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Third-generation anti-CD19 chimeric antigen receptor T-cells incorporating a TLR2 domain for relapsed or refractory B-cell lymphoma: a phase I clinical trial protocol

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Chimeric antigen receptors, CD19 Antigen, Non-Hodgkin Lymphoma, B-Cell Lymphoma, Clinical Trial Protocol

ABSTRACT

Introduction: Autologous T-cells transduced to express a chimeric antigen receptor (CAR) directed against CD19 elicit high response rates in relapsed or refractory (r/r) B-cell non-Hodgkin lymphoma (B-NHL). However, r/r B-NHL remissions are durable in fewer than half of recipients of second-generation (2G) CAR T-cells. Third-generation (3G) CARs employ two co-stimulatory domains, resulting in improved CAR T-cell efficacy *in vitro* and in animal models *in vivo* . This investigator-initiated, phase 1 dose escalation trial, termed ENABLE, will investigate the safety and preliminary efficacy of WZTL-002, comprising autologous T-cells expressing a 3G anti-CD19 CAR incorporating the intracellular signalling domains of CD28 and Toll like receptor 2 (TLR2) for the treatment of r/r B-NHL.

Methods and analysis: Eligible participants will be adults with r/r B-NHL including diffuse large B-cell lymphoma and its variants, follicular lymphoma, transformed follicular lymphoma and mantle cell lymphoma. Participants must have satisfactory organ function, and lack other curative options. Autologous T-cells will be obtained by leukapheresis. Following WZTL-002 manufacture and product release, participants will receive lymphodepleting chemotherapy comprising intravenous fludarabine and cyclophosphamide. A single dose of WZTL-002 will be administered intravenously two days later. Targeted assessments for cytokine release syndrome (CRS) and immune cell effector-associated neurotoxicity syndrome (ICANS), graded by ASTCT criteria, will be made. A modified 3 + 3 dose escalation scheme is planned starting at 5×10^4 CAR T-cells/kg with a maximum dose of 1×10^6 CAR T-cells/kg. The primary outcome of this trial is safety of WZTL-002. Secondary outcomes include feasibility of WZTL-002 manufacture and preliminary measures of efficacy.

Ethics and dissemination: Ethical approval for the study was granted by the New Zealand Health and Disability Ethics Committee (reference 19/STH/69). Trial results will be reported in a peer-reviewed journal, and results presented at scientific conferences or meetings.

Trial registration number: NCT04049513

Strengths and Limitations of this Study

- Utilises a new third-generation anti-CD19 CAR construct incorporating both CD28 and TLR2 co-stimulatory domains
- Establishes feasibility of T-cell harvest, CAR T-cell manufacture and treatment delivery at a New Zealand centre
- Employs consensus grading systems for CRS and ICANS
- Dose escalation and dosing steps similar to those employed in other 3G anti-CD19 CAR T-cell trials
- Small sample size, inclusion of several B-NHL subtypes and dose escalation design means that efficacy and exploratory outcomes will be descriptive only

INTRODUCTION

CAR T-cell therapy for B-cell non-Hodgkin lymphoma

Non-Hodgkin lymphoma (NHL) is the 7th most common malignancy worldwide, accounting for over 200,000 deaths annually [3]. Over 90% of NHLs stem from the B-cell lineage (B-NHL), and can be divided into aggressive and indolent forms [4]. While aggressive subtypes of B-NHL, exemplified by diffuse large B-cell lymphoma (DLBCL), are often cured with chemoimmunotherapy, around 20% are either refractory to treatment or will relapse [5 6 7]. For most indolent B-NHL subtypes, such as follicular lymphoma (FL), relapses after chemoimmunotherapy are the norm, and while allogeneic stem cell transplantation is curative for some patients, its use is limited by significant short- and long-term toxicities, and by the need to identify a matched haematopoietic stem cell donor.

Autologous T-cells transduced to express a chimeric antigen receptor (CAR) specific for the B-cell antigen CD19 can lyse B-NHL cells [8]. Two such 'CAR T-cell therapies' have been licensed, incorporating a single intracellular co-stimulatory domain derived from either CD28 (axicabtagene ciloleucel) or 4-1BB (tisagenlecleucel). CAR T-cell therapies lead to impressive response rates in those with relapsed or refractory (r/r) DLBCL [9 10], and with indolent B-NHL subtypes [11]. However, only 35 – 40% of recipients of currently-licensed CAR T-cells for DLBCL remain free of progression for longer than 12 months, a lack of complete metabolic response after 3 months being a major predictor of CAR T-cell treatment failure [12]. Anti-CD19 CAR T-cell therapies that exhibit improved early complete metabolic response rates and long-term disease-free survival rates could fulfil an unmet need in r/r B-NHL [9 13].

Third-generation CAR T-cells

One way of enhancing CAR T-cell efficacy is to incorporate a second intracellular co-stimulatory domain within the CAR, generating so-called 'third-generation' (3G) CAR T-cells [14]. This can lead to improved CAR T-cell proliferation, cytotoxicity and persistence *in vivo* [15 16]. Most 3G CAR T-cells in registered clinical trials combine a co-stimulatory domain derived from an immunoglobulin (Ig)

superfamily member (such as CD28 or ICOS) alongside one derived from a tumour necrosis factor receptor (TNFR) superfamily member (such as 41BB or OX40), see Table 1 [14]. Potential benefits of 3G CAR constructs over 2G CARs have been demonstrated in pre-clinical studies [17-19]. For example, Zhao et al reported that 3G CARs containing both CD28 and 41BB costimulatory domains, led to greater expansion of $CD4^+$ and $CD8^+$ T-cells, along with improved B-ALL tumour regression in xenograft models [18]. However, it is not yet clear whether 3G CAR T-cells offer improved clinical efficacy.

Activated T-cells express Toll-like receptors (TLRs), particularly TLR2, a pattern recognition receptor that recognizes bacterial cell wall components [20 21]. Ligation of TLR2 enhances Akt and Erk1/Erk2 phosphorylation in response to T-cell receptor (TCR) stimulation, enhancing TCR-induced cytokine production and proliferation [22]. T-cell intrinsic TLR2 signalling lowers the T-cell activation threshold in response to costimulatory signals received from antigen presenting cells, and enables the generation of functional memory CD8 T-cells in response to T-cell activation [23 24].

Third generation CAR T-cells incorporating the Toll/interleukin-1 receptor (TIR) domain from TLR2, which mediates the intracellular signalling of TLR2, show improved anti-tumor activity compared to second-generation (2G) CAR T-cells both *in vitro* and *in vivo* [19]. The safety and efficacy of a 3G CAR T-cell product combining CD28 and TLR2 TIR co-stimulatory domains has been explored in a Phase I clinical trial in B-cell acute lymphoblastic leukaemia (B-ALL) (ClinicalTrials.gov reference NCT02822326), in which clinical responses were observed, including among participants with extra-medullary B-ALL tumours [25].

We have modified the manufacture of 3G anti-CD19 CAR T-cells incorporating CD28 and TLR2 TIR co-stimulatory domains, to employ a third-generation self-inactivating lentiviral vector for T-cell transduction and to adopt process modifications designed to meet local Good Manufacturing Practice (GMP) requirements. We plan a phase 1 dose escalation trial to assess the safety of this product, WZTL-002, for the treatment of r/r B-NHL.

METHODS

Study design

This investigator-initiated open-label phase 1 dose escalation trial is named ENABLE: Engaging Toll-like Receptor Signalling for B-cell Lymphoma Chimeric Antigen Receptor Therapy, (ClinicalTrial.gov number: NCT04049513). The ENABLE trial aims to assess the safety of WZTL-002, comprising autologous anti-CD19 3G CAR-T cells incorporating CD28 and TLR2 TIR co-stimulatory domains, for the treatment of r/r B-NHL. The sponsor is the Malaghan Institute of Medical Research (MIMR), and the trial is conducted in collaboration with Wellington Zhaotai Therapies Limited (WZTL).

Key inclusion and exclusion criteria are presented in Box 1. Structure of the CAR employed in WZTL-002 is presented in Figure 1, and the protocol schema in Figure 2.

A modified 3 + 3 dose escalation scheme with four dose steps (5×10^4; 1×10^5; 5×10^5; 1×10^6 CAR T-cells/kg) is planned. The first dose step is two steps (10-fold) below the recommended phase II dose determined in a phase I trial of a similar product in r/r B-ALL (ClinicalTrials.gov reference NCT02822326), and is similar to that used in two reported clinical trials of 3G CAR T-cell products [1] [2]. The final dose step was selected because dose-limiting toxicities (DLTs) were observed at this level in r/r B-ALL using a similar product (ClinicalTrials.gov reference NCT02822326) and because, based on preclinical data, the dose of WZTL-002 is expected to be lower than that recommended for the licensed 2G CAR T-cell product axicabtagene ciloleucel (2×10^6 CAR T-cells/kg). Additional dose steps may be incorporated if recommended by the Data Safety Monitoring Committee (DSMC). The DLT definitions are presented in Box 2.

Study procedures

Following written informed consent and screening, eligible participants will undergo a leukapheresis procedure to harvest autologous peripheral blood mononuclear cells for WZTL-002 manufacture. Following cell harvest, bridging chemo- or radiotherapy will be permitted to provide disease control during manufacturing and treatment scheduling, and to reduce lymphoma disease bulk before WZTL-002 administration.

Once product release criteria are met, eligibility to proceed to WZTL-002 treatment is confirmed, and following any bridging chemo- or radiotherapy, a baseline PET/CT scan will be performed. This will be followed by lymphodepleting chemotherapy comprising intravenous fludarabine (30 mg/m^2/day × 3 days) and cyclophosphamide (500 mg/m^2/day × 3 days). WZTL-002 will be administered following two chemotherapy-free days as a slow intravenous push. Participants will be monitored as an inpatient for 14 days, using both regular observations and specific cytokine release syndrome (CRS) and neurotoxicity assessments, including the Immune Effector-cell Encephalopathy (ICE) score, at least twice daily [26]. Daily assessment will continue until 21 days after WZTL-002 administration. To inform treatment of the next participant, assessment of DLTs will be undertaken 21 days after WZTL-002 administration (see Box 2).

Response assessment will be by PET/CT scan three months after WZTL-002 administration using the Deauville 5-point scoring system, and response to treatment will be assigned as either Complete Response (CR), Partial Response (PR), Stable Disease (SD) or Progressive Disease (PD), according to 2014 Lugano response criteria for lymphoma [27] . A further PET/CT scan will be performed at 6 months for those with partial response at the 3 month timepoint. Trial follow-up will take place at 3 monthly intervals until 1 year, 6 monthly intervals until 2 years and annually until 5 years post-WZTL-002 administration. Participants will be registered in the Center for International Blood and Marrow Transplant Research (CIBMTR) Cellular Therapies Registry and Australasian Bone Marrow Transplant Recipient Registry (ABMTRR), in order to capture low-incidence or late treatment-related toxicities.

Study aim and outcomes

The overall aim of the ENABLE trial is to assess the safety of 3G autologous anti-CD19 CAR T-cells incorporating CD28 and TLR2 TIR co-stimulatory domains (WZTL-002) in individuals with r/r B-NHL.

The primary outcome is safety profile of WZTL-002, determined by the number and severity of adverse events assessed by CTCAE v5.0, except for Cytokine Release Syndrome and Immune Effector Cell-Associated Neurotoxicity Syndrome, which will be assessed by American Society Transplantation and Cellular Therapy (ASTCT) consensus grading criteria [28].

Secondary outcomes are as follows:

1. Feasibility of WZTL-002 manufacture, as determined by the proportion of enrolled participants undergoing at least one study leukapheresis procedure that receive WZTL-002
2. Overall response rate (ORR) as determined by complete response (CR) plus partial response (PR) 3 months after WZTL-002 administration
3. Cumulative CR rate 6 months after WZTL-002 administration
4. Relapse-free survival (RFS) for participants treated with WZTL-002 over a period of 24 months after WZTL-002 administration
5. Overall survival (OS) for participants treated with WZTL-002 over a period of 24 months after WZTL-002 administration
6. The recommended phase 2 dose of WZTL-002 for the treatment of patients with r/r B-NHL

Exploratory outcomes are:

1. Kinetics and persistence of WZTL-002 following administration, determined by peripheral blood PCR for the CAR transgene
2. Extent and duration of B-cell aplasia, determined by peripheral blood flow cytometry and serum immunoglobulin G concentration

3. Serum cytokine profile following WZTL-002 administration
4. Phenotype of the WZTL-002 CAR T-cell product before administration, and of circulating CAR T-cells following administration.

Manufacture of WZTL-002

WZTL-002 will be manufactured in the Clinical Human Immunology Laboratory at the Malaghan Institute of Medical Research in Wellington, New Zealand, which is licensed by Medsafe, the New Zealand Medicines and Medical Devices Safety Authority. Briefly, T-cells will be immunomagnetically isolated from a leukapheresis product and activated using immunomagnetic anti-CD3/anti-CD28 beads, before transduction using a third-generation self-inactivating lentiviral vector. Cells will be expanded *ex vivo* over 10 days, cryopreserved in a controlled-rate freezer and stored in the vapour phase of liquid nitrogen pending confirmation that WZTL-002 release criteria are met, and pending scheduling and administration of lymphodepleting chemotherapy.

Immune monitoring and exploratory endpoints

Kinetics of WZTL-002 will be determined by determining the CAR transgene in patient peripheral blood by quantitative PCR before and at 1, 2, 4, 7, 9, 11, 14, 16, 18, 21 and 28 days, at 2, 3, 6, 12 and 24 months, and at 3, 4 and 5 years, after WZTL-002 administration. Serum cytokine profile after WZTL-002 administration will be determined by ELISA (for IL-6) and by cytokine bead array before and at 1, 2, 4, 7, 9, 11, 14, 16, 18, 21 and 28 days after WZTL-002 administration. Depth and duration of B-cell aplasia will be established by peripheral blood flow cytometry and by nephelometric determination of serum immunoglobulin G concentration, both to inform infection risk among participants, and to serve as a surrogate measure for WZTL-002 persistence. The immunophenotype (including CD4, CD8, CD45RA and CD62L) of WZTL-002 CAR T-cells will be determined in the pre-

administration product, and within participant peripheral blood mononuclear cells after administration (if CAR T-cells are detectable).

Toxicity management

Based on clinical experience with similar constructs, CRS and ICANS are anticipated toxicities of WZTL-002. Accordingly, a CAR T-cell toxicity (CARTOX) team comprising local intensive care, neurology, haematology, immunology and infectious disease specialists and nursing representatives was formed. This team localised consensus assessment and treatment protocols for CRS and ICANS, and reviewed safety measures and training and competency assessment materials [26,29]. A summary of key measures taken to prepare for CRS and ICANS is provided in Box 3.

Monitoring and data management

A trial management committee (TMC) including the Principal Investigator, at least one Co-Investigator, the study nurse, and a representative of the Clinical Human Immunology Laboratory, will meet at least monthly during study recruitment to review recruitment rates, trial conduct, trial procedures, Adverse Events (AEs) and Serious Adverse Events (SAEs). An independent Data and Safety Monitoring Committee (DSMC) will include clinicians with experience in early phase T-cell trials and in haemato-oncology. Per the DSMC Charter, the DSMC will meet and review trial accrual, conduct and safety data a minimum of 6-monthly and before each dose step. An independent study monitor will monitor the study, and will report to the Sponsor.

Statistical analysis

This phase I trial will be analysed using descriptive statistics; no formal hypothesis testing will be undertaken. All participants who commence lymphodepleting chemotherapy will be included in the summaries of the safety outcomes.

Safety outcomes including AEs, SAEs, Suspected Unexpected Serious Adverse Reactions (SUSARs), CRS, ICANS and DLTs will be individually listed by dose group and summarised as the frequency of

events and percentages of individuals experiencing each event type. Each event summary will include details of the timing, grade, and outcome of the event. Response outcomes including ORR (defined as CR rate plus PR rate) and CR rate, will be individually listed and summarised as frequencies (%) by dose group. The survival outcomes, RFS and OS will also be summarised as frequencies (%) at 24 months and the times to events will be individually listed and may be summarised with Kaplan-Meier curves for individual dose groups if sample sizes permit. Associations between safety outcomes and presenting features will be explored in a qualitative manner.

The anticipated sample size is 12 participants, with at least 3 participants treated at each dose step. If no DLTs are observed, escalation to the next dose step may occur (see Box 2 for DLT definition). If a DLT is observed in 1 of the first 3 participants treated at a specific dose step, a further 3 participants should be treated at that dose. If 2 or more participants develops a DLT at a specific dose step, escalation to the next dose step should not occur, indicating that the Maximum Tolerated Dose (MTD) has been reached. The DMSC will be meet before each proposed dose escalation and may recommend de-escalation to a lower dose level, protocol modification, or for more participants to be treated at that dose step, based on available safety and/or efficacy data.

Ethics

The study will performed in accordance with the principles of the International Conference on Harmonisation Guidelines on Good Clinical Practise (ICH-GCP) (Step 4, dated 10th June 1996) that have their origins in the Declaration of Helsinki [30].

The trial has been approved by the New Zealand Health and Disability Ethics Committee (reference 19/STH/69), and has been endorsed by Research Advisory Group Māori at Capital & Coast District Health Board, which is mandated to provide consultation for cultural appropriateness of clinical research conducted within the region (reference RAG-M #662).

Patient and Public Involvement

The study protocol was developed after discussion at a blood cancer patient forum convened by Leukaemia & Blood Cancer New Zealand, and at meetings of the Lymphoma Network of New Zealand and the NZ Branch of the Haematology Society of Australia and New Zealand. The study protocol and consent form were developed in consultation with Research Advisory Group - Māori, a Māori relationship board, which includes lay representation, to Capital & Coast District Health Board. The participant information and consent form was reviewed by a patient representative with relevant personal experience. The study has been publicised in national media, although due to regulatory and logistical considerations, referrals must come from a relevant specialist rather than directly from potential participants. Study results will be presented in the lay media as well as in scientific journals. The patient information and consent form includes an option to request a lay summary of the study results.

Data Dissemination

Participants will be given the option to receive a summary of the trial results. Trial results will be published in a peer-reviewed journal after completion of the trial.

DISCUSSION

This manuscript describes the protocol for ENABLE, an investigator-led Phase 1 dose escalation trial evaluating a new third generation (3G) autologous anti-CD19 CAR T-cell product (WZTL-002), for the treatment of individuals with r/r B-NHL. The primary outcome is safety, which will be assessed by determining the number and severity of adverse events. Secondary outcomes will assess feasibility, efficacy and recommended WZTL-002 dose for subsequent efficacy trials.

As well as resulting in improved cytotoxicity against target cells, the incorporation of a second co-stimulatory domain can enhance CAR T-cell proliferation and cytokine production [14 19]. Thus, while 3G CAR T-cell products have the potential for increased efficacy, there is also the potential for an increased risk of toxicities including CRS and ICANS risk, compared to second-generation products. Accordingly, CRS and ICANS were identified as events of special interest early during trial development. The risk of both toxicities may relate to CAR T-cell dose and to disease burden [31 32]. Therefore, to mitigate CRS and ICANS risks, conservative starting and maximum WZTL-002 doses have been selected, based on clinical experience using similar CAR T-cell products. Targeted CRS and ICANS assessments will be performed, and a comprehensive risk mitigation plan has been developed, including the development of institutional policies and protocols, documented staff training, intensive care escalation plans and on-site tocilizumab availability. Use of the ASTCT international consensus CRS and ICANS grading system will facilitate the comparison of toxicity rates with other anti-CD19 CAR T-cell trials conducted internationally [26].

The use of 'bridging' chemo- and radiotherapy between enrolment and WZTL-002 administration is permitted. This will facilitate WZTL-002 treatment scheduling during dose escalation, and may allow reduction of disease burden before WZTL-002 therapy, potentially reducing CRS and ICANS risk [33]. To mitigate the impact of bridging therapy on efficacy assessments, baseline PET-CT scans will be conducted after completing bridging therapy and before starting lymphodepleting chemotherapy and WZTL-002 administration.

The selection of eligible lymphoma subtypes was based on evidence of efficacy from other clinical trials evaluating anti-CD19 CAR T-cell therapies in this population [9-11 34 35]. Limitations of this trial include its small size, inclusion of several B-NHL subtypes, and dose escalation design, as a result of which efficacy and exploratory outcomes will be descriptive only. In particular, secondary outcomes assessing WZTL-002 efficacy (ORR, CR rate, RFS and OS) will be preliminary, and are included to help inform the design of future Phase II trials. Similarly, the exploratory outcomes, which explore WZTL-002 kinetics, phenotype, serum cytokines and B-cell aplasia, are intended to inform outcome measure selection for future larger trials.

The published clinical experience of 3G anti-CD19 CAR T-cells for the treatment of r/r B-NHL is limited, with the final results of only two other early-phase trials published, to our knowledge [1 2]. Enblad *et al* treated 11 patients with r/r B-NHL or Chronic Lymphocytic Leukaemia (CLL) with 3G anti-CD19 CAR T-cells combining CD28 and 41BB costimulatory domains, in a Phase 1 dose escalation study [1]. Of the 11 treated participants, four did not receive lymphodepletion before CAR T-cell administration. The dose range of 3G anti-CD19 CAR T-cells administered this study was $2 \times 10^7 – 2 \times 10^8$ cells/m^2 (approximately equivalent to $5 \times 10^5 – 5 \times 10^6$ CAR T-cells/kg). A response to treatment was observed in four participants (36%), all of whom reached CR [9]. Severe CRS was reported in two participants (18%), and severe neurotoxicity in one (9%).

Ramos *et al* reported results of a Phase 1 anti-CD19 CAR T-cell trial involving simultaneous administration of autologous 2G (CD28 only) and 3G (4-1BB plus CD28) anti-CD19 CAR T-cell products to participants with r/r B-NHL. This dose escalation study treated 11 participants with active lymphoma and five in remission after autologous stem cell transplant (ASCT). All participants with active lymphoma received lymphodepletion with cyclophosphamide and fludarabine before CAR T-cell infusion, whereas no further lymphodepletion was given to those post ASCT. The dose range of total CAR T-cells administered on this study (2G + 3G CAR T-cells in 1:1 ratio), was $5 \times 10^4 – 1 \times 10^6$ CAR T-cells/kg. Six of 11 with active lymphoma (54%) responded, three (27%) reaching CR. All five recipients of CAR T-cells after ASCT remained in CR at least 9 months after CAR T-cell

administration. No cases of severe CRS, and only one of severe neurotoxicity, were reported [2]. Ramos *et al* found that the 3G anti-CD19 CARs showed superior *in vivo* expansion and persisted longer than their 2G counterparts [2], although the relative contribution of the 2G and 3G CAR T-cells to anti-tumour efficacy and to toxicity could not be assessed with this study design.

In conclusion, published phase I trials suggest that manufacture of 3G CAR T-cells is feasible, and do not yet indicate that CRS and ICANS rates are higher than for 2G products. Moreover, the Ramos *et al* study indicates that 3G CAR T-cells can exhibit improved proliferation and persistence in humans compared to 2G counterparts. However, because of the small number of reported 3G CAR T-cell recipients, and the likely suboptimal CAR T-cell dosing in the early cohorts of these dose escalation studies, conclusions cannot be drawn about the relative efficacy and safety of 3G compared with 2G CAR T-cells [1 2]. Other 3G anti-CD19 CAR T-cell trials in patients with r/r B-NHL are underway (Table 1). As well as adding to the clinical experience of 3G anti-CD19 CAR T-cell therapies for the treatment of B-NHL, the ENABLE trial will inform the clinical safety and potential utility of a new intracellular TLR2 co-stimulatory domain within CAR T-cells.

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Contributors

RW conceived the clinical trial. RW and PG designed and wrote the study protocol. ND, PG and RW drafted the manuscript. RW, PG, ND, GG, BM, EB, BA, TP, TO, CF, DR, CB and IH provided input into the study protocol. PL conceived the TLR2 costimulatory domain construct, and provided background, preclinical and clinical data regarding its use. All authors reviewed the study manuscript. RW, TP, PG and TO will conduct study procedures. RW is the Principal Investigator.

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The Malaghan Institute of Medical Research (MIMR) is the sponsor of this investigator-initiated trial. Delegated duties will be assigned to Capital and Coast District Health Board and its employees by means of the site clinical trial agreement, and to the study monitor by means of a monitor agreement.

The study is funded by philanthropic support to MIMR, an independent biomedical research institute and registered charity. A private company, WZTL, provided the rights to use the 1928T2z construct, the source plasmids for vector production, and contributed to MIMR costs for the production of WZTL-002. WZTL is not involved in study design, conduct or reporting, which are responsibilities of the Principal Investigator.

Competing Interests

PL has proprietary interest in the intellectual property of the 1928T2z construct. CB is co-Founder and Scientific Advisory Board Member of Mana Therapeutics is on the Advisory Board of Cellectis, has Stock ownership in Torque Therapeutics and Neximmune and is a Board Member of Caballeta Bio.

Patient consent

Obtained.

Ethics approval

Approved by the Southern Health and Disability Ethics Committee, New Zealand. Ethics reference: 19/STH/69.

Provenance and peer review

Not commissioned, externally peer reviewed.

Data sharing statement

The technical appendix, statistical code and data set are available from the corresponding author at rweinkove@malaghan.org.nz. The participants gave informed consent for the data sharing.

Open access

Box 1 Inclusion and Exclusion criteria for ENABLE

Inclusion criteria

- Age 16 to 75 years (inclusive)
- Biopsy-proven relapsed or treatment refractory aggressive B-cell non-Hodgkin lymphoma of the following subtypes per World Health Organisation (WHO) classification: Diffuse Large B-Cell Lymphoma (DLBCL) and its variants, Primary Mediastinal B-Cell Lymphoma (PMBCL), transformed Follicular Lymphoma (tFL), Follicular Lymphoma (FL) and Mantle Cell Lymphoma (MCL)
- Requirement for treatment in the opinion of the investigator
- No other curative treatments available, or not suitable due to patient or disease characteristics or lack of stem cell donor
- Malignancy documented to express CD19 based on flow cytometric or immunohistochemical staining
- Provision of written informed consent for this study
- Life-expectancy from non-lymphoma related causes of > 12 months
- European Cooperative Oncology Group (ECOG) performance status of 0 to 2 inclusive
- Adequate haematologic function, defined by neutrophils $\geq 1.0 \times 10^9$/L and platelets $\geq 50 \times 10^9$/L
- No serious cardiac, pulmonary, hepatic or renal disease.
    - Serum bilirubin < 2.5 times upper limit of normal (ULN)
    - Estimated creatinine clearance ≥ 50 mL/min using the modified Cockroft Gault estimation or as assessed by direct measurement
    - Cardiac Ejection Fraction ≥ 50% as determined by Echocardiogram or MUGA Scan
    - Oxygen saturations > 92% on room air
    - Diffuse Capacity of the lungs for carbon monoxide (DLCO) or Carbon monoxide transfer coefficient (KCO), Forced expiratory volume in one second (FEV1) and Forced Vital Capacity (FVC) are all ≥ 50% of predicted by spirometry after correcting for haemoglobin and/or volume on lung function testing.

Exclusion criteria

- Confirmed active or prior central nervous system (CNS) involvement by lymphoma. In patients with a clinical suspicion of CNS disease, lumbar puncture and MRI brain must be performed
- Active CNS pathology including: epilepsy, seizure within the preceding year, aphasia, paresis, stroke, dementia, psychosis within the preceding year, severe brain injury, Parkinson disease, or cerebellar disease
- Richter Syndrome

- Active autoimmune disease requiring systemic immunosuppression
- Prior solid organ transplantation
- Allogeneic stem cell transplantation within the preceding three months or still requiring systemic immunosuppression
- Current grade II – IV acute graft versus host disease (GVHD), any prior grade IV acute GVHD, or current moderate or severe chronic GVHD
- Need for systemic corticosteroids to treat a condition other than B-NHL at a daily dose of ≥ 10 mg prednisone (or equivalent)
- Peripheral blood lymphocytes < 0.5 x 10^9/L as assessed by complete blood count
- Peripheral blood $CD3^+$ T cells < 350/μL as assessed by lymphocyte subset analysis
- Pregnant or lactating female
- Women of child-bearing potential who are not willing to use highly effective methods of contraception during study participation and for at least 1 year after WZTL-002 administration
- Men who are not willing to use highly effective methods of contraception during study participation and for at least 1 year after WZTL-002 administration
- Men who have a pregnant partner and are not willing to use a condom while performing sexual activity during study participation and for at least 3 months after WZTL-002 administration
- Subjects with known sensitivity to immunoglobulin or to components of the investigational product (IP)
- History of active malignancy other than B-cell malignancy within two years prior to enrolment, with the exception of: adequately treated *in situ* carcinoma of the cervix; adequately treated basal cell carcinoma (BCC) or localized squamous cell carcinoma (SCC) of the skin; other localised malignancy surgically resected (or radically treated with another treatment modality) with curative intent
- Current or prior human immunodeficiency virus (HIV) infection
- Vaccination with a live virus within the preceding four weeks
- Treatment with a purine analogue within the preceding four weeks
- Treatment with alemtuzumab within the preceding 12 weeks
- Prior gene therapy, including prior anti-CD19 chimeric antigen receptor T-cell therapy
- Receipt of an investigational medicine within another clinical trial within the preceding four weeks
- Inadequately controlled systemic infection
- Serologic status reflecting active viral hepatitis B or any history of hepatitis C infection as follows:
- Presence of hepatitis B surface antigen (HBsAg) or hepatitis B core antibody (HBcAb). Patients with presence of HBcAb, but absence of HBsAg, are eligible if hepatitis B virus (HBV) DNA is undetectable (< 20 IU), and if they are willing to receive appropriate anti-viral prophylaxis.
- Presence of hepatitis C virus (HCV) antibody

- Presence of New York Heart Association (NYHA) class 2 or higher cardiac symptoms not related to lymphoma
- Significant concomitant illnesses which would in the investigator's opinion make the patient an unsuitable candidate for the trial
- Subjects who have diminished capacity or any circumstance that would prohibit them from understanding and providing informed consent in accordance with ICH-GCP (International Conference on Harmonisation, Good Clinical Practice)
- Subject does not provide consent to enrol onto International Cellular Therapy Registry

Box 2 Dose Limiting Toxicities

A DLT is a toxicity or AE occurring during the DLT assessment period (first 21 days after WZTL-002 administration), which is not attributable to a cause unrelated to WZTL-002 (such as underlying lymphoma, concurrent illness or concomitant medications), and meets one of the following criteria:

- Grade 4 or greater CRS or ICANS or grade 3 CRS or ICANS that does not resolve to grade 2 or lower within 7 days, both as per American Society for Transplantation and Cellular Therapy (ASTCT) criteria
- Any adverse event requiring airway intubation (including neurotoxicity requiring intubation for airway protection)
- Grade 4 neutropenia that does not resolve to grade 3 or lower within 21 days after WZTL-002 administration
- Platelet transfusion-dependent thrombocytopenia persisting for 21 days or longer after WZTL-002 administration
- All grade 4 toxicities, and grade 3 toxicities that do not resolve to grade 2 or lower within 7 days, with the exception of the following, which are not automatically considered DLTs:
  - Myelosuppression, including

- neutropenia
- bacterial infection in the setting of neutropenia with neutrophils < 1.0×10^9/L
- thrombocytopenia
- bleeding in the setting of thrombocytopenia with platelets < 50×10^9/L
- anaemia
- lymphopenia

- Hypersensitivity reactions occurring within 2 hours of WZTL-002 administration (and considered related to cell administration) that resolve to grade 2 or less within 24 hours
- Asymptomatic biochemical abnormalities that resolve to grade 2 or lower within 7 days
- Hypogammaglobulinemia

For CRS and ICANS, ASTCT grading criteria will be used. For all other toxicities, CTCAE v 5.0 will be used.

Box 3 Summary of measures taken to prepare for CRS and ICANS

- Formation of CAR T-cell Toxicity Working Group composed of Haematologists, Neurologist, ICU Physician, Immunologist and Haematology Nurses
- Attendance of principal investigator, two co-investigators, study nurse, senior haematology nurses and nurse educators at a CAR T-cell Toxicities Preceptorship Day
- Localisation of guidelines for CRS and ICANS identification, management and escalation, and the upload of these to institutional electronic treatment guide
- Education sessions and competency assessments on CAR T-cell toxicities delivered to Nursing, Patient at Risk and ICU teams
- Allowance for bridging treatment between leukapheresis and lymphodepleting chemotherapy to 'debulk' or control disease before WZTL-002 administration
- Confirmation that three doses of tocilizumab are on site before WZTL-002 administration
- Completion of clinical checklist before WZTL-002 administration
- Notification of Neurology and ICU Teams before WZTL-002 administration
- Scheduled nurse-led CRS and ICANS assessments
- Provision of participant-held wallet card and discharge summary sheet

Table 1 Other third-generation anti-CD19 CAR T-Cell trials registered on ClinicalTrials.gov

Study ClinicalTrials.gov ID	B-cell malignancy subtypes	CAR generation	Study Phase	Lymphodepletion	Study Start Date	Results published (Yes/no)
NCT02963038	B-ALL + B-NHL	3G	I+II	Not specified	June 2016	No
NCT03068416	B-ALL + B-NHL	3G	II	Not specified	September 2017	No
NCT02132624 (see discussion section in paper)	B-NHL	3G	I	Flu 25 mg/m^2 x 3d, Cy 500 mg/m^2 x 3d	April 2014	Yes †
NCT03146533	B-NHL	3G	I+II	Flu 30mg/m^2 x 3d Cy 800mg/m^2 x 3d	April 2017	No
NCT01853631 (see discussion section in paper)	B-ALL + B-NHL	3G and 2G*	I	Flu 30 mg/m^2 x 3d, Cy 500mg/m^2 x 3d	February 2014	Yes‡
NCT03676504	B-ALL + B-NHL	3G	I+II	Flu 30 mg/m^2 x 3d, Cy 500mg/m^2 x 3d	September 2018	No
NCT02822326	B-ALL	3G	I	Flu 25mg/m^2 x 3d Cy 300mg/m^2 x 3d	January 2016	No

As at 16/09/2019
B-ALL, B-cell Acute Lymphoblastic Leukaemia; B-NHL, B-cell non-Hodgkin lymphoma
*Co-infused with CD28 containing second-generation CAR and CD28 + 41BB containing third generation CAR
†See Enblad et al[1]
‡See Ramos et al[2]

Figure 1: Diagrammatic representation of WZTL-002 Anti-CD19 third generation CAR T-cell illustrating the co-stimulatory domains and components of the chimeric antigen receptor

Figure 2: Schema for the ENABLE phase 1 dose escalation study

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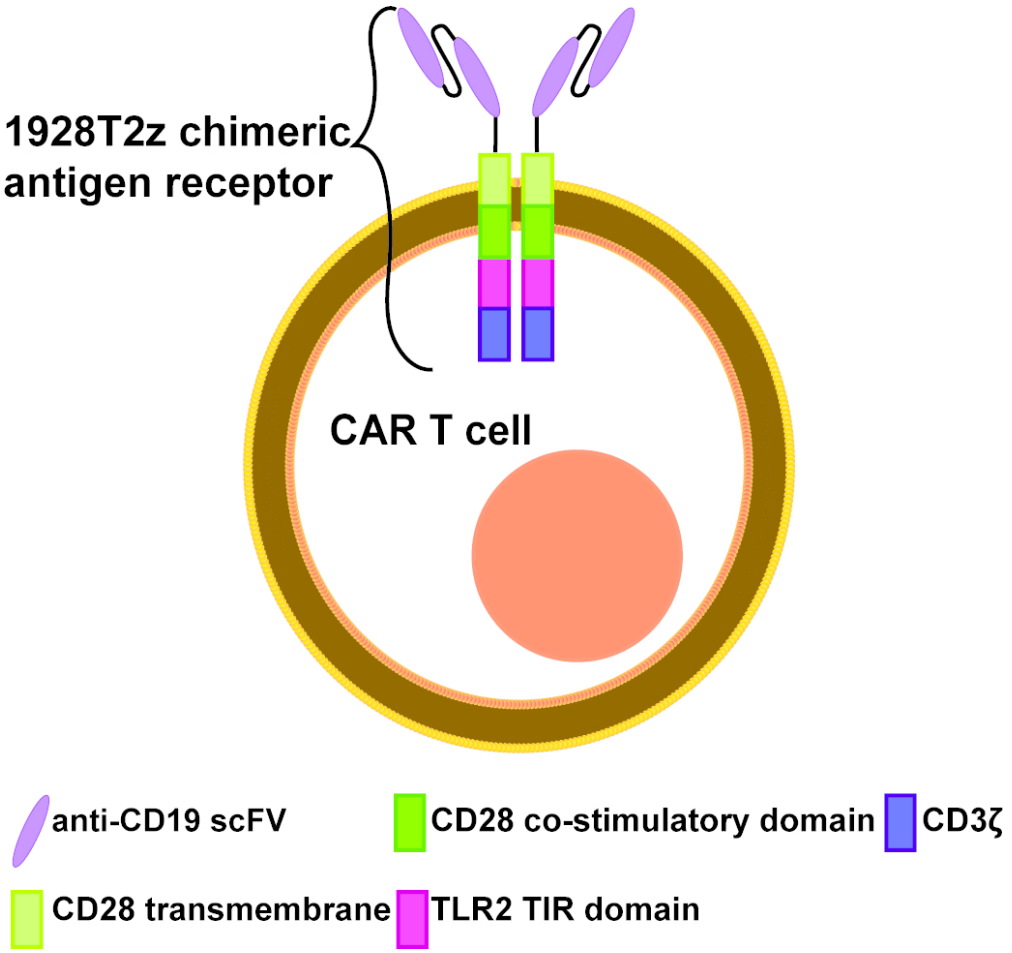
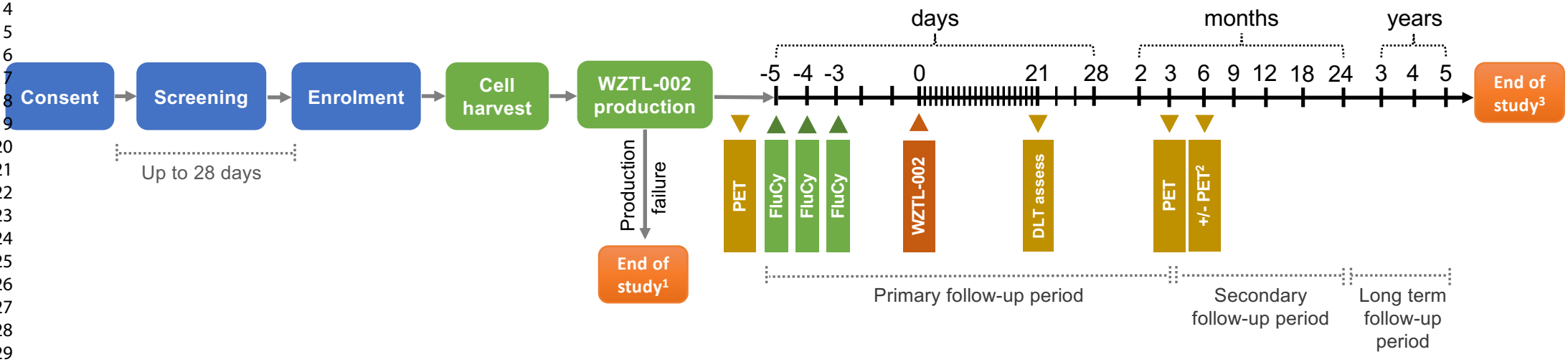


Figure 1: Diagrammatic representation of WZTL-002 Anti-CD19 third generation CAR T-cell illustrating the co-stimulatory domains and components of the chimeric antigen receptor



Notes and abbreviations:

[1] Second attempt at cell harvest and WZTL-002 production may be considered at discretion of TMC

[2] 6 month PET scan if first PET scan post WZTL-002 treatment shows partial response

[3] Long-term follow-up through bone marrow transplant clinic and Cellular Therapies Registry enrolment

FluCy, fludarabine and cyclophosphamide IV; PET, positron-emission tomography/computed tomography scan

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Third-generation anti-CD19 chimeric antigen receptor T-cells incorporating a TLR2 domain for relapsed or refractory B-cell lymphoma: a phase I clinical trial protocol (ENABLE)

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Third-generation anti-CD19 chimeric antigen receptor T-cells incorporating a TLR2 domain for relapsed or refractory B-cell lymphoma: a phase I clinical trial protocol (ENABLE)

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Chimeric antigen receptors, CD19 Antigen, Non-Hodgkin Lymphoma, B-Cell Lymphoma, Clinical Trial Protocol

ABSTRACT

Introduction: Autologous T-cells transduced to express a chimeric antigen receptor (CAR) directed against CD19 elicit high response rates in relapsed or refractory (r/r) B-cell non-Hodgkin lymphoma (B-NHL). However, r/r B-NHL remissions are durable in fewer than half of recipients of second-generation (2G) CAR T-cells. Third-generation (3G) CARs employ two co-stimulatory domains, resulting in improved CAR T-cell efficacy *in vitro* and in animal models *in vivo* . This investigator-initiated, phase 1 dose escalation trial, termed ENABLE, will investigate the safety and preliminary efficacy of WZTL-002, comprising autologous T-cells expressing a 3G anti-CD19 CAR incorporating the intracellular signalling domains of CD28 and Toll like receptor 2 (TLR2) for the treatment of r/r B-NHL.

Methods and analysis: Eligible participants will be adults with r/r B-NHL including diffuse large B-cell lymphoma and its variants, follicular lymphoma, transformed follicular lymphoma and mantle cell lymphoma. Participants must have satisfactory organ function, and lack other curative options. Autologous T-cells will be obtained by leukapheresis. Following WZTL-002 manufacture and product release, participants will receive lymphodepleting chemotherapy comprising intravenous fludarabine and cyclophosphamide. A single dose of WZTL-002 will be administered intravenously two days later. Targeted assessments for cytokine release syndrome (CRS) and immune cell effector-associated neurotoxicity syndrome (ICANS), graded by ASTCT criteria, will be made. A modified 3 + 3 dose escalation scheme is planned starting at 5×10^4 CAR T-cells/kg with a maximum dose of 1×10^6 CAR T-cells/kg. The primary outcome of this trial is safety of WZTL-002. Secondary outcomes include feasibility of WZTL-002 manufacture and preliminary measures of efficacy.

Ethics and dissemination: Ethical approval for the study was granted by the New Zealand Health and Disability Ethics Committee (reference 19/STH/69) on 23rd June 2019 for Protocol Version 1.2. Trial results will be reported in a peer-reviewed journal, and results presented at scientific conferences or meetings.

Trial registration number: NCT04049513
Trial opened to recruitment on 30th September 2019.

Strengths and Limitations of this Study

- Utilises a new third-generation anti-CD19 CAR construct incorporating both CD28 and TLR2 co-stimulatory domains
- Establishes feasibility of T-cell harvest, CAR T-cell manufacture and treatment delivery at a New Zealand centre
- Employs consensus grading systems for CRS and ICANS
- Dose escalation and dosing steps similar to those employed in other 3G anti-CD19 CAR T-cell trials
- Small sample size, inclusion of several B-NHL subtypes and dose escalation design means that efficacy and exploratory outcomes will be descriptive only

INTRODUCTION

CAR T-cell therapy for B-cell non-Hodgkin lymphoma

Non-Hodgkin lymphoma (NHL) is the 7th most common malignancy worldwide, accounting for over 200,000 deaths annually [1]. Over 90% of NHLs stem from the B-cell lineage (B-NHL), and can be divided into aggressive and indolent forms [2]. While aggressive subtypes of B-NHL, exemplified by diffuse large B-cell lymphoma (DLBCL), are often cured with chemoimmunotherapy, around 20% are either refractory to treatment or will relapse [3 4 5]. For most indolent B-NHL subtypes, such as follicular lymphoma (FL), relapses after chemoimmunotherapy are the norm, and while allogeneic stem cell transplantation is curative for some patients, its use is limited by significant short- and long-term toxicities, and by the need to identify a matched haematopoietic stem cell donor.

Autologous T-cells transduced to express a chimeric antigen receptor (CAR) specific for the B-cell antigen CD19 can lyse B-NHL cells [6]. Two such 'CAR T-cell therapies' have been licensed, incorporating a single intracellular co-stimulatory domain derived from either CD28 (axicabtagene ciloleucel) or 4-1BB (tisagenlecleucel). CAR T-cell therapies lead to impressive response rates in those with relapsed or refractory (r/r) DLBCL [7 8], and with indolent B-NHL subtypes [9]. However, only 35 – 40% of recipients of currently-licensed CAR T-cells for DLBCL remain free of progression for longer than 12 months, a lack of complete metabolic response by six months being a major predictor of CAR T-cell treatment failure [10]. Anti-CD19 CAR T-cell therapies that exhibit improved early complete metabolic response rates and long-term disease-free survival rates could fulfil an unmet need in r/r B-NHL [7 11].

Third-generation CAR T-cells

One way of enhancing CAR T-cell efficacy is to incorporate a second intracellular co-stimulatory domain within the CAR, generating so-called 'third-generation' (3G) CAR T-cells [12]. This can lead to improved CAR T-cell proliferation, cytotoxicity and persistence *in vivo* [13 14]. Most 3G CAR T-cells in registered clinical trials combine a co-stimulatory domain derived from an immunoglobulin (Ig)

superfamily member (such as CD28 or ICOS) alongside one derived from a tumour necrosis factor receptor (TNFR) superfamily member (such as 41BB or OX40), see Table 1 [12]. Potential benefits of 3G CAR constructs over 2G CARs have been demonstrated in pre-clinical studies [15-17]. For example, Zhao et al reported that 3G CARs containing both CD28 and 41BB costimulatory domains, led to greater expansion of $CD4^+$ and $CD8^+$ T-cells, along with improved B-ALL tumour regression in xenograft models [16]. However, it is not yet clear whether 3G CAR T-cells offer improved clinical efficacy.

Activated T-cells express Toll-like receptors (TLRs), particularly TLR2, a pattern recognition receptor that recognizes bacterial cell wall components [18 19]. Ligation of TLR2 enhances Akt and Erk1/Erk2 phosphorylation in response to T-cell receptor (TCR) stimulation, enhancing TCR-induced cytokine production and proliferation [20]. T-cell intrinsic TLR2 signalling lowers the T-cell activation threshold in response to costimulatory signals received from antigen presenting cells, and enables the generation of functional memory CD8 T-cells in response to T-cell activation [21 22].

Third generation CAR T-cells incorporating the Toll/interleukin-1 receptor (TIR) domain from TLR2, which mediates the intracellular signalling of TLR2, show improved anti-tumor activity compared to second-generation (2G) CAR T-cells both *in vitro* and *in vivo* [17]. The safety and efficacy of a 3G CAR T-cell product combining CD28 and TLR2 TIR co-stimulatory domains has been explored in a Phase I clinical trial in B-cell acute lymphoblastic leukaemia (B-ALL) (ClinicalTrials.gov reference NCT02822326), in which clinical responses were observed, including among participants with extra-medullary B-ALL tumours [23].

We have modified the manufacture of 3G anti-CD19 CAR T-cells incorporating CD28 and TLR2 TIR co-stimulatory domains, to employ a third-generation self-inactivating lentiviral vector for T-cell transduction and to adopt process modifications designed to meet local Good Manufacturing Practice (GMP) requirements. We plan a phase 1 dose escalation trial to assess the safety of this product, WZTL-002, for the treatment of r/r B-NHL.

METHODS

Study design

This investigator-initiated open-label phase 1 dose escalation trial is named ENABLE: Engaging Toll-like Receptor Signalling for B-cell Lymphoma Chimeric Antigen Receptor Therapy, (ClinicalTrial.gov number: NCT04049513). The ENABLE trial aims to assess the safety of WZTL-002, comprising autologous anti-CD19 3G CAR-T cells incorporating CD28 and TLR2 TIR co-stimulatory domains, for the treatment of r/r B-NHL. The sponsor is the Malaghan Institute of Medical Research (MIMR), and the trial is conducted in collaboration with Wellington Zhaotai Therapies Limited (WZTL). The Study Site is Wellington Hospital, Capital & Coast District Health Board, New Zealand.

Key inclusion and exclusion criteria are presented in Box 1. In addition to the inclusion and exclusion criteria presented in Box 1, immunosuppressive therapies, with the exception of lymphodepleting chemotherapy, must be avoided during the week before WZTL-002 administration (72 hours for systemic corticosteroids). Prior autologous and allogeneic stem cell recipients are eligible to participate in the Study. Structure of the CAR employed in WZTL-002 is presented in Figure 1, and the protocol schema in Figure 2.

A modified 3 + 3 dose escalation scheme with four dose steps (5×10^4; 1×10^5; 5×10^5; 1×10^6 CAR T-cells/kg) is planned. The first dose step is two steps (10-fold) below the recommended phase II dose determined in a phase I trial of a similar product in r/r B-ALL (ClinicalTrials.gov reference NCT02822326), and is similar to that used in two reported clinical trials of 3G CAR T-cell products [24] [25]. The final dose step was selected because dose-limiting toxicities (DLTs) were observed at this level in r/r B-ALL using a similar product (ClinicalTrials.gov reference NCT02822326) and because, based on preclinical data, the dose of WZTL-002 is expected to be lower than that recommended for the licensed 2G CAR T-cell product axicabtagene ciloleucel (2×10^6 CAR T-cells/kg). Additional dose steps may be incorporated if recommended by the Data Safety Monitoring Committee (DSMC). The DLT definitions are presented in Box 2.

Study procedures

All potential participants will be assessed at a lymphoma multidisciplinary team meeting to confirm that no other curative treatment options are available. Following written informed consent and screening, eligible participants will undergo a leukapheresis procedure to harvest autologous peripheral blood mononuclear cells for WZTL-002 manufacture. Following cell harvest, bridging therapy will be permitted to provide disease control during manufacturing and treatment scheduling, and to reduce lymphoma disease bulk before WZTL-002 administration. Anti-microbial prophylaxis and tumour lysis prophylaxis will be given as per standard of care for patients receiving treatment for haematological malignancies.

Once product release criteria are met, eligibility to proceed to WZTL-002 treatment is confirmed, and following any bridging chemo- or radiotherapy, a baseline PET/CT scan will be performed. This will be followed by lymphodepleting chemotherapy comprising intravenous fludarabine (30 mg/m^2/day × 3 days) and cyclophosphamide (500 mg/m^2/day × 3 days). WZTL-002 will be administered following two chemotherapy-free days as a slow intravenous push. Participants will be monitored as an inpatient for 14 days, using both regular observations and specific cytokine release syndrome (CRS) and neurotoxicity assessments, including the Immune Effector-cell Encephalopathy (ICE) score, at least twice daily [26]. Daily assessment will continue until 21 days after WZTL-002 administration. To inform treatment of the next participant, assessment of DLTs will be undertaken 21 days after WZTL-002 administration (see Box 2).

Response assessment will be by PET/CT scan three months after WZTL-002 administration using the Deauville 5-point scoring system, and response to treatment will be assigned as either Complete Response (CR), Partial Response (PR), Stable Disease (SD) or Progressive Disease (PD), according to 2014 Lugano response criteria for lymphoma [27] . A further PET/CT scan will be performed at 6 months for those with partial response at the 3 month timepoint. Additional imaging to assess or confirm treatment response, to investigate toxicities, or to seek potential disease progression, may be carried out at any time, as clinically indicated. Trial follow-up will take place at 3 monthly intervals until 1 year, 6 monthly intervals until 2 years and annually until 5 years post-WZTL-002 administration. Participants

will be registered in the Center for International Blood and Marrow Transplant Research (CIBMTR) Cellular Therapies Registry and Australasian Bone Marrow Transplant Recipient Registry (ABMTRR), in order to capture low-incidence or late treatment-related toxicities.

Study aim and outcomes

The overall aim of the ENABLE trial is to assess the safety of 3G autologous anti-CD19 CAR T-cells incorporating CD28 and TLR2 TIR co-stimulatory domains (WZTL-002) in individuals with r/r B-NHL.

The primary outcome is safety profile of WZTL-002, determined by the number and severity of adverse events assessed by CTCAE v5.0, except for Cytokine Release Syndrome and Immune Effector Cell-Associated Neurotoxicity Syndrome, which will be assessed by American Society Transplantation and Cellular Therapy (ASTCT) consensus grading criteria [28].

Secondary outcomes are as follows:

1. Feasibility of WZTL-002 manufacture, as determined by the proportion of enrolled participants undergoing at least one study leukapheresis procedure that receive WZTL-002
2. Overall response rate (ORR) as determined by complete response (CR) plus partial response (PR) 3 months after WZTL-002 administration
3. Cumulative CR rate 6 months after WZTL-002 administration
4. Relapse-free survival (RFS) for participants treated with WZTL-002 over a period of 24 months after WZTL-002 administration
5. Overall survival (OS) for participants treated with WZTL-002 over a period of 24 months after WZTL-002 administration
6. The recommended phase 2 dose of WZTL-002 for the treatment of patients with r/r B-NHL

Exploratory outcomes are:

1. Kinetics and persistence of WZTL-002 following administration, determined by peripheral blood PCR for the CAR transgene
2. Extent and duration of B-cell aplasia, determined by peripheral blood flow cytometry and serum immunoglobulin G concentration
3. Serum cytokine profile following WZTL-002 administration
4. Phenotype of the WZTL-002 CAR T-cell product before administration, and of circulating CAR T-cells following administration.

Manufacture of WZTL-002

WZTL-002 will be manufactured in the Clinical Human Immunology Laboratory at the Malaghan Institute of Medical Research in Wellington, New Zealand, which is licensed by Medsafe, the New Zealand Medicines and Medical Devices Safety Authority. Briefly, peripheral blood mononuclear cells (PBMCs) are isolated from the leukapheresis using a density gradient medium. T-cells are selected and activated using immunomagnetic CD3/CD28 microbeads and genetically modified using a third-generation generation self-inactivating non-replication competent lentiviral vector (manufactured in-house and tested according to EMEA guidelines). After washing to remove lentiviral vector and microbeads, CAR T-cells are expanded in a GMP-grade medium supplemented with IL-2 and human serum for 7 days. The CAR T-cell product is harvested and formulated in a cryopreservation medium containing 10% DMSO. Release criteria for the CAR T-cell product include product sterility, identity, purity and absence of residual lentiviral vector.

Immune monitoring and exploratory endpoints

Kinetics of WZTL-002 will be determined by determining the CAR transgene in patient peripheral blood by quantitative PCR before and at 1, 2, 4, 7, 9, 11, 14, 16, 18, 21 and 28 days, at 2, 3, 6, 12 and 24 months, and at 3, 4 and 5 years, after WZTL-002 administration. Serum cytokine profile after

WZTL-002 administration will be determined by ELISA (for IL-6) and by cytokine bead array before and at 1, 2, 4, 7, 9, 11, 14, 16, 18, 21 and 28 days after WZTL-002 administration. At each timepoint 25 mL of blood is taken for study-specific analyses. In subjects with neurotoxicity, CSF samples may be stored for assessment of cytokine and CAR transgene levels. Depth and duration of B-cell aplasia will be established by peripheral blood flow cytometry and by nephelometric determination of serum immunoglobulin G concentration, both to inform infection risk among participants, and to serve as a surrogate measure for WZTL-002 persistence. The immunophenotype (including CD4, CD8, CD45RA and CD62L) of WZTL-002 CAR T-cells will be determined in the pre-administration product, and within participant peripheral blood mononuclear cells after administration (if CAR T-cells are detectable).

Samples of the CAR T-cell product and recipient PBMCs post-administration (at each protocolised timepoint) will be cryopreserved. The timepoints for exploratory analyses will be selected based on pharmacokinetics determined by PCR for the CAR transgene. The outcomes of exploratory analyses are not expected to be definitive, and are included to inform design of a subsequent efficacy trial.

Toxicity management

Based on clinical experience with similar constructs, CRS and ICANS are anticipated toxicities of WZTL-002. Accordingly, a CAR T-cell toxicity (CARTOX) team comprising local intensive care, neurology, haematology, immunology and infectious disease specialists and nursing representatives was formed. This team localised consensus assessment and treatment protocols for CRS and ICANS, and reviewed safety measures and training and competency assessment materials [26,29]. A summary of key measures taken to prepare for CRS and ICANS is provided in Box 3.

Monitoring and data management

A trial management committee (TMC) including the Principal Investigator, at least one Co-Investigator, the study nurse, and a representative of the Clinical Human Immunology Laboratory, will meet at least monthly during study recruitment to review recruitment rates, trial conduct, trial procedures, Adverse Events (AEs) and Serious Adverse Events (SAEs). An independent Data and Safety Monitoring Committee (DSMC) will include clinicians with experience in early phase T-cell trials and in haemato-oncology. Per the DSMC Charter, the DSMC will meet and review trial accrual, conduct and safety data a minimum of 6-monthly and before each dose step. An independent study monitor will monitor the study, and will report to the Sponsor.

The Study site will hold responsibility for the confidentiality of electronic and paper clinical records held for the study participant. To maintain confidentiality of trial participants, study data or samples sent to collaborating investigators or external contractors for analysis or review will be labelled with study-specific codes, and not with patient identifiers. The Principal Investigator will hold responsibility for ensuring that presentations and publications of the study findings do not contain identifiable information. Laboratory records will be kept for a minimum of fifteen years. Clinical data (including Case Report Forms) will be stored securely for a minimum of fifteen years.

Statistical analysis

This phase I trial will be analysed using descriptive statistics; no formal hypothesis testing will be undertaken. All participants who commence lymphodepleting chemotherapy will be included in the summaries of the safety outcomes.

Safety outcomes including AEs, SAEs, Suspected Unexpected Serious Adverse Reactions (SUSARs), CRS, ICANS and DLTs will be individually listed by dose group and summarised as the frequency of events and percentages of individuals experiencing each event type. Each event summary will include details of the timing, grade, and outcome of the event. Response outcomes including ORR (defined as CR rate plus PR rate) and CR rate, will be individually listed and summarised as frequencies (%) by

dose group. The survival outcomes, RFS and OS will also be summarised as frequencies (%) at 24 months and the times to events will be individually listed and may be summarised with Kaplan-Meier curves for individual dose groups if sample sizes permit. Associations between safety outcomes and presenting features will be explored in a qualitative manner.

The study sample size will depend upon dose-limiting toxicities observed during dose escalation, and is estimated at 12 participants, with at least 3 participants treated at each dose step. If no DLTs are observed, escalation to the next dose step may occur (see Box 2 for DLT definition). If a DLT is observed in 1 of the first 3 participants treated at a specific dose step, a further 3 participants should be treated at that dose. If 2 or more participants develops a DLT at a specific dose step, escalation to the next dose step should not occur, indicating that the Maximum Tolerated Dose (MTD) has been reached. The DMSC will be meet before each proposed dose escalation and may recommend de-escalation to a lower dose level, protocol modification, or for more participants to be treated at that dose step, based on available safety and/or efficacy data.

Ethics

The study will performed in accordance with the principles of the International Conference on Harmonisation Guidelines on Good Clinical Practise (ICH-GCP) (Step 4, dated 10th June 1996) that have their origins in the Declaration of Helsinki [30].

The trial has been approved by the New Zealand Health and Disability Ethics Committee (reference 19/STH/69), and has been endorsed by Research Advisory Group Māori at Capital & Coast District Health Board, which is mandated to provide consultation for cultural appropriateness of clinical research conducted within the region (reference RAG-M #662).

Patient and Public Involvement

The study protocol was developed after discussion at a blood cancer patient forum convened by Leukaemia & Blood Cancer New Zealand, and at meetings of the Lymphoma Network of New

Zealand and the NZ Branch of the Haematology Society of Australia and New Zealand. The study protocol and consent form were developed in consultation with Research Advisory Group - Māori, a Māori relationship board, which includes lay representation, to Capital & Coast District Health Board. The participant information and consent form was reviewed by a patient representative with relevant personal experience. The study has been publicised in national media, although due to regulatory and logistical considerations, referrals must come from a relevant specialist rather than directly from potential participants. Study results will be presented in the lay media as well as in scientific journals. The patient information and consent form includes an option to request a lay summary of the study results.

Data Dissemination

Participants will be given the option to receive a summary of the trial results. Trial results will be published in a peer-reviewed journal after completion of the trial.

DISCUSSION

This manuscript describes the protocol for ENABLE, an investigator-led Phase 1 dose escalation trial evaluating a new third generation (3G) autologous anti-CD19 CAR T-cell product (WZTL-002), for the treatment of individuals with r/r B-NHL. The primary outcome is safety, which will be assessed by determining the number and severity of adverse events. Secondary outcomes will assess feasibility, efficacy and recommended WZTL-002 dose for subsequent efficacy trials.

As well as resulting in improved cytotoxicity against target cells, the incorporation of a second co-stimulatory domain can enhance CAR T-cell proliferation and cytokine production [12 17]. Thus, while 3G CAR T-cell products have the potential for increased efficacy, there is also the potential for an increased risk of toxicities including CRS and ICANS risk, compared to second-generation products. Accordingly, CRS and ICANS were identified as events of special interest early during trial development. The risk of both toxicities may relate to CAR T-cell dose and to disease burden [31 32]. Therefore, to mitigate CRS and ICANS risks, conservative starting and maximum WZTL-002 doses have been selected, based on clinical experience using similar CAR T-cell products. Targeted CRS and ICANS assessments will be performed, and a comprehensive risk mitigation plan has been developed, including the development of institutional policies and protocols, documented staff training, intensive care escalation plans and on-site tocilizumab availability. Use of the ASTCT international consensus CRS and ICANS grading system will facilitate the comparison of toxicity rates with other anti-CD19 CAR T-cell trials conducted internationally [26].

The use of 'bridging' chemo- and radiotherapy between enrolment and WZTL-002 administration is permitted. This will facilitate WZTL-002 treatment scheduling during dose escalation, and may allow reduction of disease burden before WZTL-002 therapy, potentially reducing CRS and ICANS risk [33]. To mitigate the impact of bridging therapy on efficacy assessments, baseline PET-CT scans will be conducted after completing bridging therapy and before starting lymphodepleting chemotherapy and WZTL-002 administration.

The selection of eligible lymphoma subtypes was based on evidence of efficacy from other clinical trials evaluating anti-CD19 CAR T-cell therapies in this population [7-9 34 35]. Limitations of this trial include its small size, inclusion of several B-NHL subtypes, and dose escalation design, as a result of which efficacy and exploratory outcomes will be descriptive only. In particular, secondary outcomes assessing WZTL-002 efficacy (ORR, CR rate, RFS and OS) will be preliminary, and are included to help inform the design of future Phase II trials. Similarly, the exploratory outcomes, which explore WZTL-002 kinetics, phenotype, serum cytokines and B-cell aplasia, are intended to inform outcome measure selection for future larger trials.

The published clinical experience of 3G anti-CD19 CAR T-cells for the treatment of r/r B-NHL is limited, with the final results of only two other early-phase trials published, to our knowledge[24 25]. Enblad *et al* treated 11 patients with r/r B-NHL or Chronic Lymphocytic Leukaemia (CLL) with 3G anti-CD19 CAR T-cells combining CD28 and 41BB costimulatory domains, in a Phase 1 dose escalation study[25]. Of the 11 treated participants, four did not receive lymphodepletion before CAR T-cell administration. The dose range of 3G anti-CD19 CAR T-cells administered this study was 2×10^7 – 2×10^8 cells/m^2 (approximately equivalent to 5×10^5 – 5×10^6 CAR T-cells/kg). A response to treatment was observed in four participants (36%), all of whom reached CR[25]. Severe CRS was reported in two participants (18%), and severe neurotoxicity in one (9%).

Ramos *et al* reported results of a Phase 1 anti-CD19 CAR T-cell trial involving simultaneous administration of autologous 2G (CD28 only) and 3G (4-1BB plus CD28) anti-CD19 CAR T-cell products to participants with r/r B-NHL[24]. This dose escalation study treated 11 participants with active lymphoma and five in remission after autologous stem cell transplant (ASCT). All participants with active lymphoma received lymphodepletion with cyclophosphamide and fludarabine before CAR T-cell infusion, whereas no further lymphodepletion was given to those post ASCT. The dose range of total CAR T-cells administered on this study (2G + 3G CAR T-cells in 1:1 ratio), was 5×10^4 – 1×10^6 CAR T-cells/kg. Six of 11 with active lymphoma (54%) responded, three (27%) reaching CR. All five recipients of CAR T-cells after ASCT remained in CR at least 9 months after CAR T-cell

administration. No cases of severe CRS, and only one of severe neurotoxicity, were reported[24]. Ramos *et al* found that the 3G anti-CD19 CARs showed superior *in vivo* expansion and persisted longer than their 2G counterparts, although the relative contribution of the 2G and 3G CAR T-cells to anti-tumour efficacy and to toxicity could not be assessed with this study design[24].

In conclusion, published phase I trials suggest that manufacture of 3G CAR T-cells is feasible, and do not yet indicate that CRS and ICANS rates are higher than for 2G products. Moreover, the Ramos *et al* study indicates that 3G CAR T-cells can exhibit improved proliferation and persistence in humans compared to 2G counterparts. However, because of the small number of reported 3G CAR T-cell recipients, and the likely suboptimal CAR T-cell dosing in the early cohorts of these dose escalation studies, conclusions cannot be drawn about the relative efficacy and safety of 3G compared with 2G CAR T-cells[24 25]. Other 3G anti-CD19 CAR T-cell trials in patients with r/r B-NHL are underway (Table 1). As well as adding to the clinical experience of 3G anti-CD19 CAR T-cell therapies for the treatment of B-NHL, the ENABLE trial will inform the clinical safety and potential utility of a new intracellular TLR2 co-stimulatory domain within CAR T-cells.

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Contributors

RW conceived the clinical trial. RW and PG designed and wrote the study protocol. ND, PG and RW drafted the manuscript. RW, PG, ND, GG, BM, EB, BA, TP, TO, CF, DR, CB and IH provided input into the study protocol. PL conceived the TLR2 costimulatory domain construct, and provided background, preclinical and clinical data regarding its use. All authors reviewed the study manuscript. RW, TP, PG and TO will conduct study procedures. RW is the Principal Investigator.

Funding

The Malaghan Institute of Medical Research (MIMR) is the sponsor of this investigator-initiated trial. Delegated duties will be assigned to Capital and Coast District Health Board and its employees by means of the site clinical trial agreement, and to the study monitor by means of a monitor agreement.

The study is funded by philanthropic support to MIMR, an independent biomedical research institute and registered charity. A private company, WZTL, provided the rights to use the 1928T2z construct, the source plasmids for vector production, and contributed to MIMR costs for the production of WZTL-002. WZTL is not involved in study design, conduct or reporting, which are responsibilities of the Principal Investigator.

Competing Interests

Trial Principal Investigator RW and Co-Investigator PG, are employees of the Malaghan Institute of Medical Research, a charitable research institute and study sponsor. The other Co-Investigators have no competing interests to declare. PL has proprietary interest in the intellectual property of the 1928T2z construct. CB is co-Founder and Scientific Advisory Board Member of Mana Therapeutics is on the Advisory Board of Cellectis, has Stock ownership in Torque Therapeutics and Neximmune and is a Board Member of Caballeta Bio.

Patient consent

Obtained.

Ethics approval

Approved by the Southern Health and Disability Ethics Committee, New Zealand. Ethics reference: 19/STH/69.

Provenance and peer review

Not commissioned, externally peer reviewed.

Data sharing statement

The technical appendix, statistical code and data set are available from the corresponding author at rweinkove@malaghan.org.nz. The participants gave informed consent for the data sharing.

Open access

Box 1 Inclusion and Exclusion criteria for ENABLE

Inclusion criteria

- Age 16 to 75 years (inclusive)
- Biopsy-proven relapsed or treatment refractory B-cell non-Hodgkin lymphoma of the following subtypes per World Health Organisation (WHO) classification: Diffuse Large B-Cell Lymphoma (DLBCL) and its variants, Primary Mediastinal B-Cell Lymphoma (PMBCL), transformed Follicular Lymphoma (tFL), Follicular Lymphoma (FL) and Mantle Cell Lymphoma (MCL)
- Requirement for treatment in the opinion of the investigator
- No other curative treatments available, or not suitable due to patient or disease characteristics or lack of stem cell donor
- Malignancy documented to express CD19 based on flow cytometric or immunohistochemical staining
- Provision of written informed consent for this study
- Life-expectancy from non-lymphoma related causes of > 12 months
- European Cooperative Oncology Group (ECOG) performance status of 0 to 2 inclusive
- Adequate haematologic function, defined by neutrophils $\geq 1.0 \times 10^9$/L and platelets $\geq 50 \times 10^9$/L
- No serious cardiac, pulmonary, hepatic or renal disease.
    - Serum bilirubin < 2.5 times upper limit of normal (ULN)
    - Estimated creatinine clearance ≥ 50 mL/min using the modified Cockroft Gault estimation or as assessed by direct measurement
    - Cardiac Ejection Fraction ≥ 50% as determined by Echocardiogram or MUGA Scan
    - Oxygen saturations > 92% on room air
    - Diffuse Capacity of the lungs for carbon monoxide (DLCO) or Carbon monoxide transfer coefficient (KCO), Forced expiratory volume in one second (FEV1) and Forced Vital Capacity (FVC) are all ≥ 50% of predicted by spirometry after correcting for haemoglobin and/or volume on lung function testing.

Exclusion criteria

- Confirmed active or prior central nervous system (CNS) involvement by lymphoma. In patients with a clinical suspicion of CNS disease, lumbar puncture and MRI brain must be performed

- Active CNS pathology including: epilepsy, seizure within the preceding year, aphasia, paresis, stroke, dementia, psychosis within the preceding year, severe brain injury, Parkinson disease, or cerebellar disease
- Richter Syndrome
- Active autoimmune disease requiring systemic immunosuppression
- Prior solid organ transplantation
- Allogeneic stem cell transplantation within the preceding three months or still requiring systemic immunosuppression
- Current grade II – IV acute graft versus host disease (GVHD), any prior grade IV acute GVHD, or current moderate or severe chronic GVHD
- Need for systemic corticosteroids to treat a condition other than B-NHL at a daily dose of ≥ 10 mg prednisone (or equivalent)
- Peripheral blood lymphocytes < 0.5 x 10^9/L as assessed by complete blood count
- Peripheral blood $CD3^+$ T cells < 350/μL as assessed by lymphocyte subset analysis
- Pregnant or lactating female
- Women of child-bearing potential who are not willing to use highly effective methods of contraception during study participation and for at least 1 year after WZTL-002 administration
- Men who are not willing to use highly effective methods of contraception during study participation and for at least 1 year after WZTL-002 administration
- Men who have a pregnant partner and are not willing to use a condom while performing sexual activity during study participation and for at least 3 months after WZTL-002 administration
- Subjects with known sensitivity to immunoglobulin or to components of the investigational product (IP)
- History of active malignancy other than B-cell malignancy within two years prior to enrolment, with the exception of: adequately treated *in situ* carcinoma of the cervix; adequately treated basal cell carcinoma (BCC) or localized squamous cell carcinoma (SCC) of the skin; other localised malignancy surgically resected (or radically treated with another treatment modality) with curative intent
- Current or prior human immunodeficiency virus (HIV) infection
- Vaccination with a live virus within the preceding four weeks
- Treatment with a purine analogue within the preceding four weeks
- Treatment with alemtuzumab within the preceding 12 weeks
- Prior gene therapy, including prior anti-CD19 chimeric antigen receptor T-cell therapy
- Receipt of an investigational medicine within another clinical trial within the preceding four weeks
- Inadequately controlled systemic infection
- Serologic status reflecting active viral hepatitis B or any history of hepatitis C infection as follows:

- Presence of hepatitis B surface antigen (HBsAg) or hepatitis B core antibody (HBcAb). Patients with presence of HBcAb, but absence of HBsAg, are eligible if hepatitis B virus (HBV) DNA is undetectable (< 20 IU), and if they are willing to receive appropriate anti-viral prophylaxis.
- Presence of hepatitis C virus (HCV) antibody
- Presence of New York Heart Association (NYHA) class 2 or higher cardiac symptoms not related to lymphoma
- Significant concomitant illnesses which would in the investigator's opinion make the patient an unsuitable candidate for the trial
- Subjects who have diminished capacity or any circumstance that would prohibit them from understanding and providing informed consent in accordance with ICH-GCP (International Conference on Harmonisation, Good Clinical Practice)
- Subject does not provide consent to enrol onto International Cellular Therapy Registry

Box 2 Dose Limiting Toxicities

A DLT is a toxicity or AE occurring during the DLT assessment period (first 21 days after WZTL-002 administration), which is not attributable to a cause unrelated to WZTL-002 (such as underlying lymphoma, concurrent illness or concomitant medications), and meets one of the following criteria:

- Grade 4 or greater CRS or ICANS or grade 3 CRS or ICANS that does not resolve to grade 2 or lower within 7 days, both as per American Society for Transplantation and Cellular Therapy (ASTCT) criteria
- Any adverse event requiring airway intubation (including neurotoxicity requiring intubation for airway protection)
- Grade 4 neutropenia that does not resolve to grade 3 or lower within 21 days after WZTL-002 administration
- Platelet transfusion-dependent thrombocytopenia persisting for 21 days or longer after WZTL-002 administration

- All grade 4 toxicities, and grade 3 toxicities that do not resolve to grade 2 or lower within 7 days, with the exception of the following, which are not automatically considered DLTs:
  - Myelosuppression, including
    - neutropenia
    - bacterial infection in the setting of neutropenia with neutrophils $< 1.0 \times 10^9$/L
    - thrombocytopenia
    - bleeding in the setting of thrombocytopenia with platelets $< 50 \times 10^9$/L
    - anaemia
    - lymphopenia
  - Hypersensitivity reactions occurring within 2 hours of WZTL-002 administration (and considered related to cell administration) that resolve to grade 2 or less within 24 hours
  - Asymptomatic biochemical abnormalities that resolve to grade 2 or lower within 7 days
  - Hypogammaglobulinemia

For CRS and ICANS, ASTCT grading criteria will be used. For all other toxicities, CTCAE v 5.0 will be used.

Box 3 Summary of measures taken to prepare for CRS and ICANS

- Formation of CAR T-cell Toxicity Working Group composed of Haematologists, Neurologist, Intensivist, Immunologist and Haematology Nurses
- Attendance of principal investigator, co-investigators, study nurse, senior haematology nurses and nurse educators at a CAR T-cell Toxicities Preceptorship Day
- Localisation of guidelines for CRS and ICANS identification, management and escalation, and the upload of these to institutional electronic treatment guide
- Education sessions and competency assessments on CAR T-cell toxicities delivered to Nursing, Patient at Risk and ICU teams
- Allowance for bridging treatment between leukapheresis and lymphodepleting chemotherapy to ‘debulk’ or control disease before WZTL-002 administration
- Confirmation that three doses of tocilizumab are on site before WZTL-002 administration
- Levetiracetam 750mg BID to be given for 28 days following WZTL-002 adminsitration for anti-seizure prophylaxis
- Completion of clinical checklist before WZTL-002 administration

- Notification of Neurology and Intensive Care Unit teams before WZTL-002 administration
- Scheduled nurse-led CRS and ICANS assessments
- Provision of participant-held wallet card and discharge summary sheet

Table 1 Other third-generation anti-CD19 CAR T-Cell trials registered on ClinicalTrials.gov

Study ClinicalTrials.gov ID	B-cell malignancy subtypes	CAR generation	Study Phase	Lymphodepletion	Study Start Date	Results published (Yes/no)
NCT02963038	B-ALL + B-NHL	3G	I+II	Not specified	June 2016	No
NCT03068416	B-ALL + B-NHL	3G	II	Not specified	September 2017	No
NCT02132624 (see discussion section in paper)	B-NHL	3G	I	Flu 25 mg/m^2 x 3d, Cy 500 mg/m^2 x 3d	April 2014	Yes [†]
NCT03146533	B-NHL	3G	I+II	Flu 30mg/m^2 x 3d Cy 800mg/m^2 x 3d	April 2017	No

NCT01853631 (see discussion section in paper)	B-ALL + B-NHL	3G and 2G*	I	Flu 30 mg/m^2 x 3d, Cy 500mg/m^2 x 3d	February 2014	Yes‡
NCT03676504	B-ALL + B-NHL	3G	I+II	Flu 30 mg/m^2 x 3d, Cy 500mg/m^2 x 3d	September 2018	No
NCT02822326	B-ALL	3G	I	Flu 25mg/m^2 x 3d Cy 300mg/m^2 x 3d	January 2016	No

As at 16/09/2019
B-ALL, B-cell Acute Lymphoblastic Leukaemia; B-NHL, B-cell non-Hodgkin lymphoma
*Co-infused with CD28 containing second-generation CAR and CD28 + 41BB containing third generation CAR
†See Enblad et al[24]
‡See Ramos et al[25]

Figure 1: Diagrammatic representation of WZTL-002 Anti-CD19 third generation CAR T-cell illustrating the co-stimulatory domains and components of the chimeric antigen receptor

Figure 2: Schema for the ENABLE phase 1 dose escalation study

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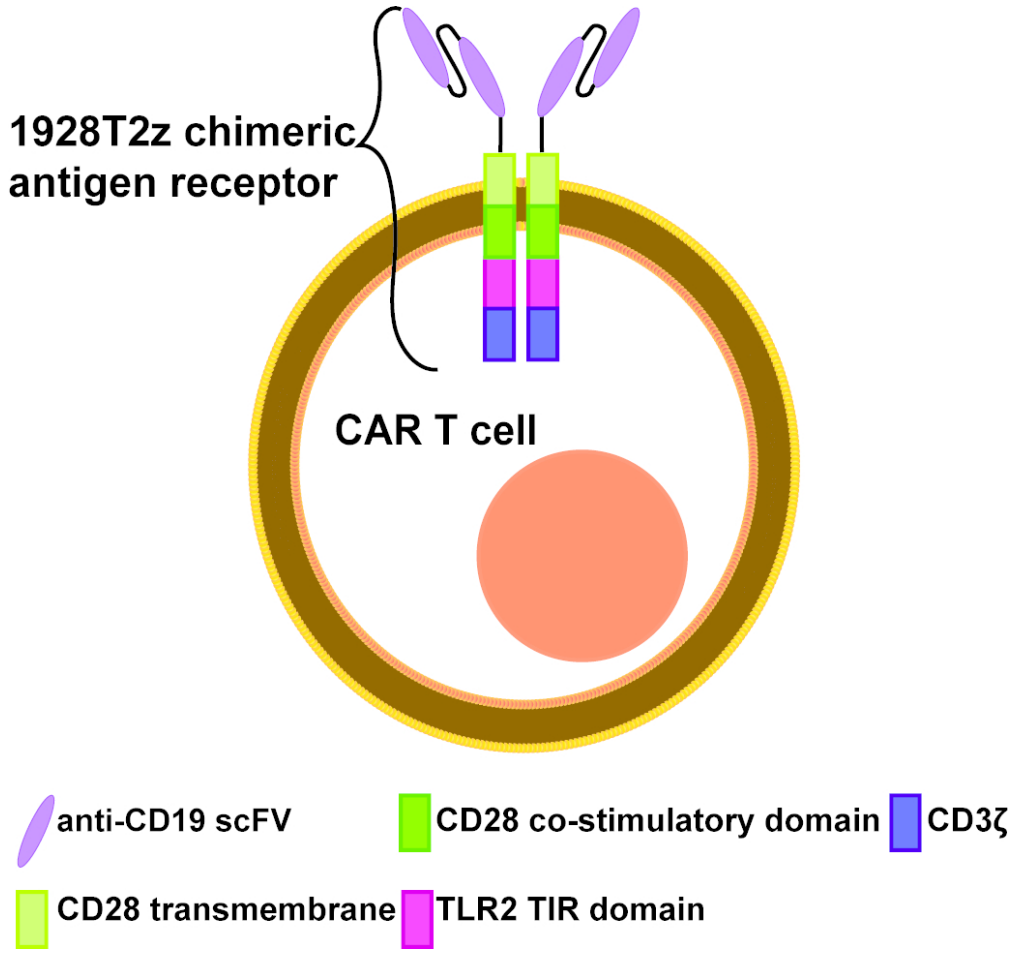
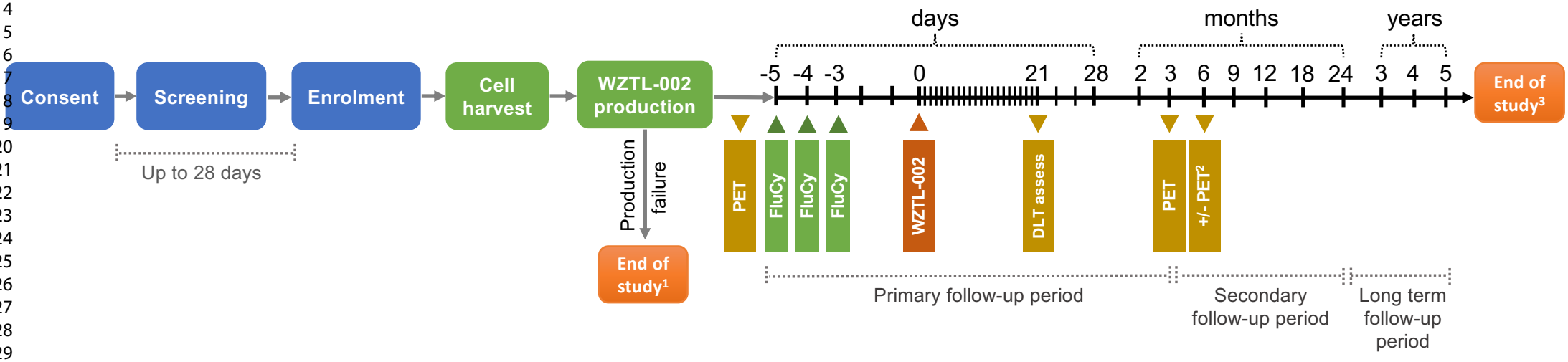


Figure 1: Diagrammatic representation of WZTL-002 Anti-CD19 third generation CAR T-cell illustrating the co-stimulatory domains and components of the chimeric antigen receptor



Notes and abbreviations:

[1] Second attempt at cell harvest and WZTL-002 production may be considered at discretion of TMC

[2] 6 month PET scan if first PET scan post WZTL-002 treatment shows partial response

[3] Long-term follow-up through bone marrow transplant clinic and Cellular Therapies Registry enrolment

FluCy, fludarabine and cyclophosphamide IV; PET, positron-emission tomography/computed tomography scan

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym **"Third-generation anti-CD19 chimeric antigen receptor T-cells incorporating a TLR2 domain for relapsed or refractory B-cell lymphoma: a phase I clinical trial protocol (ENABLE)" –** Page 1 of manuscript
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Clinicaltrials.gov registration number: **NCT04049513**– page 3
	2b	All items from the World Health Organization Trial Registration Data Set: **Included in the ClinicalTrials.gov registration**
Protocol version	3	Date and version identifier: **23rd June 2019, Protocol Version 1.2** – page 3
Funding	4	Sources and types of financial, material, and other support: **"The study is funded by philanthropic support to MIMR, an independent biomedical research institute and registered charity. A private company, WZTL, provided the rights to use the 1928T2z construct, the source plasmids for vector production, and contributed to MIMR costs for the production of WZTL-002. WZTL is not involved in study design, conduct or reporting, which are responsibilities of the Principal Investigator" –** see page 19 of manuscript.

Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors : Names and affiliations of authors as outlined on Page 1 of manuscript under descriptive title. Roles of contributors: **"RW conceived the clinical trial. RW and PG designed and wrote the study protocol. ND, PG and RW drafted the manuscript. RW, PG, ND, GG, BM, EB, BA, TP, TO, CF, DR, CB and IH provided input into the study protocol. PL conceived the TLR2 costimulatory domain construct, and provided background, preclinical and clinical data regarding its use. All authors reviewed the study manuscript. RW, TP, PG and TO will conduct study procedures. RW is the Principal Investigator" –** see page 19 of study manuscript.
	5b	Name and contact information for the trial sponsor – "**Malaghan Institute of Medical Research is the trial sponsor with the following contact email address"**: enable@malaghan.org.nz – page 1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities. **"The study is funded by philanthropic support to MIMR, an independent biomedical research institute and registered charity. A private company, WZTL, provided the rights to use the 1928T2z construct, the source plasmids for vector production, and contributed to MIMR costs for the production of WZTL-002. WZTL is not involved in study design, conduct or reporting, which are responsibilities of the Principal Investigator" –** see page 19-20
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) **"An independent Data and Safety Monitoring Committee (DSMC) will include clinicians with experience in early phase T-cell trials and in haemato-oncology. Per the DSMC Charter, the DSMC will meet and review trial accrual, conduct and safety data a minimum of 6-monthly and before each dose step" –** see page 13
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention – See pages 4-6 under sections**: "CAR T-cell therapy in B-cell Non Hodgkin Lymphoma" and "Third generation CAR T-cells."**

	6b	Explanation for choice of comparators – **N/A**
Objectives	7	Specific objectives or hypotheses **See Study aims and outcomes on pages 9-11**
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) **Included – this is a Phase 1 3+3 dose escalation trial**

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained. "**The Study Site is Wellington Hospital, Capital & Coast District Health Board, New Zealand" –** this statement has been added to page 7 of the manuscript under **'Study Design.'**
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) **Inclusion and exclusion criteria are provided in Box 1** – page 21
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered – **"Once product release criteria are met, eligibility to proceed to WZTL-002 treatment is confirmed, and following any bridging chemo- or radiotherapy, a baseline PET/CT scan will be performed. This will be followed by lymphodepleting chemotherapy comprising intravenous fludarabine (30 mg/m^2/day × 3 days) and cyclophosphamide (500 mg/m^2/day × 3 days). WZTL-002 will be administered following two chemotherapy-free days as a slow intravenous push"** – see 'Study Procedures' Section on page 8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) – **N/A – this is a one-off intervention**
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) **None applicable for this one-off intravenous hospital-supervised therapy**

	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial: "**Anti-microbial prophylaxis and tumour lysis prophylaxis will be given as per standard of care for patients receiving treatment for haematological malignancies" –** this sentence has been added to the manuscript in the 'Study Procedures' Section on page 8.
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended. **Full primary, secondary and exploratory outcomes, as approved by the Ethics Committee and listed in trial registration are provided on pages 9-10**.
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure): **See figure 2: Schema for the ENABLE phase 1 dose escalation study**
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations: **See Statistical analysis Section on pages 13-14**
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size: **Not applicable; this is a Phase 1 dose escalation trial with sequential enrolment; the number of participants enrolled will depend on dose-limiting toxicities observed at each dose step**

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions: **N/A – not a controlled trial**
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned: **N/A**
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions: **N/A**

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how: **N/A**
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial: **N/A**

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol: **"Safety outcomes including AEs, SAEs, Suspected Unexpected Serious Adverse Reactions (SUSARs), CRS, ICANS and DLTs will be individually listed by dose group and summarised as the frequency of events and percentages of individuals experiencing each event type. Each event summary will include details of the timing, grade, and outcome of the event. Response outcomes including ORR (defined as CR rate plus PR rate) and CR rate, will be individually listed and summarised as frequencies (%) by dose group. The survival outcomes, RFS and OS will also be summarised as frequencies (%) at 24 months and the times to events will be individually listed and may be summarised with Kaplan-Meier curves for individual dose groups if sample sizes permit. Associations between safety outcomes and presenting features will be explored in a qualitative manner."** – See pages 13-14
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols. **"Participants will be registered in the Center for International Blood and Marrow Transplant Research (CIBMTR) Cellular Therapies Registry and Australasian Bone Marrow Transplant Recipient Registry (ABMTRR), in order to capture low-incidence or late treatment-related toxicities."** – See page 9. These international cellular therapies registries will follow up participants lifelong to collect data on long-term toxicities, and includes participants who discontinue study follow up. As a condition of trial approval by the applicable regulatory body, study participants must consent to registration on International Cellular Therapies registries in order to meet trial eligibility (See inclusion/ exclusion criteria).

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol. All data will be collected using electronic case report forms (e-CRFs). The data from the CRFs will be entered onto an electronic database for targeted data analysis and extraction. This e-CRF database system is an international, web based, electronic data capture system. Further information can be requested from the corresponding author on rweinkove@malaghan.org.nz.
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol. **See Statistical Analysis Section on pages 13-14.**
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) **N/A**
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) **N/A**
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed **"An independent Data and Safety Monitoring Committee (DSMC) will include clinicians with experience in early phase T-cell trials and in haemato-oncology. Per the DSMC Charter, the DSMC will meet and review trial accrual, conduct and safety data a minimum of 6-monthly and before each dose step**." – See page 13. Further information regarding the DSMC Charter for this trial can be obtained by contacting the corresponding author on rweinkove@malaghan.org.nz

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial. This study follows a '3+3' dose escalation design. In the Statistics Section on page 13 we state: **"If 2 or more participants develops a DLT at a specific dose step, escalation to the next dose step should not occur, indicating that the Maximum Tolerated Dose (MTD) has been reached."** The full list of Dose Limiting Toxicities (DLTs) is provided in Box 2. The Data and Safety Monitoring Committee (DSMC) will use these criteria to determine whether DLTs have occurred and will **"review trial accrual, conduct and safety data a minimum of 6-monthly and before each dose step"** (see page 13).
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct Please see Monitoring and Data Management Section of manuscript on page 13 of manuscript which states: **"A trial management committee (TMC) including the Principal Investigator, at least one Co-Investigator, the study nurse, and a representative of the Clinical Human Immunology Laboratory, will meet at least monthly during study recruitment to review recruitment rates, trial conduct, trial procedures, Adverse Events (AEs) and Serious Adverse Events (SAEs)."** Furthermore, **"an independent study monitor will monitor the study, and will report to the Sponsor."** More detailed information regarding the collection, assessment and reporting of adverse events is provided in the full Study Protocol. Further information can be obtained from the corresponding author: rweinkove@malaghan.org.nz.
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor. Page 13 of the Study manuscript states that, **"an independent study monitor will monitor the study, and will report to the Sponsor."** A Study Monitoring Plan which details frequencies and procedures for auditing trial conduct has been agreed between the sponsor and the independent study monitor. Further information regarding the Study Monitoring Plan can be obtained from the sponsor at enable@malaghan.org.nz.

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval **"Ethical approval for the study was granted by the New Zealand Health and Disability Ethics Committee (reference 19/STH/69) on 23rd June 2019 for Protocol Version 1.2"** – see page 3 of Study manuscript.
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) Important protocol modifications must be approved by the New Zealand Health and Disability Ethics Committee. The full study protocol states: "The study protocol and the Patient Information Sheet and Informed Consent Form (PICF), and all subsequent amendments must be reviewed and approved by HDEC before being implemented." It also states that "The investigator and study staff are responsible for maintaining a comprehensive and centralised filing system including protocol amendments." The Principal Investigator is responsible for disseminating information regarding to protocol amendments to all relevant parties, but these duties may be delegated to other members of the study team.
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) Study investigators will obtain "**written informed consent"** from potential trial participants prior to them under-going any trial-specific procedures or enrolment in the trial (see page 8 of the manuscript in the Study Procedures section). Further information on the consent process is provided in the full Study Protocol which states that "All patients whose first language is not English will be offered an interpreter. All patients will be given at least 24 hours to consider their decision. Patients will be encouraged to discuss the matter with their GP and/or their oncologist (if their primary oncologist is not one of the investigators)." Further information about the consent process for this trial can be obtained from the corresponding author on rweinkove@malaghan.org.nz.

	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable Subject to informed consent from the study subject, samples may be taken as part of a study specific procedure and stored in the in the Malaghan Institute Immune Tissue Bank for future unspecified research. Study subjects would need to provide additional informed consent for this ethics approved Tissue Bank in order for participant data and biological specimens to be stored for future ancillary studies. This information is provided in the full Study Protocol and in the Participant Information Sheet and Consent Form, but is not included in this manuscript.
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial **"The Study site will hold responsibility for the confidentiality of electronic and paper clinical records held for the study participant. To maintain confidentiality of trial participants, study data or samples sent to collaborating investigators or external contractors for analysis or review will be labelled with study-specific codes, and not with patient identifiers. The Principal Investigator will hold responsibility for ensuring that presentations and publications of the study findings do not contain identifiable information. All laboratory records will be kept for a minimum of fifteen years. Clinical data (including Case Report Forms) will be stored securely for a minimum of fifteen years"** – this statement has been added to page 13-14 in Monitoring and Data Management Section.

Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site See the Competing Interests section of the manuscript on page 20 which has been modified on the revised manuscript: **"Trial Principal Investigator RW and Co-Investigator PG, are employees of the Malaghan Institute of Medical Research, a charitable research institute and study sponsor. The other Co-Investigators have no competing interests to declare. Scientific collaborator PL has proprietary interest in the intellectual property of the 1928T2z construct, and is not involved in protocol design or data analysis. Independent DSMC member CB is co-Founder and Scientific Advisory Board Member of Mana Therapeutics is on the Advisory Board of Cellectis, has Stock ownership in Torque Therapeutics and Neximmune and is a Board Member of Caballeta Bio."** PL is a scientific collaborator for the of the Study Team and CB has provided advise on the Study design and sits on the Data and Safety Monitoring Committee for this trial.
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators Please 'Data Sharing Statement on page 21 of the manuscript: **"The technical appendix, statistical code and data set are available from the corresponding author at rweinkove@malaghan.org.nz."**
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation The full study protocol and the participant information and consent form states that study participants that have been injured in this study would be eligible to apply for compensation from Accident Compensation Corporation (ACC) in New Zealand.

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions **"Participants will be given the option to receive a summary of the trial results. Trial results will be published in a peer-reviewed journal after completion of the trial"** – see Data Dissemination Section on page 15 of the manuscript. **"Study results will be presented in the lay media as well as in scientific journals. The patient information and consent form includes an option to request a lay summary of the study results"** – see Patient and Public involvement section on Page 15 of the manuscript.
	31b	Authorship eligibility guidelines and any intended use of professional writers N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code Please 'Data Sharing Statement on page 20 of the manuscript: **"The technical appendix, statistical code and data set are available from the corresponding author at rweinkove@malaghan.org.nz."**
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates All study participants will be provided with the New Zealand Ethics Committee approved Participant Information Sheet and Consent Form (PICF). The PICF was **"developed in consultation with Research Advisory Group - Māori, a Māori relationship board, which includes lay representation, to Capital & Coast District Health Board. The participant information and consent form was reviewed by a patient representative with relevant personal experience"** – see Patient and Public Involvement Section on Page 15 of manuscript.

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable **"Kinetics of WZTL-002 will be determined by determining the CAR transgene in patient peripheral blood by quantitative PCR before and at 1, 2, 4, 7, 9, 11, 14, 16, 18, 21 and 28 days, at 2, 3, 6, 12 and 24 months, and at 3, 4 and 5 years, after WZTL-002 administration. Serum cytokine profile after WZTL-002 administration will be determined by ELISA (for IL-6) and by cytokine bead array before and at 1, 2, 4, 7, 9, 11, 14, 16, 18, 21 and 28 days after WZTL-002 administration"** – See the Immune monitoring and exploratory endpoints Section of the Protocol on page 11 of the manuscript.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.