CLINICAL STUDY REPORT

T4 Trial

Phase 1 Trial: T4 Immunotherapy of Head and Neck Cancer

Sponsor Protocol Code:	T4
EudraCT Number:	2012-001654-25
ClinicalTrials.gov Identifier:	NCT01818323
ISRCTN number:	ISRCTN81726461
REC Number:	12/LO/1834
IRAS Number:	81143
Investigational Drugs (IMPs):	T4 immunotherapy; fludarabine; cyclophosphamide; nivolumab
Indication:	Squamous cell carcinoma of head and neck
Development Phase:	Phase I
Study Begin (FPFV):	05.06.2015
Study End (LPLV):	07.06.2022
Early Termination Date:	15.10.2024
Report Version & Issue Date:	Version 1.0 issued on 29.11.2025
Co-sponsor Name and Address:	Sponsor 1: Guy's and St Thomas' NHS Foundation Trust, Great Maze Pond, London SE1 9RT Sponsor 2: King's College London, Strand, London WC2R 2LS
Co-sponsor contact details:	Ann Marie Murtagh: King's Health Partners Clinical Trials Office 16th floor Tower Wing, Guy's Hospital London SE1 9RT Tel: 020 7188 5732 Fax: 020 7188 8330 E-mail: QM.KHPCTO@kcl.ac.uk
Chief Investigator:	John Maher

SIGNATURE PAGE

By signing below, I approve the contents of this Clinical Study Report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study. The clinical trial reported herein was conducted in accordance with the principles contained in the Declaration of Helsinki, Good Clinical Practice (GCP) and all applicable laws and regulations.

This was a non-commercial academic trial, the results of this study are not intended to be used or a licensing application.

Chief Investigator:

Printed name John Maher

Signature  **Date 29.11.2025**

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APPENDICES

1. Ethics

Independent Ethics Committee or Institutional Review Board
The study protocol and amendments were reviewed and approved by the National Research Ethics Committee London (West London).

Ethical conduct of the study
The trial was conducted according to the protocol and in compliance with the principles of the Declaration of Helsinki (1996) as amended, the principles of Good Clinical Practice (GCP) and in accordance with Medicines for Human Use (Clinical Trials) Regulations 2004, as amended, the Research Governance Framework for Health and Social Care, the Data Protection Act 1998 and other regulatory requirements as appropriate. The trial protocol and substantial amendments were reviewed by the United Kingdom (UK) Medicines and Healthcare products Regulatory Agency (MHRA)

Subject information and consent
Subjects were recruited from referrals made to the Phase 1 oncology unit at Guy's and St Thomas NHS Foundation Trust. Patients who were considered for enrollment had locally advanced or locally recurrent head and neck cancer that was measurable, amenable to intra-tumoural injection and considered to be sufficiently fit to participate in a Phase 1 oncology clinical trial. Subjects received a participant information sheet which outlined the rationale for the study and all associated procedures. This information was then discussed with a member of the Phase 1 team prior to obtaining informed consent to participate and completion of a consent form.

2. Data Monitoring
A combined data monitoring and trial steering committee oversaw the trial, monitoring safety as well as data quality and acting under the terms described in an accompanying charter. Members included the trial chief and co-investigators as well as an independent chair, deputy chair, representative of the funding body and one or more trial statisticians.

3. Sponsors, Investigators and Trial Sites

Co-Sponsors	Sponsor 1: Guy's and St Thomas' NHS Foundation Trust, Great Maze Pond, London SE1 9RT Sponsor 2: King's College London, Strand, London WC2R 2LS Contact details for both: Anne Marie Murtagh, King's Health Partners Clinical Trials Office 16th floor Tower Wing, Guy's Hospital London SE1 9RT Tel: 020 7188 5732 Fax: 020 7188 8330 E-mail: QM.KHPCTO@kcl.ac.uk

Chief Investigator	Dr John Maher King's College Hospital, Third floor Bermondsey Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT E-mail: john.maher@kcl.ac.uk

4. Co-Investigator(s), Statistician, Laboratories, Database Management

Co-investigator	Prof James Spicer Department of Research Oncology, Division of Cancer Studies, Third Floor Bermondsey Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT e-mail: james.spicer@kcl.ac.uk
Co-investigator	Mr Jean-Pièrre Jeannon, Department of Ear, Nose and Throat/ Head & Neck Surgery, Guy's Hospital, Great Maze Pond, London SE1 9RT e-mail: Jean-Pierre.Jeannon@gstt.nhs.uk
Co-investigator	Dr Sophie Papa School of Cancer & Pharmaceutical Medicine, Third Floor Bermondsey Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT e-mail: sophie.papa@kcl.ac.uk
Co-investigator	Dr Teresa Urbano-Guerrero Department of Clinical Oncology, Oncology Offices, Ground Floor, Lambeth Wing St Thomas' Hospital, Westminster Bridge Rd London SE1 7EH e-mail: Teresa.GuerreroUrbano@gstt.nhs.uk

Co-investigator Dr Cienne Morton

Department of Medical Oncology, Fourth Floor Bermondsey Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT

e-mail: Cienne.Morton@gstt.nhs.uk

Statisticians Rachel Phillips Rachel.phillips@kcl.ac.uk

Fiona Reid Fiona.reid@kcl.ac.uk

Nick Beckley-Hoelscher Nicholas.beckley-hoelscher@kcl.ac.uk

Maria Elstad maria.elstad@kcl.ac.uk

Marc Delord marc.delord@kcl.ac.uk

Rachel Burman Rachel.c.burman@kcl.ac.uk

Abdel Douiri Abdel.douiri@kcl.ac.uk

5. Study Synopsis

Title of clinical trial	Phase 1 Trial: T4 Immunotherapy of Head and Neck Cancer
Protocol Short Title/Acronym	T4 Immunotherapy
Study Phase	I
Sponsor name	Guy's and St Thomas' NHS Foundation Trust, and King's College London
Chief Investigator	John Maher
Eudract number	2012-001654-25
REC number	12/LO/1834
IRAS project ID:	81143
Medical condition or disease under investigation	Squamous cell carcinoma of head and neck
Purpose of clinical trial	Evaluation of safety of T4 immunotherapy
Primary objective	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.
Secondary objective (s)	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.
Trial Design	Dose finding open label 3+3 trial
Endpoints	*Primary*: Dose limiting toxicity of T4 immunotherapy graded according to NCI Common Terminology Criteria for Adverse Events, Current Version. *Secondary*: 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 and 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.

Planned number of subjects	22 – 29 subjects
Summary of eligibility criteria	*Inclusion*: Histologically and/ or cytologically confirmed SCCHN 18 years or older 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 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 and 8, as specified in this protocol. In the presence of metastatic disease, recent short-course palliative radiotherapy to non-target site(s) is allowed Those who refuse palliative treatment may be eligible for participation. However, their reasons for not opting for palliative treatment must be explored thoroughly 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 Eastern Co-operative Oncology Performance Status of 0-2 (0-1 for cohorts 6-8) 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 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 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) 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. Written informed consent prior to any trial procedure and registration (enrolment; i.e. day of blood harvest) *Exclusion*: The presence of or imminent occurrence of airway obstruction, unless tracheostomy in place The presence of or imminent occurrence of tumour-mediated infiltration of major blood vessels. History of HIV-1, HIV-2, HTLV-1, HTLV-2, Hepatitis B, Hepatitis C or syphilis infection Prior splenectomy Clinically active autoimmune disease or interstitial lung disease. Sub-clinical or quiescent autoimmune disease does not exclude from participation 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 Concurrent use of anticoagulant therapy is not permissible 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 The presence of any psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule Cyclophosphamide or fludarabine allergy or contraindication (Cohorts 6-8 only) Nivolumab allergy (Cohorts 7-8 only) Pregnancy Breastfeeding Prior T4 immunotherapy. However, prior immune checkpoint blockade does not preclude participation

	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 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
IMP, dosage and route of administration	T4 immunotherapy: single intra-tumoural doses of 10, 30, 100, 300 and 1000 x 10^6 CAR T-cells Fludarabine: Three intravenous doses of 25mg/m^2 Cyclophosphamide: Three intravenous doses of 250mg/m^2 Nivolumab; Three intravenous doses of 480mg
Active comparator product(s)	Not applicable
Maximum duration of treatment of a subject	9 weeks if fludarabine, cyclophosphamide, T4 immunotherapy and nivolumab all administered as planned.
Version and date of protocol amendments	V1.0 04.08.2012 V1.1 04.09.2012 V2.0 24.11.2012 V2.1 03.12.2012 V2.2 05.04.2013 V2.3 13.02.2015 V2.4 30.03.2015 V2.5 10.05.2015 V3.0 04.10.2015 V3.1 13.12.2015 V4.0 10.12.2016 V5.0 01.01.2018 V6.0 26.04.2018 V6.1 11.09.2018 V7.0 02.01.2019 V7.1 16.04.2019 V8.0 15.04.2021 V8.1 19.07.2021 V9.0 15.10.2022 V10.1 14.05.2024

6. Glossary of terms

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 4$\alpha\beta$ co-expressed with T1E28z
TGF-β	-	Transforming Growth Factor-β
TMF	-	Trial Master File
TNF	-	Tumour Necrosis Factor
Treg	-	Regulatory T-Cells
TSC/ DMC	-	Combined Trial Steering and Data Monitoring Committee
TUNEL	-	Terminal Deoxynucleotidyl Transferase dUTP Nick End Labelling
UAR	-	Unexpected Adverse Reaction

U&E - Urea and Electrolytes

WOCB - Women of Childbearing Potential

7. Publications (references)

van Schalkwyk MCI, Papa S, Jeannon JP, Guerrero Urbano T, Spicer JF, *Maher J* (2013) Design of a phase 1 clinical trial to evaluate intra-tumoral delivery of ErbB-targeted CAR T-cells in locally advanced or recurrent head and neck cancer. **Human Gene Therapy Clinical Development**. 24(3):134-142.

Papa S, Adami A, Metoudi M, Achkova D, van Schalkwyk M, Parente Pereira A, Bosshard-Carter L, Whilding L, van der Stegen S, Davies DM, Farzaneh F, Guerrero Urbano T, Jeannon JP, Spicer JF, Maher J (2018) A Phase 1 trial of T4 CAR T-cell immunotherapy in head and neck squamous cell cancer (HNSCC). **Journal of Clinical Oncology**. 36(15_S) 3046.

van Schalkwyk MCI, van der Stegen SJC, Bosshard-Carter L, Graves H, Papa S, Parente-Pereira AC, Farzaneh F, Fisher CD, Hope A, Adami A, Maher J (2021) Development and validation of a good manufacturing process for IL-4-driven expansion of chimeric cytokine receptor-expressing CAR T-cells. **Cells** 10(7) 1797.

Papa S, Adami A, Metoudi M, Beatson R, George MS, Achkova D, Williams E, Arif S, Reid F, Elstad M, Beckley-Hoelscher N, Douri A, Delord M, Lyne M, Shivapatham D, Fisher C, Hope A, Gooljar S, Mitra A, Gomm L, Morton C, Henley-Smith R, Thavaraj S, Santambrogio A, Andoniadou C, Allen S, Gibson V, Cook GJR, Parente-Pereira AC, Davies DM, Farzaneh F, Schurich A, Guerrero-Urbano T, Jeannon JP, Spicer J, Maher J (2023) Intratumoral pan-ErbB targeted CAR-T for head and neck squamous cell carcinoma: interim analysis of the T4 immunotherapy study. **Journal for Immunotherapy of Cancer**. 11(6) e007162.

Morton C, Wang F, Papa S, Adami A, Metoudi M, Achkova D, Reid F, Elstad M, Beckley-Hoelscher N, Douiri A, Delord M, Lyne M, Shivapatham D, Al-Rawi A, Fisher C, Hope A, Gooljar S, Mitra A, Gomm L, Parente-Pareira AC, Davies DM, Farzaneh F, Guerrero-Urbano T, Jeannon J, Spicer J, Maher J (2025) Final results of a first-in-human (FIH) study of intratumoral panErbB-targeted CAR T cell therapy for head and neck squamous cell carcinoma (HNSCC): T4 immunotherapy. **Cancer Research.** 85 (8_S2): CT508.

8. Study period (years)

The study ran from June 2015 to June 2022.
FPFV 05.06.2105.
LPLV 07.06.2022.
Recruitment completed on 29.03.2022.
Early Termination Date 15.10.2024.
The trial was paused during the covid 19 pandemic and terminated early owing to recruitment challenges and lack of sufficient therapeutic efficacy.

9. Phase of development

Phase I

10. Objectives

Primary objective	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.
Secondary objective (s)	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.

11. Background and Context

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%. At the time of design of this clinical trial, there had been little improvement in patient survival over the past 30 years (1, 3). In patients with recurrent or metastatic disease the median survival time was a mere six months (4, 5).

In designing this clinical trial, three key areas of unmet need were identified:

(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 (e.g. 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) Metastatic disease

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 set out 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.

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).

The ErbB family of receptor tyrosine kinases was targeted with a second generation CAR named T1E28z (40). In this clinical trial, the T1E28z CAR was co-expressed in human T-cells together with a chimeric cytokine receptor named 4$\alpha\beta$ (41). Efficacy and safety of this approach had been established in pre-clinical studies as follows. 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 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 (40). 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.

Pre-clinical safety testing of human T4 immunotherapy was conducted in mice for the following reasons.

- Human T-cells can elicit profound toxicity in mice (42).
- We have shown that human T-cells engrafted with the human T1E28z fusion receptor can recognize and destroy mouse $ErbB^{+}$ SCCHN tumour cells (40).

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. Dose-dependent toxicity has been demonstrated following intraperitoneal administration of larger doses of $T4^{+}$ T-cells. Toxicity was 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).

12. Methodology

Purpose

The purpose of the study was to determine the safety and maximum tolerated dose of intra-tumoural T4 immunotherapy in patients with relapsed SCCHN. T4 immunotherapy was administered alone (cohorts 1-5), following lymphodepletion with fludarabine and cyclophosphamide (cohorts 6-7), or following combined lymphodepletion and PD-1 immune checkpoint blockade (cohort 7/ cohort 8). Patients remained on study for 6 (cohorts 1-6) or 12 weeks (cohort 7/ cohort 8) post T4 immunotherapy.

Subjects

Eligible subjects were $\geq$18 years of age with locally advanced and/ or recurrent SCCHN, with or without metastatic disease (excluding brain metastases) for whom no standard therapy remained or was suitable.

CAR design and expression

Patient derived T-cells engineered to express the combination of T1E28z and 4$\alpha\beta$ are referred to as "T4 immunotherapy". Expression of T1E28z and 4$\alpha\beta$ was achieved using the SFG onco-retroviral expression vector. In the T1E28z CAR, targeting of ErbB dimers was achieved with a chimeric peptide named T1E (43-45). 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 as well as ErbB3 and ErbB4 containing dimers. To engineer the T1E28z fusion receptor (40), 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 4αβ chimeric cytokine receptor consists of a fusion in which the human interleukin (IL)-4 receptor (IL-4R) α ectodomain (amino acids 1-233) was 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 (41). Binding of IL-4 leads to approximation of the 4αβ chimeric cytokine receptor with the common γ chain. By this means, a potent IL-2-like growth signal was 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 IL-4 (41).

Manufacture and release of T4 immunotherapy
The manufacturing process has been described in (46). T4 immunotherapy was manufactured over a period of up to 14 days using anticoagulated whole blood (40-120mL) as starting material. Manufacture was undertaken at the Guy's and St Thomas' NHS Foundation Trust (GSTT) GMP manufacturing facility using immobilised CD3 and CD28 antibodies to elicit T-cell activation in closed gas permeable bags. Gene transfer was achieved using the SFG T4 retroviral vector, produced by EUFETS (now BioNTech; Iadr-Oberstein, Germany) from a PG13 master cell bank. A vector multiplicity of infection of 4.2 was used to achieve gene transfer into activated T-cells. Thereafter, T4 batches were expanded in a closed process within gas permeable bags. Interleukin-4 was the sole cytokine support provided after gene transfer (41). Drug product was suspended in X-VIVO medium (Lonza, Basel Switzerland) + 1X GlutaMAX (ThermoFisher Scientific, Horsham, UK) + 10% human AB serum (BioIVT, West Sussex, UK). Cells were formulated in 1-4mL according to dose on the final day of manufacture. Products were released by a QP (qualified person) if sterile (absence of bacterial or mycoplasma contamination), sufficiently potent (>10% $T4^+$ T-cells), sufficiently expanded (>10^7 T4 CAR T-cells, representing a >2 fold expansion) and sufficiently viable (>70%).

Trial design and dosing
The study was an uncontrolled open-label investigator initiated single centre (GSTT) non-randomised Phase 1 clinical trial. No reference group was included. A traditional 3+3 dose escalation design was adopted with 5 dose levels, administered initially without lymphodepleting chemotherapy (**Table 1**). This approach was followed in order to determine the maximum tolerated dose (MTD) and aid in the definition of the recommended dose for phase 2 testing (RDPT). If a MTD had been identified, this would define the upper limit for the RDPT of T4 immunotherapy.

Thereafter, cohort 6 entailed the pre-conditioning of subjects with fludarabine (25mg/m^2) and cyclophosphamide (250mg/m^2), each administered for 3 days. Lymphodepletion was undertaken between 2-11 days prior to CAR T-cell treatment. The CAR T-cell dose was reduced to 10^8 cells in this cohort.

In cohort 7, anti-PD1 blockade was added to this regimen. Nivolumab (480mg IV) was administered on the day before CAR T-cell treatment and at 4 and 8 weeks later.

An eight cohort had also been planned in which an increased T4 dose was combined with lymphodepletion and nivolumab. However, the trial was suspended after treatment of the first patient

in cohort 7 on the recommendation of the combined Trial Steering Committee and Data Monitoring Committee (TSC/ DMC) owing to lack of sufficient efficacy of the drug product.

CAR T-cell dosing entailed the intra-tumoural delivery of a single dose of freshly formulated T4 immunotherapy, administered by multiple injections to one or more target lesions. Local anaesthesia was applied first to the target lesion and ultrasound guidance to enable accurate dose delivery was used in 1 case. Planned dosing cohorts of T4 immunotherapy, lymphodepletion (cyclophosphamide and fludarabine) and nivolumab are shown in **Table 1**. Although a dose range of T4 immunotherapy was permitted, the target dose was achieved in all cases.

Table 1. Planned T4 dosing cohorts

Notes	Dose Level (Cohort No.)	Target dose	Acceptable dose range of $T4^+$ cells	Volume for injection (mL)	No. 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 immunotherapy	6	1 x 10^8 cells	3.1 – 10 x10^7 cells	4 ± 0.8	3
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	3
Fludarabine, cyclophosphamide and nivolumab as above	8	1 x 10^9 cells**	3.1 – 10 x10^8 cells	4 ± 0.8	3

* -1 dose level, to be used in the event of 2 DLTs in cohort 1
** Total T-cell dose

Dose escalation and toxicity

The rate of subject entry and escalation to the next dose level was dependent on safety profile assessment of patients entered at the previous dose level. Toxicity and dose limiting toxicities (DLT) related to T4 immunotherapy were evaluated according to the currently active version of the NCI Common Terminology Criteria for Adverse Events (CTCAE). The DLT period was 28 days and the following were defined as DLTs:

1. Cytokine release syndrome (CRS, as distinct from uncomplicated pyrexia) was considered to represent a DLT in cohorts 1-5. Grade 3 CRS that lasted for 3 days or more was considered a DLT in cohort 6-8 patients who received Flu/Cy lymphodepleting chemotherapy prior to T4 immunotherapy.
2. Haematological:

- Febrile neutropenia (Absolute neutrophil count < 1.0 x 10^9/L with fever ≥ 38.5^{0}C; 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 were not considered DLTs in cohorts 6-8. Instead, the following events constituted 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: A decline in ejection fraction of ≥10% between ECHO investigations was considered a DLT if the resulting ejection fraction fell 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 was not a DLT unless patients were grade 0 or 1 at baseline.

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

Enrolment within each cohort was consecutive. After treatment, patients in cohorts 1-5 were intentionally hospitalised for 24 hours. Patients in cohort 6 onwards (lymphodepletion cohorts) were hospitalised for a mandatory 7 day minimum period after intra-tumoural administration of T4 immunotherapy. If no DLT had occurred within the two-week period following CAR T-cell treatment of the first patient in any cohort, recruitment was opened for the next two patients in that cohort (meaning that they would not receive T4 immunotherapy for at least a further 2 weeks). This interval was extended to 4 weeks in cohort 7/ cohort 8 to account for the addition of nivolumab to the therapeutic regimen. However, since only one patient cell product could be manufactured at a time, this meant that patients 2 and 3 within each cohort could be treated with a minimum gap of 2 weeks. Once three patients had been enrolled in a cohort, all were evaluated for DLT for 28 days before escalation to the next dose level. In the case of cohort 7/ cohort 8, this 28 day monitoring period commenced on the day that patient 3 received the final (third) dose of nivolumab.

The maximum administered dose was defined when 2 out of 3 patients (or 2 out of 6 with cohort expansion) experienced a DLT at a given dose level. In this event, the MTD would have been exceeded. The MTD would then have been taken as the dose level administered to the cohort below the maximum administered dose. If one DLT occurred in a cohort of 3 patients, cohort expansion would proceed,with at least three further patients treated at that dose level. If a second DLT occurred in that cohort, the MTD would have been exceeded and the next lowest dose level would be expanded to establish the MTD. Thus, the MTD was defined as the highest dose at which <33% of subjects experience a DLT. If two DLT occurred in the first three patients enrolled in any cohort, the MTD would also have been exceeded. If this had occurred in cohort 1 (**Table 1**), de-escalation to a –1 dosing regimen would have been implemented. Before opening the next dose level, all adverse events recorded at the previous dose level (within 28 days of dosing) were reviewed and discussed by the TSC/DMC. Since the MTD was not reached in this clinical trial, it was intended that the trial would complete when the last patient in cohort 8 has completed final evaluation at their last visit, 12 weeks after receiving T4 immunotherapy. However, the trial was terminated following approval for cohort 8 recruitment. This decision was taken by the TSC/ DMC owing to recruitment challenges and since no objective responses had been observed in any subject, with stable disease the best outcome reported.

Subject replacement was considered under some circumstances. In the event of T4 batch failure, patient would be offered a second opportunity for treatment. Should they agree, the patient would 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.

Endpoints

The primary endpoint was occurrence of DLTs induced by T4 immunotherapy within 6 weeks of treatment. In cohort 7/ cohort 8, this observation period was extended to 12 weeks. Secondary endpoints included serum cytokine levels, presence of $T4^+$ cells in the circulation and tumour biopsies (where available post therapy), effect of T4 immunotherapy on reactivity to MAGE-A3 and MAGE-A4 tumour antigens, objective tumour response and time to progression.

Trafficking of T4 immunotherapy was assessed in a single subject, utilising a radiotracer consisting of [^{111}In] Indium passively labelled $T4^+$ T-cells (30 x 10^6 cells containing 5MBq [^{111}In] Indium). The radiotracer was administered on the same day as the T4 drug product, but as a separate procedure. The radiotracer was not considered to constitute a component of the drug product or therapeutic dose. A single photon emission computed tomography (SPECT) – computed tomography (CT) scan was performed approximately 1 hour after radiotracer delivery while additional SPECT scans were performed at 24 and 48 hours and were co-registered with the initial CT scan.

Response Criteria

Response was assessed utilising response evaluation criteria in solid tumors (RECIST) (current version) (47). Up to five accessible and measurable tumours were documented at baseline prior to injection with T4 immunotherapy. Baseline CT imaging was carried out a maximum of four weeks prior to T4 immunotherapy injection and response was evaluated by CT scanning six weeks after administration of T4 immunotherapy. Confirmation of any partial or complete response was required at least four weeks after initial response imaging. Ongoing evaluation after progression was by the referring clinician's standard practice.

Subject monitoring

The schedule of events for each cohort recruited to the study is shown as **Table 2a-c**. Actual completion of patient visits is shown in **Table 3**.

Table 2a. Schedule of events – 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	

Table 2b. Schedule of events – cohort 6

LYMPHODEPLETION COHORT
COHORT 6

	Screen	Lymphodepletion 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

Table 2c. Schedule of events – cohorts 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	

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

1. CT scanning of head, neck, thorax and abdomen.
2. Three patients had tumour 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. Optional: head, neck and half-body PET-CT scan was not performed on any subjects.
4. One patient received an a intra-tumoural radiotracer in which T4-engineered T-cells had been labelled with ^{111}Indium.
5. Tests performed more than once on this day.
6. A 48-hour window operated for visits at Days 8, 15, 28, 29, 43, 56, 71 and 85 to allow for weekend breaks.
7. SPECT alone was conducted at physician's discretion.
8. Patients were discharged if well and apyrexial for 24 hours.
9. Patients were asked to attend or make contact with the treating team daily until day 10 post CAR T-cell treatment.
10. Patients were 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 were instructed to make immediate contact with the Phase I oncology team if they felt unwell or developed pyrexia.

Table 3 Completion status of visits

Patient ID	Consent Date	Intended Cohort	Actual Cohort	V1	V2	V3	V4	V5	V6	V7	V8	V9	V9a	V10	V10a	V11	V12	V13	V14	V15	V16	V17
1	05/06/2015	1	1	x	x	x	x	x	x	x	x	x		x		x						
2	04/08/2015	1	1	x	x	x	x	x	x	x	x	x		x		x						
3	01/09/2015	1	1	x	x	x	x	x	x	x	x	x		x		x						
4	17/11/2015	2	2	x	x	x	x	x	x	x	x	x		x		x						
5	12/01/2016	2	2	x	x	Patient died before T4 immunotherapy																
6	15/03/2016	2	2	x	x	x	x	x	x	x	x	x		*								
7	03/05/2016	2	2	x	x	x	x	x	x			x		x		**						
8	12/07/2016	3	3	x	x	x	x	x	x	x	x	x		x		x						
9	23/08/2016	3	3	x	x	x	x	x	x	x	x	x		x***		x						
10	25/10/2016	3	3	x	x	x	x	x	x	x	x	x		x		x						
11	17/01/2017	4	4	x	x	x	x	x	x	x	x	x		x		x						
12	28/02/2017			x		Patient ineligible																
13	21/03/2017	4	4	x	x	x	x	x	x	x	x	x		x		x						
14	27/06/2017	4	4	x	x	x	x	x	x	x	x	x		x		x						
15	08/09/2017	5	5	x	x	x	x	x	x	x	x	x		x		x						
16	25/05/2018	5	5	x	x	x	x	x	x	x	x	x		x		x						
17	07/09/2018	5	5	x	x	x	x	x	x	x	x	x		x		x						

Patient ID	Consent Date	Intended Cohort	Actual Cohort	V1	V2	V3	V4	V5	V6	V7	V8	V9	V9a	V10	V10a	V11	V12	V13	V14	V15	V16	V17
18	12/11/2019	6	6	x	X (3 days)		x	x	x	x	x	x	x	x	x	x						
19	18/05/2021	6	6	x	X (3 days)		x	x	x	x	x	x	x	x	x	x						
20	15/06/2021	6	6	x	X (3 days)		x	x	x	x	x	x	x	x	x	x						
21	29/03/2022	7	7		X (3 days)		x	x	x	x	x			x		x	x	x	x	x		

* Patient 06 died on Day 29, hence did not attend for V10 follow-up. But AE information has been collected.

** Patient 07 only partially attended the V11 follow-up visit (for imaging and echo scans, but not blood tests or clinical exam)

*** Patient 09 attended V10 at 25 days (not 29 days) due to holiday

Long term follow up and trial completion
Trial completion occurred after database lock (following completion of monitoring of the last patient undergoing the trial) and completion of analysis of laboratory samples collected from patients. Safety follow-up was undertaken for least two years as per pharmacovigilance regulations. Patients were followed up periodically after treatment for general health and survival by direct patient contact or telephoning of general practitioner. All subjects treated in this clinical trial are now deceased.

Translational studies

Multiplex cytokine bead array

Serum levels of a custom panel of 29 cytokines were measured before, and at 30 minutes and 1, 4, 24, 48-96 and 120-168 hours post T4 immunotherapy. Frozen sera were thawed, vortexed, centrifuged at 1000 x g for 10 minutes and analysed undiluted using a ProcartaPlex Immunoassay Kit (ThermoFisher), according to manufacturer's instructions. Data were analysed using a Luminex xMAP Intelliflex (ThermoFisher).

Flow cytometric assays

All analysis was performed on a Fortessa™ flow cytometer (BD Biosciences, Franklin Lakes NJ) using FACSDIVA™ or FlowJo™ v10 software (both BD Biosciences).

1. Immunophenotyping of T4 immunotherapy product

Immunophenotyping was performed for information only on 9 batches of T4 immunotherapy using the following panels. Compensation beads were prepared fresh for every run and used as per manufacturer's instructions, including fluorescence minus one controls. All incubations were performed on ice in the dark.

Drug substance (2mL) was collected, centrifuged (200g, 5 minutes) and re-suspended at $10x10^6$/ml in PBS. For antibody panel 1, 100µL of cells ($1x10^6$) were stained with 3µL biotinylated anti-hEGF antibody (R&D systems, code BAF236, Minneapolis, MN). Phosphate buffered saline (PBS; 2mL) was then added, and the cells were washed (200g for 3 minutes). After discarding supernatant, cells were resuspended in 100µL PBS and incubated with 1µL Streptavidin-PE (ThermoFisher, code S866) for 15 minutes. Wash was repeated and cells were resuspended in 100µL PBS with 3µL of each antibody (all from BioLegend, San Diego, CA; antiCD3 APC-Cy7 [HIT3a], antiCD4 AF700 [RPA-T4], antiCD8 FITC [SK1], antiCD62L PerCPCy5.5 [DREG-56], antiCCR7 BV605 [GOG3H7], antiCD57 APC [QA17A04], antiCD28 BV421 [CD28.2], antiCD45RO PE-Cy7 [UCHL1]) before being incubated for 25 minutes. Cells were washed and resuspended in 300µL PBS for analysis. For panel 2, cells were similarly stained with anti-hEGF and Streptavidin-PE followed by 3µL of each antibody (all from BioLegend; antiCD3 APC-Cy7 [HIT3a], antiCD4 AF700 [RPA-T4], antiCD16 FITC [3G8], antiCD25 PE [BC96], antiCD45RO PerCPCy5.5 [UCHL1], antiCD45RA BV605 [HI100], antiPD-1 APC [EH12.2H7], antiCD19 BV421 [HIB19], antiNKG2D PE-Cy7 [1D11]), incubated for 25 minutes, washed and resuspended in 300µL PBS. For the unstained control, 50µL of cells ($5x10^5$) were taken washed once in PBS as per the above conditions, before being resuspended in 300µL PBS for analysis.

Additional immunophenotyping was performed on a subset of T4 batches where sufficient retention samples were available. In these subjects, best RECIST response following T4 immunotherapy (without lymphodepletion or nivolumab) was stable disease (n=8) or progressive disease (n=3).

Cryopreserved cells were thawed rapidly at 37°C, then washed with 1x PBS. Cells were surface stained with the following antibodies in 1x PBS for 30 minutes at 4°C (protected from light): CD4-APC-Cy7[RPA-T4), CD8-Alexa Fluor 700[SK1], PD-1-PerCP-CY5.5[EH12.2H7], CD71-Per-CP-Cy7[CY1G4], CD39-FITC[A1] (all BioLegend) and CD98-PE-Vio770[REA387] (Miltenyi Biotec). CAR was stained with a biotinylated anti-hEGF antibody (R&D systems, code BAF236) followed by streptavidin-BV510. Cells were stained concurrently with 1µL/mL Live/Dead dye (ThermoFisher). Cells were washed with 1x PBS, then fixed with FOXP3 fix buffer (ThermoFisher) for 20 minutes at room temperature (protected from light). Cells were stained for intracellular markers using the following antibodies in FOXP3 perm buffer: Glut1-APC (Abcam, Cambridge, UK), PGC1a-rabbit (Novus Biologicals, Bio-Techne) followed by APC-rabbit-IgG (BioLegend). Donor cells from a single healthy donor were analyzed in each run as a control for consistency.

2. CAR-T cell effector molecule production assay

Functional analysis was also performed on T4 retention samples described in the previous paragraph. Thawed CAR T-cells were resuspended at 1 million/mL in RPMI 1640 medium (ThermoFisher) supplemented with 10mM glucose, 0.1 mM non-essential amino acids, 10 mM HEPES buffer, 1 mM sodium-pyruvate and 10% fetal calf serum (FCS; all Sigma-Aldrich). Brefeldin A (1 µg/mL; BioLegend) was added to block export of effector proteins. Cells were either rested or activated with plate bound CD3 (OKT3 1µg/mL) and CD28 (CD28.2, 0.5µg/mL) antibodies (all BioLegend) in a 96 well plate in an incubator overnight at 37°C, 5% CO_2. After washing in 1x PBS, cells were surface stained as before with: biotinylated anti-hEGF antibody (R&D systems, code BAF236), Streptavidin-BV510, CD4-APC-Cy7, CD8-Alexa Fluor 700, and live/dead dye. After fixation with Cytofix™ (BD Biosciences) for 20 minutes at 4°C, cells were stained with Granzyme-B-AF488 (GRZB; BioLegend), tumour necrosis factor (TNF)-α-APC and IFN-γ-BV421 (all BD Biosciences) in permeabilisation buffer (1% FCS, 0.1% saponin in 1x PBS) for 30 minutes.

3. Flow cytometric monitoring of circulating $T4^+$ CAR T-cells

EDTA anticoagulated blood (50µL) was added to a FACS tube to which 4µL biotinylated anti-hEGF antibody (R&D systems, code BAF236) was added/ mixed for 15 minutes. Next, 1µL of Streptavidin-PE (ThermoFisher, code S866) was added/ mixed for 15 minutes. Then, 450µL of red blood cell lysis buffer (Biolegend 420301) was added, mixed and incubated for 15 minutes. Pre-mixed Countbright absolute counting beads (50µL; C36950, Invitrogen, Waltham, MA) were then added. Comparison was made to a positive control ($T4^+$ T-cells) and a negative control in which primary antibody had been omitted.

<u>MAGE-A3/A4 Interferon-γ ELISPOT Assay</u>

Analysis was performed using a human interferon (IFN)-*γ* T-cell Elispot assay (U-CyTech, Utrecht, The Netherlands). Thawed peripheral blood mononuclear cells (PBMC) were resuspended in RPMI (ThermoFisher Scientific)+10% AB serum (Sigma-Aldrich, Gillingham, UK). Triplicates of $1x10^6$ PBMC per well were co-cultured with peptide pools of MAGE-A3/A4 (Miltenyi Biotec, Bergisch Gladbach, Germany) at 2µg, 1µg and 0.5µg of each peptide/mL. As positive controls, 1 or 5µL CEF (cytomegalovirus, Epstein Barr virus, influenza) viral peptide pool (Mabtech, Stockholm, Sweden) and 1 or 0.1µL of Infanrix -IPV+ Hib (GlaxoSmithKline UK Ltd, Brentford, UK) were used. Cultures (including unstimulated controls) were incubated overnight at 37°C. Cells were harvested, washed in RPMI +10% AB serum and transferred to an ELISPOT plate coated with the capture antibody. The

plate was incubated at 37°C for 20-22 hours and cells were removed by washing as per manufacturer's instructions. Areas of cytokine capture were detected by addition of the biotinylated detection antibody. After washing, j-labelled anti-biotin antibody (GABA) was added to each well and incubated for 1 hour at 37°C. Next, freshly prepared Activator I/II solution was added to each well and incubated at room temperature in the dark. Spot development was monitored every 5 minutes and the reaction was stopped once clear spots were visible by rinsing the wells with demineralized water. The plate was air dried at room temperature and spots counted using an immunospot analyzer (Bioreader®/EazyReader, Miami, FL). Mean values in test wells were compared with values in unstimulated control wells to calculate a stimulation index.

Quantitative polymerase chain reaction (PCR) analysis of circulating $T4^+$ T-cells

Peripheral blood samples were collected in EDTA tubes and genomic (g)DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Venlo, The Netherlands), according to manufacturer's instructions. A standard curve for transcript copy number was established by the amplification of linearised SFG T4, serially diluted from 10^7 to 10^2 copies of plasmid. The number of transgene copies per µg gDNA was determined using an Applied Biosystems 7500 Fast real-time PCR instrument (ThermoFisher) using labelled probes and primers, as described.

Analysis of core tumour biopsies

Core tumour biopsies were obtained from 4 patients prior to and after CAR T-cell administration. Specimens were fixed in 10% (v/v) buffered formal saline and embedded in paraffin wax. Following review of the diagnostic hematoxylin and eosin section by a specialist head and neck pathologist for tissue adequacy, 4µM paraffin sections were routinely prepared. EGF receptor extracellular domain (Clone 3C6, Catalog No. 790-2988, Roche Tissue Diagnostics, Oro Valley AZ), EGF receptor intracellular domain (Clone 5B7, 790-4347, Roche Tissue Diagnostics, immunohistochemical staining was undertaken using prediluted proprietary kits (Ventana Medical Systems, Roche Tissue Diagnostics) on a Ventana Benchmark Autostainer (Ventana Medical Systems, Roche Tissue Diagnostics) according to manufacturer's instructions. EGF receptor expression was quantified using the H-score method. CD3 and cleaved caspase 3 (Cell Signalling Technologies UK) were detected similarly. CAR T-cell presence was analysed using mRNA in situ hybridization according to manufacturer instructions using a 2.5HD Assay-Brown (ACD, Bio-Techne, Abingdon, UK) and custom-designed probe against the SFG retroviral vector. Positive and negative control probes used were Homo Sapiens Ubiquitin C and dapB (dihydrodipicolinate reductase gene of *Bacillus subtilis*; both ACD, Bio-Techne), respectively. Target retrieval time was optimised to 12 minutes and protease digestion to 30 minutes. Sections were counterstained with Hematoxylin QS (H-3404-100, Vector Laboratories, Newark, CA) and slides were mounted in VectaMount Permanent Mounting Medium (H-5000-60, Vector Laboratories). Cleaved caspase 3 and CD3

Trial Medication

Investigational medicinal products evaluated in this clinical trial were T4 immunotherapy, cyclophosphamide, fludarabine and nivolumab.

Dosing Regimen
See **Table 1** above.

13. Number of patients (planned and analysed)

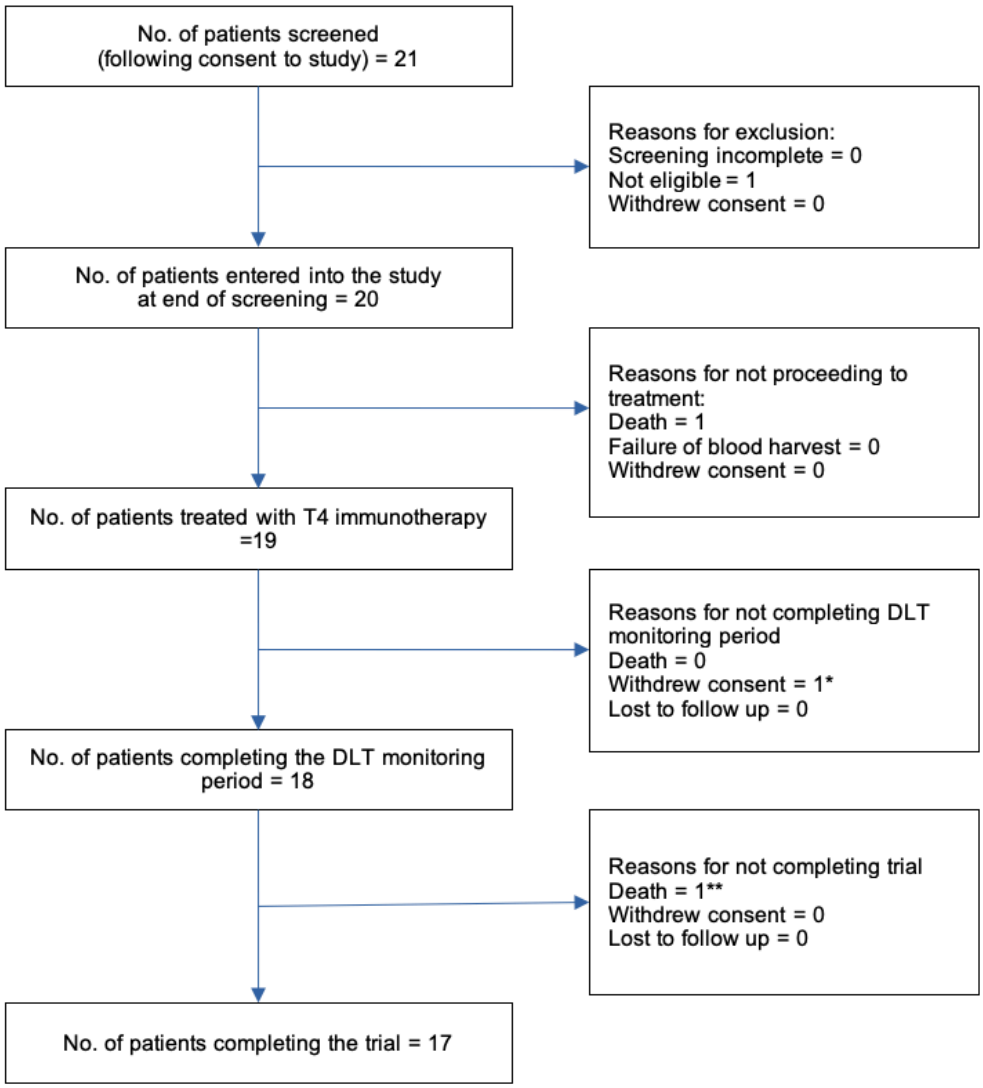
13.1 Planned

When originally planned, the expected sample size in this study was 30. This was later revised to 22-29 subjects.

13.2 Analysed

Allocation of screened patients across the entire study is shown in CONSORT (consolidated standards of reporting trials) diagram shown in **Figure 1**.

Figure 1. Progression of screened participants in T4 clinical trial



*Patient 21 (cohort 7) withdrawn after Day 56 due to disease progression

**Patient 06 (cohort 2) died on Day 29 not collected, but AE information obtained from hospice including blood tests

Twenty one subjects consented to participate and were enrolled in the T4 immunotherapy Phase 1 clinical trial between June 2015 and March 2022. Recruitment timelines are summarised in **Table 4**.

Table 4. Summary of recruitment by month

Month	Screened†	Enrolled††	Cohort (actual)
2015:			
June	1	1	Cohort 1
July			
August	1	1	Cohort 1
September	1	1	Cohort 1
October			
November	1	1	Cohort 2
December			
2016:			
January	1	1	Not applicable as not treated*
February			
March	1	1	Cohort 2
April			
May	1	1	Cohort 2
June			
July	1	1	Cohort 3
August	1	1	Cohort 3
September			
October	1	1	Cohort 3
November			
December			
2017:			
January	1	1	Cohort 4
February	1	Not eligible**	Not applicable
March	1		
April		1	Cohort 4
May			
June	1	1	Cohort 4
July			
August			
September	1	1	Cohort 5
October			
November			
December			
2018:			
January			

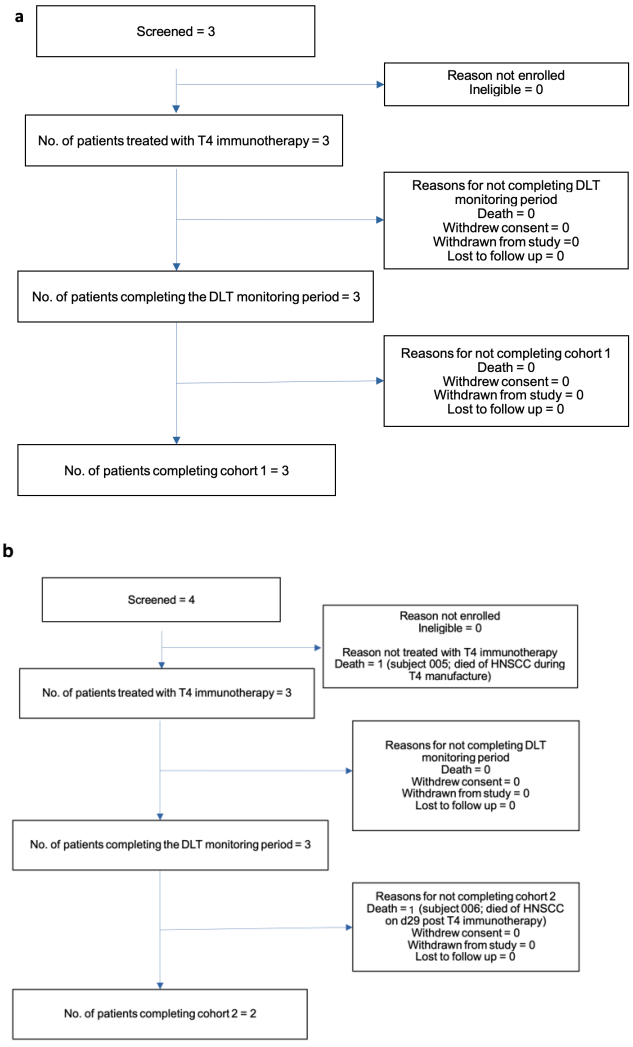
February			
March			
April			
May	1		
June		1	Cohort 5
July			
August			
September	1	1	Cohort 5
October			
November			
December			
2019:			
January			
February			
March			
April			
May			
June			
July			
August			
September			
October			
November	1	1	Cohort 6
December			
2020[¥]			
2021			
January			
February			
March			
April			
May	1	1	Cohort 6
June	1	1	Cohort 6
July			
August			
September			
October			
November			
December			
2022			
January			

February			
March	1	1	Cohort 7
Total	**21**	**20**	

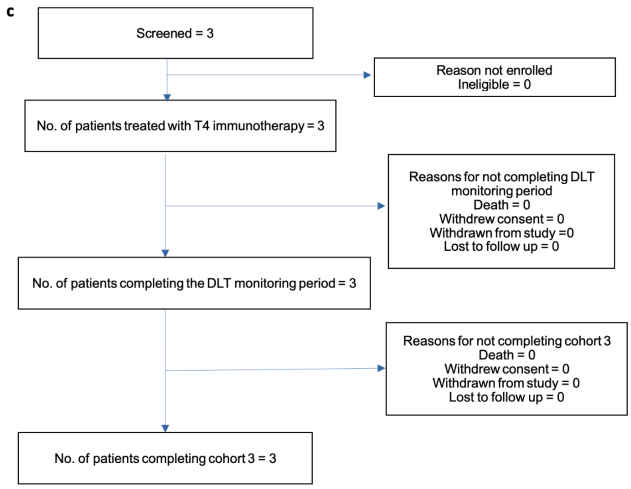
† Screened: month of consent to screening. †† Enrolled: month confirmed eligible and entered into the study.
¥ No recruitment in 2020 due to COVID-19.
*Patient 05 enrolled into cohort 2 (intended) but died before treatment**.** **Patient 12 found to be ineligible during screening.

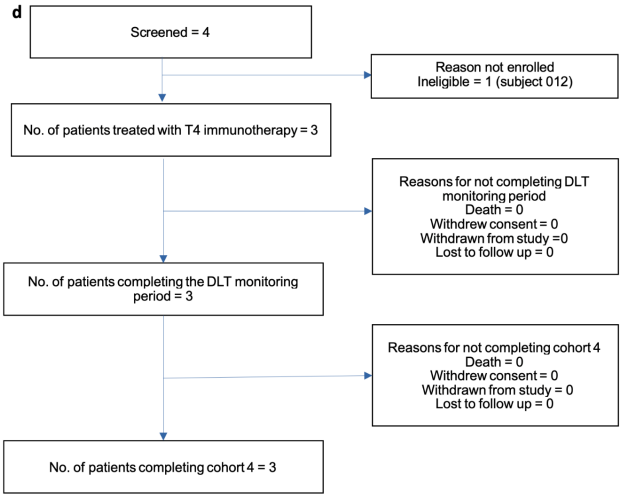
Recruitment by cohorts 1-7 is summarised in **Figure 2a-g** respectively. Nineteen subjects were treated across seven dose cohorts ranging from $1x10^7$ to $1x10^9$ $T4^+$ autologous cells. Two subjects did not receive T4 immunotherapy. One subject in cohort 2 (005) died due to progressive SCCHN during the 14 day manufacturing process and a second subject in cohort 4 (012) failed screening due to tumour proximity to major vessels.

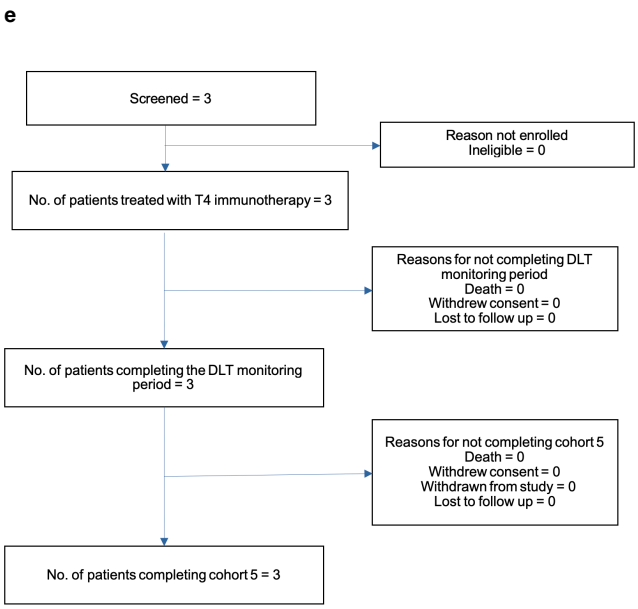
Figure 2. Screened participants in T4 clinical trial. (a) Cohort 1; (b) Cohort 2; (c) Cohort 3; (d) Cohort 4; (e) Cohort 5; (f) Cohort 6 and (g) Cohort 7.



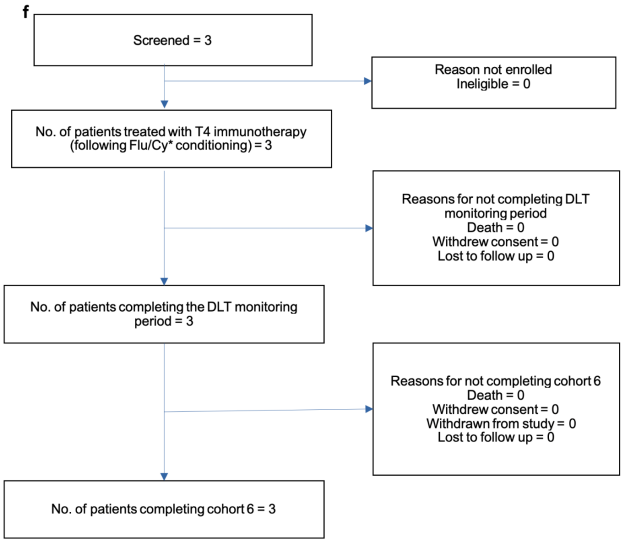
* Patient 6 (cohort 2) dies on day 29 before final visit


c
Screened = 3
Reason not enrolled
Ineligible = 0
No. of patients treated with T4 immunotherapy = 3
Reasons for not completing DLT monitoring period
Death = 0
Withdrew consent = 0
Withdrawn from study =0
Lost to follow up = 0
No. of patients completing the DLT monitoring period = 3
Reasons for not completing cohort 3
Death = 0
Withdrew consent = 0
Withdrawn from study = 0
Lost to follow up = 0
No. of patients completing cohort 3 = 3

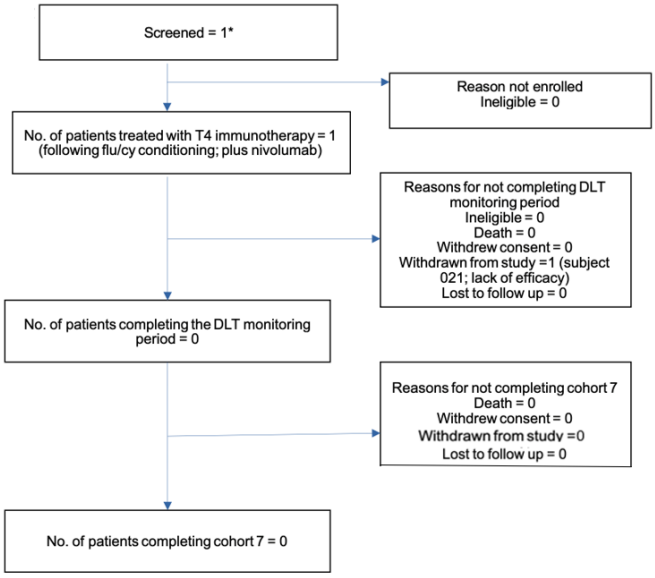

d
Screened = 4
Reason not enrolled
Ineligible = 1 (subject 012)
No. of patients treated with T4 immunotherapy = 3
Reasons for not completing DLT monitoring period
Death = 0
Withdrew consent = 0
Withdrawn from study =0
Lost to follow up = 0
No. of patients completing the DLT monitoring period = 3
Reasons for not completing cohort 4
Death = 0
Withdrew consent = 0
Withdrawn from study = 0
Lost to follow up = 0
No. of patients completing cohort 4 = 3


e
Screened = 3
Reason not enrolled
Ineligible = 0
No. of patients treated with T4 immunotherapy = 3
Reasons for not completing DLT monitoring period
Death = 0
Withdrew consent = 0
Lost to follow up = 0
No. of patients completing the DLT monitoring period = 3
Reasons for not completing cohort 5
Death = 0
Withdrew consent = 0
Withdrawn from study = 0
Lost to follow up = 0
No. of patients completing cohort 5 = 3

f


Screened = 3
Reason not enrolled
Ineligible = 0
No. of patients treated with T4 immunotherapy (following Flu/Cy* conditioning) = 3
Reasons for not completing DLT monitoring period
Death = 0
Withdrew consent = 0
Lost to follow up = 0
No. of patients completing the DLT monitoring period = 3
Reasons for not completing cohort 6
Death = 0
Withdrew consent = 0
Withdrawn from study = 0
Lost to follow up = 0
No. of patients completing cohort 6 = 3

g


Screened = 1*
Reason not enrolled
Ineligible = 0
No. of patients treated with T4 immunotherapy = 1 (following flu/cy conditioning; plus nivolumab)
Reasons for not completing DLT monitoring period
Ineligible = 0
Death = 0
Withdrew consent = 0
Withdrawn from study = 1 (subject 021; lack of efficacy)
Lost to follow up = 0
No. of patients completing the DLT monitoring period = 0
Reasons for not completing cohort 7
Death = 0
Withdrew consent = 0
Withdrawn from study =0
Lost to follow up = 0
No. of patients completing cohort 7 = 0

Disease history of screened patients is shown in **Table 5**. Baseline investigation results in screened patients are shown in **Table** 6. Ages of treated subjects was 39-82 years and PS ranged from 0-2. Subjects had received up to 9 lines of prior treatment with surgery, radiotherapy, and chemotherapy, in line with current clinical practice. Further details are provided in section 1.3 of the final analysis provided by the trial statisticians.

Table 5. Disease history of screened patients

Patient ID	Age	Sex	Primary diagnosis	Previous treatment received
Patient 01	56	Male	Nasal Cavity	Surgical procedures: n=2 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=1 Courses of other disease modifying treatment: n=1
Patient 02	59	Female	Oral Cavity (Tongue)	Surgical procedures: n=1 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=1 Courses of other disease modifying treatment: n=1
Patient 03	59	Male	Oral Cavity (Tongue)	Surgical procedures: n=1 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=1 Courses of other disease modifying treatment: n=1
Patient 04	57	Female	Oral Cavity (Tongue)	Surgical procedures: n=4 Courses of radiotherapy: n=2 Discrete courses of chemotherapy: n=2 Courses of other disease modifying treatment: n=1
Patient 05	50	Male	Oral Cavity (Tongue)	Surgical procedures: n=0 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=1 Courses of other disease modifying treatment: n=1
Patient 06	55	Male	Oral Cavity (Tongue)	Surgical procedures: n=1 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=2 Courses of other disease modifying treatment: n=1
Patient 07	78	Male	Pharynx	Surgical procedures: n=0 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=0 Courses of other disease modifying treatment: n=1
Patient 08	62	Male	Squamous cell carcinoma of the right neck	Surgical procedures: n=3 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=4 Courses of other disease modifying treatment: n=1
Patient 09	81	Male	Oral Cavity (Other: unspecified)	Surgical procedures: n=4 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=0 Courses of other disease modifying treatment: n=1
Patient 10	64	Male	Pharynx	Surgical procedures: n=1 Courses of radiotherapy: n=1

Patient ID	Age	Sex	Primary diagnosis	Previous treatment received
				Discrete courses of chemotherapy: n=2 Courses of other disease modifying treatment: n=1
Patient 11	61	Female	Oral Cavity (Other: unspecified)	Surgical procedures: n=0 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=0 Courses of other disease modifying treatment: n=1
Patient 12	36	Male	Oral Cavity (Tongue)	Surgical procedures: n=1 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=2 Courses of other disease modifying treatment: n=1
Patient 13	66	Male	Pharynx	Surgical procedures: n=1 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=1 Courses of other disease modifying treatment: n=1
Patient 14	82	Male	Oral Cavity (Tongue)	Surgical procedures: n=2 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=0 Courses of other disease modifying treatment: n=1
Patient 15	39	Male	Pharynx	Surgical procedures: n=1 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=2 Courses of other disease modifying treatment: n=1
Patient 16	57	Female	Oral Cavity (Other: unspecified)	Surgical procedures: n=2 Courses of radiotherapy: n=2 Discrete courses of chemotherapy: n=1 Courses of other disease modifying treatment: n=1
Patient 17	61	Male	Pharynx	Surgical procedures: n=0 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=3 Courses of other disease modifying treatment: n=1
Patient 18	46	Male	Oral Cavity (Other: unspecified)	Surgical procedures: n=0 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=2 Courses of other disease modifying treatment: n=1
Patient 19	68	Male	Pharynx	Surgical procedures: n=1 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=1 Courses of other disease modifying treatment: n=1
Patient 20	63	Male	Oral Cavity (Other: unspecified)	Surgical procedures: n=2 Courses of radiotherapy: n=2 Discrete courses of chemotherapy: n=2 Courses of other disease modifying treatment: n=1
Patient 21	71	Male	Oral Cavity (Palate)	Surgical procedures: n=2 Courses of radiotherapy: n=1 Discrete courses of chemotherapy: n=1 Courses of other disease modifying treatment: n=1

Table 6. Baseline investigation results in screened patients.

Patient ID	Full blood count††	CT Tumour imaging results (Summary)	ECG	Echocardiogram
Patient 01	Hb=119 WCC=8.6 Lymph=0.9 Plts=315	1.4 x 1.3cm soft tissue right nasolabial fold 3.5cm right facial nodal mass 1.9 x 1.3cm Pleural nodule PACS report indicates small pleural effusion. There is a 24 x 15mm soft tissue nodule in left chest wall in keeping with a metastasis.	normal	normal
Patient 02	Hb=109 WCC=8 Lymph=1 Plts=289	Target lesion 1 -right base of tongue lesion-3 cm Non-target disease: Abnormal soft tissue thickening related to the left vallecula and oropharynx as described. Left axillary FDG avid node - not confirmed as metastatic therefore NOT included. Baseline RECIST = 3 cm	Normal	Normal LV cavity size and function. Estimated visula EF of ~60%.
Patient 03	Hb=134 WCC=10.8 Lymph=1.3 Plts=309	Baseline Marker lesion tumour in the floor of the mouth 30mm Non marker lesion: subclavicular lymph node 10mm	Normal	Normal LV cavity size with normal wall thickness, Good left ventricular systolic function. Estimated EF by Simpsons biplane is 59%.
Patient 04	Hb=140 WCC=5.9 Lymph=0.5 Plts=240	Marker lesion 1 - anterior neck subcutaneous metastasis Non-marker lesions, small nodules within buccal soft tissues and other neck subcutaneous nodules.	Normal Sinus rhythm	Normal LV cavity size and wall thickness. No regional wall motion abnormalities. Normal systolic function with a visually estimated EF ~ 55-60%. Normal RV cavity size and function. Small pericardial effucion (max 1.4cm in systole) with no obvious evidence to suggest heamodunamic compromise.
Patient 05	Hb=123 WCC=8.7	T3 oral right tongue tumour. Diffuse thickening of supra glottis and glottis. Paratracheal LN 1.8cm	normal	Booked but patient died 23/1/16 before administration of

Patient ID	Full blood count††	CT Tumour imaging results (Summary)	ECG	Echocardiogram
	Lymph=0.7 Plts=345			IMP and before ECHO visit
Patient 06	Hb=110 WCC=7.6 Lymph=0.6 Plts=165	Marker lesions 1-Left Submandibular lesion Non Marker lesions Right level II adenopathy	Sinus bradycardia Otherwise normal ECG	Normal left ventricular size and systolic function EF 50-55%
Patient 07	Hb=113 WCC=8.5 Lymph=0.8 Plts=252	Marker lesion 1 - right tongue base/tonsil Marker lesion 2 - right level II neck node, adjacent to primary	Normal Sinus rhythm Normal ECG	Normal LV cavity size and function Estimated visula EF of ~60% Normal RV size and function
Patient 08	Hb=110 WCC=4.4 Lymph=1 Plts=211	Baseline Resist 1.1 Single phase post contrast scans performed through the chest abdomen and pelvis. CT neck and brain included. Right carotid stent noted. There is a benign cyst in the left lobe of thyroid. No hilar or mediastinal lymphadenopathy is seen. Normal pulmonary arteries. Elevation the right hemidiaphragm with some right lower lobe consolidation. No parenchymal lung metastases are identified. The liver, gallbladder and biliary tree appear normal. Normal spleen. The right adrenal gland is mildly enlarged in keeping with a benign adenoma. Normal kidneys and retroperitoneum. No para-aortic lymphadenopathy is seen. No pelvic lymph nodes are demonstrated. No metastatic bone disease is seen. CT brain. Post contrast scans performed. No intracranial abnormality identified. No extracerebral collection is noted. No bony disease is seen. The paranasal sinuses appear normal. CT neck. Right carotid stent noted. There is a large exophytic soft tissue mass on the right side of the neck extending from the ramus of the mandible down to the thyroid cartilage. Maximum transverse diameter (image 85/series 5) measures 5 x 3.9 cm, previously measuring up to 33 mm. The soft tissue mass extends posteriorly onto the vertebral bodies and medially onto the thyroid cartilage. There is a enlarged submental lymph node measuring 10 mm (image 84/series 5) which was not PET avid, and has not progressed significantly over the past 10 weeks, but should be observed on followup imaging. Opinion. Large locally recurrent soft tissue mass on the right side of the neck. Single marker lesion as recorded above. No non-marker lesions or evidence	Normal sinus rhythm. ECG misplaced. Repeated on 26th July to confirm eligibility. Repeat ECG: Normal sinus rhythm.	Good LV systolic function w/est EF 60-65% midly impaired and dilated RV. Mild disastolic dysfunction. No significant valvular abnormalities

Patient ID	Full blood count††	CT Tumour imaging results (Summary)	ECG	Echocardiogram
		of metastatic disease. Indeterminate sub mental node. Baseline resist 1.1 = 5		
Patient 09	Hb=140 WCC=3.3 Lymph=0.6 Plts=155	Small subcentimetre mediastinal nodes are seen, which do not meet size criteria for significance. No significant axillary adenopathy. Biapical pleural thickening. Patchy atelectasis. Lungs and pleural spaces are otherwise clear. Below the diaphragm, PEG tube noted in situ. Simple liver cyst. Small gallstones in a thin-walled gallbladder. Solid organs otherwise unremarkable. 8 mm rounded short axis upper abdominal node (series 11, image 119) of dubious significance. No significant para-aortic adenopathy. Bony review shows degenerative change.	Not clinically significant	1. Non-dilated LV with low normal LVEF (visual estimate 50-55%) 2. Non-dilated RV with normal long-axis function. Trivial TR, RSVP is not less than 19mmHg + RAP. 3. Normal atria size 4. No significant valvular abnormalities
Patient 10	Hb=117 WCC=4.5 Lymph=0.4 Plts=126	CT Head : Non-contrast study. There is no intracranial haemorrhage or acute large vessel infarct. There is normal grey-white matter differentiation and the ventricles and basal cisterns are preserved. No suspicious osseous lesion seen. Conclusion: No features of intracranial metastatic disease within the limits of the study. CT Neck : Comparison is made to CT dated 5/10/15. There are stable appearances to the abnormal soft tissue involving the soft palate (bilaterally) and extending to the left lateral and left dorsal aspects of the nasopharynx, measuring 4.6 x 2.5cm (s3/130). There is some soft tissue extension along the graft at the level of the oropharynx, also unchanged from previous. There is stable soft tissue extension into the left parapharyngeal space and abutting the left internal carotid artery without encasement. There remains stable attenuation of the the right medial pterygoid plate and sclerosis of the left pterygoid plate. There is no cervical adenopathy. Conclusion: Stable appearances of the soft palate recurrence. Baseline RECIST 1.1: Soft palate lesion = 4.6cm (3/130). CT Thorax & abdo & pelvis with contrast : Comparison is made with the CT dated 5/10/16. There is no mediastinal, hilar or axillary adenopathy. No focal parenchymal lung lesions seen. There are no pleural or pericardial effusions. Imaging of the abdomen has been performed in the urographic phase. The solid abdominal viscera have normal, unenhanced appearance. The unprepared small and large bowel are grossly normal. No abdominal or pelvic adenopathy or free fluid is seen. No	Sinus rhythm	EF 60%

Patient ID	Full blood count††	CT Tumour imaging results (Summary)	ECG	Echocardiogram
		suspicious osseous lesion seen. Conclusion: No thoracic or abdominal metastatic disease demonstrated.		
Patient 11	Hb=127 WCC=6.3 Lymph=0.8 Plts=335	Left level 1b node	NCS	Normal
Patient 12	Hb=93 WCC=7.9 Lymph=0.6 Plts=478	Conclusion: Large heterogeneous enhancing mass in the right subclavicular fossa which may represent necrotic nodal mass and/or collection. Clinical correlation advised. Smaller volume subpectoral, axillary and mediastinal adenopathy. No assessable disease elsewhere in the chest/abdomen. Thrombus in the right subclavian vein and brachiocephalic vein extending into the SVC. Please see separate neck report.	ECG not reviewed	study within normal limits
Patient 13	Hb=102 WCC=15 Lymph=1.4 Plts=427	Clinical History : Clinical Details: Oropharyngeal cancer. Please scan on 18/04/17 as per trial requirements. Screening scan for T4. Infection risk 2: None known Bleep Number: 88103 Clinical Details: Oropharyngeal cancer. Please scan on 18/04/17 as per trial requirements. Screening scan for T4. Infection risk 2: None known Bleep Number: 88103 Clinical Details: Oropharyngeal cancer. Please scan on 18/04/17 as per trial requirements. Screening scan for T4. Please scan full brain for trial purposes Infection risk 2: None known Bleep Number: 88103 CT Head : Pre and postcontrast scans through the brain. There is no pathological contrast enhancement. Normal appearances of the brain parenchyma, ventricles and extra-axial CSF spaces. Conclusion: No evidence of intracranial metastatic disease. AN ADDENDUM HAS BEEN ENTERED AT THE END OF THIS REPORT CT Neck : There is no previous imaging available for comparison. There is a very large heterogeneous mass centered on the oral cavity/tongue base straddling the midline, mainly involving/infiltrating the deep tongue musculature and tongue base. It shows thick irregular peripheral enhancement and large central non-enhancing large non-enhancing areas - the lesion is partly obscured by dental amalgam artefact in the upper portion. Inferiorly, there is extension to the sublingual spaces and extrinsic tongue muscles. It extends to the surgical clips laterally in the left submandibular region and there is some extension to the left lateral oropharyngeal wall.	NCS	NCS

Patient ID	Full blood count††	CT Tumour imaging results (Summary)	ECG	Echocardiogram
		It measures approximately 6.7 x 4.5 cm in long and short axial diameter (measurements obtained on image 87 of series 11 - image saved to PACS). Note is made of previous laryngectomy and reconstruction - the tumour reaching the anterior margin of the neopharynx. There some further irregular thickening of the anterior wall of the neopharynx below this level (just above the level of the tracheostomy). There are a few small rounded right paratracheal and pretracheal lymph nodes, largest of which measures 15 x 11 mm in long/short axial diameter. There is generalised stranding of fat and thickening of fascial planes, likely treatment related. Impression: Large tongue/tongue base mass with thickening of the lateral oropharyngeal wall and anterior wall of the neopharynx, and small lower cervical (level VI/VII) nodes, as described. Comparison with previous imaging is advised. Reviewed in trials meeting: For baseline RECIST v1.1: Marker lesion 1 - 6.7 x 4.5 cm (I 87/s11) Baseline RECIST v1.1 - 6.7 cm CT Thorax & abdo & pelvis with contrast : Post intravenous contrast scan through the chest, abdomen and pelvis. Previous laryngectomy and tracheostomy are noted. There are no enlarged hilar or mediastinal nodes. No suspicious lung abnormality. There are multiple low attenuation foci within the liver measuring up to 1 cm. These are too small to further characterise on CT. Normal appearances of the gallbladder, pancreas, spleen, adrenal glands and kidneys. There is an appropriately sited gastrostomy tube. The unprepared bowel is otherwise unremarkable. There are no enlarged abdominal or pelvic lymph nodes. Previous posterior column lumbar spinal surgery is noted. There is no suspicious skeletal abnormality. Conclusion: No evidence of thoracic metastatic disease. Multiple low attenuation foci within the liver are likely cysts or biliary hamartomas but not fully characterised and metastatic foci cannot be excluded. These should be monitored on followup imaging.		
Patient 14	Hb=133 WCC=10 Lymph=1.2 Plts=452	Clinical Details: Lingual tonsillar cancer. Please scan using recist 1.1, Baseline scan for T4 clinical trial. Please scan by 3/7/17. Please scan whole brain as per protocol Infection risk 2: None known Bleep Number: 2825 Clinical Details: Lingual tonsillar cancer. Please scan using recist 1.1, Baseline scan for T4 clinical trial. Please scan by 3/7/17 Infection risk 2: None known Bleep Number: 2825	NCS	NCS

Patient ID	Full blood count††	CT Tumour imaging results (Summary)	ECG	Echocardiogram
		03/07/2017, 14:52, CT Thorax & abdo & pelvis with contrast 03/07/2017, 14:52, CT Head Scans of the chest, abdomen and pelvis were performed following IV contrast. Post IV contrast imaging of the brain also acquired. CT head: There is no intra or extra-axial collection. No sulcal effacement. Grey-white matter differentiation is preserved. No abnormal enhancement. No midline shift. Basal cisterns are patent. Foramen magnum is capacious. Partial soft tissue thickening within the maxillary sinuses likely inflammatory. No evidence of intracranial disease. CT thorax, abdomen and pelvis: Chest: There is no axillary, hilar or mediastinal nodal enlargement. The oesophagus is somewhat distended and fluid filled above the level of the carina but no focal lesion is demonstrated. Abdomen pelvis: The liver enhances uniformly with no focal lesion. No biliary dilatation. The portal vein is patent. The gallbladder is thin-walled. The spleen is not enlarged. The adrenal glands and pancreas are unremarkable. Both kidneys are nonobstructed with no suspicious lesion. There is a 6 mm non complicating calculus in the inferior pole of the right kidney. No calcification within the left kidney. The non prepared bowel is of normal calibre throughout its length with no obvious intraluminal lesion. Diverticulosis of the sigmoid noted. Imaging of the pelvis is somewhat degraded by artefact from bilateral total hip replacements. Allowing for this, there is no intra-abdominal or pelvic nodal enlargement. No free intra-abdominal fluid. No suspicious lesion in the visualised skeleton. Conclusion: No measurable disease within the chest, abdomen or pelvis. Inflammatory changes at both lung bases. Date Reported: 04/07/2017, 12:18 03/07/2017, 14:52, CT Thorax & abdo & pelvis with contrast 03/07/2017, 14:52, CT Head Scans of the chest, abdomen and pelvis were performed following IV contrast. Post IV contrast imaging of the brain also acquired. CT head: There is no intra or extra-axial collection. No sulcal effacement. Grey-white matter differentiation is preserved. No abnormal enhancement. No midline shift. Basal cisterns are patent. Foramen magnum is capacious. Partial soft tissue thickening within the maxillary sinuses likely inflammatory. No evidence of intracranial disease. CT thorax, abdomen and pelvis: Chest: There is no axillary, hilar or mediastinal nodal enlargement. The oesophagus is somewhat distended and fluid filled above the level of the carina but no focal lesion is		

Patient ID	Full blood count††	CT Tumour imaging results (Summary)	ECG	Echocardiogram
		demonstrated. Abdomen pelvis: The liver enhances uniformly with no focal lesion. No biliary dilatation. The portal vein is patent. The gallbladder is thin-walled. The spleen is not enlarged. The adrenal glands and pancreas are unremarkable. Both kidneys are nonobstructed with no suspicious lesion. There is a 6 mm non complicating calculus in the inferior pole of the right kidney. No calcification within the left kidney. The non prepared bowel is of normal calibre throughout its length with no obvious intraluminal lesion. Diverticulosis of the sigmoid noted. Imaging of the pelvis is somewhat degraded by artefact from bilateral total hip replacements. Allowing for this, there is no intra-abdominal or pelvic nodal enlargement. No free intra-abdominal fluid. No suspicious lesion in the visualised skeleton. Conclusion: No measurable disease within the chest, abdomen or pelvis. Inflammatory changes at both lung bases. Date Reported: 04/07/2017, 12:18 END OF REPORT / 03/07/2017, 14:52, CT Neck Post IV contrast. There is a 3.2 cm soft tissue mass at the left lateral tongue base, series for image 44 [link], for use as single marker lesion. Non marker/non measurable disease: Nil. Baseline RECIST=3.2 cm. The remainder of the upper aerodigestive tract shows no significant abnormality. No enlarged neck nodes. Incidental bilateral para nasal sinus inflammatory disease is identified. No evidence of bone metastases within the skull base or neck.		
Patient 15	Hb=144 WCC=4.8 Lymph=0.7 Plts=303	CT: Extensive, confluent, left deep and superficial neck soft tissue enhancement (involving retropharyngeal, parapharyngeal, prevertebral, perivertebral, carotid, masticator and parotid spaces) with marked associated soft tissue thickening, extending from the periorbital region to the clavicle, with marked buccal engorgement.	NCS	NCS
Patient 16	Hb=127 WCC=5.8 Lymph=0.8 Plts=235	Right sided oral tumour	NCS	Normal LV size and systolic function. Normal RV size and function. Normal size atria. no significant valvular abnormalities
Patient 17	Hb=134 WCC=2.7	Target and non-target lesions in neck and anterior and posterior chest. Left axillary node.	NCS	NCS

Patient ID	Full blood count††	CT Tumour imaging results (Summary)	ECG	Echocardiogram
	Lymph=0.9 Plts=211			
Patient 18	Hb=125 WCC=5.2 Lymph=1.1 Plts=217	Pre and postcontrast acquisition through the brain. Postcontrast acquisitions through the neck, thorax and abdomen. Comparison is made with a previous study dated 22/10/2019. Head: No enhancing intracranial lesions demonstrated. There is minor white matter hypoattenuation within the right frontal lobe, which may be on a small vessel basis. Incidental note is made of cutaneous and subcutaneous thickening posterior to the vertex, which may reflect scarring and would be better correlated clinical examination findings. Neck: There is a centrally necrotic and subtly peripherally enhancing lesion within the right masticator space, which is associated with extensive erosion of the right mandibular body, angle and a ascending ramus with dehiscence of the ID canal. It extends cranially to reach the undersurface of the greater wing of the sphenoid, but there is no transcortical/intracranial extension. Accurate and reproducible measurement of this lesion is likely to be challenging owing to its limited enhancement on the current study. Nevertheless, it measures in the order of 4.2 cm in maximum CC dimension (see image 167 of additional reformatted sagittal stack), which is approximately stable relative to the prior study. Note is again made of anterior bowing of the posterior wall of the maxillary antrum and partial erosion of the lateral pterygoid plate. No new soft tissue within the orbital fissure. The right level 1b node measures 9 mm in short axis (unchanged). No pathologically enlarged cervical lymph nodes are demonstrated elsewhere within the neck. There are diffuse post radiotherapy changes within the pharyngeal and laryngeal mucosa. Thorax: No suspicious pulmonary parenchymal lesions. No thoracic lymphadenopathy. Small hiatus hernia noted. Abdomen and pelvis: Normal appearances of the liver. There is the impression of minor nodular thickening of the wall of the gallbladder in the region of the medial body/neck (image 127 of series 15 unremarkable appearances of the pancreas, spleen, adrenal glands and kidneys. Unremarkable appearances of the unprepared bowel. Percutaneous gastrostomy noted. No abdominal or pelvic lymphadenopathy. Skeletal review: No unequivocal distant skeletal metastases are seen. There is a heterogeneous appearance to the marrow within the iliac bones	NCS, QTcF 409 ms	ECG: SR (70-80 bpm). Normal LV cavity size and ejection fraction. Biplane EF: 59%. No evidence of LVH. No obvious RWMA. Normal LV filling pressures. Mild left atrial dilatation. Normal RV size with good systolic function. No significant intracardiac valve pathology.

Patient ID	Full blood count††	CT Tumour imaging results (Summary)	ECG	Echocardiogram
		bilaterally, but appearances are not significantly changed. This can be kept under review. There is grade 1 retrolisthesis of L5 on S1, which is likely to be on a degenerative basis. Conclusion: 1. Stable appearances relative to the prior imaging. 2. Incidental nodular thickening of the wall of the gallbladder in the region of the medial body/neck. Further assessment with a focus ultrasound study is recommended.		
Patient 19	Hb=131 WCC=5.1 Lymph=0.5 Plts=226	Head: No enhancing intracranial lesions. Neck: The previous left-sided neck dissection and pectoralis major free flap reconstruction is again noted. The lobulated cutaneous lesion involving the skin of the left lower neck superficial to the flap reconstruction demonstrates greater ulceration and measures approximately 3 cm in maximum axial dimension (im 283 of s3). A further tiny nodule is seen inferomedially. At its deep aspect, it demonstrates encroachment and possibly early invasion of the flap. There is a non-specific peripherally enhancing and centrally low density lesion deep to the left upper common carotid artery measuring 0.9 cm in short axis (im 250). There is extensive post treatment change within the neck, with diffuse left cervical or soft tissue oedema as well as distortion of the endolarynx and hypopharynx, in keeping with the site of the previously treated hypopharyngeal primary. No focal lobulated mass is seen in this region. Thorax: There is a necrotic subcarinal node measuring 1 cm in short axis. No pathologically enlarged or appearing nodes are seen elsewhere in the thorax. There is a 3.5 cm subpleural nodule within the right lower lobe (image 221 of thoracic s3), which appears slightly more conspicuous than on the PET CT and was not present on the thoracic CT of 02/07/20. Abdomen: Unremarkable appearances of the imaged abdominal viscera. There is extensive the aortic calcifications. Skeletal review: No suspicious osseous lesions. Conclusion: Slight increase in the maximum dimension of the left-sided left inferior cervical necrotic cutaneous deposit. There is a non-specific partially enhancing retrocarotid lesion on the left, which can be kept under review. The subcarinal node seen on CT has pathological appearances and is compatible with metastatic involvement. The right subpleural pulmonary nodule is suspicious, but nonspecific and can be kept under review	NCS	Suboptimal pictures throughout -> Limited study -> Mainly visual assessment: - LV: Undilated. Impression of mildly increased wall thickness (+/- 12 mm). Visually normal global systolic function (EF estimated @ +/- 60%). Unable to accurately comment on regional wall motion. No signs of LVOT obstruction at rest. - RV: Visually undilated with normal global systolic function. (TAPSE = 2.8 cm) - Atria: Visually normal in size. - Suboptimal/Limited pictures of all valvular structures, but no signs of significant dysfunction was found. - Normal IVC diameter and variation. - No effusions of note were seen.

Patient ID	Full blood count††	CT Tumour imaging results (Summary)	ECG	Echocardiogram
Patient 20	Hb=101 WCC=7.7 Lymph=2.1 Plts=220	22/06/2021, 13:37, CT Neck with contrast Comparison is made with the previous CT dated 8605/21. Postcontrast volumetric acquisition through the head and neck. The previous right-sided segmental mandibulectomy and free flap reconstruction are noted. There is no significant loosening in association with the screw and plate fixation. The left level 1 mass (spanning levels 1A and 1B) measures 4.7 cm in short axis compared with 3.8 cm previously. There is ipsilateral level 2a lymph lymphadenopathy measuring up to 1.5 cm, compared to 0.4 cm previously. There is a suspicious left level 5 node (it is closely associated with inferior branches of the external jugular vein) measuring 0.6 cm in short axis (image 355 of s3). Unchanged appearances of the I have at upper aerodigestive tract. No enhancing intracranial lesions. No suspicious osseous lesions. Conclusion: There has been interval disease progression, notably and left levels 1 and 2, as described above. Measurable disease: Target lesions: Lesion 1 (left level 1 nodal disease): 4.7 cm short axis (im. 318 of se 3) Lesion 2 (left level 2 node): 1.5 cm short axis (im. 280 of se 3) Non measurable disease: Smaller pathological or suspicious nodes at left levels 2 and level 5. No brain metastases are seen. Current RECIST v1.1 = 6.2 cm 22/06/2021, 13:37, CT Thorax & abdomen with contrast Portal venous phase images. Comparison to previous study of 06/05/2021 and 16/12/2020. The partially-imaged left submandibular mass has increased and demonstrates ulceration and contains gas (please see separate CT neck report). Small left supraclavicular fossa and left axillary nodes have increased slightly, a left axillary node measuring up to 7 mm compared to 4 mm. No size significantly enlarged mediastinal, hilar or axillary lymph nodes. The nodule related to the horizontal fissure is unchanged and there are minor dependent changes within the lungs. No suspicious parenchymal lung lesion. No pleural effusion. No central pulmonary embolus. Coronary calcification noted. The right-sided PICC line is appropriately sited. No focal hepatic lesion and normal spleen, pancreas, right adrenal gland both kidneys that are not obstructed. Nodularity of the left adrenal gland is unchanged. There are no enlarged abdominal lymph nodes. Unremarkable appearances of the imaged bowel. No suspicious bone lesion. Interpretation: Marginal	'Normal - Check Potassium' Potassium value was in range.	Summary Normal LV dimensions with good global and regional systolic function. BP MOD LV EF 62 % Normal LV filling pattern with a lateral E/E' ratio of 6 Normal RV size and systolic function.

Patient ID	Full blood count††	CT Tumour imaging results (Summary)	ECG	Echocardiogram
		increase of a small left supraclavicular and left axillary nodes, non-specific and not enlarged by RECIST criteria. No evidence of metastases within the thorax or abdomen. No measurable/non measurable disease within the thorax or abdomen.		
Patient 21	Hb=120 WCC=6 Lymph=0.7 Plts=132	Interval progression of the left maxillary recurrence with skin and oral mucosal ulceration and invasion of the masseter. Stable ipsilateral periparotid, retropharyngeal and lateral neck nodes. No distant disease.	Primary AV block NCS	Normal LV size and systolic function.Biplane EF 62%. No obvious RWMA. Upper limit of normal wall thickness. Normal RV size and systolic function. LA appears dilated. Impression of tricuspid aortic valve with thickened and restricted motion of the cusps. Moderate AS and mild AR (peak vel 3.4m/s, 30mmHg, DVI 0.33, AVA 1,2cm^2). No pericardial effusion

††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 was not performed on any patients

*Patient 12 found to be ineligible during screening; hence ECG was not checked

Reasons for patient withdrawal from the study are listed in **Table 7**.

Table 7. Reasons for patient withdrawal from the study

Patient	Comments
5	This subject died of progressive disease post blood harvest but prior to infusion of T4 immunotherapy.
6	This subject died of progressive disease on day 29 post T4 immunotherapy.
12	This subject failed screening due to a vascular invasive tumour

21	This subject was withdrawn from the study on day 56 post T4 immunotherapy owing to progressive disease.

14. Diagnosis and main criteria for inclusion

Diagnosis: Locally advanced or recurrent SCCHN unsuited to conventional therapy.

Inclusion criteria:
Histologically and/ or cytologically confirmed SCCHN

18 years or older

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

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 cohort 7, as specified in this protocol. In the presence of metastatic disease, recent short-course palliative radiotherapy to non-target site(s) is allowed

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

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

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

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

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

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)
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.

Written informed consent prior to any trial procedure and registration (enrolment; i.e. day of blood harvest)

Exclusion criteria:
The presence of or imminent occurrence of airway obstruction, unless tracheostomy in place

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

History of HIV-1, HIV-2, HTLV-1, HTLV-2, Hepatitis B, Hepatitis C or syphilis infection

Prior splenectomy

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

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

Concurrent use of anticoagulant therapy is not permissible

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

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

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

Nivolumab allergy (Cohorts 7-8 only)

Pregnancy

Breastfeeding

Prior T4 immunotherapy. However, prior immune checkpoint blockade does not preclude participation
With respect to cohorts 6-8 (fludarabine and cyclophosphamide pre-treatment), patients who had received a live vaccine four weeks or fewer before enrolment were ineligible for recruitment to the study. During treatment and for three months after treatment with fludarabine, administration of live vaccines was prohibited

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

15. Test product, dose and mode of administration

Baseline therapy

Patients could not receive any other disease-modifying therapy while on study. Unless prohibited by contra-indications or drug interactions, patients also received 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 was intended to mitigate local inflammatory reactions due to T4 immunotherapy. Subjects who received lymphodepletion also received infection prophylaxis at physician's discretion.

Dates of treatment administration and interval between treatment of sequentially enrolled patients are shown in **Table 8**.

Table 8. Date of treatment of patients*

Patient ID	Cohort (actual)	Date of treatment	Time from treatment of previous patient (days)
Patient 01	1	20/07/2015	
Patient 02	1	04/09/2015	46
Patient 03	1	22/09/2015	18
Patient 04	2	08/12/2015	77
Patient 06	2	05/04/2016	119
Patient 07	2	24/05/2016	49
Patient 08	3	09/08/2016	77
Patient 09	3	27/09/2016	49
Patient 10	3	22/11/2016	56
Patient 11	4	07/02/2017	77
Patient 13	4	09/05/2017	91
Patient 14	4	18/07/2017	70
Patient 15	5	26/09/2017	70
Patient 16	5	03/07/2018	280
Patient 17	5	25/09/2018	84
Patient 18	6	10/12/2019	441
Patient 19	6	14/06/2021	552
Patient 20	6	19/07/2021	35
Patient 21	7	12/04/2022	267

*The protocol (version 10, legend to Figure 3) specified that:

There is a minimum 6 week gap between the treatment of patients 1 and 2 within a cohort. This interval was extended to 8 weeks in cohort 7 and cohort 8.

The minimum interval is 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).

T4 dosing (**Table 9**) and doses of all investigational medicinal products (IMP) administered to each patient (**Table 10**) are shown.

Table 9. T4 dosing and radiotracer administration

Patient ID	Cohort (actual)	CAR+ T-cell dose injected	Total T-cell dose injected	Radiolabelled cells administered (Yes/No)
Patient 01	1	1.0x10*7	1.8x10*7	No
Patient 02	1	1.0x10*7	1.3x10*7	No
Patient 03	1	9.8x10*6	1.3x10*7	No
Patient 04	2	3.0x10*7	4.0x10*7	No
Patient 06	2	2.9x10*7	6.7x10*7	No
Patient 07	2	3.0x10*7	5.9x10*7	No
Patient 08	3	1.0x10*8	1.3x10*8	No
Patient 09	3	9.6 x 10*7	1.8x10*8	No
Patient 10	3	1.0x10*8	1.2x10*8	No
Patient 11	4	2.9x10*8	4.8x10*8	No
Patient 13	4	3x10*8	4.4x10*8	No
Patient 14	4	3x10*8	3.7x10*8	No
Patient 15	5	1x10*9	1.3x10*9	No
Patient 16	5	1x10*9	1.3x10*9	Yes
Patient 17	5	1x10*9	1.3x10*9	No
Patient 18	6	1x10^8	1.22x10^8	No
Patient 19	6	1x10^8	1.24x10^8	No
Patient 20	6	1x10^8	1.23x10^8	No
Patient 21	7	1x10^8	1.40x10^8	No

Table 10. Dose of IMP administered to each study participant

Subject number	CAR T-cell dose	Total T-cell dose	Cyclophosphamide (mg; intravenous)	Fludarabine (mg; intravenous)	Nivolumab (mg; intravenous)
1	10×10^6	18×10^6	-	-	-
2	10×10^6	13×10^6	-	-	-
3	9.8×10^6	13×10^6	-	-	-
4	30×10^6	40×10^6	-	-	-
5	Not treated		-	-	-
6	29×10^6	67×10^6	-	-	-
7	30×10^6	59×10^6	-	-	-
8	100×10^6	130×10^6	-	-	-
9	96×10^6	180×10^6	-	-	-
10	100×10^6	120×10^6	-	-	-

11	290 x 10^6	480 x 10^6	-	-	-
12	Not treated		-	-	-
13	300 x 10^6	440 x 10^6	-	-	-
14	300 x 10^6	370 x 10^6	-	-	-
15	1000 x 10^6	130 x 10^6	-	-	-
16*	1000 x 10^6	130 x 10^6	-	-	-
17	1000 x 10^6	130 x 10^6	-	-	-
18	100 x 10^6	122 x 10^6	480mg x 3 doses	45mg x 3 doses	-
19	100 x 10^6	124 x 10^6	400mg x 3 doses	40mg x 3 doses	-
20	100 x 10^6	123 x 10^6	480mg x 3 doses	50mg x 3 doses	-
21	100 x 10^6	140 x 10^6	480mg x 3 doses	50mg x 3 doses	480mg x 3 doses*

* This subject was withdrawn on day 56 post T4 immunotherapy due to progressive disease and only received 2 doses of nivolumab.

16. Duration of treatment

T4 immunotherapy was administered as a single dose.

Fludarabine and cyclophosphamide (cohorts 6-7) were each administered daily for 3 days, 2-11 days prior to T4 immunotherapy.

Nivolumab (cohort 7 only) was administered every 4 weeks (3 doses only), commencing 1 day prior to T4 immunotherapy.

17. Reference therapy, dose and mode of administration

Not applicable.

18. Criteria for evaluation: Endpoints

18.1 Primary endpoint: Safety

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

18.2 Secondary endpoints

18.2.1 Efficacy

Evidence of response was evaluated by CT imaging at 6 weeks post T4 immunotherapy. In the case of patients in cohorts 7-8, it was intended to repeat imaging at 12 weeks post therapy. Clinical response was assessed according to RECIST 1.1 criteria. All eligible patients were included in the response rate calculation. Objective tumour response and time of progression was measured according to RECIST criteria (47).

18.2.2 Serum cytokine analysis

Cytokine levels were 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.

18.2.3 Intra-tumoural CAR T-cell analysis

Presence of persistent $T4^+$ T-cells in tumour biopsies was measured in some subjects using RNAScope.

18.2.4 CAR T-cell pharmacokinetics

The presence of $T4^+$ T-cells in the circulation was monitored in serial blood samples analysed by flow cytometry and quantitative (q)PCR.

Effects of lymphodepletion with fludarabine and cyclophosphamide or the combination of lymphodepletion and nivolumab on T4 immunotherapy were evaluated by measurement of dose-limiting toxicity and objective tumour response rate.

The effect of T4 immunotherapy upon immune reactivity against endogenous tumour antigens was assessed by measurement of T-cell reactivity by ELISpot against overlapping peptides derived from MAGE-A3 and MAGE-A4 (cohorts 3-5 only).

Evidence of immunomodulation by cyclophosphamide and fludarabine was measured by quantification of circulating numbers of $CD4^+$ $CD25^{HIGH}$ $CD127^{DIM/NEG}$ regulatory T-cells and myeloid-derived suppressor cells in three and two subjects respectively.

It was intended that effects of T4 immunotherapy on gene expression in the tumour microenvironment would be assessed in serial tumour biopsies by RNA sequencing. However, no suitable biopsy samples were collected for this analysis.

Trafficking of T4 immunotherapy was assessed in one subject (016) by SPECT-CT imaging, following administration of an aliquot of T4 immunotherapy that has been radiolabelled with Indium-111.

18.3 Additional translational studies

Not applicable.

19. Statistical Methods

All subjects who consented to treatment are described in this report. All subjects who received T4 immunotherapy completed the minimum 28-day DLT analysis period and were included in the safety

analysis. Descriptive statistics are presented to address the primary and secondary study aims. In exploratory comparative analyses, between-group comparisons were conducted by unpaired *t*-test, and within-group comparisons by paired *t*-test. For analysis of multiple groups, statistical analysis was performed using one-way or two-way ANOVA test, when there were one or two independent variables respectively, followed by Tukey's multiple comparisons test. All statistical analyses were performed using GraphPad Prism, versions 9.5 – 10.1 (GraphPad software, Boston, MA).

Analysis of Efficacy Variables

Serum cytokine levels are presented using median and ranges. Evolution in time is shown.

Presence of $T4^+$ T-cells in tumour biopsies was performed by generating tables of frequency compared with dose level.

Presence of $T4^+$ T-cells in the circulation was performed by generating tables of frequency compared with dose level. Data are also presented as a graph showing median and range at individual timepoints.

Disease status at six weeks after administration of T4 immunotherapy was analysed by generating tables of frequency compared with dose level. Percentage change in RECIST score is also shown. Patients were followed up for survival after leaving the study.

Endogenous T-cell reactivity against MAGE antigens is presented using median and ranges. Evolution in time is shown.

In cohorts who received lymphodepletion with fludarabine and cyclophosphamide, frequency of circulating Treg cells and myeloid-derived suppressor cells is presented prior to and after completion of lymphodepleting chemotherapy. Evolution in time is shown.

Toxic effects of nivolumab and/ or lymphodepleting chemotherapy, in conjunction with T4 immunotherapy was analysed by tables of frequency compared with dose level. All other documented toxicity of any grade according to NCI CTCAE, Current Version was similarly analysed. Toxic side effects of all grades are given by grade and dose level.

RNA-seq analysis was written into the protocol but was not undertaken on any samples.

Trafficking of T4 immunotherapy: Signal intensity within the tumour and at other sites is presented in a descriptive manner, based upon the reports of the nuclear medicine physician/ radiologist for each scan.

Analysis of Safety Variables

The presence of DLT was analysed by tables of frequency compared with dose level. All other documented toxicity of any grade according to NCI CTCAE, Current Version were similarly analysed. Toxic side effects of all grades are given by grade and dose level.

20. Changes in the Trial Plan

These are summarised in **Table 11.**

Table 11. Amendments to the clinical trial protocol

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
10.1	May 2024	Updated sponsor contact, personnel and CI signature page. Reduction in blood volumes taken for circulating T1E28z+ cell analysis (exploratory assays). Change in reporting responsibilities of SUSARs: KHP CTO to report all SUSARs to MHRA and ethics committee. Minor administrative correction – addition of cohort 8 details to some sections.

20.1 Protocol Deviations

There were no serious breaches or major protocol deviations in the trial.

21. Summary – Conclusions

21.1 Demographic data

The following tables summarise the demographics of the study population.

Table 12. Age and sex distribution of enrolled subjects

Number of Subjects			
Age (years)	**Male**	**Female**	**Total**
Pre-term new-born infants (<37 weeks)	0	0	0
New-borns (0-27 days)	0	0	0
Infants and toddlers (28 days – 23 months)	0	0	0

Children (2-11 years)	0	0	0
Adolescents (12-17 years)	0	0	0
Adults (18-64 years)	10	4	14
From 65-84 years	6	0	6
85 years and over	0	0	0
Total	**16**	**4**	**20**

Ethnicity of the study population was not recorded

21.2 Primary outcome

No DLTs were induced by T4 immunotherapy in this clinical trial. Data are summarised in **Table 13**.

Table 13. 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	1	No*	-	-	-	-	-
Patient 02	1	No	-	-	-	-	-
Patient 03	1	No	-	-	-	-	-
Patient 04	2	No	-	-	-	-	-
Patient 06	2	No	-	-	-	-	-
Patient 07	2	No	-	-	-	-	-
Patient 08	3	No	-	-	-	-	-
Patient 09	3	No	-	-	-	-	-
Patient 10	3	No	-	-	-	-	-
Patient 11	4	No	-	-	-	-	-
Patient 13	4	No	-	-	-	-	-
Patient 14	4	No	-	-	-	-	-
Patient 15	5	No	-	-	-	-	-
Patient 16	5	No	-	-	-	-	-
Patient 17	5	No	-	-	-	-	-
Patient 18	6	No	-	-	-	-	-
Patient 19	6	No	-	-	-	-	-
Patient 20	6	No	-	-	-	-	-
Patient 21	7*	-**	-	-	-	-	-

*A DLT was initially recorded for Patient 01 but subsequently downgraded from SUSAR to IME as deemed not 'likely' to be related to T4, and hence not a DLT. See minutes of the unscheduled meeting of the T4 Trial Steering Committee on 3 September 2015.** Patient 21 did not complete the DLT period as withdrawn early due to disease progression.

Follow up summary and decision of the TSC/ DMC to recommend dose escalation/ commencement of recruitment to the next cohort following completion of each cohort is summarized in **Table 14**.

Table 14. Cohort progression and dose escalation.

Cohort (actual)	Number of patients treated	Number of DLTs	Number withdrawn or lost to follow-up (reasons other than DLT)	Decision to escalate*	Date of TSC/ DMC meeting
1	3	0	0**	Yes	13/11/15
2	3	0	1***	Yes	13/06/16
3	3	0	0	Yes	17/01/17
4	3	0	0	Yes	07/09/17
5	3	0	0	N/A*****	21/11/2018
6	3	0	0	N/A*****	5/10/2021
7	1	0	1****	N/A*****	11/07/2022
8	0	0	N/A	N/A	N/A***

* 'Decision to escalate' and 'Date of decision' are recorded and signed off at the joint (TSC / DMC meetings, as part of the TSC charter. ** A DLT was initially recorded for Patient 01 but subsequently downgraded from SUSAR to a not related IME (important medical event) as deemed not 'likely' to be related to T4, and hence not a DLT. See minutes of the unscheduled meeting of the T4 TSC/ DMC on 3 September 2015.*** Patient 06 died on Day 29, hence follow-up data for Day 29 not collected, but AE information obtained. **** Patient 21 was withdrawn from the study on day 56 due to lack of efficacy. ***** Cohort 5 received the maximum planned study dose. The TSC made a recommendation to continue the study with an amended protocol through cohorts 6 and 7 (see Protocol 7) and recommended termination of the study following approval for cohort 8 recruitment. Patient 21 (cohort 7) was withdrawn from the study due to disease progression prior to completing the DLT period.

21.3 Safety results

Table 15 presents a summary of adverse events identified in all treated patients, including serious adverse events, which are summarised in **Table 16**. The commonest adverse reactions noted were pain and swelling of the injected target lesion, frequently accompanied pyrexia by within the first 24 hours. In patients 18, 20 and 21, this event was recorded as grade 1 cytokine release syndrome (CRS). Two of three patients treated with the highest dose of T4 immunotherapy (cohort 5 – 1 x 10^9 CAR T-cells) had persisting fever at 24 hours and were kept in hospital on a precautionary bases, rendering these severe adverse reactions. In both cases, pyrexia had resolved by 48 hours, permitting discharge at that time.

All treatment-emergent adverse events, treatment-emergent adverse reactions (ranked as possibly or definitely), treatment-emergent serious adverse events and treatment-emergent serious adverse reactions are listed in **Appendix 1-4** respectively.

Table 15. Listing of Adverse Events for all treated patients, including serious adverse events.

Category	Events	Patients
Serious adverse events (SAE)*	8	5
Serious Adverse Reactions (SAR)	4	3
Suspected Unexpected Serious Adverse Reactions (SUSAR)	0	0
Adverse events (not serious) (AE)	221	19
CTCAE grade: 1 mild	154	17
CTCAE grade: 2 moderate	59	15
CTCAE grade: 3 severe	11	6
CTCAE grade: 4 life threatening	3	3
CTCAE grade: 5 death	1**	1**

*These include the 4 SARs

**Patient 5 died before receiving T4 immunotherapy.

Table 16. Listing of Serious Adverse Events for all patients

Serious Adverse Events	e.g. Treatment Arm	e.g. Placebo
Total Number of SAEs per Study Arm	8	Not applicable
Total number of all cause deaths per Study Arm	1	Not applicable
Total number of deaths resulting from adverse events per Study Arm	0	Not applicable

Within the per protocol population (n= 21 of whom 19 were treated), a total of 229 AEs, including 8 SAE, were identified as treatment-emergent and included in the safety analysis. Summary tables for AEs and SAEs are presented in the appendix of this synopsis.

Overall, 19 patients (100%) patients experienced at least one AE. The proportion that experienced at least one SAE was 26% (n=5).

Incidence of adverse drug reactions (ADRs): 117 / 229 AEs (51%) were assessed as related to at least one study drug and 18 / 20 patients (90 %) experienced 117 ADR.

There were 4 Serious Adverse Reactions (SARs) and 0 SUSARs.

21.4 Secondary endpoint results

21.4.1 Efficacy

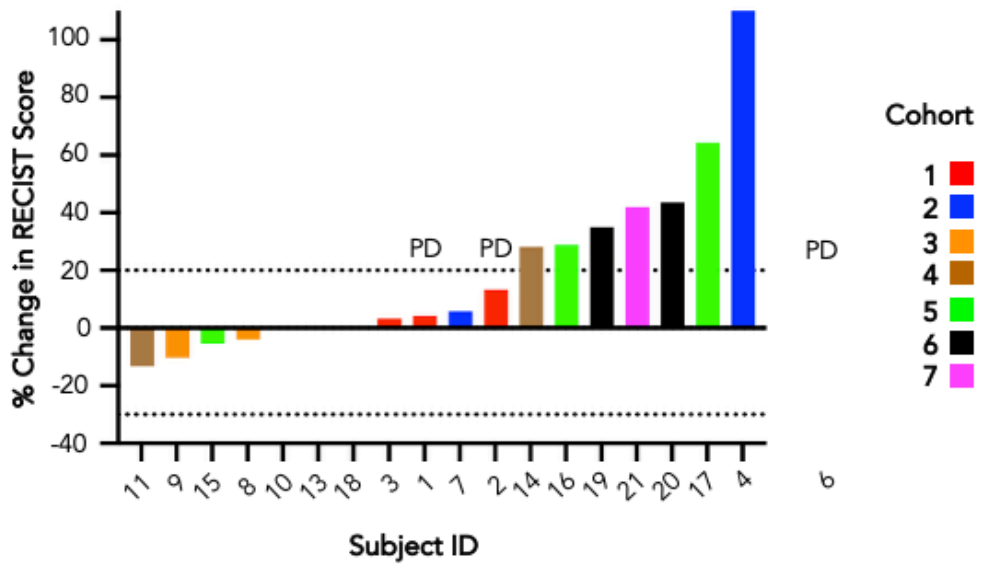
No clinical responses by RECIST 1.1 at day 43 were observed. Stable disease (SD) as best response was observed in 9/19 subjects while progressive disease (PD) was observed the in remaining 11 subjects, giving a disease control rate (DCR) of 47% at day 43 (**Figure 3**).

Figure 3. Clinical response of treated patients. (A) RECIST response overall and in the injected lesions is shown. SD – stable disease; PD – progressive disease. * indicates that assessment was also planned for day 85 in cohort 7 but was not carried out since the single patient enrolled into that cohort was withdrawn from the study on day 56. PD was not confirmed radiologically in patient 6 but the cause of death was disease progression. (B) Waterfall plot indicating percentage change in RECIST score on day 43. PD indicates that PD occurred outside the RECIST target lesion.

A

Patient ID	Cohort	RECIST response at day 43 - overall*	RECIST response at d43 - first injected lesion*	RECIST response at d43 - second injected lesion*
001	1	PD	PD	-
002	1	PD	SD	-
003	1	SD	SD	-
004	2	PD	PD	-
006	2	---------- Died on day 29 due to PD ----------		
007	2	SD	SD	-
008	3	SD	SD	-
009	3	SD	SD	-
010	3	SD	SD	-
011	4	SD	SD	-
013	4	SD	SD	-
014	4	PD	PD	-
015	5	SD	SD	-
016	5	PD	PD	-
017	5	PD	PD	PD
018	6	SD	SD	-
019	6	PD	PD	PD
020	6	PD	PD	PD
021	7	PD	PD	-

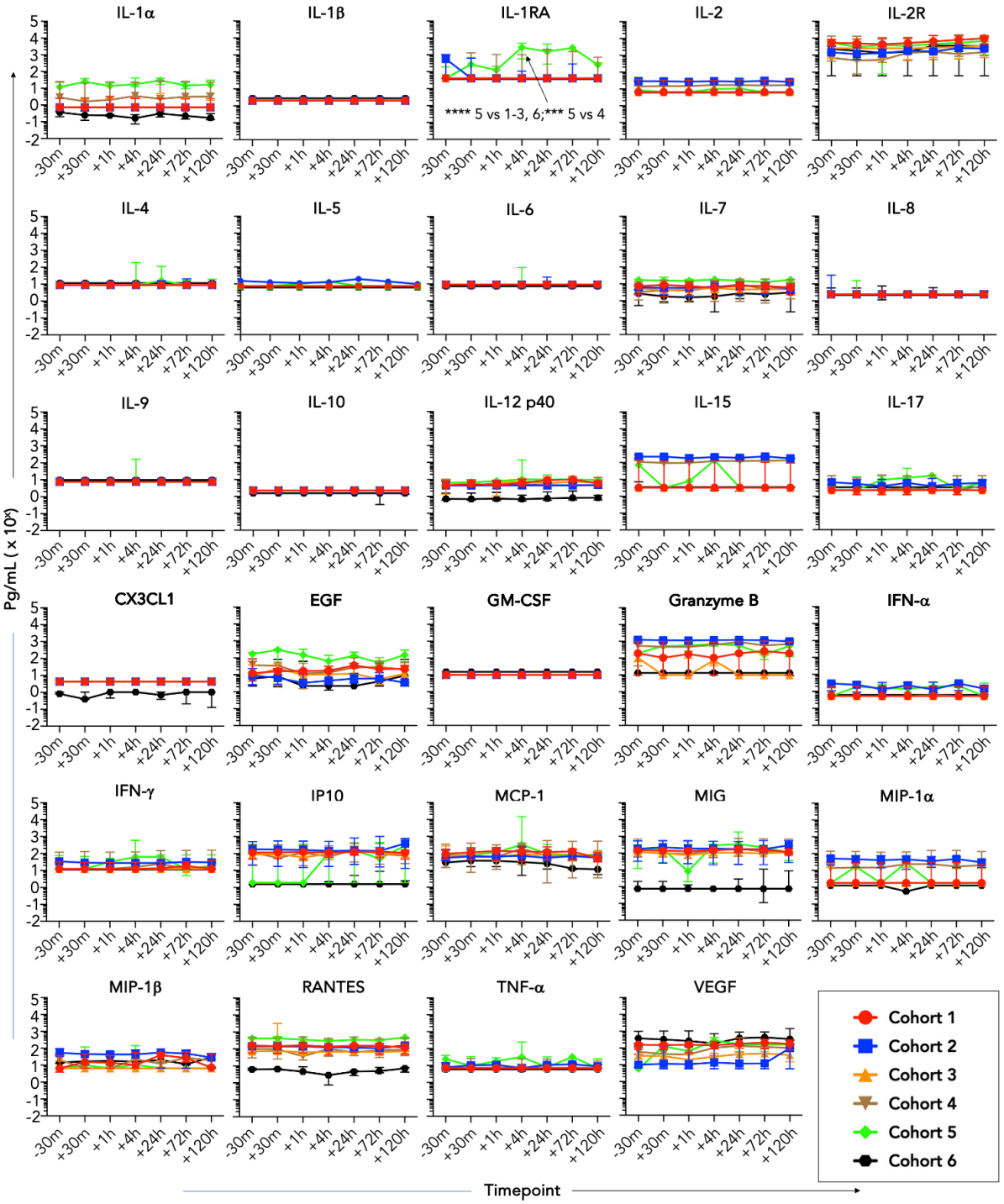
B



21.4.2 Serum cytokine levels

Serum cytokine levels immediately before and at timepoints after T4 immunotherapy are shown in **Figure 4**. Within hours of CAR T-cell immunotherapy treatment, elevated circulating IL-1RA levels were detected in two of 18 treated subjects, both of whom were in the highest (1×10^9 cells) dose cohort (cohort 5). No other significant differences were noted, consistent with the lack of clinical evidence of CRS other than transient pyrexia. Samples were not collected from the cohort 7 patient. Individual cytokine levels are presented in tabular form in section 1.7.3 of the final analysis report.

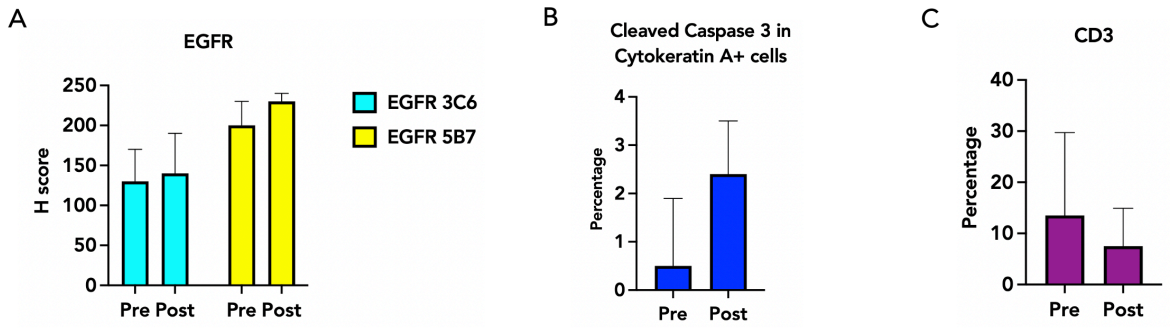
Figure 4. Serum cytokine levels in patients 1-20 (median ± range). Indicated statistical analysis by two-way ANOVA whereby individual timepoints are sequentially numbered.



21.4.3 Tumour biopsy analysis

The presence of $T4^+$ CAR T-cells was assessed in tumour biopsies collected from four patients (008, 009, 010 and 017). Pre-treatment biopsies were consistent with SCCHN in three out of four cases (008-010; 17 was reported as high-grade carcinoma) and residual tumour was identified in the post treatment biopsy collected from patient 8 (day 8) and patient 10 (day 15), but not patient 9 (day 8) or patient 17 (day 15). Using RNAScope, CAR T-cells were not detected in any of the four post-treatment biopsies (Final Analysis Report, section 1.7.6). All other analyses were performed on three (008-010) pre-treatment and two post treatment (008, 010) biopsies. Expression of EGF receptor expression was not altered following CAR T-cell treatment (**Figure 5A**) while a non-significant increase in cytokeratin A^+ (tumour) cells that contained cleaved caspase 3 (**Figure 5B**) and reduction in $CD3^+$ cells (**Figure 5C**) was noted post treatment. Biopsy test results are summarised in **Appendix 5**.

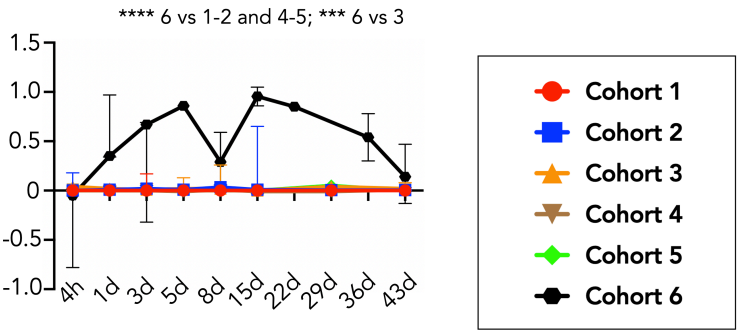
Figure 5. Tumour biopsy analysis pre- and post-T4 immunotherapy. (A) Expression of epidermal growth factor receptor (a key target of T4 immunotherapy) was assessed using the indicated monoclonal antibodies. (B) Percentage cleaved caspase 3 was measured in cytokeratin A^+ tumour cells to provide a marker of tumour cell apoptosis. (C) Percentage infiltrating $CD3^+$ T-cell was determined bein tumour biopies collected before and after treatment (median + range, n= 2-3 biological replicates).



21.4.4 Circulating CAR T-cell pharmacokinetics

Significantly elevated, albeit very low levels of circulating CAR T-cells were detected by qPCR in subjects enrolled in cohort 6 who received pre-conditioning with fludarabine and cyclophosphamide (**Figure 6** and **Table 18**). CAR T-cells were not detected by qPCR in any other cohort. Samples were not collected from the cohort 7 patient.

Figure 6. Circulating CAR T-cell analysis by cohort (median and range, n=3). Statistical analysis was by two-way ANOVA, whereby individual timepoints are sequentially numbered..



CAR T-cells ranged between 0 – 0.6 cells/mL by flow cytometry (**Table 17**).

Table 17. Circulating CAR T-cell levels by flow cytometry and qPCR

Patient ID	Cohort (actual)	Time (post-injection)	CAR T-Cells (no./mL blood – flow cytometry)	qPCR (vector copy no. per gram DNA)
Patient 01	1	4 hours	0	ND insufficient sample*
		24 hours	0	ND insufficient sample*
		48-96 hours	0	0.17
		120-168 hours	0	0
		8 days	0	0
		15 days	0	0
		29 days	0	0
		43 days	0	0
Patient 02	1	4 hours	0	0
		24 hours	0	0
		48-96 hours	0	0
		120-168 hours	0	0
		8 days	0	0
		15 days	0	0
		29 days	0	0.01
		43 days	0	0
Patient 03	1	4 hours	0	0
		24 hours	0	0
		48-96 hours	0	0
		120-168 hours	0.1	0
		8 days	0	0
		15 days	0	0
		29 days	0	0
		43 days	0	0
Patient 04	2	4 hours	0	0
		24 hours	0	0
		48-96 hours	0	0
		120-168 hours	0	0
		8 days	0.1	0.01
		15 days	0	0.65

Patient ID	Cohort (actual)	Time (post-injection)	CAR T-Cells (no./mL blood – flow cytometry)	qPCR (vector copy no. per gram DNA)
		29 days	0	0
		43 days	0.6	0.01
Patient 06	**2**	4 hours	0	0.18
		24 hours	0	0.04
		48-96 hours	0	0.05
		120-168 hours	0	0.02
		8 days	0	0.06
		15 days	0	0.01
		29 days	-	no sample available*
		43 days	-	-
Patient 07	**2**	4 hours	0	0
		24 hours	0	0.01
		48-96 hours	0	0.02
		120-168 hours	no sample available*	no sample available*
		8 days	no sample available*	no sample available*
		15 days	0.1	0
		29 days	0	0.01
		43 days	0	0
Patient 08	**3**	4 hours	0	0.04
		24 hours	0	0.02
		48-96 hours	0	0.07
		120-168 hours	0	0.01
		8 days	0	0.26
		15 days	0	0.02
		29 days	0	0.04
		43 days	0	0.08
Patient 09	**3**	4 hours	0	0.04
		24 hours	0	0.05
		48-96 hours	0	0
		120-168 hours	0.5	0.13
		8 days	0.2	0.02

Patient ID	Cohort (actual)	Time (post-injection)	CAR T-Cells (no./mL blood – flow cytometry)	qPCR (vector copy no. per gram DNA)
		15 days	0	0
		29 days	0	0.04
		43 days	0	0
Patient 10	**3**	4 hours	0	0.02
		24 hours	0	0.01
		48-96 hours	0	0.01
		120-168 hours	0	0.02
		8 days	0	0.01
		15 days	0	0
		29 days	0	0.01
		43 days	0	0.02
Patient 11	**4**	4 hours	0	0.01
		24 hours	0	0
		48-96 hours	0	0
		120-168 hours	0	0.02
		8 days	0	0.01
		15 days	0	0.02
		29 days	0	0.02
		43 days	0	0.01
Patient 13	**4**	4 hours	0	0.01
		24 hours	0	0.01
		48-96 hours	0	0
		120-168 hours	0	0
		8 days	0	0.01
		15 days	0	0
		29 days	0	0
		43 days	0	0
Patient 14	**4**	4 hours	0	0
		24 hours	0	0
		48-96 hours	0	0.01
		120-168 hours	0	0

Patient ID	Cohort (actual)	Time (post-injection)	CAR T-Cells (no./mL blood – flow cytometry)	qPCR (vector copy no. per gram DNA)
		8 days	0	0.05
		15 days	0	0
		29 days	0	0
		43 days	0	0.03
Patient 15	**5**	4 hours	0	0
		24 hours	0	0
		48-96 hours	0	0
		120-168 hours	0	0.01
		8 days	0	0.01
		15 days	0	0.01
		29 days	0.3	0.04
		43 days	0	0
Patient 16	**5**	4 hours	0	0
		24 hours	0	0
		48-96 hours	0	0
		120-168 hours	0	0
		8 days	0	0
		15 days	0	0
		29 days	0	0.05
		43 days	0	0
Patient 17	**5**	4 hours	0	0
		24 hours	0	0
		48-96 hours	0	0
		120-168 hours	0	0
		8 days	0	0
		15 days	0	0
		29 days	0	0.05
		43 days	0	0
Patient 18	**6**	4 hours	0	0
		24 hours	0	0.35
		48-96 hours	0	0.69

Patient ID	Cohort (actual)	Time (post-injection)	CAR T-Cells (no./mL blood – flow cytometry)	qPCR (vector copy no. per gram DNA)
		120-168 hours	0	undetermined*
		8 days	0	0.29
		15 days	Not Done	Not Done
		22 days	Not Done	Not Done
		36 days	0	0.3
		43 days	0	0
Patient 19	**6**	4 hours	0	0
		24 hours	0	0.34
		48-96 hours	0	0
		120-168 hours	0	undetermined*
		8 days	0	0
		15 days	0	0.86
		22 days	0	0.85
		36 days	0	undetermined*
		43 days	0	0.14
Patient 20	**6**	4 hours	0	0.01
		24 hours	0	0.97
		48-96 hours	0	0.67
		120-168 hours	0	0.86
		8 days	0	0.59
		15 days	0	1.05
		22 days	0	undetermined*
		36 days	0	0.78
		43 days	0	0.47
Patient 21	**7**	4 hours	Not Done	Not Done
		24 hours	Not Done	Not Done
		48-96 hours	Not Done	Not Done
		120-168 hours	Not Done	Not Done
		8 days	Not Done	Not Done
		15 days	Not Done	Not Done
		22 days	Not Done	Not Done

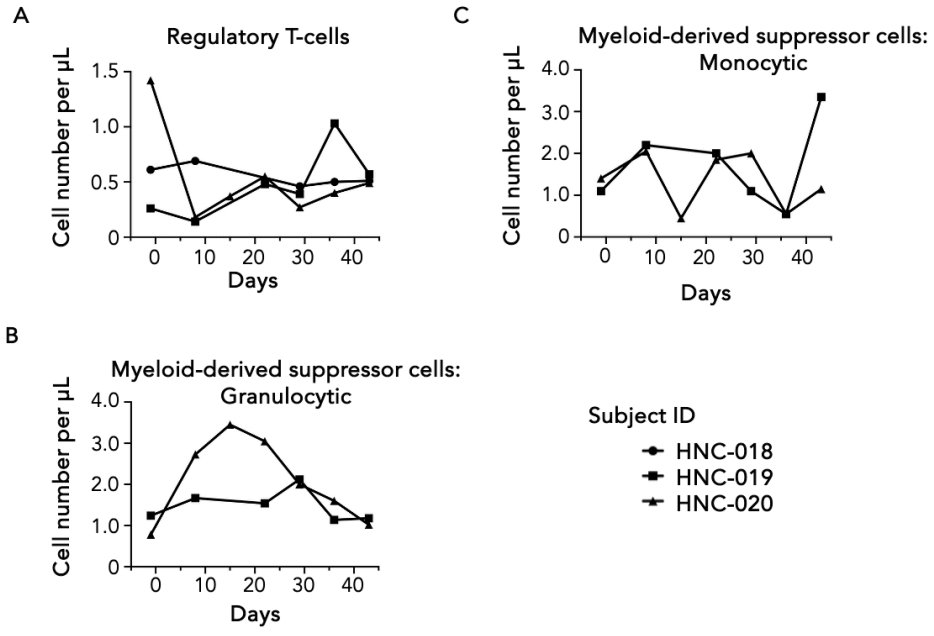
Patient ID	Cohort (actual)	Time (post-injection)	CAR T-Cells (no./mL blood – flow cytometry)	qPCR (vector copy no. per gram DNA)
		28 days	Not Done	Not Done
		36 days	Not Done	Not Done
		43 days	Not Done	Not Done
		56 days	Not Done	Not Done
		71 days	Not Done	Not Done
		85 days	Not Done	Not Done

21.4.5 Effects of lymphodepletion

We did not observe any DLTs in patients who received these additional interventions. Only 1 of 4 subjects who received lymphodepletion achieved stable disease, indicating that no measurable improvement in efficacy was noted in these groups (**Figure 3**).

Evidence of immunomodulation by cyclophosphamide and fludarabine was measured by circulating numbers of $CD4^{+}$ $CD25^{HIGH}$ $CD127^{DIM/NEG}$ regulatory T-cells and myeloid-derived suppressor cells. Cells using flow cytometry in patients enrolled into cohort 6. A reduction of circulating Tregs was only seen in 1 of 3 treated subjects (**Figure 7A**). Granulocytic myeloid-derived suppressor cells (MDSC) demonstrated a rebound increased in one of two treated subjects (**Figure 7B**) while no change in monocytic MDSC was seen in two treated subjects.

Figure 7 Enumeration of circulating immunosuppressive leukocytes in patients treated in cohort 6. Subjects who received lymphodepleting chemotherapy with fludarabine and cyclophosphamide were monitored for circulating regulatory T-cells (A), granulocytic myeloid-derived suppressor cells (MDSCs, B) and monocytic MDSCs (C). Analysis was performed on all 3 subjects (A) or in 2 subjects (B-C).



21.4.6 MAGE A3 and MAGE A4-reactive T-cells

No definitive effect of intra-tumoural T4 immunotherapy on endogenous MAGE A3 or MAGE A4-reactive T-cells was observed in ELISpot analysis, undertaken using overlapping peptides derived from these antigens (**Figure 8**).

Figure 8. MAGE-A3 and MAGE-A4 ELISPOT analysis. PBMC from the indicated subjects were isolated prior to and 29 days after T4 immunotherapy. Samples were stimulated with MAGE-A3 and MAGE-A4 peptide pools, making comparison with unstimulated control wells. Interferon-γ spots were enumerated and data expressed as a stimulation index with respect to unstimulated control wells. Data show mean + SD from n=9 biological replicates collected from subjects enrolled into cohorts 3-5. Data are shown for each cohort in **Table 18**.

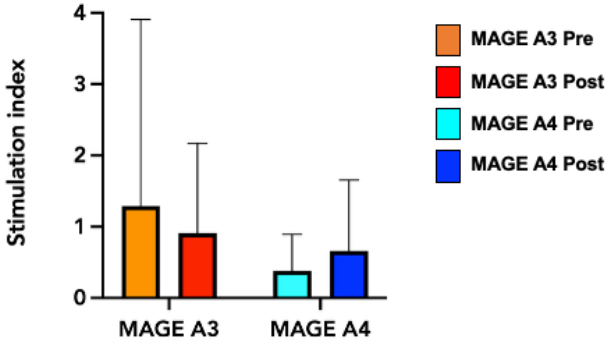


Table 18. EliSPOT analysis of MAGE A3 and MAGE A4-reactive T-cells before and after T4 immunotherapy.

Cohort (actual)	Number of patients treated	Time (days post-injection)	MAGE A3	MAGE A4
			Spot forming cells per million PBMC, median (range)	
			MAGE A3	**MAGE A4**
1	**3**	-3	*	*
		29	*	*
2	**3**	-3	*	*
		29	*	*
3	**3**	-3	0.3 (0.1-0.5)	0.4 (0.1-0.9)
		29	0.4 (0.2-0.6)	0.1 (0.1-0.2)
4	**3**	-3	0.4 (0.0-2.3)	0.4 (0.1-1.5)
		29	0.7 (0.0-4.0)	0.4 (0.0-1.0)
5	**3**	-3	0.0 (0.0-8.0)	0.0 (0.0-0.0)
		29	0.7 (0.0-1.6)	1.0 (0.0-3.1)

ELISpot analysis measures T-cell responses to MAGE A3 and A4 antigens.

Day -3: Baseline measurement prior to T4 immunotherapy.

Day 29: Post-treatment measurement.

* Insufficient sample available for analysis.

21.4.8 Trafficking of T4 immunotherapy

Intra-tumoural injection of an [^{111}In] passively labelled T4^{+} CAR-T radiotracer was undertaken on the same day as T4 immunotherapy administration in a single subject (CAR- HNC- 016; **Figure 9**). The subject's tumour was locally progressive in the right buccal mucosa. SPECT imaging was performed after approximately 1 hour and on days 2 and 3 postinjection. A CT scan was performed on day 1 and was co-registered with all three SPECT images. Imaging demonstrated focal retention of signal in the right buccal mucosa over 48 hours. Whole body imaging showed no signal in other organs other than stomach (attributed to swallowed tracer, which had leaked from the site of injection). These data are consistent with the qPCR and flow cytometry data presented in 21.4.4 and indicate that T4 immunotherapy is not appreciably absorbed from the target lesion injection site into the systemic circulation. However, no evidence of infiltration of the tracer into the tumour mass was observed.

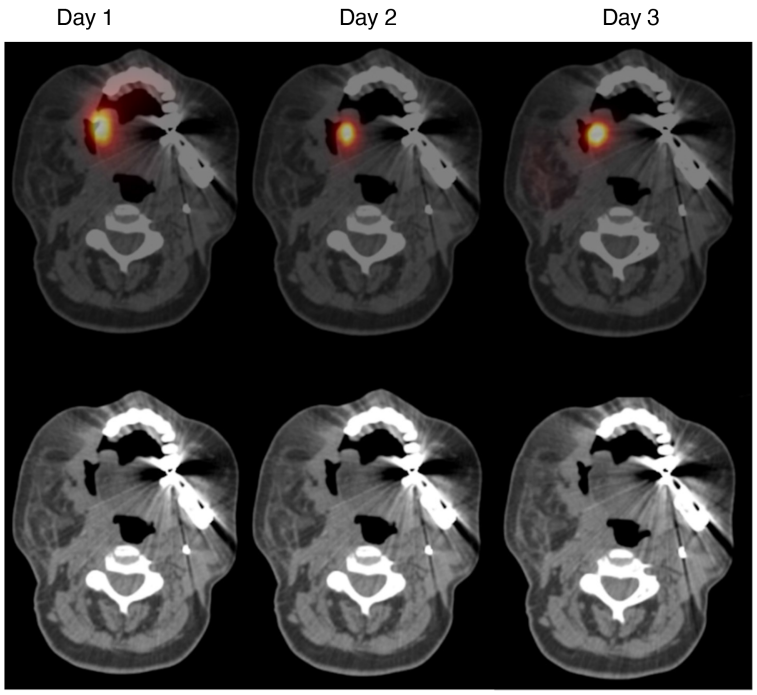


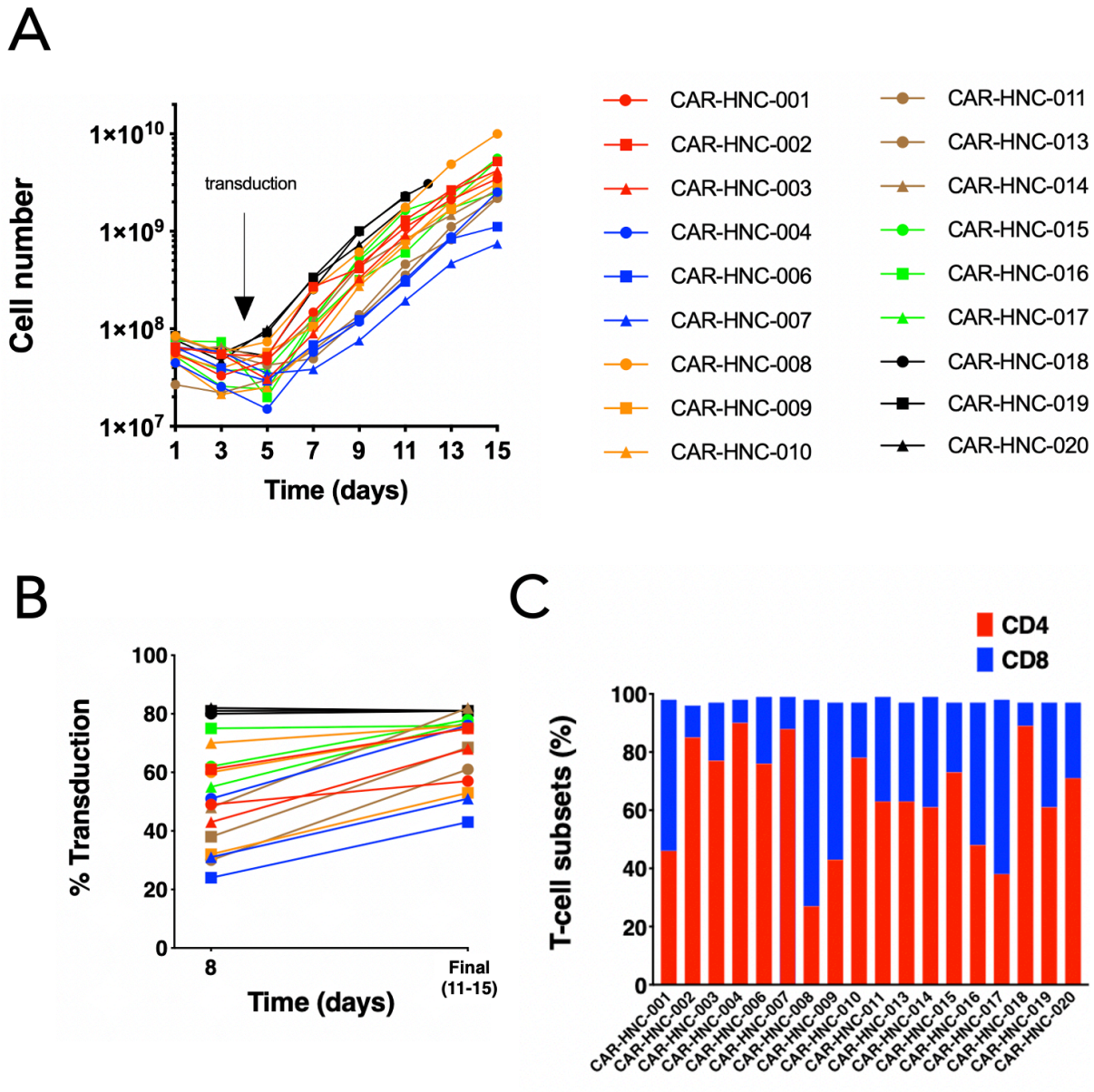
Figure 9. SPECT-CT imaging of [111In] radiolabelled T4 CAR T- cells. A radiotracer was prepared in which 30×10^{6} autologous T4^{+} CAR cells were labelled with 5MBq [^{111}In] indium. The tracer was injected into a target lesion in the right buccal mucosa in a single subject (CAR- HNC- 016). SPECT scans were performed at the indicated intervals and were co-registered with a CT scan (shown below) performed on day 1. CAR T- cells, chimeric antigen receptor T- cells; SPECT- CT, single photon emission- CT.

21.5 Manufacture of T4 immunotherapy

T4 immunotherapy was successfully manufactured from whole blood (40-120mL) in all cases, despite lymphopenia in 12 of 15-treated subjects. There were no batch manufacturing failures. Robust expansion (**Figure 10A**) and enrichment (**Figure 10B**) of transduced cells in response to the bespoke 4$\alpha\beta$-cytokine receptor + IL-4 culture system was consistently observed, irrespective of starting material blood volume or lymphocyte count.

Phenotyping of the T4 immunotherapy product indicated that CD4/ CD8 T-cell ratio varied significantly across batches (**Figure 10C**).

Figure 10. Manufacture of T4 immunotherapy. (A) Expansion, (B) Percentage CAR positivity on day 8 and on the final day of manufactire (days 11-15) and (C) CD4/ CD8 ratio of the indicated batches of T43 immunotherapy.

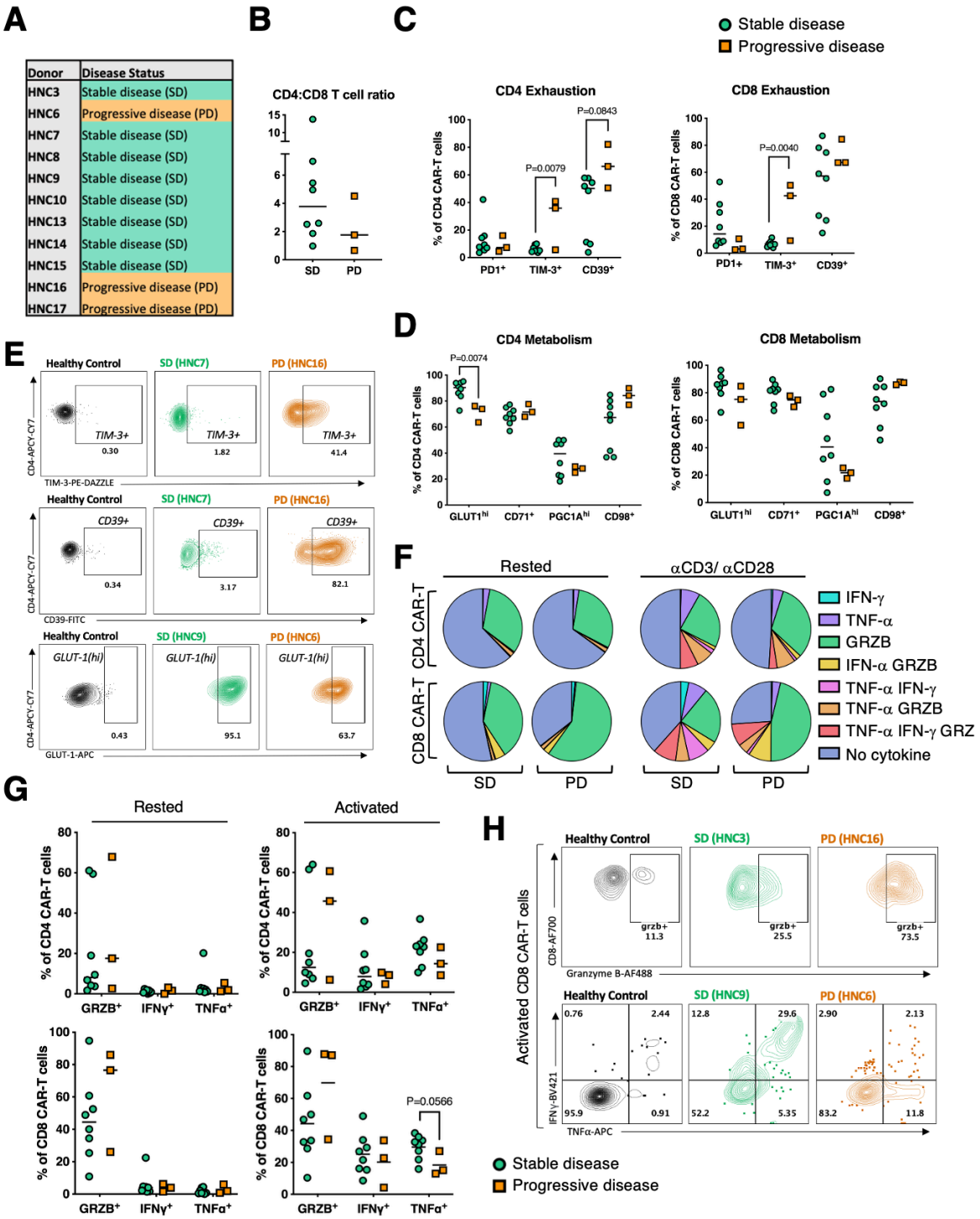


21.6 Additional translational studies

Additional immunophenotypic analysis was carried out on T4 batches administered to patients wwho achieved stable disease (SD) or progressive disease (PD; **Figure 11A**).The CD4/CD8 T-cell ratio in the T4 CAR-expressing population (e.g. $CD3^+$ CAR^+) tended to be higher in the SD population (**Figure 11B**). A more exhausted phenotype was observed at baseline in the 3 subjects with PD versus the 8 who achieved SD (**Figure 11C**). This was indicated by a trend towards higher CD39 expression by $CD4^+$ CAR T-cells from the PD group, together with significantly elevated TIM-3 levels in both $CD4^+$ and $CD8^+$ CAR T-cells.

Figure 11. Immunophenotypic analysis of T4 CAR T-cell batches in patients with stable (SD) or progressive disease (PD) (overleaf). (A) Outcomes of 11 patients whose pre-infusion CAR-T phenotype was assessed in surplus retention samples. (B) Cryopreserved PBMCs from the infusion product were analyzed by flow cytometry, with gating on live $CD4^+$ and $CD8^+$ CAR-T cells. Ratio of CD4 to CD8 CAR-T cells in PD and SD patients is shown. (C) Frequency of $PD1^+$, $TIM\text{-}3^+$ and $CD39^+$ $CD4^+$ and $CD8^+$ CAR-T cells. (D) Frequency of $GLUT\text{-}1^{hi}$, $CD71^+$, peroxisome proliferator-activated receptor-γ coactivator $(PGC1A)^{hi}$ and $CD98^+$ $CD4^+$ and $CD8^+$ CAR-T cells. (E) Representative flow

cytometry gating of TIM-3$^+$, CD39$^+$ and GLUT-1^{hi} CD4$^+$ CAR-T cells in SD and PD subjects with gating on healthy donor T-cells as a control. CAR-T effector molecule production was assessed by incubating cells overnight in the presence of Brefeldin-A ± plate-bound αCD3/αCD28 activation before staining for intracellular proteins. (F) Mean proportion of CD4$^+$ and CD8$^+$ CAR-T cells in SD and PD subjects that are single, double, or triple positive for Granzyme B (GRZB), IFN-γ and TNF-α; each pie chart totals 100%. (G) Total frequency of GRZB$^+$, IFN-γ$^+$ and TNF-α$^+$ CD4$^+$ and CD8$^+$ CAR-T cells. (H) Representative flow cytometry gating of GRZB$^+$, IFN-γ$^+$ and TNF-α$^+$ CD8$^+$ CAR-T cells in an SD and PD subject with gating on healthy donor T cells as a control. Statistics: SD and PD groups were compared by unpaired *t*-test.



Next, markers of metabolite transportation and mitochondrial biogenesis in $T4^+$ CAR T-cell products was examined. Significantly reduced GLUT1 expression was seen in the $CD4^+$ CAR T cells of the PD population, suggestive of a reduced ability to uptake glucose for glycolysis (**Figure 11D**). Differences in GLUT1 expression were not observed in the $CD8^+$ CAR T-cells, while expression of CD71 (transferrin receptor) and CD98 (neutral amino acid transporter) were also similar in CAR T-cells from PD and SD groups. Peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α promotes mitochondrial biogenesis and a trend towards increased expression of this factor was noted in $CD4^+$ and $CD8^+$ CAR T-cells from SD subjects (**Figure 11D**). Representative dot plots of $CD4^+$ CAR T-cells from a healthy control, a subject with SD and a subject with PD are shown for TIM-3, CD39 and GLUT1 (**Figure 11E**).

Effector capacity of $T4^+$ CAR T-cells was evaluated through analysis of GRZB, IFN-*γ* and TNF-α levels at rest and after overnight anti-CD3/CD28 stimulation. In SD subjects, we observed a trend towards an increase in $CD8^+$ CAR T-cells that were double positive for these effector markers following overnight stimulation (**Figure 11F**). In keeping with this, SD subjects also demonstrated a trend towards greater IFN-*γ* and TNF-α production by both CAR T-cell subsets after activation (**Figure 11G**). Representative plots for $CD8^+$ CAR T-cells that contain GRZB alone or co-produce IFN-*γ* and TNF-α are shown for a healthy donor, an SD and a PD subject (**Figure 11H**). While sample size is small, these data collectively suggest that more favorable clinical outcome is associated with a less exhausted and more functional CAR T-cell product.

22. Conclusion

This first in human dose escalation study was undertaken to investigate the safety and tolerability of a panErbB-targeted CAR T-cell product, T4 immunotherapy, in subjects with relapsed refractory HNSCC. We believe that this is the first published clinical trial of CAR T-cell immunotherapy for this indication. Despite previous issues with on-target off-tumor toxicity when ErbB family members have been targeted using CAR T-cell immunotherapy,[1] safety of the intra-tumoral approach described here has been robustly demonstrated at doses of up to $1x10^9$ $T4^+$ cells. The patient population was representative of relapsed refractory HNSCC with subjects over 80 years of age and with ECOG-PS up to 2 treated on study. The rapidly progressive nature of terminal stage HNSCC was exemplified by one disease-related death which occurred during the two week T4 manufacturing process.

No objective responses at 43 days were observed, but 60% of subjects obtained disease control, with 9/15 having a best response of stable disease. There was no clear evidence of a dose-response effect, although target lesion size varied considerably, complicating the assessment of this relationship. Notably, the overall survival outcomes of the whole cohort were favorable when compared to historical controls, suggestive of an efficacy signal in this study.

Following intra-tumoral delivery of up to 1 billion CAR T-cells, leakage into the circulation was not detected. Moreover, a radiotracer prepared from the CAR T-cells remained at the site of injection for 48 hours. This is very likely to account for the lack of significant toxicity of intra-tumoral T4 immunotherapy. This also provides a strong rationale for further potentiation of this approach, for example with cytokine armoring strategies[2] since it is unlikely that such cells would gain access to the circulation in significant numbers.

Manufacture utilizing a bespoke semi-closed fresh product platform was successful for all subjects from whole blood as starting material.[3] Vein to treatment time was only 14 days owing to the delivery of a fresh product in which final sterility testing data was only available post treatment.

The CAR T-cell drug product demonstrated considerable donor to donor variability in immunophenotype, which is a recognized limitation of autologous CAR T-cell immunotherapy.[4, 5] Importantly however, improved outcome was linked to the administration of products with greater functionality and lower exhaustion marker expression.

Collectively these data also deliver a strong rationale for the addition of lymphodepleting chemotherapy as preconditioning of HNSCC subjects prior to administration of T4 immunotherapy. This next phase of the T4 immunotherapy trial has received regulatory approval and is currently ongoing.

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24. Date of Report

This is version 1.0 of the Clinical Study Report synopsis, dated 29/11/2025.

APPENDICES

i) Summary of treatment-emergent AEs in the per protocol population

(coded using MedDRA 28.1 by the chief investigator as a subjective exercise, based on information (often incomplete) in the AE list. MedDRA coding was not recorded in the trial database).

System Organ Class	Preferred Term	Number/% of Subjects Experiencing the AE in Active Arm	Total Number of Occurrences of the AE	Subject IDs	Number of Subjects Experiencing the AE in Placebo Arm	Total Number of Occurrences of the AE
Blood and lymphatic system disorders	Anaemia	6/19 (32%)	9	8, 10, 17, 18, 20, 21	0	9
	Lymphopenia	4/19 (21%)	7	18, 19, 20, 21	0	7
	Neutropenia	2/19 (11%)	2	20, 21	0	2
	Neutrophilia	2/19 (11%)	2	17, 20	0	2
	Thrombocytopenia	2/19 (11%)	4	18, 21	0	4
Cardiac disorders	Tachycardia	1/19 (5%)	1	15	0	1
Congenital, familial and genetic disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Ear and labyrinth disorders	Otorrhoea	1/19 (5%)	1	15	0	1
	Tinnitus	1/19 (5%)	1	10	0	1
	Ear pain	1/19 (5%)	1	10	0	1
	Periorbital oedema	2/19 (11%)	2	15, 21	0	2
	Eyelid ptosis	1/19 (5%)	1	15	0	1
	Vision blurred	1/19 (5%)	1	15	0	1
Gastrointestinal disorders	Abdominal pain	1/19 (5%)	1	18	0	1
	Constipation	5/19 (26%)	5	3, 14, 17, 18, 19	0	5
	Diarrhoea	6/19 (32%)	6	2, 3, 7, 13, 14, 18	0	6

	Dyspepsia	2/19 (11%)	2	18, 20	0	2
	Dysphagia	6/19 (32%)	10	4, 10, 11, 13, 14, 15	0	9
	Gastrooesophageal reflux disease	1/19 (5%)	1	7	0	1
	Gastritis	1/19 (5%)	1	4	0	1
	Oral pain	1/19 (5%)	2	13	0	2
	Tongue erosion	1/19 (5%)	1	13	0	1
	Nausea	2/19 (11%)	2	16, 18	0	2
	Oropharyngeal pain	1/19 (5%)	1	16	0	1
	Glossodynia	1/19 (5%)	1	13	0	1
General disorders and administration site conditions	Administration site odour	1/19 (5%)	1	16	0	1
	Chills	4/19 (21%)	4	8, 10, 11, 21	0	4
	Death*	1/19 (5%)	1	5	0	1
	Fatigue	13/19 (68%)	17	1, 4, 7, 8, 9, 11, 13, 15, 16, 17, 18, 19, 20	0	14
	Headache	3/19 (16%)	3	4, 14, 18	0	3
	Injection site pain	5/19 (26%)	5	4, 7, 13, 17, 18	0	5
	Oedema peripheral	1/19 (5%)	1	19	0	1
	Malaise	1/19 (5%)	1	7	0	1
	Night sweats	1/19 (5%)	1	15	0	1
	Pain	2/19 (11%)	3	8, 17	0	3
	Pyrexia	6/19 (32%)	11	9, 13, 15, 17, 18, 20	0	11
	Swelling	1/19 (5%)	2	19	0	2
Hepatobiliary disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Immune system disorders	Cytokine release syndrome**	3/19 (16%)	3	18, 20, 21	0	3
Infections and infestations	Bronchial infection	1/19 (5%)	1	2	0	1
	Paronychia	1/19 (5%)	1	18	0	1
	Lower respiratory tract infection	1/19 (5%)	1	7	0	1
	Oral candidiasis	3/19 (16%)	3	1, 14, 18	0	3

	Sepsis	1/19 (5%)	1	20	0	1
	Streptococcal infection	1/19 (5%)	1	2	0	1
Injury, poisoning and procedural complications	Fall	1/19 (5%)	1	7	0	1
	Limb injury	1/19 (5%)	1	7	0	1
	Gastrostomy tube site complication	1/19 (5%)	2	7	0	2
Investigations	C-reactive protein increased	1/19 (5%)	1	16	0	1
	Serum ferritin increased	1/19 (5%)	1	20	0	1
	Liver function test abnormal	1/19 (5%)	1	1***	0	1
	Hyponatraemia	2/19 (11%)	4	2, 20	0	4
	Weight decreased	5/19 (26%)	5	1, 7, 14, 17, 20	0	5
Metabolism and nutrition disorders	Decreased appetite	2/19 (11%)	2	14, 20	0	2
	Iron deficiency	1/19 (5%)	1	20	0	1
	Hypercalcaemia	1/19 (5%)	1	20	0	1
Musculoskeletal and connective tissue disorders	Musculoskeletal chest pain	1/19 (5%)	2	1	0	2
	Arthralgia	1/19 (5%)	1	8	0	1
	Jaw pain	2/19 (11%)	2	7, 13	0	2
	Neck pain	1/19 (5%)	1	19	0	1
	Trismus	2/19 (11%)	2	15, 16	0	2
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	Tumour inflammation	7/19 (37%)	10	7, 8, 11, 13, 14, 16, 17	0	9
	Tumour discharge	1/19 (5%)	1	7	0	1
	Tumour haemorrhage	4/19 (21%)	5	8, 10, 16, 21	0	5
	Tumour pain	10/19 (53%)	18	1, 6, 7, 9, 11, 13, 14, 16, 17, 18	0	16
	Infected neoplasm	4/19 (21%)	7	6, 16, 20, 21	0	7
Nervous system disorders	Insomnia	1/19 (5%)	1	18	0	1
	Paraesthesia	1/19 (5%)	1	4	0	1

	Hypoaesthesia	1/19 (5%)	1	18	0	1
Pregnancy, puerperium and perinatal conditions	Not applicable	0/19 (0%)	0	Not applicable	0	0
Product issues	Not applicable	0/19 (0%)	0	Not applicable	0	0
Psychiatric disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Renal and urinary disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Reproductive system and breast disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Respiratory, thoracic and mediastinal disorders	Aspiration	1/19 (5%)	1	2	0	1
	Bronchial obstruction	1/19 (5%)	1	21	0	1
	Cough	4/19 (21%)	4	2, 3, 4, 17	0	4
	Dysphonia	1/19 (5%)	1	2	0	1
	Dyspnoea	3/19 (16%)	3	7, 8, 14	0	3
	Orthopnoea	1/19 (5%)	1	15	0	1
	Throat tightness	1/19 (5%)	1	15	0	1
	Bronchial secretion retention	1/19 (5%)	1	10	0	1
	Rhinorrhoea	2/19 (11%)	2	3, 15	0	2
Skin and subcutaneous tissue disorders	Swelling face	3/19 (16%)	5	13, 15, 17	0	5
	Lip swelling	2/19 (11%)	2	4, 15	0	2
	Pruritus	2/19 (11%)	2	17, 18	0	2
	Rash	2/19 (11%)	3	4, 10	0	3
	Urticaria	1/19 (5%)	1	4	0	1
Social circumstances	Not applicable	0/19 (0%)	0	Not applicable	0	0
Surgical and medical procedures	Gastrostomy tube insertion	1/19 (5%)	1	7	0	1
Vascular disorders	Thrombosis	1/19 (5%)	1	20	0	1

* Due to progressive SCCHN; ** Grade 1 CRS; *** classified as important medical event

ii) Summary of treatment-emergent ARs in the per protocol population

(coded using MedDRA 28.1 by the chief investigator as a subjective exercise, based on information (often incomplete) in the AE list. MedDRA coding was not recorded in the trial database).

System Organ Class	Preferred Term	Number/% of Subjects Experiencing the AR in Active Arm	Total Number of Occurrences of the AR	Subject IDs	Number of Subjects Experiencing the AR in Placebo Arm	Total Number of Occurrences of the AR
Blood and lymphatic system disorders	Anaemia	5/19 (26%)	6	10, 17, 18, 20, 21	0	6
	Lymphopenia	4/19 (21%)	7	18, 19, 20, 21	0	7
	Neutropenia	2/19 (11%)	2	20, 21	0	2
	Thrombocytopenia	2/19 (11%)	4	18, 21	0	4
Cardiac disorders	Tachycardia	1/19 (5%)	1	15	0	1
Congenital, familial and genetic disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Ear and labyrinth disorders	Tinnitus	1/19 (5%)	1	10	0	1
	Ear pain	1/19 (5%)	1	10	0	1
	Periorbital oedema	2/19 (11%)	2	15, 21	0	2
	Eyelid ptosis	1/19 (5%)	1	15	0	1
Gastrointestinal disorders	Constipation	2/19 (11%)	2	14, 19	0	2
	Diarrhoea	1/19 (5%)	1	3	0	1
	Dyspepsia	1/19 (5%)	1	20	0	1
	Dysphagia	2/19 (11%)	3	11, 14	0	3
	Oral pain	1/19 (5%)	1	13	0	1
	Tongue erosion	1/19 (5%)	1	13	0	1
	Nausea	2/19 (11%)	2	16, 18	0	2
	Oropharyngeal pain	1/19 (5%)	1	16	0	1
General disorders and administration site conditions	Chills	4/19 (21%)	4	8, 10, 11, 21	0	4
	Fatigue	7/19 (37%)	10	1, 8, 9, 11, 13, 17, 18	0	10
	Headache	1/19 (5%)	1	14	0	1

	Injection site pain	2/19 (11%)	2	4, 17	0	2
	Oedema peripheral	1/19 (5%)	1	19	0	1
	Night sweats	1/19 (5%)	1	15	0	1
	Pain	2/19 (11%)	2	8, 17	0	2
	Pyrexia	5/19 (26%)	9	9, 13, 15, 17, 20	0	9
	Swelling	1/19 (5%)	2	19	0	2
Hepatobiliary disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Immune system disorders	Cytokine release syndrome	3/19 (16%)	3	18, 20, 21	0	3
Infections and infestations	Bronchial infection	1/19 (5%)	1	2	0	1
	Paronychia	1/19 (5%)	1	18	0	1
Injury, poisoning and procedural complications	Not applicable	0/19 (0%)	0	Not applicable	0	0
Investigations	C-reactive protein increased	1/19 (5%)	1	16	0	1
	Serum ferritin increased	1/19 (5%)	1	20	0	1
	Hyponatraemia	1/19 (5%)	1	2	0	1
Metabolism and nutrition disorders	Decreased appetite	1/19 (5%)	1	20	0	1
Musculoskeletal and connective tissue disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	Tumour inflammation	7/19 (37%)	10	7, 8, 11, 13, 14, 16, 17	0	10
	Tumour haemorrhage	1/19 (5%)	1	21	0	1
	Tumour pain	6/19 (32%)	11	9, 11, 13, 14, 16, 18	0	11
	Infected neoplasm	1/19 (5%)	2	16	0	2
Nervous system disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0

Pregnancy, puerperium and perinatal conditions	Not applicable	0/19 (0%)	0	Not applicable	0	0
Product issues	Not applicable	0/19 (0%)	0	Not applicable	0	0
Psychiatric disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Renal and urinary disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Reproductive system and breast disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Respiratory, thoracic and mediastinal disorders	Orthopnoea	1/19 (5%)	1	15	0	1
	Throat tightness	1/19 (5%)	1	15	0	1
	Bronchial secretion retention	1/19 (5%)	1	10	0	1
	Rhinorrhoea	1/19 (5%)	1	15	0	1
Skin and subcutaneous tissue disorders	Swelling face	2/19 (11%)	3	13, 15	0	3
	Lip swelling	1/19 (5%)	1	15	0	1
	Pruritus	2/19 (11%)	2	17, 18	0	2
	Rash	1/19 (5%)	1	4	0	1
Social circumstances	Not applicable	0/19 (0%)	0	Not applicable	0	0
Surgical and medical procedures	Not applicable	0/19 (0%)	0	Not applicable	0	0
Vascular disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0

iii) Summary of treatment-emergent SAEs in the study population*

(coded using MedDRA 28.1 by the chief investigator as a subjective exercise, based on information (often incomplete) in the AE list. MedDRA coding was not recorded in the trial database).

System Organ Class	Preferred Term	Number/% of Subjects Experiencing the SAE in Active Arm	Total Number of Occurrences of the SAE	Subject IDs	Number of Subjects Experiencing the SAE in Placebo Arm	Total Number of Occurrences of the SAE
Blood and lymphatic system disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Cardiac disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Congenital, familial and genetic disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Ear and labyrinth disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Gastrointestinal disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
General disorders and administration site conditions	Fatigue	1/19 (5%)	1	20	0	1
Hepatobiliary disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Immune system disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Infections and infestations	Sepsis	1/19 (5%)	1	20	0	1
Injury, poisoning and procedural complications	Not applicable	0/19 (0%)	0	Not applicable	0	0
Investigations	Not applicable	0/19 (0%)	0	Not applicable	0	0
Metabolism and nutrition disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0

Musculoskeletal and connective tissue disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	Tumour haemorrhage	1/19 (5%)	1	10	0	1
	Infected neoplasm	1/19 (5%)	1	20	0	1
Nervous system disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Pregnancy, puerperium and perinatal conditions	Not applicable	0/19 (0%)	0	Not applicable	0	0
Product issues	Not applicable	0/19 (0%)	0	Not applicable	0	0
Psychiatric disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Renal and urinary disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Reproductive system and breast disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Respiratory, thoracic and mediastinal disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Skin and subcutaneous tissue disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Social circumstances	Not applicable	0/19 (0%)	0	Not applicable	0	0
Surgical and medical procedures	Not applicable	0/19 (0%)	0	Not applicable	0	0
Vascular disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0

* This table excludes SAEs that were classed as SARs, all of which are shown in the Table that follows.

iv) Summary of treatment-emergent SARs in the study population

(coded using MedDRA 28.1 by the chief investigator as a subjective exercise, based on information (often incomplete) in the AE list. MedDRA coding was not recorded in the trial database).

System Organ Class	Preferred Term	Number/% of Subjects Experiencing the SAE in Active Arm	Total Number of Occurrences of the SAE	Subject IDs	Number of Subjects Experiencing the SAE in Placebo Arm	Total Number of Occurrences of the SAE
Blood and lymphatic system disorders	Anaemia	1/19 (5%)	1	21	0	1
	Neutropenia	1/19 (5%)	1	21	0	1
Cardiac disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Congenital, familial and genetic disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Ear and labyrinth disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Gastrointestinal disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
General disorders and administration site conditions	Pyrexia	2/19 (11%)	2	15, 17	0	2
Hepatobiliary disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Immune system disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Infections and infestations	Not applicable	0/19 (0%)	0	Not applicable	0	0
Injury, poisoning and procedural complications	Not applicable	0/19 (0%)	0	Not applicable	0	0
Investigations	Not applicable	0/19 (0%)	0	Not applicable	0	0

Metabolism and nutrition disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Musculoskeletal and connective tissue disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	Not applicable	0/19 (0%)	0	Not applicable	0	0
Nervous system disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Pregnancy, puerperium and perinatal conditions	Not applicable	0/19 (0%)	0	Not applicable	0	0
Product issues	Not applicable	0/19 (0%)	0	Not applicable	0	0
Psychiatric disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Renal and urinary disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Reproductive system and breast disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Respiratory, thoracic and mediastinal disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Skin and subcutaneous tissue disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0
Social circumstances	Not applicable	0/19 (0%)	0	Not applicable	0	0
Surgical and medical procedures	Not applicable	0/19 (0%)	0	Not applicable	0	0
Vascular disorders	Not applicable	0/19 (0%)	0	Not applicable	0	0

v) Tumour biopsy results

Patient ID	Cohort (actual)	Visit	Number of days after T4 immunotherapy	H&E staining	Immunochemistry	RNA scope	RNA sequencing performed?
008	3	Screening	-18	Collected on: 22/07/2016 00:00 Accession: T4-08 BL Received on: 22/07/2016 00:00 Clinical Information: Study specific biopsy for clinical trial before treatment. Cores from lymph node metastases. Macroscopic Description: 2 cores 15x2x2mm and 18x2x2mm. Both embed whole. Microscopic Description Core biopsy of squamous cell carcinoma arranged as broad interconnecting strands and islands. Diagnosis: Squamous cell carcinoma.	Supplementary report 1 #EGFR (3C6) H score = 170 EGFR (5B7) H score = 230 Percentage cleaved caspase-3 = 1.9 (mean 10 high-power fields) Percentage CD3 = 29.7 (mean 10 high-power fields)	Negative	No
		Visit 8	10	Collected on: 19/08/2016 00:00 Accession: T4-08 AT Received on: 19/08/2016 00:00 Clinical Information: Study specific biopsy for T4 clinical trial post infusion. Cores from lymph node metastases. Macroscopic Description: 2 cores 6x2x2mm and 11x2x2mm. Both processed whole. Thrid core 15x2x2mm sent fresh for molecular studies. Microscopic Description:	EGFR (3C6) H score = 190 EGFR (5B7) H score = 240 Percentage cleaved caspase-3 = 3.5 (mean 10 high-power fields) Percentage CD3 = 14.9 (mean 10 high-power fields)	Negative	No

				Core biopsy of fibrin, extravasated erythrocytes and fibrous tissue infiltrated by squamous cell carcinoma arranged as variably sized islands. Diagnosis: Squamous cell carcinoma.			
009	3	Screening	-19	Collected on: 08/09/2016 00:00 Accession: T4-09 BL Received on: 08/09/2016 00:00 Clinical Information: Study specific biopsy for T4 clinical trial before treatment. Biopsy from recurrent primary tumour. Macroscopic Description: 2 punches of mucosa 4x3x3mm and 5x2x4mm. All embedded Microscopic Description: Superficial biopsies comprising fibrous tissue covered by fibrinous ulcer slough and moderately dyplastic squamous epithelium. Both pieces are infiltrated by an undifferentiated malignant neoplasm. The tumour abuts the squamous epithelium in places, but there is no evidence of gradation or continuity between the two. Diagnosis: Undifferentiated high-grade malignant neoplasm. Definitive diagnosis of squamous carcinoma cannot be made on this biopsy. Review of the pre-treatment sections and block are necessary	EGFR (3C6) H score = 110 EGFR (5B7) H score = 120 Percentage cleaved caspase-3 = 0.5 (mean 10 high-power fields) Percentage CD3 = 13.5 (mean 10 high-power fields)	Negative	No
		Visit 8	9	Collected on: 06/10/2016 00:00 Accession: T4-09 AT Received on: 06/10/2016 00:00 Clinical Information:	Not Performed	Negative	No

				Study specific biopsy for T4 clinical trial post treatment. Biopsy from recurrent primary tumour. Macroscopic Description: Ellipse of mucosa 7x3x3mm. Serially sliced into 3. Central slice fresh to molecular studies. Two ends all embed. Microscopic Description: Superficial biopsies comprising surface squamous epithelium only. A moderate chronic inflammatory host response is present within the lamina propria, but there is no evidence of invasive carcinoma nor overt epithelial dysplasia. Diagnosis: No carcinoma present			
010	3	Screening	-11	Collected on: 11/11/2016 00:00 Accession: T4-10 BL Received on: 11/11/2016 00:00 Clinical Information: Study specific biopsy for T4 clinical trial before treatment. Biopsy from recurrent primary tumour Macroscopic Description: 2 mucosal pieces 7x3x5mm and 6x4x3mm. Both embed whole. Microscopic Description: Infiltrative moderately differentiated squamous cell carcinoma lies in continuity with dysplastic surface epithelium.	Supplementary report 1 EGFR (3C6) H score = 130 EGFR (5B7) H score = 200 Percentage cleaved caspase-3 = 0.5 (mean 10 high-power fields) Percentage CD3 = 0 (mean 10 high-power fields)	Negative *	No

				Diagnosis: Squamous cell carcinoma.			
		Visit 9	16	Clinical Information: Study specific biopsy for T4 clinical trial post treatment. Biopsy from recurrent primary treatment. Macroscopic Description: 2 mucousal pieces 4x3x2mm and 5x3x4. Both embed whole. Third mucosa piece 4x3x3mm fresh for molecular studies. Microscopic Description: Infiltrative moderately differentiated squamous cell carcinoma lies in continuity with dysplastic surface epithelium. Diagnosis: Squamous cell carcinoma.	Supplementary Report: EGFR (3C6) H score = 90 EGFR (5B7) H score = 220 Percentage cleaved caspase-3 = 1.3 (mean 10 high-power fields) Percentage CD3 = 0.01 (mean 10 high-power fields)	Negative	No
017	5	Screening	-14	Clinical Information: Right chest metastasis excised Macroscopic Description: 1 piece of skin bearing ST received in theatre, cut into three. 1 into neutral buffered formalin, 2 into RNALater (RNALater to Antonella in Research oncology) (RHS) Microscopic Description: Skin, the dermis of which contains islands and broad interconnecting trabeculae of a malignant neoplasm. Tumour cells demonstrate ovoid and partly vesicular nuclei, together with moderate anisonucleosis and pleomorphism. There is a brisk mitotic rate and frequent apoptotic tumour cells are evident. Possible duct-like structures containing eosinophilic material are present, and there is no definite evidence of intercellular bridges. The features are those of a high-grade carcinoma. There	Supplementary report 1 No Immunohistochemistry performed. Percentage cleaved caspase-3 = 8.0 (mean 10 high-power fields) Percentage CD3=0 (mean 10 high-power fields)	Negative*	No

				is no definite evidence of squamous differentiation. Confirmation of squamous origin requires further workup. Diagnosis: High-grade carcinoma			
		Visit 9	17	Clinical Information: Left neck metastasis Macroscopic Description: 3 cores of tan and haemorrhagic tissue, 2 into RNALater (RNALater to Antonella in Research oncology). Third core 12mm in length, 2mm diameter embedded whole. (RHS) Microscopic Description: Core biopsy of fibrous tissue, together with a fibrin exudate and extravasated erythrocytes. No carcinoma is evident in the section examined. Diagnosis: No carcinoma present.	No Immunohistochemistry performed.	Negative*	No

* Entered in database as “No transcripts detectable on RNA in situ hybridisation”