PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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)
AUTHORS	George, Philip; Dasyam, Nathaniel; Giunti, Giulia; Mester, Brigitta; Bauer, Evelyn; Andrews, Bethany; Perera, Travis; Ostapowicz, Tess; Frampton, Chris; Li, Peng; Ritchie, David; Bollard, Catherine M; Hermans, Ian F; Weinkove, Robert

VERSION 1 – REVIEW

REVIEWER	Julio Chavez Moffitt Cancer Center, United States
REVIEW RETURNED	28-Oct-2019

GENERAL COMMENTS	Dr George and Colleagues are presenting a Phase I clinical trial dose escalation of a third generation CART using costimnulatory domains with CD28 and top-like receptor(TLR) 2 given the enhanced T-cell activity provided by TLR in several studies. This is a novel and interesting concept. The study is well described and provides a rationale too us it in lymphomas. I have several minor comments that are mostly clarifications. Page 6 Ln 41: While CR patients do better, between 40-50% of PR after CART infusion will eventually convert into CR Page 9 Ln 8: I would change for bringing therapy as physicians may elect to do novel agents as a bridge (such as Ibrutinib or lenalidomide) or steroids Page 9 Ln 38: While may argue a 30 day PET scan assessment in CART therapy, it may be worthy for patients with aggressive lymphomas. 3 month assessment post CART infusion seem a bit long especially for DLBCL. Perhaps adding imaging as needed? Page 9 Ln 46: Seems that patients in CR will get only one scan after CART infusion? While current guidelines do not necessarily support surveillance imaging for lymphomas, it is not known whether that is applicable yet for CART therapies, specially for a newconstruct. At least should be mentioned that imaging should be done on as needed basis Page 10 Ln 23: I would add as a secondary endpoint the long term complications of the investigational CART product Page 10 Ln 52: I am nor sure whether this is a fully humanized CAR product. If not there should be determination of immunogenicity Page 11 Ln 39: I would suggest CSF studies (including cytokines) in patients that develop neurotoxicity. Page 20 Inclusion Criteria: How many lines of therapy patients should have failed prior to study entry?

	Page 20: In general I do not exclude older patients based on older age. As longs as PS is adequate, it should be fine for patients to participate Page 20 Inclusion Criteria: must include presence of measurable disease per Lugano criteria Page 20 Inclusion Criteria: Should mention washout period from last therapy (2 weeks or half-live should be acceptable for instance) Page 21 Exclusion criteria: I suspect post autologous HCT are candidates but it is not mention. Should mention it for clarification purposes Page 21 Ln 16: Exclusion criteria: While proper CD3+ T cells ar essential for CAR manufacturing, setting a minimum of 350 CD3+ may be difficult to achieve in refractory patients with several prior lines of therapy. Page 21 Ln 41: 4 weeks washout from another trial is a bit long for refractory aggressive lymphomas. I would use half-lives and add that G3-4 toxicity from prior trial should have resolved I would work better with the statistics. Provided that there will be some DLTs, some patients may need to be added in some of the dose levels. For instance: it is not unusual to see grade 4 neutropenia pass 21 days will require adding 3 more patients to the respective DL according to the 3+3 rules. Thus it is very possible a sample seize > 12 patients

REVIEWER	Priv.-Doz. Dr. med. Anita Schmitt University Hospital Heidelberg Germany
REVIEW RETURNED	26-Nov-2019

GENERAL COMMENTS	The third-generation anti-CD19 CAR T cell study for r/r B cell lymphoma is a phase I dose escalation study to evaluate safety and preliminary data concerning efficacy. This study is innovative, well planned and due to the multidisciplinary aspect of clinical CAR T cell therapy very well organized. Minor revisions: 1) Dates of the study should be included in the manuscript. 2) In the material and method part only little is mentioned concering the Isolation procedure, the Stimulation with beads, self-inactivating lentiviral vector, release criteria, potency assay. Please include the company of the used materials. Who produces the vector Plasmid, how is it tested, (RCL)? 3) How much blood is taken daily from the patient, if you do PCR, Serum, FACS analyzes? 4) When do you perform FACS analysis, if you do a subtyping only when CARTs are measurable?

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

- ***Page 6 Ln 41: While CR patients do better, between 40-50% of PR after CART infusion will eventually convert into CR***

Thank you for highlighting that a fraction of CAR T-cell recipients convert from PR to CR between 3 and 6 months. We have changed the sentence from, "*a lack of complete metabolic response after 3*

months being a major predictor of CAR T-cell treatment failure" to, "*a lack of complete metabolic response by 6 months being a predictor of CAR T-cell treatment failure*"

- ***Page 9 Ln 8: I would change for bringing therapy as physicians may elect to do novel agents as a bridge (such as Ibrutinib or lenalidomide) or steroids***

We agree, and would add that radiotherapy is also an option. We have changed this text to, "*Following cell harvest, bridging* ***therapy*** *will be permitted to provide disease control during manufacturing and treatment scheduling.*"

- ***Page 9 Ln 38: While may argue a 30 day PET scan assessment in CART therapy, it may be worthy for patients with aggressive lymphomas. 3 month assessment post CART infusion seem a bit long especially for DLBCL. Perhaps adding imaging as needed?***
- ***Page 9 Ln 46: Seems that patients in CR will get only one scan after CART infusion? While current guidelines do not necessarily support surveillance imaging for lymphomas, it is not known whether that is applicable yet for CART therapies, specially for a new construct. At least should be mentioned that imaging should be done on as needed basis***

We agree; the protocol-mandated PET scanning in this trial is limited because the primary outcome at phase I is safety, not efficacy. If we can establish a recommended phase II dose within this trial, we envision more frequent PET scanning within a future efficacy trial.

For this trial, we had always anticipated that additional scans will be performed as needed to aid clinical management - these are not excluded in study protocol. To clarify this, we have added the following sentence to the manuscript: ***"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."***

- ***Page 10 Ln 23: I would add as a secondary endpoint the long term complications of the investigational CART product***

Thank you for this suggestion. We are unable to add a new secondary endpoint as the study protocol has already been approved by the ethics committee and gene technology advisory committee, and recruitment has commenced. Safety events occurring during the trial (which includes adverse events up to 5 years after therapy) will be reported as part of the primary outcome. To capture complications after completion of the study, all participants are required to consent to enrolment in the CIBMTR Cellular Therapy Registry (per the inclusion/exclusion criteria). This registry, in conjunction with ClinicalTrials.gov registration of our trial and publication of this protocol manuscript will ensure long-term follow-up is possible many years after completion of this trial (see Study Procedures Section of the manuscript).

- ***Page 10 Ln 52: I am not sure whether this is a fully humanized CAR product. If not there should be determination of immunogenicity***

This is not a fully humanized CAR; the single chain variable antibody fragment (scFv) is derived from the murine anti-CD19 monoclonal antibody, FMC63. However, all other CAR sequences, including the novel TLR2 domain, are fully human. This is the same murine scFv employed in the three commercial anti-CD19 CAR T-cell products axicabtagene ciloleucel, tisagenlecleucel and lisocabtagene maraleucel, and we do not expect a substantially different immunogenicity profile.

Nonetheless, we acknowledge that regulatory agencies will likely expect immunogenicity data before licensing a product. To this end, we are gathering CAR T-cell persistence data within the trial, and storing serum samples from recipients at multiple time points until 5 years after administration. If the data from this phase I trial, and the extant competitive and commercial environment, favour progressing this product to a registration study, we would expect to formally assess immunogenicity then.

- ***Page 11 Ln 39: I would suggest CSF studies (including cytokines) in patients that develop neurotoxicity.***

We agree that CSF studies, including cytokine and CAR levels, would be useful in participants that develop neurotoxicity. However, after liaise with other clinicians involved in investigator-initiated CAR T-cell trials, we elected not to mandate these in the Study Protocol as we are mindful that there are often practical difficulties and risks in obtaining CSF samples during severe neurotoxicity (such as cerebral oedema, seizures, coagulopathy, thrombocytopenia). Our institutional ICANS policy recommends CSF analysis, including storage of a CSF sample for cytokine and CAR level analyses, and this is accounted for in the participant information and consent form. We have clarified the manuscript to add new text (**in bold**) as follows:

"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. ***In subjects with neurotoxicity, CSF samples may be stored for assessment of cytokine and CAR transgene levels."***

- ***Page 20 Inclusion Criteria: How many lines of therapy patients should have failed prior to study entry?***

We have not stipulated a specific number of prior lines of therapy, as this will depend on histology, refractory versus relapsed status, and the available funded and unfunded (e.g. trial) therapies. The inclusion criteria states subjects will have ***"No other curative treatments available, or not suitable due to patient or disease characteristics or lack of stem cell donor;"*** and we have added a statement at the start of the ***Study Procedures*** on page 8 section stating ***"*****All potential participants will be assessed at a lymphoma multidisciplinary team meeting to confirm that no other curative treatment options are available."**

- ***Page 20: In general I do not exclude older patients based on older age. As long as PS is adequate, it should be fine for patients to participate***

We opted to set an upper age limit of 75 years for this phase 1 trial after discussion within the trial management committee, with our transplant physicians and with other CAR T-cell treating clinicians, and this limit was agreed-upon by the ethics committee. While we agree with this Reviewer that highly-selected patients above this age might be candidates for licensed or later-phase CAR T-cell products, we harbour concern about recruiting those older than 75 for this first-in-human trial due to the established impact of age on transplant-related mortality. We expect to review the need for this upper age limit during future efficacy studies, if a safe dose is established in this phase I trial.

- ***Page 20 Inclusion Criteria: must include presence of measurable disease per Lugano criteria***

We have included **'requirement for treatment in the opinion of the investigator**' as an inclusion criterion, which we anticipate will encompass the need for measurable disease in lymphoma. We agree that the suggested criterion is more explicit, and would certainly be critical for an efficacy trial. The inclusion/exclusion criteria already agreed to by the ethics committee cannot be changed, but we do plan to submit a protocol amendment after treatment of at least two subjects, and have added this to the list of planned changes.

- ***Page 20 Inclusion Criteria: Should mention washout period from last therapy (2 weeks or half-live should be acceptable for instance)***

We agree. The exclusion criteria state that patients '**should not have received treatment a purine analogue within the preceding four weeks or treatment with alemtuzumab within the previous 12 weeks.'** In addition, the approved study protocol states that, **'with the exception of lymphodepleting chemotherapy, immunosuppressive therapies, including systemic corticosteroids, must be avoided during the week before WZTL-002 administration (72 hours for systemic corticosteroids).' –** this was not mentioned in the submitted manuscript, so we have now added this to the **Study design** section of the manuscript.

- ***Page 21 Exclusion criteria: I suspect post autologous HCT are candidates but it is not mention. Should mention it for clarification purposes***

Yes, prior autologous stem cell transplant recipients are eligible. We have added a comment to the text of the manuscript in the **Study design** section on page 7 to make this clear.

- ***Page 21 Ln 16: Exclusion criteria: While proper CD3+ T cells are essential for CAR manufacturing, setting a minimum of 350 CD3+ may be difficult to achieve in refractory patients with several prior lines of therapy.***

Thank you for raising this point. We based this threshold on a paper by Allen *et al* which reported high rates of CAR T-cell production failure in those with T-cell numbers lower than this threshold[1]. We agree that this will exclude some refractory patients, and have a modification to this threshold on the list for potential protocol amendments once we have accrued additional screening and manufacturing experience.

- ***Page 21 Ln 41: 4 weeks washout from another trial is a bit long for refractory aggressive lymphomas. I would use half-lives and add that G3-4 toxicity from prior trial should have resolved.***

This time period is consistent with inclusion criteria for the JULIET Trial (ClinicalTrials.gov reference NCT02445248). We believe that existing eligibility criteria would exclude a participant with ongoing grade 3-4 toxicities. For this dose-finding first-in-human safety trial, we harbour concerns about enrolling recipients who have received another investigational therapy within the preceding four weeks, as this may lead to erroneous safety signals. We could review this if we proceed to a phase II efficacy study.

- ***I would work better with the statistics. Provided that there will be some DLTs, some patients may need to be added in some of the dose levels. For instance: it is not unusual to see grade 4 neutropenia pass 21 days will require adding 3 more patients to the respective DL according to the 3+3 rules. Thus it is very possible a sample seize > 12 patients***

To clarify this point, we have added the following text in **bold** in the Statistical Analysis Section on page 14 of the manuscript: "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)."

Comments from reviewer 2:

- ***1) Dates of the study should be included in the manuscript.***

We have included the dates of the study on page 3 under **'Ethics and dissemination:' "**Ethical approval for the study was granted by the New Zealand Health and Disability Ethics Committee **on 23rd June 2019."**

We have also added the following line under **'Trial registration number'** on page 3:

"Trial opened to recruitment on 30th September 2019."

Study completion dates will be reported in a future manuscript reporting study outcomes.

- ***2) In the material and method part only little is mentioned concerning the Isolation procedure, the Stimulation with beads, self-inactivating lentiviral vector, release criteria, potency assay. Please include the company of the used materials. Who produces the vector Plasmid, how is it tested, (RCL)?***

In response to this suggestion, we have added to the details for WZTL-002 manufacture in the revised manuscript, and summarised the release criteria. We cannot provide full manufacturing supplier details in this clinical trial protocol manuscript, as these are confidential to WZTL. These details have been made available to the applicable regulator, MedSafe New Zealand, which has provided the license to manufacture this medicinal product.

We have included the following information in the **'Manufacture of WZTL-002 Section:'**

***"*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 AB serum for 7 days. The CAR T-cell product is then 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**."*

- ***3) How much blood is taken daily from the patient, if you do PCR, Serum, FACS analyzes?***

We take study-specific blood tests for exploratory endpoints before CAR T-cell administration and at: days 1, 2, 4, 7, 9, 11, 14, 16, 18, 21 and 28; at 2, 3, 6, 12 and 24 months; and at 3, 4 and 5 years (after WZTL-002 administration). We have added the following statement in the **'Immune monitoring and exploratory endpoints Section':**

"At each timepoint 25 mL of blood is taken for study-specific analyses."

- ***4) When do you perform FACS analysis, if you do a subtyping only when CARTs are measurable?***

CAR T-cell pharmacokinetics are analysed by peripheral blood PCR for the CAR transgene, and are batch-analysed after day 21, with earlier analyses only if required to aid toxicity management. To explain when we plan to perform FACS analysis, we have added the following paragraph to the **'Immune Monitoring and exploratory endpoints'** section:

"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."

In addition to the revisions to meet Reviewer comments, we have also made the following revisions to meet the SPIRIT checklist requirements:

- Addition of the trial acronym **"ENABLE"** to the study manuscript.
- Addition of sponsor contact details on page 1 of manuscript.
- Clarification of the study site on page 7 under 'Study Design' Section: **"The Study Site is Wellington Hospital, Capital & Coast District Health Board, New Zealand."**
- Addition of **"Anti-microbial prophylaxis and tumour lysis prophylaxis will be given as per standard of care for patients receiving treatment for haematological malignancies"** in the Study Procedures Section on page 8 of the manuscript.
- Addition of **"Levetiracetam 750mg twice daily to be given for 28 days following WZTL-002 administration for anti-seizure prophylaxis"** in Box 3, Institutional Preparations for CRS and ICANS.

- Addition of **"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"** in Monitoring and Data Management Section.
- Addition of **"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"** to Competing Interest Section on page 20.

References:

1. Allen ES, Stroncek DF, Ren J, et al. Autologous lymphapheresis for the production of chimeric antigen receptor T cells. *Transfusion* 2017;57(5):1133-41. doi: 10.1111/trf.14003 [published Online First: 2017/02/27]

VERSION 2 – REVIEW

REVIEWER	Julio Chavez Moffitt Cancer Center USA
REVIEW RETURNED	15-Jan-2020

GENERAL COMMENTS	The authors responded appropriately to prior comments. I do not have additional concerns for this paper

REVIEWER	PD Dr. med. Anita Schnitt University Hospital Heidelberg Germany
REVIEW RETURNED	05-Dec-2019

GENERAL COMMENTS	The reviewer completed the checklist but made no further comments.