In-vivo B-cell maturation antigen CAR T-cell therapy for relapsed or refractory multiple myeloma

Chimeric antigen receptor (CAR) T-cell therapy has shown impressive efficacy in treating relapsed or refractory multiple myeloma. Nonetheless, its availability is substantially restricted by complex manufacturing procedures, high logistical requirements, protracted waiting time, and prohibitive costs.[1] In-vivo CAR T delivers CAR transgene directly to endogenous T cells, reprogramming them into CAR T cells in situ. It is more a ready-to-use product than a customised drug, which obviates the need for apheresis, CAR T-cell manufacturing and lymphodepletion.[2] Although some preclinical studies have used lentiviral vectors for in-vivo CAR T-cell engineering,[2] clinical evidence is still scarce.

ESO-T01 is a nanobody-targeted, immune-shielded lentiviral vector for in-vivo T-cell engineering with a humanised anti-B-cell maturation antigen (BCMA) single-domain-antibody CAR. To circumvent lentivirus's extensive tropism to mammalian cells, the pivotal residues in vesicular stomatitis virus glycoprotein G were mutated. The viral membrane is engineered to overexpress CD47, thus suppressing phagocytosis of mononuclear phagocytic system,[3] and to include an anti-T-cell receptor nanobody to attain T-cell targeting. Additionally, the major histocompatibility complex class-I is knocked out to mitigate immunogenicity.[4] With the upstream region containing a T cell-specific synthetic promoter, the CAR is composed of an anti-BCMA variable domain of the heavy chain, human CD8 hinge and transmembrane domains, 4–1BB costimulatory and CD3 zeta activation domains (CN109134665B; appendix p 1). ESO-T01 showed a good safety and efficacy profile in preclinical mouse models (appendix pp 47–54). Here, we present a case series of the first-in-human data of four patients with relapsed or refractory multiple myeloma treated with the lowest dose of ESO-T01 (EsoBiotec, Mont-Saint-Guibert, Belgium; appendix pp 10–11) in an ongoing single-arm, open-label, dose-escalation, phase 1 study to assess safety, tolerability, and preliminary efficacy. This study is registered with ClinicalTrials.gov (NCT06691685) and the full protocol is available in the appendix (pp 12–123).

In this case series, four adult (aged ≥18 years) patients with multiple myeloma (according to the International Myeloma Working Group response criteria), confirmed BCMA expression, who have received at least two previous lines of therapy and demonstrated disease progression or are refractory to immunomodulators and proteasome inhibitors, and have measurable disease, were enrolled from a single hospital in Wuhan, China between Nov 19, 2024 and Jan 20, 2025. The baseline characteristics and previous treatments are presented in the appendix (pp 2, 6). Patient 1 had developed penta-refractory disease and multisite extramedullary disease. Patient 2 had tandem autologous hematopoietic stem-cell transplantation before developing two paraosseous masses. Patient 3, refractory to four previous therapies, presented with extensive extramedullary disease. Patient 4 did not respond to BCMA-G-protein-coupled receptor class C group 5 member D CAR T-cell therapy. All patients received a single intravenous infusion of ESO-T01 at a starting dose of $2{\cdot}0 \times 10^8$ transduction units without previous apheresis and lymphodepletion. Pre-emptive promethazine hydrochloride was administered in advance. Diclofenac suppositories were administered at the clinicians' discretion to prevent a sharp rise in temperature following infusion, although this was not specified in the protocol. For patients 2–4, an additional 20 mg of dexamethasone was administered before the infusion based on the post-infusion reactions of patient 1. After ESO-T01 infusion, all patients received electrocardiographic monitoring for 24 h and were isolated from others for the first 48 h as a safety precaution. This measure was not pre-specified in the protocol but was taken due to concerns about potential cross-infection.

Immediately after the infusion, all patients developed acute inflammatory reactions within the first day. They all had similar temporal patterns: chills emerged first, followed by a fever within 3 h, which persisted for 6–18 h (figure A). Hypotension requiring vasopressor support developed in patients 1, 2, and 4. Patients 1 and 4 developed hypoxemia requiring supplemental oxygen. Patient 1 also exhibited mild disturbance of consciousness and tremors in both upper limbs. Cranial CT revealed no clinically significant abnormalities. After the administration of dexamethasone (10 mg) and gamma globulin, all symptoms resolved within 48 h in patient 1. Patient 3 reported headache and leg muscle soreness. All clinical manifestations subsided after symptomatic treatments. Laboratory tests revealed a transient increase in cytokines, C-reaction protein, ferritin, and liver enzymes (figure B; appendix p3). Cytokine Release Syndrome (CRS) was assessed in all patients, with grade 3 CRS observed in patients 1, 2, and 4, and grade 1 in patient 3. Lymphocyte counts sharply decreased within the first 12 h, and then gradually recovered by 48 h post-infusion (appendix p 3). Between days 8 and 12, grade 1 CRS occurred in all patients, characterised by fever, and lasted for 1–4 days. A second peak was observed in IL-6 and IL-10. Notably, patient 4, who presented with the highest tumour burden in the CSF, developed grade 1 Immune Effector Cell-Associated Neurotoxicity

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Syndrome (ICANS) on day 8. Both CRS and ICANS were fully controlled by the administration of glucocorticoids. Grade 3–4 haematological toxicities developed after infusion (appendix p 3) and included neutropenia (patients 1, 2, and 4), leukopenia (patients 2 and 4), thrombocytopenia (patients 3 and 4), and lymphopenia (patients 2, 3, and 4), but most of these toxicities recovered to baseline during follow-up (appendix p 6–7). Pulmonary infections were observed in patients 2 and 4 within the 28 days, and were controlled by antibiotics. The comprehensive adverse events are summarised in the appendix (p 8). After infusion, no virus was detected in patients' urine, saliva, and CSF, and the viral titre in peripheral blood peaked within the first 12 h and decreased to virtually undetectable concentrations by 48 h after infusion (figure C). Notably, patient 4 exhibited the highest peak of viral titre, which coincided with the elevated cytokines on day 1.

As of April 1, 2025, all patients completed the 2-month follow-up, with the first two patients completing the 3-month follow-up. Patient 1 attained a stringent complete response with resolution of all medullary and extramedullary lesions by month 2 (appendix p 4), whereas patient 2 had a stringent complete response with complete lesion resolution by day 28 (appendix p 4). Patients 3 and 4 had partial responses, with tumour lesions reduced and minimal residual disease negativity reached in the bone marrow by day 28 (appendix p 4, 9). At the 2-month follow-up, serum protein electrophoresis and free light chain concentrations (appendix p 9) returned to normal in patient 3 and were further reduced in patient 4.

CAR T cells in peripheral blood were first detected on days 4–8 and peaked on days 10–17, and were also detectable in the bone marrow, tumour tissues, pleural effusions, and CSF (figure D; appendix pp 5, 9). In patient 2,

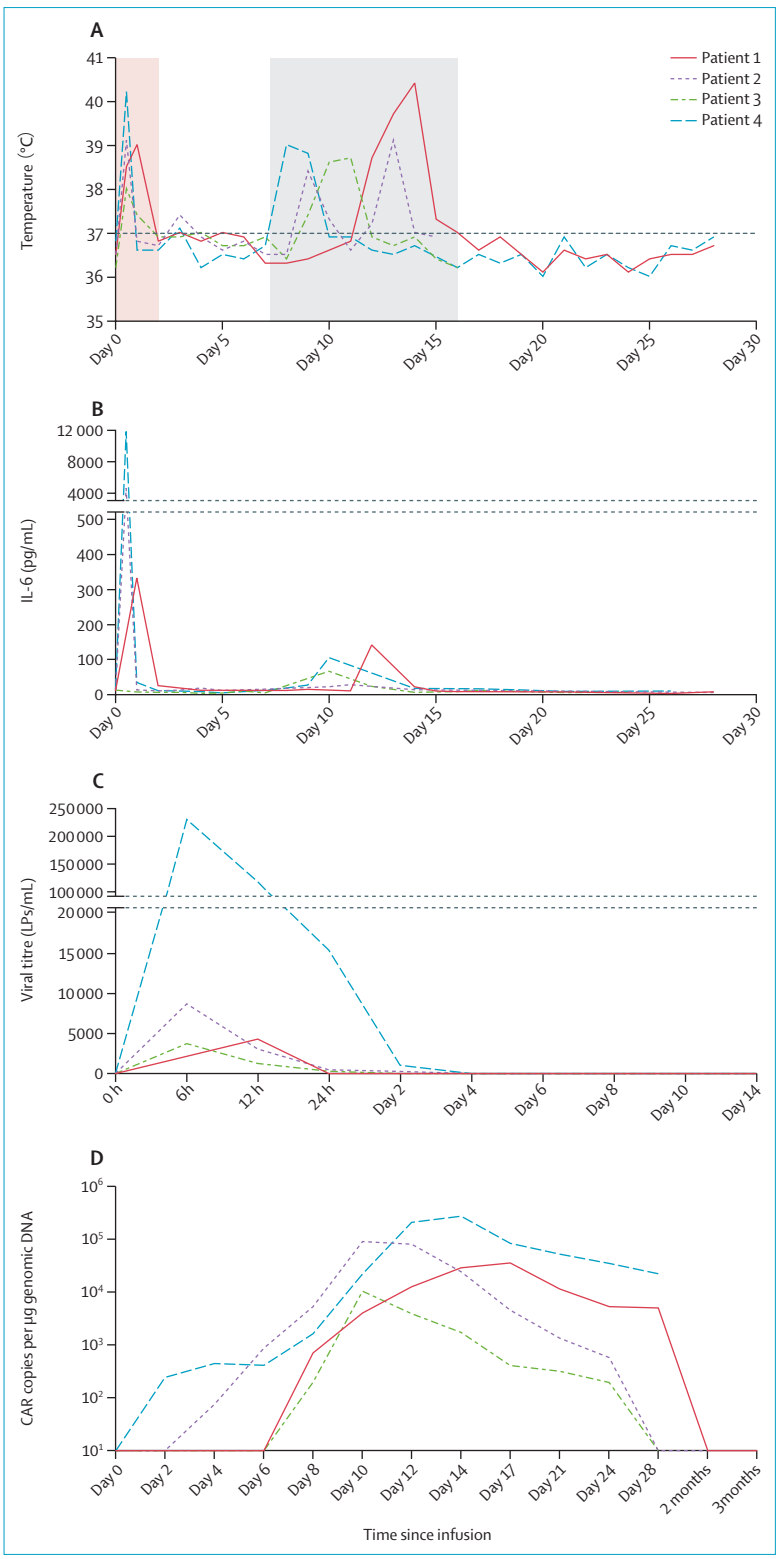

Figure: **Overview of the safety, efficacy, and transduction of ESO-T01**
Post-infusion changes of (A) body temperature, (B) IL-6, (C) viral titre in peripheral blood. 0 in the abscissa axis refers to the day of infusion (before infusion, also a baseline timepoint). (D) CAR T-cell kinetics detected by quantitative polymerase chain reaction. CAR=chimeric antigen receptor. LP=lentiviral particle.

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immunohistochemistry of baseline and day 10 tumour biopsies revealed marked reduction of $CD38^{+}CD138^{+}BCMA^{+}$ tumour cells alongside significant $CD3^{+}$ T-cell infiltration in the tumour microenvironment (appendix p 5). We then analysed the phenotypic profile of CAR^{+} T cells at the expansion peak (appendix p 5). Patient 1 had a higher proportion of CAR+ T cells with a central memory phenotype, whereas patients 2 and 4 had a greater abundance of naive and central memory CAR^{+} T cells. Effector and $CD28^{-}CD57^{+}$ senescent T cells were enriched in CAR^{+} T cells of patient 3, which corresponded to unfavourable CAR T-cell expansion. Flow cytometry of CAR T cells in peripheral blood showed a low CAR expression in $CD3^{-}$ lymphocytes and natural killer cells (appendix p 5). Nonetheless, off-target transduction in haematopoietic cells and other immune cells requires further study, and tumourigenicity needs to be monitored through long-term follow-up.

So far, only one case of B-cell non-Hodgkin lymphoma treated with in vivo-produced CAR T cells has been reported. In this case, lentivirus was infused together with T cells for in-vivo transduction, while preserving apheresis and lymphodepletion.[5] Therefore, to our knowledge our study was the first multiple myeloma case series treated with in-vivo CAR T cells. The starting dose of ESO-T01 was set at one-tenth of the human equivalent dose, calculated from the effective dose in mice by bodyweight. It was significantly lower than those previously reported in non-human primates, which used a dose of $7{\cdot}5 \times 10^{9}$ transducing units per kg with a good tolerability.[3] Nonetheless, it yielded a maximum concentration and area under the curve from 0 to 28 days after infusion that was comparable to those of idecabtagene vicleucel and ciltacabtagene autoleucel.[6–9] After the infusion, lymphocytes sharply decreased, which was probably due to T-cell migration from the circulation after being activated by the anti-T-cell receptor nanobody. The reactions in the early phase most probably resulted from virus-mediated acute immune activation, as previously reported in primates.[3] In the late phase, the development of CRS was in line with CAR T-cell expansion.

The prognosis of patients with multiple myeloma and extramedullary disease remains poor, especially in those with extraosseous plasmacytomas.[10-13] In this case series, ESO-T01 appeared to eradicate extramedullary disease. CAR T cells within the tumour site suggested enhanced infiltration, possibly due to the smaller viral particle size, allowing for their better penetration into tumour tissues. Moreover, in-vivo CAR T cells might possess better tumour-homing abilities with an improved tumour microenvironment. These mechanisms warrant further investigation. Nevertheless, a larger cohort and longer follow-up are needed to further look into the persistence of in vivo-produced CAR T cells and their efficacy, and a randomised controlled design is required for more convincing results. Collectively, the results of this case series highlighted the potential therapeutic effect of ESO-T01 on relapsed or refractory multiple myeloma. This case series could provide useful data that might inform future studies on in vivo-generated CAR T-cell therapy.

The data collected in this study are uploaded in the appendix. Any other underlying data are not publicly available due to privacy and ethical restrictions, but are available from the corresponding author through email on reasonable request. JX, LL, and PP contributed equally. HM, YH, and CL contributed equally to the study conception and design. Data collection, analysis, and interpretation were performed by JX, LL, WX, ZC, and CS. JX, LL, and HM were involved in the writing. HM, PP, and JZ took part in the revision of the manuscript. Statistical analysis was conducted by JX, LL, and HM. PP is employed by and owns stocks in EsoBiotec. JZ is employed by and owns stocks in Shenzhen Pregene Biopharma Company. All other authors declare no competing interests. This case series was supported by grants from the National Natural Science Foundation of China (grant numbers 82425003, 82350103, and 82330005) and Shenzhen Pregene Biopharma. We thank the patients, who gave consent for the study; Jing Wang and Chunxia Qin for professional assistance in medical imaging; Jin'e Zheng and Li Cai for flow cytometry detection; all doctors and nurses at the Department of Hematology, who provided clinical care; and the staff at Shenzhen Pregene Biopharma, who offered technical support to this study.

*Jia Xu, Lin Liu, Philippe Parone, Wei Xie, Chunyan Sun, Zhaozhao Chen, Jishuai Zhang, Chunrui Li, Yu Hu, *Heng Mei*

hmei@hust.edu.cn

Institute of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China (JX, LL, WX, CS, ZC, YH, HM); EsoBiotec, Mont-Saint-Guibert, Belgium (PP), Shenzhen Pregene Biopharma, Shenzhen, China (JZ); Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China (CL)

1 Al Hadidi S, Szabo A, Esselmann J, et al. Clinical outcome of patients with relapsed refractory multiple myeloma listed for BCMA directed commercial CAR-T therapy. *Bone Marrow Transplant* 2023; **58:** 443–45.

2 Michels A, Ho N, Buchholz CJ. Precision medicine: In vivo CAR therapy as a showcase for receptor-targeted vector platforms. *Mol Ther* 2022; **30:** 2401–15.

3 Milani M, Annoni A, Moalli F, et al. Phagocytosis-shielded lentiviral vectors improve liver gene therapy in nonhuman primates. *Sci Transl Med* 2019; **11:** 11.

4 Milani M, Annoni A, Bartolaccini S, et al. Genome editing for scalable production of alloantigen-free lentiviral vectors for *in vivo* gene therapy. *EMBO Mol Med* 2017; **9:** 1558–73.

5 Chen X-F, Huang K, Liu J-Y, et al. 1482 In-vivo CAR-T cells produced by specific virus in refractory B cell lymphoma. *J Immunother Cancer* 2024; **12:** A1712.

6 Raje N, Berdeja J, Lin Y, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. *N Engl J Med* 2019; **380:** 1726–37.

7 Berdeja JG, Madduri D, Usmani SZ, et al. Ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy in patients with relapsed or refractory multiple myeloma (CARTITUDE-1): a phase 1b/2 open-label study. *Lancet* 2021; **398:** 314–24.

8 Celgene Corporation. ABECMA. Package Insert and Medication Guide. US Food and Drug Administration, 2024.

9 Janssen Biotech. CARVYKTI. Package Insert and Medication Guide. US Food and Drug Administration, 2024.

10 Bhutani M, Foureau DM, Atrash S, Voorhees PM, Usmani SZ. Extramedullary multiple myeloma. *Leukemia* 2020; **34:** 1–20.

11 Zanwar S, Ho M, Lin Y, et al. Natural history, predictors of development of extramedullary disease, and treatment outcomes for patients with extramedullary multiple myeloma. *Am J Hematol* 2023; **98:** 1540–49.

12 Touzeau C, Moreau P. How I treat extramedullary myeloma. *Blood* 2016; **127:** 971–76.

13 Li C, Xu J, Luo W, et al. Bispecific CS1-BCMA CAR-T cells are clinically active in relapsed or refractory multiple myeloma. *Leukemia* 2024; **38:** 149–59.