

Table 16: ELISpot analysis: T-cell responses to MAGE A3 and A4 antigens

Table 19: Biopsy test results of Selected Patients

Patient ID	Cohort (actual)	Visit	Number of days after T4 immunotherapy	H&E staining	Immuno-chemistry	RNA scope	RNA sequence performed?
Patient 01	#	Screening	-#				
		Visit X	#				
Patient 02	#	Screening	-#				
		Visit X	#				
Patient 03	#	Screening	-#				
		Visit X	#				
Patient 04	#	Screening	-#				
		Visit X	#				

Table 17: Biopsy Test Results of Selected Patients

*X –biopsy post T4 Immunotherapy is taken at visit 8/9 for cohorts 1-6 and visit 8/10 for cohorts 7-8.