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ORAL ABSTRACTS

704.CELLULAR IMMUNOTHERAPIES: EARLY PHASE AND INVESTIGATIONAL THERAPIES

A Phase 1 Dose Escalation Trial of Third-Generation CD19-Directed CAR T-Cells Incorporating CD28 and Toll-like Receptor 2 (TLR2) Intracellular Domains for Relapsed or Refractory B-Cell Non-Hodgkin Lymphomas (ENABLE)

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Introduction:
Chimeric antigen receptor (CAR) T cell therapies directed against CD19 and incorporating either CD28 or 4-1BB intracellular co-stimulatory domains are a standard of care for relapsed or refractory (r/r) B-cell lymphomas. CD19-directed CAR T-cell products employing CD28 co-stimulation yield high response rates, but have been associated with higher rates of immune effector cell-associated neurotoxicity syndrome (ICANS) and severe cytokine release syndrome (CRS) than 4-1BB co-stimulated products. There is a need for CAR T-cell products that combine high efficacy with low toxicity.
Toll-like receptor 2 (TLR2) is expressed by T-cells, and its engagement enhances T-cell expansion, modulates cytokine production, and promotes long-lived T-cell memory. In preclinical studies, interposition of an intracellular domain from TLR2 between CD28 and CD3ζ resulted in reduced CAR T-cell production of ICANS-associated cytokines GM-CSF and IFN-γ, while maintaining production of the homeostatic cytokine IL-7. We investigated the safety and efficacy of a novel third generation CD19-directed CAR T-cell product, which combines CD28 and TLR2 co-stimulatory domains (**Figure**).
Methods:
We completed a first-in-human phase 1 dose escalation trial of WZTL-002, comprising autologous 1928T2z CAR T-cells ('ENABLE', NCT04049513) for patients with r/r B-cell non-Hodgkin lymphomas (B-NHL). A 3+3 dose escalation design was used, with doses from 5×10^4 to 1×10^6 viable CAR T-cells/kg body weight. Eligible participants had radiologically assessable disease, satisfactory organ function and no central nervous system (CNS) involvement by lymphoma. Bridging therapy was permitted after leukapheresis and pending CAR T-cell manufacture and release. WZTL-002 CAR T-cells were administered intravenously after 3 days of fludarabine (30mg/m^2/day) and cyclophosphamide (500mg/m^2/day) lymphodepletion. Adverse events (AEs) were graded by CTCAE 5.0 except CRS and ICANS (graded by American Society for Transplantation and Cellular Therapy criteria). Response assessment was by PET/CT at month 3, per Lugano 2014 criteria. Pharmacokinetic analyses were by droplet digital PCR for CAR transgenes in blood mononuclear cells.
Results:
Of 21 patients treated within the dose escalation trial, median age was 57 years (range 23 - 70); 10 (48%) were female; 4 (19%) Māori. Lymphomas were of large cell histology in 17 (81%, **Table**). Participants had received a median of 4 prior lines of therapy, including autograft in 11 (52%) and allograft in 1. Product phenotyping showed WZTL-002 CAR T-cells were median 49% CD4$^+$, 41% CD8$^+$ and 34% CD62L$^+$. As of June 14 2023, all patients had reached the primary follow-up timepoint (3 months

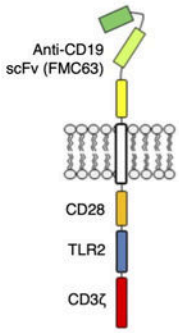
after WZTL-002 administration). Grade ≥ 3 AEs occurring in ≥ 10% of recipients were: neutropenia (95%), lymphopenia (57%), hypogammaglobulinemia (57%), anaemia (43%), febrile neutropenia (43%), thrombocytopenia (24%) and tumor pain (19%). Grade 1 - 2 CRS occurred in 13 patients (62%); 6 received tocilizumab and 3 dexamethasone. No CRS of grade ≥ 3, and no ICANS of any grade, occurred. Two grade 4 cytopenia dose limiting toxicities occurred at day 21 (one each at 5×10^5 and 1×10^6 cells/kg), both resolved to grade 2 by day 90. Maximum tolerated dose was not reached. Responses were seen at all dose levels with complete metabolic response in 11 (52%) at month 3. WZTL-002 CAR T-cells expanded at all dose levels. Among recipients of 0.5 - 1.0 $\times 10^6$ CAR T-cells/kg (n = 12), median peak CAR T-cell level (C_{max}) was 93,950 transgene copies/μg genomic DNA (reached at median day 10) and CAR T-cells persisted in 9 of 11 assessed (82%) at day 90. A recommended phase 2 dose (RP2D) range of 0.5 - 1 $\times 10^6$/kg was selected.

Conclusions:

In this dose escalation trial of a novel third-generation CD19-directed CAR T-cell product that combines CD28 and TLR2 co-stimulatory domains for r/r B-NHL, we observed no severe CRS, no ICANS of any grade and complete responses at all dose levels. At RP2D, *in vivo* CAR T-cell expansion was similar to or greater than other CD19-directed products. In conjunction with preclinical findings, this study suggests interposition of a TLR2 domain between CD28 and CD3ζ domains may reduce CAR T-cell-related ICANS risk while retaining efficacy. Enrolment to a dose expansion cohort has commenced and will assess outpatient management and the safety and efficacy of WZTL-002 CAR T-cells manufactured using a closed automated process.

Disclosures Weinkove: *BioOra:* Research Funding; *AbbVie:* Honoraria; *Janssen:* Honoraria, Research Funding. **George:** *AbbVie:* Honoraria. **Fyfe:** *Janssen:* Research Funding. **Dasyam:** *BioOra:* Research Funding. **Mester:** *BioOra:* Research Funding. **Giunti:** *BioOra:* Research Funding. **Bollard:** *Cabaletta Bio, Catamaran Bio:* Current equity holder in private company, Current equity holder in publicly-traded company, Current holder of *stock options* in a privately-held company, Membership on an entity's Board of Directors or advisory committees, Patents & Royalties: Patent applications in CAR-NKs; *Roche:* Consultancy. **Ritchie:** *Takeda:* Consultancy, Honoraria; *Novartis:* Honoraria, Research Funding; *MSD:* Honoraria; *Amgen:* Research Funding; *BMS:* Research Funding. **Perret:** *BioOra:* Research Funding. **Li:** *Wellington Zhaotai Therapies Limited:* Current equity holder in private company; *Guangdong Zhaotai Biomedicine Ltd:* Current equity holder in private company, Patents & Royalties. **Hermans:** *Avalia Immunotherapies:* Ended employment in the past 24 months, Membership on an entity's Board of Directors or advisory committees, Patents & Royalties: Patents related to vaccine design; *BioOra:* Research Funding.

Figure: Schematic of the third-generation 1928T2z CAR expressed by WZTL-002 T-cells



scFv, single chain variable fragment

Table: Dose levels, key toxicities and 3 month response for patients treated with WZTL-002

Participant	Lymphoma subtype	Dose level (WZTL-002 cells/kg)	CRS (highest grade)	ICANS (highest grade)	Dose-limiting toxicities	3 month response
EN1-01	FL	5×10^4	1	0	0	PD
EN1-02	MCL	5×10^4	2	0	0	PD
EN1-03	DHL	5×10^4	0	0	0	**CR**
EN1-04	FL	1×10^5	0	0	0	**CR**
EN1-05	DLBCL	1×10^5	1	0	0	**CR**
EN1-07	DHL	1×10^5	0	0	0	**CR**
EN1-12	DHL	2×10^5	2	0	0	PD
EN1-13	DHL	2×10^5	1	0	0	PD
EN1-14	DLBCL	2×10^5	1	0	0	**CR**
EN1-08	DLBCL	5×10^5	1	0	Neutropenia	PD
EN1-15	tFL	5×10^5	0	0	0	**PR**
EN1-16	PMBCL	5×10^5	0	0	0	**CR**
EN1-17	tFL	5×10^5	0	0	0	**CR**
EN1-18	DLBCL	5×10^5	0	0	0	PD
EN1-20	THRLBCL	5×10^5	2	0	0	PD
EN1-21	DLBCL	1×10^6	1	0	Thrombocytopenia	**CR**
EN1-22	DLBCL	1×10^6	1	0	0	**CR**
EN1-23	FL	1×10^6	1	0	0	**CR**
EN1-24	DLBCL	1×10^6	0	0	0	**CR**
EN1-25	DLBCL	1×10^6	1	0	0	PD
EN1-26	DLBCL	1×10^6	1	0	0	PD

CR, complete response; CRS, cytokine release syndrome; DHL, 'double hit lymphoma' (high grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements); DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; ICANS, immune effector cell-associated neurotoxicity syndrome; MCL, mantle cell lymphoma; PD, progressive disease; PMBCL, primary mediastinal large B-cell lymphoma; PR, partial response; tFL, transformed follicular lymphoma; THRLBCL, T-cell/histiocyte rich large B-cell lymphoma

Figure 1

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