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POSTER ABSTRACTS

704.CELLULAR IMMUNOTHERAPIES: EARLY PHASE AND INVESTIGATIONAL THERAPIES

Intracellular Retention of Tcr$\alpha\beta$/CD3 to Generate Novel Allogeneic CAR-T Cells (ThisCART19A) with Enhanced Antitumor Potency for Treating B-ALL

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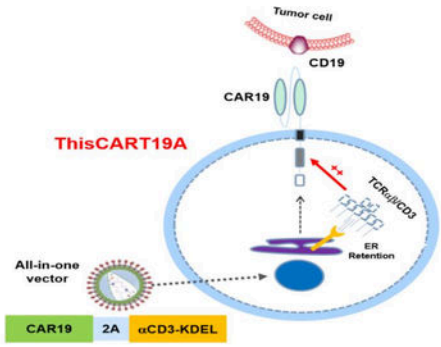
Background: Autologous CAR-T therapy has been complicated by long production time, high-cost and risks of manufacturing failure. Allogeneic CAR-T cells can overcome these hurdles, but would subsequently require specific strategies to inhibit allogeneic TCR responses and GvHD. Gene-editing technologies can efficiently deplete endogenous TCR, but also leads to off-target edits and chromosomal abnormality. Furthermore, genetic depletion of TCR disrupts the intracellular T cell activation signal and may compromise CAR-T cytotoxicity. It is thus necessary to develop non-gene-editing allogeneic CAR-T platforms and enhance the potency of allogeneic CAR-T cells.
Methods: We developed a novel non-gene-editing platform named ThisCART (**T**CR$\alpha\beta$/CD3 and/or HLA-I **i**ntracellular **s**equestered) to manufacture allogeneic CAR-T cells. The platform was based on the intracellular retention of TCR$\alpha\beta$/CD3 complex, allowing for allogeneic CAR-T production with a single lentiviral vector without genetic depletion of TCR. Allogeneic CD19 CAR-T cells (ThisCART19A) was a prototypic product for the platform. The construct contains a CD19-targeted CAR and a KDEL-tagged anti-CD3 single chain antibody (scFv) which prevents TCR$\alpha\beta$/CD3 from being secreted from the endoplasmic reticulum (ER) (Figure A). The efficacy and safety of ThisCART19A were tested in xenograft models. Finally, a phase I study was conducted to assess the safety, efficacy and pharmacokinetics in patients with relapsed or refractory (R/R) B-ALL (NCT05350787). All patients received intravenous fludarabine (30mg/m ^{2}/d), cyclophosphamide (300mg/m ^{2}/d) and etoposide (100mg/d) for 5 days followed by a single infusion of thisCART19A.
Results: The manufacturing platform of ThisCART19A was able to achieve over 150-folds of ex vivo CAR-T expansion in all batches, with the purity of products (CAR-positive/TCR$\alpha\beta$-negative) above 99%. In preclinical models, ThisCART19A did not induce GvHD, and exhibited superior antitumor function compared to conventional CD19 CAR-T cells. In the Phase I study (Figure B), 10 patients were enrolled and 8 received thisCART19A at doses of 3 (n =5) and 5 (n = 3)$\times 10^6$/kg. All patients were diagnosed as relapse/refractory acute B cell leukemia (R/R B-ALL). Three patients previously received CD19-or CD22-targeted therapies (autologous CAR-T, BiTE or ADC). Grade 3-4 treatment-related adverse events were reported in 8/8 (100%) patients, the most frequent being neutropenia (100%) and thrombocytopenia (87.5%), which most likely related to lymphodepletion. Grade 3-4 CRS was reported in 2/8(25%) patients, and ICANS was reported in 3/8 (37.5%) patients which were all reversible with steroid treatment. 7 patients were evaluable for efficacy analyses (one died from CRS and infection at 5 days post infusion), and MRD-negative CR/CRi was achieved in 100% of these patients. With a median follow-up of 146 days (range, 56 to 407), 4/7 patients remained MRD-negative. Two patients were bridged to allo-HSCT. The mean peak of CAR-T number by FCM was 5908.8(0.51-17457.9) cells/μL , which occurred on day 9 (7-9).
Conclusions: We report for the first time that intracellular retention of TCR$\alpha\beta$/CD3 complex can successfully manufacture allogeneic CAR-T cells, with superior activation potential. In patients with R/R B-ALL, ThisCART19A demonstrated acceptable safety, robust expansion and encouraging clinical response profiles. With streamlined single-vector-based production and

enhanced CAR signaling, ThisCART platform represents an attractive alternative to gene-editing-based allogeneic CAR-T platforms.

Disclosures No relevant conflicts of interest to declare.

A



B

Patient	Age	Gender	Previous lines of therapy	CAR-T source	BM blasts prior to conditioning, %	Dose level	CRS Grade	ICANS grade	Best response	PFS/OS (Days)
1	48	male	5	Donor 1	62	DL1	2	0	CR, MRD-	56/56, lost follow-up
2	55	male	4	Donor 1	40	DL1	2	0	CR, MRD-	407*/407*,
3	51	female	5	Donor 1	64	DL1	2	1	CRi, MRD-	192/192，died of COVID19 infection
4	43	female	6	Donor 1	62	DL2	2	0	CR, MRD-	86/146, died of disease progression
5	49	female	5	Donor 1	8.5	DL2	4	0	NE	NE
6	21	female	7	Donor 1	38	DL2	2	4	CR, MRD-	60/330*
7	25	male	4	Donor 2	83	DL1	3	4	CR, MRD-	104*/104*
8	67	female	7	Donor 2	35	DL1	2	0	CR, MRD-	75*/75*

*: ongoing follow-up NE: Not evaluable DL: dose level

Figure 1

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