

GPC3-specific dnTGFβRII-armoured CAR T cells for hepatocellular carcinoma

<https://doi.org/10.1038/s41586-026-10786-z>

Received: 12 February 2025

Accepted: 9 June 2026

Published online: 15 July 2026

 Check for updates

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Glypican-3 (GPC3) is highly expressed in hepatocellular carcinoma (HCC), making it an attractive target for chimeric antigen receptor (CAR) T cell therapy; however, this approach has previously shown limited clinical efficacy, potentially owing to high levels of transforming growth factor-β (TGFβ) in the tumour microenvironment^{1–4}. We therefore engineered CAR T cells with a dominant-negative TGFβ receptor II, which showed enhanced antitumour activity in preclinical studies⁵. Here we report findings from a first-in-human trial evaluating the safety and efficacy of C-CAR031 in patients with advanced, treatment-refractory HCC (NCT05155189). Thirty-six patients received CAR T infusions at four dose levels (from 0.75×10^6 to 4.0×10^6 cells per kg). Cytokine release syndrome was reported in 34 patients, of which two cases were grade 3. Nine patients had non-haematological adverse events of grade 3 or higher. Tumour regression was observed in 32 patients, with a median best tumour reduction from baseline of 41.6% (range: 3.4–94.4%) in target lesions. The objective response rate was 44.4%, and the median duration of response was 4.4 months (95% confidence interval: 2.9–7.4). Median progression-free survival and overall survival were 4.2 months (95% confidence interval: 2.9–4.8) and 14.2 months (95% confidence interval: 10.1 to not evaluable), respectively. High-throughput analyses of tumour samples and functional validation suggested that GPC3 antigen loss and increased TGFβ levels may contribute to C-CAR031 resistance. Collectively, these results indicate that C-CAR031 has a manageable safety profile and encouraging antitumour activity in heavily pretreated patients with advanced HCC.

Hepatocellular carcinoma (HCC) is an important cause of cancer-related mortality worldwide, with a particularly high incidence in Asia⁶. Current effective treatments for unresectable HCC remain limited. Recommended first-line regimens such as atezolizumab plus bevacizumab have demonstrated efficacy with an objective response rate (ORR) of 35% and a 1-year overall survival rate of 67.2%⁷, whereas the STRIDE regimen (comprising single-dose tremelimumab with durvalumab at regular intervals) yielded an 18-month overall survival rate of 48.7% in systemic-treatment-naïve patients⁸. However, considerable proportions of patients either do not respond to these therapies or develop resistance; thus, there is an urgent need for new therapeutics^{9,10}.

Although there are well-established global guidelines for first- and second-line treatment of advanced HCC, recommendations are sparse for cases in which multiple treatments have failed. Chimeric antigen receptor (CAR) T cell therapies have demonstrated substantial clinical benefits with manageable safety profiles in haematological malignancies^{11,12}, leading to the approval of many CAR T cell therapies by major regulatory authorities. However, several factors limit the efficacy of CAR T cell therapies in solid tumours, including the complex immunosuppressive tumour microenvironment (TME) and the challenges of identifying tumour-associated antigens for effective trafficking of CAR T cells to the tumour site^{1,13–15}.

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GPC3 is highly expressed on HCC cells and is associated with poor prognosis¹⁶, but it is rarely found in normal cells, making it an appealing target for CAR T cell therapy¹⁷. GPC3-targeting CAR T cells have shown limited efficacy in trials involving unresectable HCCs^{2,3}; this has been attributed partly to the TME¹. Armouring CAR T cells with immunosuppressive cytokine antagonists can protect them from the TME and enhance their efficiency¹⁸. Transforming growth factor- β (TGF β), a potent immunosuppressor, shows elevated levels in tumour and blood samples from patients with HCC compared with those with chronic hepatitis or cirrhosis^{19–21}, and TGF β inhibition with dominant-negative TGF β receptor II (dnTGF β RII)-armoured CAR T cells has demonstrated promising clinical outcomes in a prostate cancer phase I trial²². However, whether this approach can improve the antitumour efficacy of CAR T cells in tumour of other types, including HCC, has remained unknown.

Here we report on safety and efficacy from a first-in-human trial of dnTGF β RII-armoured GPC3-specific CAR T cells with affinity-tuned single-chain variable fragments (C-CAR031)⁵ in patients with advanced HCC. We also present preclinical and translational findings with respect to C-CAR031 expansion kinetics, TME modulation and potential resistance mechanisms.

GPC3 and TGF β expression in HCC

GPC3 is highly expressed in HCC but not in normal liver samples, as demonstrated by the immunohistochemical analysis we used to identify patients for the trial (Extended Data Fig. 1a). TGF β signalling is widely activated in HCC, as evidenced by TGF β and phosphorylated SMAD2 (pSMAD2) expression in both tumour cells and stroma (Extended Data Fig. 1b,c). TGF β levels were also significantly higher in HCC than in cirrhotic tissues, whereas pSMAD2 levels were similar (Extended Data Fig. 1d–f). In addition, *TGFB1* gene expression and TGF β gene signature expression levels were significantly higher in HCC than in normal liver (Extended Data Fig. 1g,h). These findings suggest that targeting GPC3 while inhibiting TGF β represents a rational strategy for CAR T cell therapy in HCC.

TGF β -resistant armoured CAR T cells

Truncated TGF β RII functions as a dominant-negative receptor by blocking TGF β signalling and its immunosuppressive effects in T cells. Therefore, we coexpressed dnTGF β RII and GPC3-specific CAR under the same promoter to generate C-CAR031. Coexpression of dnTGF β RII with CAR resulted in a bona fide dominant-negative receptor, as neither unarmoured CAR T cells nor untransduced T cells exhibited detectable surface expression of TGF β RII (Fig. 1a). TGF β -induced rapid phosphorylation of SMAD2/3 proteins in CAR T cells was blocked by dnTGF β RII (Fig. 1b,c). Both unarmoured and armoured GPC3-specific CAR T cells underwent a progressive decline in their ability to kill GPC3-positive cells upon repeated tumour cell challenges; however, whereas exogenous TGF β accelerated this decline in unarmoured cells, its inhibitory effect was attenuated in dnTGF β RII-armoured cells (Extended Data Fig. 2a). Consistently, exogenous TGF β inhibited antigen-dependent proliferation only in unarmoured CAR T cells (Extended Data Fig. 2b). In the presence of exogenous TGF β , dnTGF β RII preserved interferon- γ (IFN γ) production in armoured CAR T cells, whereas this effector cytokine response was suppressed in unarmoured cells (Fig. 1d). Similar results were observed under chronic plate-bound GPC3 stimulation (Extended Data Fig. 2c,d). Therefore, we conclude that dnTGF β RII successfully blocks TGF β signalling and preserves CAR T cell function in vitro in the presence of suppressive levels of TGF β .

In a xenograft mouse model with GPC3-positive HCC cells overexpressing TGF β (Huh-7-oeTGF β), armoured CAR T cells were more potent than unarmoured cells in inducing tumour regression and increased numbers of CAR-positive cells in the tumours 7 days postinjection

(Extended Data Fig. 2e–h). Furthermore, expression levels of programmed cell death protein 1 (PD1) and lymphocyte-activation gene 3 (LAG3), both of which can be upregulated by TGF β ²³, were significantly reduced in CD4⁺ and CD8⁺ tumour-infiltrating lymphocytes from mice treated with armoured CAR T cells (Extended Data Fig. 2i,j), indicating enhanced immune responses in the TME. Serum IFN γ levels were significantly higher, whereas serum α -fetoprotein (AFP) levels were reduced to a greater extent in mice treated with armoured CAR T cells compared mice with unarmoured cells (Extended Data Fig. 2k,l), consistent with observed tumour shrinkage. In patient-derived xenograft mouse models, armoured CAR T cells induced tumour regression regardless of TGF β levels, with preservation of IFN γ production, whereas unarmoured CAR T cells were less effective in high-TGF β models (Fig. 1e–h). Thus, dnTGF β RII protected CAR T cells from TGF β -mediated immunosuppression in preclinical models.

Patient characteristics

We then launched a clinical trial of dnTGF β RII-armoured CAR T cells (C-CAR031) in HCC. Between December 2021 and November 2024, 118 patients were prescreened for GPC3 expression by immunohistochemical analysis. Prescreening failed for 74 of these (50 owing to low GPC3 levels), and 44 patients entered screening (Extended Data Fig. 3a,b). Thirty-six patients underwent leukapheresis and completed lymphodepletion chemotherapy followed by C-CAR031 infusion. Ultimately, one patient received C-CAR031 infusion at 0.75×10^6 cells kg^{-1} (dose level 1; DL1), six received 1.5×10^6 cells kg^{-1} (dose level 2; DL2), 18 received 2.5×10^6 cells kg^{-1} (dose level 3; DL3) and 11 received 4.0×10^6 cells kg^{-1} (dose level 4; DL4) (Extended Data Fig. 3c).

Baseline characteristics of the 36 infused patients are presented in Supplementary Table 1 (overall) and Supplementary Table 2 (per individual). Among the 36 patients, 31 (86.1%) were male, and the median age was 51 years (range: 25–70). Median GPC3 expression level at baseline was 80.0% (range: 25–100%) in tumour cells overall and 47.5% (range: 10–100%) on tumour cell membranes. Most patients had an Eastern Cooperative Oncology Group performance status score of 1 (80.6%), Barcelona Clinic Liver Cancer stage C (94.4%) and Child–Pugh scores of 5–6 (94.4%). Twenty-five (69.4%) patients had intrahepatic lesions, and 30 (83.3%) had extrahepatic lesions, predominantly in the lung (26 of 36; 72.2%), followed by the peritoneum (5 of 36; 13.9%) and bone (3 of 36; 8.3%). Most patients had a history of hepatitis B virus (HBV) infection (35; 97.2%), cirrhosis (25; 69.4%) and abnormal serum AFP levels (greater than 40 ng ml^{-1}) (31; 86.1%), with 25 (69.4%) having levels of 400 ng ml^{-1} or higher. All patients received targeted therapy, and 33 (91.7%) received immunotherapy; 25 (69.4%) had received three or more lines of previous therapy (median of four lines for the entire cohort). Twenty-nine (80.6%) patients had previous hepatectomy and 32 (88.9%) had transcatheter arterial (chemo)embolization. Twenty (55.6%) patients received bridging therapy for a median duration of 15.5 days (range: 6–24).

Safety

As of the data cut-off on 31 August 2025, all 36 evaluable patients had received C-CAR031 single-dose infusions; two of these patients had received a second dose. Lymphodepletion was satisfactorily administered before C-CAR031 infusion. The manufacturing success rate was 100%, and the median cell viability of C-CAR031 was 91% (range: 73–95%) before freezing and 87% (range: 63–97%) after thawing, showing good cell quality of the infused cells.

Overall safety profiles are shown in Table 1, with dose-level details in Supplementary Table 3. All patients experienced treatment-emergent adverse events (TEAEs) of any grade, including TEAEs of grade 3 or higher. TEAEs related to C-CAR031 (any grade) occurred in all patients, with the most common being cytokine release syndrome (CRS)

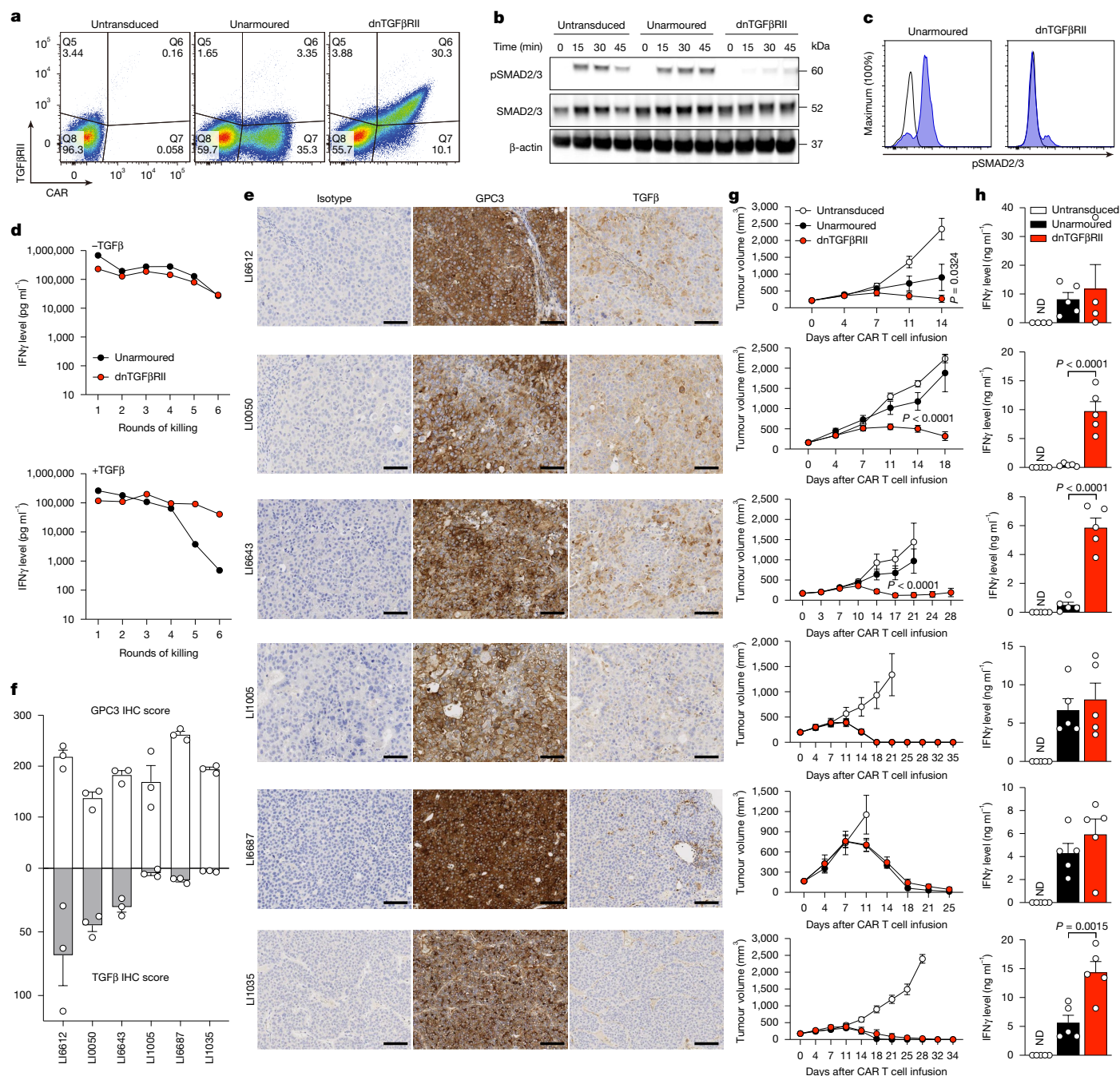


Fig. 1 | dnTGFβRII-armoured GPC3-specific CAR T cells overcome TGFβ immunosuppressive effects in preclinical models. a, Dot plots depicting expression of CAR and TGFβRII/dnTGFβRII gated on CD8⁺ T cells. **b**, SMAD2/3 and pSMAD2/3 expression levels were analysed by immunoblotting in untransduced T cells or CAR T cells stimulated with TGFβ (1 ng ml⁻¹) or left unstimulated. For gel source data, see Supplementary Fig. 1. One of two independent experiments is shown. **c**, pSMAD2/3 expression was analysed by flow cytometry in unarmoured or armoured CAR T cells left unstimulated or stimulated with TGFβ (5 ng ml⁻¹). **d**, CAR T cells were incubated repetitively with PLC/PRF/5 cells in the presence or absence of TGFβ (10 ng ml⁻¹). IFNγ levels in the supernatants were analysed at the indicated time points. One of two independent experiments is shown in **b–d**. **e,f**, GPC3 and TGFβ expression levels were evaluated by immunohistochemical

(IHC) staining in six HCC samples used for patient-derived xenograft (PDX) mouse models. Isotype staining was used as a control. $n = 3$ biologically independent samples. Representative images are shown in **e**, and IHC scores are shown in **f**. Scale bars, 100 μm. **g**, PDX-bearing mice were treated with 5×10^6 unarmoured or armoured CAR T cells ($n = 5$ mice per group). Untransduced T cells were infused as a control. Tumour volume was measured twice weekly. **h**, Mouse serum IFNγ levels were evaluated by Meso Scale Discovery assays 7 days after cell infusion; $n = 5$ mice per group. One mouse per group did not have sufficient serum volume for analysis in the untransduced and dnTGFβRII groups for the LI6612 PDX model. **f–h**, Data are mean $\pm$ s.e.m. Two-way analysis of variance (ANOVA) with Šidák's multiple comparisons test was used in **g**; one-way ANOVA with Tukey's honest significant difference test was used in **h**. ND, not detected.

(94.4%), increased levels of fibrin D-dimer (55.6%), abnormal coagulation test (52.8%) and alopecia (52.8%). CRS events were mostly of grade 1–2, with two patients (5.6%) receiving DL4 having grade 3 CRS (Extended Data Table 1). CRS episodes were managed with tocilizumab in 26 (72.2%) patients and corticosteroids in 5 patients (13.9%), with

4 patients receiving both. Common TEAEs of grade 3 or higher related to C-CAR031 included thrombocytopenia (11.1%) and increased aspartate aminotransferase levels (11.1%) (Table 1). Serious adverse events (SAEs) related to C-CAR031 were observed in three (8.3%) patients. One patient at DL4 developed grade 3 CRS-related interstitial pneumonitis, which

Table 1 | Summary of safety profile events in the entire cohort

Adverse event category	Total (n=36)	
	Any grade	Grade≥3
All TEAEs	36 (100.0)	36 (100.0)
TEAEs related to C-CAR031 infusion	36 (100.0)	13 (36.1)
TEAEs related to C-CAR031 infusion leading to study withdrawal	0	0
Adverse event of special interest		
CRS	34 (94.4)	2 (5.6)
ICANS	0	0
IEC-HS	0	0
Interstitial lung disease	2 (5.6)	1 (2.8)
SAE	9 (25.0)	8 (22.2)
SAE related to C-CAR031 infusion	3 (8.3)	3 (8.3)
Common ^a TEAEs related to C-CAR031 by preferred terms		
Haematologic toxicities		
Platelet count decreased	5 (13.9)	4 (11.1)
Neutrophil count decreased	2 (5.6)	2 (5.6)
Non-haematologic toxicities		
Fibrin D-dimer increased	20 (55.6)	0
Coagulation test abnormal	19 (52.8)	0
Alopecia	19 (52.8)	0
Aspartate aminotransferase increased	13 (36.1)	4 (11.1)
HBV DNA increased	12 (33.3)	0
Pleural effusion	11 (30.6)	1 (2.8)
Diarrhoea	8 (22.2)	0
Blood pressure decreased	7 (19.4)	0
Cytomegalovirus test positive	7 (19.4)	1 (2.8)
Protein urine present	6 (16.7)	0
Pyrexia	6 (16.7)	0
Myalgia	5 (13.9)	0
Vomiting	5 (13.9)	0
Alanine aminotransferase increased	4 (11.1)	1 (2.8)
Abdominal distension	4 (11.1)	0
Weight decreased	4 (11.1)	0
C-reactive protein increased	4 (11.1)	0
Procalcitonin increased	4 (11.1)	0
Ascites	3 (8.3)	2 (5.6)

Data are n (%). ^aIncidence of 10% or more of any-grade TEAEs related to C-CAR031 or at least 2 patients with TEAEs of grade 3 or higher related to C-CAR031 in the total population.

resolved within 1 week with tocilizumab and corticosteroids, and fully recovered after 1 month. Another patient at DL3 developed grade 4 myelosuppression with thrombocytopenia fluctuating between grades 1 and 4, which fully resolved within 6 months; although this patient was initially unresponsive to platelet-elevating agents (including thrombopoietin, thrombopoietin receptor agonists and interleukin-11 (IL-11)), he ultimately achieved normal platelet counts with romiplostim. A third patient at DL4 developed grade 3 CRS and tumour lysis syndrome (TLS) on day 5 postinfusion, leading to veno-occlusive liver disease (resolved with defibrotide) and hepatic and renal dysfunction. Although the acute symptoms of CRS and TLS eventually resolved, the patient succumbed to renal failure 6 weeks following infusion after declining dialysis. No dose-limiting toxicities, immune effector cell-associated neurotoxicity syndrome (ICANS) or immune effector cell-associated haemophagocytic lymphohistiocytosis-like syndrome (IEC-HS) were reported.

Table 2 | Efficacy per RECIST v.1.1 across C-CAR031 dose levels in full analysis set

Variables	0.75×10 ⁶ cells/kg ⁻¹ (DL1) n=1	1.5×10 ⁶ cells/kg ⁻¹ (DL2) n=6	2.5×10 ⁶ cells/kg ⁻¹ (DL3) n=18	4.0×10 ⁶ cells/kg ⁻¹ (DL4) n=11	Total n=36
Best overall response ^a , n (%)					
Complete response	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PR	0 (0)	3 (50.0)	7 (38.9)	6 (54.5)	16 (44.4)
Stable disease	1 (100)	2 (33.3)	11 (61.1)	3 (27.3)	17 (47.2)
Progressive disease	0 (0)	1 (16.7)	0 (0)	1 (9.1)	2 (5.6)
NE	0 (0)	0 (0)	0 (0)	1 (9.1)	1 (2.8)
ORR, n (%)	0	3 (50.0)	7 (38.9)	6 (54.5)	16 (44.4)
DCR, n (%)	1 (100)	5 (83.3)	18 (100)	9 (81.8)	33 (91.7)
DOR, months					
Median	NE	7.4	3.4	4.4	4.4
95% CI	NE	2.9–NE	2.8–NE	2.8–NE	2.9–7.4
PFS, months					
Median	NE	4.3	4.2	4.3	4.2
95% CI	NE	1.5–NE	2.8–4.8	1.7–5.8	2.9–4.8
Overall survival, months					
Median	7.0	10.4	14.2	17.7	14.2
95% CI	NE	7.6–NE	10.1–NE	4.4–NE	10.1–NE
DOR follow-up time, months					
Median	NE	NE	NE	NE	NE
95% CI	NE	NE–NE	4.2–NE	NE–NE	4.2–NE
PFS follow-up time, months					
Median	2.8	NE	NE	NE	NE
95% CI	NE	2.1–NE	5.6–NE	NE–NE	NE–NE
Overall survival follow-up time, months					
Median	7.0	30.4	22.1	19.6	22.1
95% CI	NE	NE–NE	8.6–27.1	13.2–NE	13.2–24.0

^aBest overall response was defined as the best response outcome from the first cell infusion to disease progression, death or new antitumour therapy, whichever occurred first. NE, not evaluable.

Antitumour efficacy

All 36 patients were included in the efficacy analysis by the intention-to-treat principle (full analysis set) (Table 2). Objective responses (by Response Evaluation Criteria in Solid Tumours (RECIST) v.1.1) were seen in 16 (44.4%) patients: 3 (50.0%) at DL2, 7 (38.9%) at DL3 and 6 (54.5%) at DL4, all of which were confirmed to be partial responses (PR) (Fig. 2a and Table 2). The median time to response was 1.43 months, and the disease control rate (DCR) was 91.7% overall (83.3% at DL2, 100% at DL3 and 81.8% at DL4).

Thirty-two (88.9%) patients experienced tumour reduction, with a median best reduction from baseline of 41.6% (range: 3.4–94.4%) in target lesions (Fig. 2b,c). As an example of early response, one DL2 patient exhibited marked regression of both intrahepatic lesions and lung metastases and achieved PR at week 6; as an example of durable response, one DL3 patient had PR lasting for more than 20 months; as an example of response in a case involving multimetastatic lesions, one DL4 patient demonstrated rapid and substantial resolution of extensive pulmonary lesions (Extended Data Fig. 4). These patients showed marked AFP level decline and CAR T cell expansion. Among the 31 patients with abnormal baseline AFP levels, 27 (87.1%) experienced a marked decrease (more than 50% reduction or normalization) (Fig. 2d,e).

Median progression-free survival (PFS) was 4.2 months (95% confidence interval (CI): 2.9–4.8); the median overall survival was

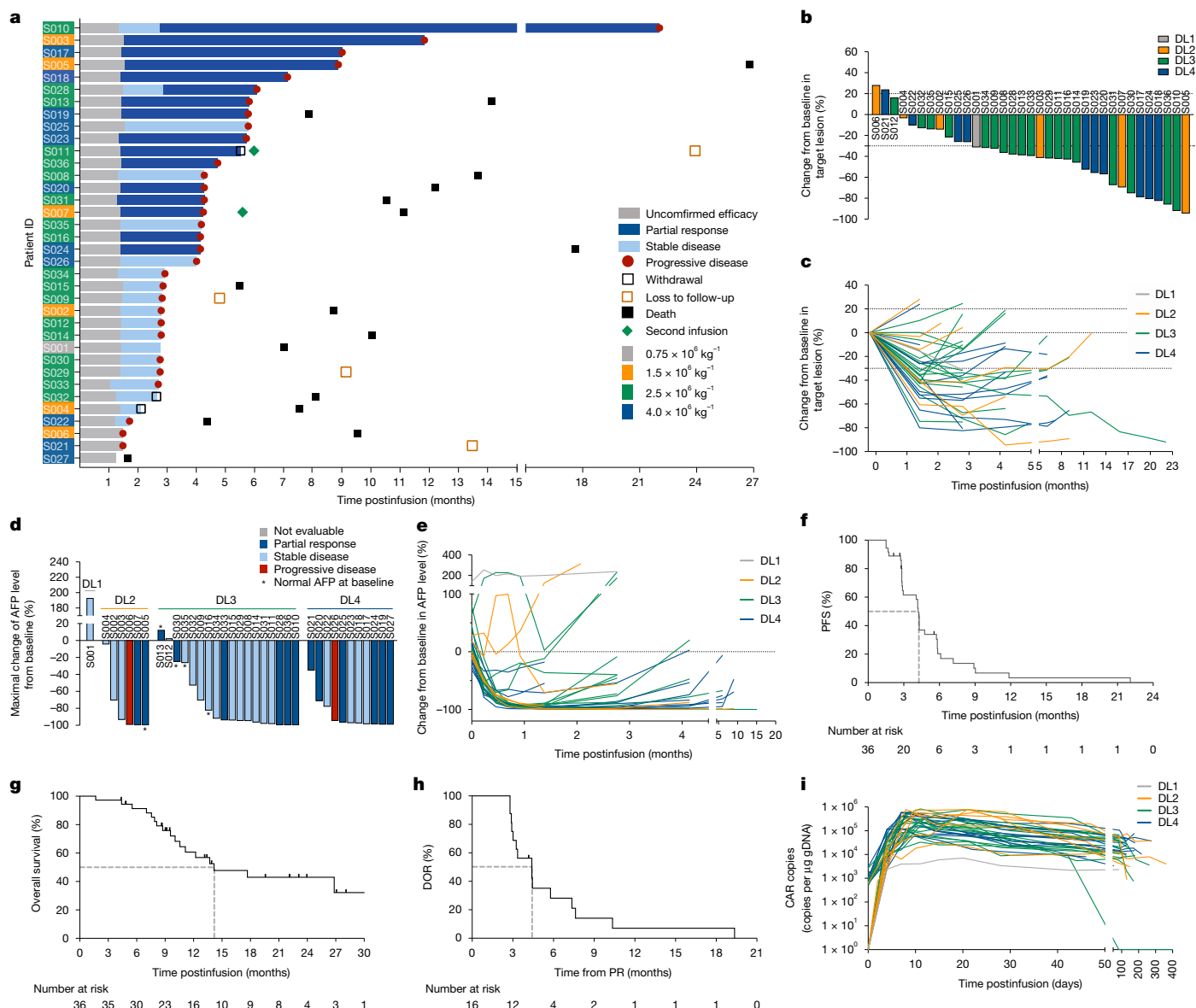


Fig. 2 | Clinical responses in patients with unresectable HCC administered C-CAR031. **a**, Swimmer plot showing tumour response over time following C-CAR031 infusion ($n = 36$). **b**, Waterfall plot of percentage change in target lesions determined as the best overall response ($n = 35$; 1 patient did not have tumour assessment). **c**, Spider plot of change in sum of longest diameters of target lesions evaluated from baseline ($n = 35$). **d, e**, Changes in serum AFP levels following C-CAR031 infusion, shown by waterfall plot of dose levels ($n = 35$,

1 patient with major protocol violation was excluded) (**d**); and spider plot of changes over time ($n = 30$, 1 patient was excluded owing to deviation from the protocol, as well as 5 patients with normal baseline AFP levels) (**e**). **f–h**, Kaplan–Meier curves for PFS (**f**), overall survival (**g**) and DOR (**h**); all 36 patients were included, and data were based on RECIST v.1.1. **i**, Cellular kinetics of C-CAR031 in peripheral blood of each patient ($n = 35$; 1 patient was excluded owing to major protocol violation). ID, identifier.

14.2 months (95% CI: 10.1 to not evaluable) (Fig. 2f,g, Table 2 and Supplementary Fig. 2a,b) after a median follow-up of 22.1 months (95% CI: 13.2–24.0). The 6-month and 12-month overall survival rates were 91.3% (95% CI: 75.4–97.1%) and 60.7% (95% CI: 40.8–75.7%), respectively. The median duration of response (DOR) was 4.4 months (95% CI: 2.9–7.4) (Fig. 2h and Supplementary Fig. 2c). Even at the time of progression, the average tumour shrinkage from baseline was 24.6%, and the median reduction in AFP levels from baseline was 53.0%. Similar findings were seen for response evaluated by modified RECIST (Extended Data Table 2).

Cellular kinetics and cytokine profiles

Robust and sustained expansion of C-CAR031 in blood was observed in all evaluable patients (one was excluded owing to protocol deviation

involving preinfusion steroid overuse) (Fig. 2i). C-CAR031 transgene DNA was detectable in blood as early as 2 h postinfusion in 20 (57.1%) patients, with a median of 771 (range: 465–2,998) copies per microgram genomic DNA (gDNA). The median maximum observed concentration (C_{max}) was 332,278 (range: 7,083–826,492) copies per microgram gDNA, and the median time to maximum observed concentration (T_{max}) was 10 (range: 7–31) days postinfusion. The median area under the curve for the first 28 days postinfusion was 3,035,803 (range: 125,077–14,372,706) copies per microgram gDNA $\times$ day. C-CAR031 remained detectable in 34 of 35 patients at the last follow-up, with time to the last measurable concentration (T_{last}) ranging from 38 to 361 days. Cellular kinetic parameters were not associated with C-CAR031 dose, except T_{max} , which decreased significantly at higher doses. No significant differences in cellular kinetic parameters were observed between responders and non-responders. In addition, patients with CRS of grade 2 or higher

exhibited higher peak C-CAR031 levels than those with CRS of grade less than 2 (median: 439,887 versus 244,872 copies per microgram gDNA, $P = 0.031$).

Twelve plasma cytokines were monitored during treatment. IL-6, IL-8 and IFN γ levels increased significantly and rapidly after CRS onset, peaking at a median of 4 days (Extended Data Fig. 5a–l). Levels of other cytokines, including IL-2, IL-4, IL-5, IL-10, interferon- α , tumour necrosis factor, IL-12P70, IL-1 β and IL-17 α , also increased, although to a lesser extent. Significantly higher peak levels of IFN γ (median: 466.2 versus 47.2 pg ml $^{-1}$, $P = 0.025$) and IL-2 (median: 8.0 versus 3.3 pg ml $^{-1}$, $P = 0.045$) were seen in patients with CRS grade 2 or higher compared with those with grade 1 or no CRS on day 4 postinfusion (Extended Data Fig. 5m,n).

Correlative analyses of ORR

We used logistic regression models to investigate associations of patient or C-CAR031 characteristics with tumour response (Supplementary Fig. 3). Variables for inclusion were selected on the basis of the literature, clinical relevance and statistical prescreening. Notably, absence of cirrhosis at baseline and IFN γ below median levels in the CAR T cell potency assay were significantly associated with improved ORR in univariate and multivariate analyses. Although infused C-CAR031 dose and overall GPC3 expression levels did not show any significant association with ORR in the univariate analysis, they emerged as significant predictors in the multivariate analysis, suggesting potential dose- and target-dependent effects when we accounted for other variables.

TME analysis

To explore C-CAR031 resistance mechanisms, we performed digital spatial profiler on 35 tumour samples from 20 patients before and after infusion (Fig. 3a). Compared with baseline, *GPC3* expression was downregulated in both PR and non-PR samples, whereas *TGFB1* levels were specifically elevated in non-PR samples (Fig. 3b), suggesting elimination of GPC3-positive tumour cells and feedback upregulation of TGF β . Differential gene expression analysis of tumour cells revealed that non-PR samples were enriched in IFN γ response, oxidative phosphorylation signatures and MYC activation, whereas PR samples showed upregulation of genes involved in antigen presentation, apoptosis and DNA repair (Fig. 3c). Gene ontology and pathway enrichment analyses highlighted immune evasion mechanisms linked to antigen presentation, extracellular matrix structure, cell adhesion and oxidative phosphorylation (Fig. 3d). Terminally exhausted T cell scores and *PDCD1* and *LAG3* expression levels in T cells were downregulated in PR samples (Fig. 3e,f), whereas C-C motif chemokine ligand 3 (*CCL3*) and *CCL5* showed increased expression in tumour cells of PR samples (Fig. 3c,g), suggesting that CCL3 and/or CCL5 may play a part in promoting antitumour immunity.

Xenium in situ technology was used to further profile the TME differences between PR and non-PR samples. We identified 15 cell types and found that only T cells and hepatic stellate cells showed significantly decreased proportions in non-PR samples (Fig. 3h,i and Extended Data Fig. 6a,b). Tumour-infiltrating T cell composition differed between the PR and non-PR groups, with non-PR samples showing lower proportions of effector T cells and higher proportions of exhausted T cells (Fig. 3j and Extended Data Fig. 6c–h). Notably, the expression of *CCR5*, a predominant receptor of CCL3/CCL5, was significantly higher in PR samples (Fig. 3k), suggesting a role of the CCL3/CCL5–CCR5 axis in tumour response to C-CAR031. *TGFB1* expression was higher in tumour cells in non-PR samples compared with PR samples (Fig. 3l), and TGF β signalling was more activated across most immune cell types in non-PR samples, driving distinct pathway alterations (Extended Data Fig. 7a–e). Furthermore, macrophages and neutrophils showed noticeable immunosuppressive features in non-PR samples (Extended Data Fig. 7a,b),

whereas a specific cellular neighbourhood (tumour C0), surrounded by abundant immune cells, was enriched in PR samples (Extended Data Fig. 7f–h). Generally, PR and non-PR samples exhibited distinct T cell infiltration and tumour cell aggregation patterns: T cells were abundantly distributed in the TME, with upregulated *CCR5* expression in PR samples, whereas tumour cells showed denser aggregation with *TGFB* overexpression in non-PR samples (Fig. 3m,n).

To confirm that the observed TME changes were direct effects of C-CAR031, we established CAR T cell refractory mouse models using GPC3-positive HCC cells. Refractory tumours were typically negative for GPC3 (with respect to both protein and mRNA levels) and had low levels of CCL3/CCL5 and high levels of TGF β (Fig. 4a–e). Another mouse model, using low-dose GPC3-specific CAR T cell infusion to mimic resistance, recapitulated GPC3 transcriptional inhibition (Supplementary Fig. 4). IFN γ substantially induced expression of CCL3 and CCL5 in HCC cells, and these chemokines showed potent ability to recruit CAR T cells (Fig. 4f,g). In vitro, excessive TGF β induced more exhausted T cells and fewer effector T cells in C-CAR031 (Fig. 4h). In vivo, TGF β -overexpressing HCC cells showed greater resistance to C-CAR031 compared with HCC cells without TGF β overexpression (Fig. 4i–l), suggesting that excessive levels of TGF β could override dnTGF β RII-mediated protection on CAR T cells (Fig. 4m). In addition, when CCL3 and CCL5 were knocked out in HCC cells, CAR T cells showed significantly weakened antitumour efficacy (Extended Data Fig. 8a–f), as well as inhibited infiltration and cytotoxicity, with more exhausted features and less IFN γ production (Extended Data Fig. 8g–l). The tumour cell origin of CCL3 and CCL5 was supported by analysis of public single-cell transcriptomics datasets, patient samples and cell-derived xenografts (Extended Data Fig. 8m–o). These findings indicate that downregulation of GPC3 in tumour cells and increased TGF β levels in the TME may contribute to C-CAR031 resistance, whereas the CCL3/CCL5-mediated secondary immune response may enhance antitumour efficacy (Fig. 4n).

Discussion

In this study, we report preclinical, clinical and translational outcomes for C-CAR031, a GPC3-specific dnTGF β RII-armoured CAR T cell product. C-CAR031 showed a manageable safety profile and promising antitumour activity in heavily pretreated patients with advanced HCC. Although CRS was reported in most patients, grade 3 CRS was seen only at DL4 in 2 of 11 patients. The three SAEs related to C-CAR031 reported were a grade 3 CRS and TLS, a grade 3 CRS-related interstitial pneumonitis and a grade 4 myelosuppression. In addition, all 161 blood samples from the 36 patients were negative for vesicular stomatitis virus-G protein sequence, suggesting the absence of replication-competent lentivirus. Insertion site analysis of the CAR gene also demonstrated no evidence of overrepresented dominant clones integrating into cancer-related genes.

Patients with advanced HCC have limited treatment options; this represents an unmet clinical need. The ORR of 44.4% seen with C-CAR031 is thus promising, particularly given that most patients had three or more previous therapies and extrahepatic lesions. By contrast, current guideline-recommended second-line therapies such as regorafenib, cabozantinib and ramucirumab achieve ORRs of only 4–11%^{24–26}. Deep responses were noted especially for patients at DL3 and DL4 in our study, with 4 and 6 patients, respectively, achieving 50% or more best tumour shrinkage. A median overall survival of 14.2 months was achieved with C-CAR031 treatment, indicating that its performance was consistent with that of first-line benchmarks (13.6 and 12.3 months for lenvatinib and sorafenib, respectively) and superior to that of standard second-line therapies (8.5–10.6 months) for advanced HCC^{24–28}. In addition, the median PFS was 4.2 months with C-CAR031 treatment, compared with 3.1 months for regorafenib, 5.2 months for cabozantinib and 2.8 months for ramucirumab^{24–26}. Notably, C-CAR031 responders demonstrated significantly longer PFS and overall survival compared

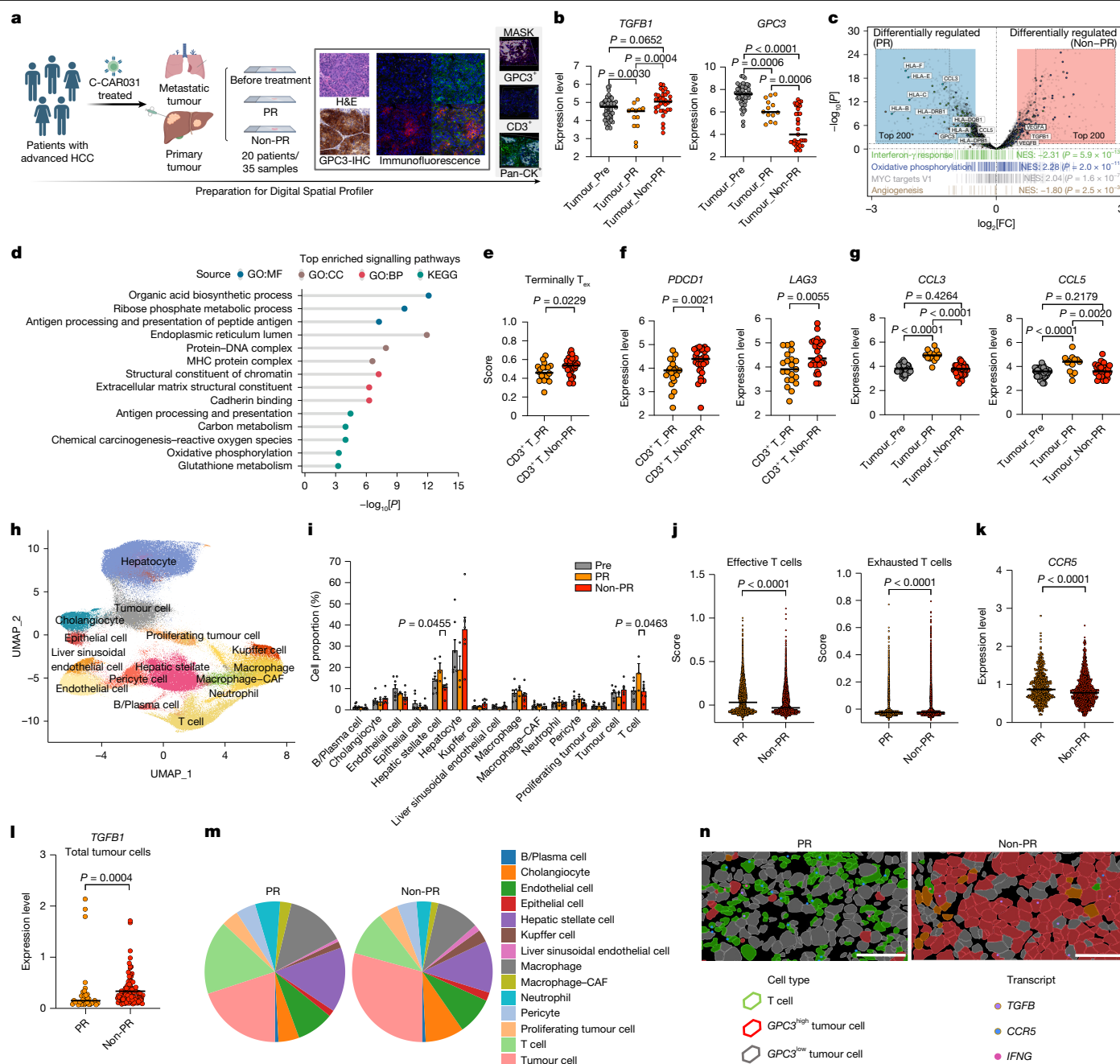


Fig. 3 | High-throughput analyses of TME following C-CAR031 infusion. **a**, Digital Spatial Profiler analysis study workflow. **b**, *TGFB1* and *GPC3* expression in tumour cells across prescreening (pre), PR, and stable or progressive disease (non-PR) samples. **c**, Volcano plot illustrating differentially expressed genes of tumour cells between PR and non-PR samples. **d**, Functional enrichment of tumour differentially expressed genes. **e, f**, Terminally exhausted state scores for CD3⁺ T cells (**e**), and *PDCD1* and *LAG3* expression in CD3⁺ T cells (**f**) for the two groups. **g**, *CCL3* and *CCL5* expression in tumour cells. For **b** and **g**, $n = 52$, 13 and 33 independent regions of interest for pre, PR and non-PR samples, respectively; for **e** and **f**, $n = 21$ and 28 independent regions of interest for PR and non-PR samples, respectively. **h**, Main cell types identified by Xenium Spatial Analysis. **i**, Comparison of proportions of cell types from **h** among pre ($n = 7$), PR ($n = 3$) and non-PR ($n = 7$) samples. **j–l**, Effective T cell scores, exhausted T cell scores ($n = 5,641$ and 11,264 cells) (**j**), *CCR5* expression in T cells ($n = 1,072$ and

2,088 cells) (**k**) and *TGFB1* expression in tumour cells ($n = 33$ and 113 cells) (**l**) in the PR and non-PR groups. **m**, Cellular compositions of 100 adjacent cells of each tumour cell. **n**, Subcellular spatial mapping of transcripts for three genes in the PR and non-PR groups. Results are representative of five independent comparisons. Scale bars, 50 μm . Data are mean $\pm$ s.e.m. in **i** and median in **b, e–g** and **j–l**. Statistical analyses used two-sided *t*-test with multiple testing correction using Benjamini–Hochberg false discovery rate (**c**), two-sided hypergeometric test with multiple testing correction using Benjamini–Hochberg false discovery rate (**d**), two-sided unpaired Student's *t*-test (**e**), two-sided Mann–Whitney U test (**f** and **j–l**) or one-way ANOVA with Tukey's honest significant difference test (**b, g** and **i**). CAF, carcinoma-associated fibroblast; FC, fold change; H&E, haematoxylin and eosin; NES, normalized enrichment score; T_{ex}, exhausted T cells; UMAP, uniform manifold approximation and projection. Workflow in **a** created in BioRender; Jin, G. <https://biorender.com/t90d471> (2026).

with non-responders, suggesting that the profound tumour reductions contributed to a meaningful survival advantage (Supplementary Fig. 5a,b). Furthermore, the pattern of progression in many patients was characterized by limited tumour size increase and mixed responses

(Supplementary Fig. 5c), explaining the observed dissociation between DOR/PFS and overall survival. Although cross-trial comparisons have been limited, C-CAR031 seems to offer a higher ORR and a more favourable safety profile than other GPC3-specific CAR T cells without TGF β

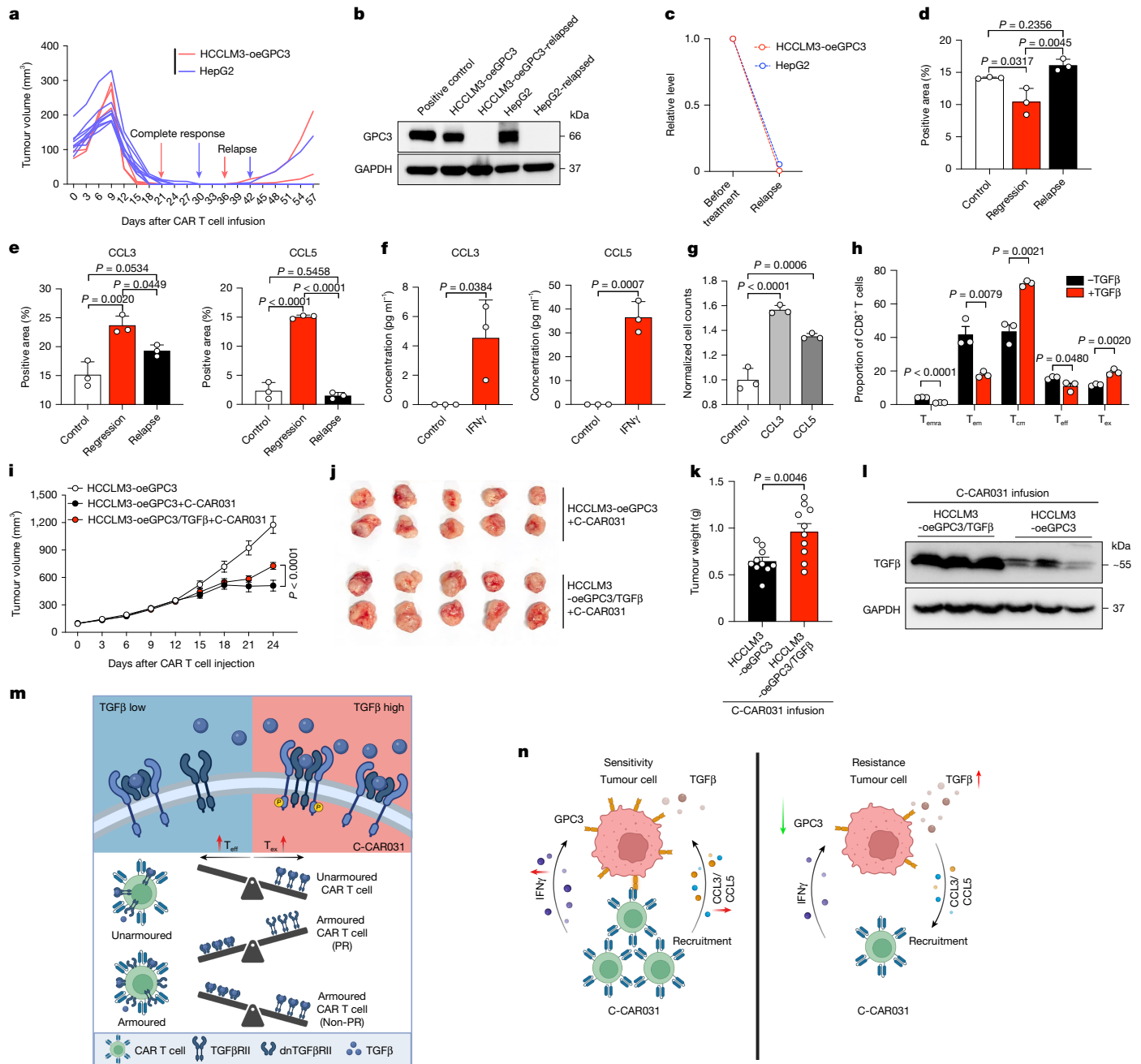


Fig. 4 | Reduced GPC3 and excessive TGFβ confer resistance to C-CAR031.

a, Establishment of CART cell-resistant mouse models using HepG2 ($n = 9$) and GPC3-overexpressing HCCLM3 (HCCLM3-oeGPC3, $n = 3$) cells. **b,c**, GPC3 protein (**b**) and mRNA (**c**) expression levels in untreated versus relapsed tumours. Results are representative of two independent experiments. **d,e**, Immunohistochemical analysis of TGFβ (**d**) and CCL3 and CCL5 (**e**) ($n = 3$ technical replicates) during tumour regression versus relapse. Results are representative of three independent experiments. **f**, CCL3 and CCL5 levels in HepG2 supernatants with and without IFNγ treatment (20 ng ml⁻¹, 36 h). $n = 3$ independent samples. **g**, CART cell migration towards CCL3 and CCL5 (100 ng ml⁻¹, 8 h) quantified using transwell assays. $n = 3$ independent assays. **h**, C-CAR031 CD8⁺ T cell subsets after coculture with tumour cells and TGFβ (20 ng ml⁻¹, 24 h). $n = 3$ independent samples. **i–k**, CART cell-resistant mouse model established using TGFβ-overexpressing HCCLM3 (HCCLM3-oeGPC3/TGFβ) cells. $n = 10$ and $n = 3$ mice for C-CAR031-treated and untreated groups,

respectively. Tumour growth curves are shown in **i**, tumour images are shown in **j** and tumour weights are shown in **k**. **l**, TGFβ expression in xenografts. Results are representative of two independent experiments. **m**, Hypothesis that excessive TGFβ inactivates C-CAR031 and induces tumour resistance. **n**, Schematic of tumour resistance mechanisms by GPC3 downregulation in tumour cells and excess TGFβ in the TME. Data are mean ± s.d. in **d–g**, and mean ± s.e.m. in **h**, **i** and **k**. One-way ANOVA with Tukey's honest significant difference test was used in **d** and **e**, one-way ANOVA with Dunnett's test in **g**, two-way ANOVA with Šidák's multiple comparisons test in **i** and two-sided unpaired Student's *t*-test in **f**, **h** and **k**. For gel source data, see Supplementary Fig. 1. T_{cm}, central memory T cells; T_{em}, effector memory T cells; T_{emra}, effector memory CD45RA⁺ T cells; T_{eff}, effector T cells. Diagrams in **m** and **n** created in BioRender; Jin, G. <https://biorender.com/o38hmg4> (**m**) and <https://biorender.com/ndk58by> (**n**) (2026).

blockade in patients with advanced HCC. For instance, an unarmoured GPC3-specific CART T cell product (CT017) achieved an ORR of 16.7% and a DCR of 50%; 50% of patients had grade 3 CRS². Another unarmoured

GPC3-specific CART T cell product (Y035) resulted in an ORR of 15.4% and a DCR of 30.4%, with 1 patient experiencing grade 5 CRS³. Furthermore, the peak copy number of C-CAR031 seemed to be substantially higher

than that of CT017 and Y035, although these numbers were not directly comparable owing to differences in assays^{2,3}. Thus, the presence of dnTGFβRII may be important for enhancing the expansion, antitumour activity and therapeutic index of C-CAR031. This was supported by our preclinical data, which demonstrated that dnTGFβRII on C-CAR031 could inhibit pSMAD2/3 in the presence of TGFβ, thereby blocking the immunosuppressive effects of TGFβ and leading to an enhanced antitumour response. Moreover, genes related to antigen presentation and chemokines were upregulated in responders, suggesting a secondary immune response following C-CAR031-mediated tumour lysis. In vivo experiments further demonstrated that tumour-derived CCL3/CCL5 facilitated CAR T cell-mediated tumour control. These findings indicate that in addition to directly protecting CAR T cells, dnTGFβRII can alleviate TGFβ-mediated suppression of secondary immune response. Collectively, these results provide a mechanistic rationale for combining CAR T cell therapy with immune checkpoint inhibitors. C-CAR031 showed promising antitumour activity and survival in advanced HCC, plausibly owing to its unique design with dnTGFβRII. However, excessive TGFβ could still impair the function of C-CAR031; future strategies such as incorporation of a TGFβ switch receptor that converts the immunosuppressive signal into a prosurvival and activation signal in CAR T cells may help to overcome this limitation.

We longitudinally monitored peripheral blood biomarkers to assess drug exposure and activity. C-CAR031 expansion was correlated with CRS of grade 2 or higher but not with clinical efficacy, infused cell dose or baseline GPC3 expression. The risk of CRS of grade 2 or higher showed a dose-dependent increase (33%, 44% and 64% at DL2, DL3 and DL4, respectively), with no other baseline variables, including tumour burden and GPC3 expression, showing significant associations with CRS risk in the multivariable analysis (Supplementary Fig. 6). We found no correlation between soluble GPC3 levels and efficacy, nor between changes in biopsied lesion size and GPC3 expression (Supplementary Fig. 7), suggesting the complexity of CAR T cell-mediated tumour killing. On-treatment AFP reduction predicted treatment response and improved PFS, and rebound of AFP levels reliably preceded radiographic progression (Supplementary Fig. 8). Notably, in the two patients with repeat infusions, we did not observe substantial CAR T cell re-expansion or the expected therapeutic effect; the reasons for this are unclear (Supplementary Fig. 9 and Supplementary Table 4). Nonetheless, given the small sample size, tumour heterogeneity and potential confounders, these correlative findings were exploratory. Further studies are needed to identify potential predictors of response.

Despite the high ORR of C-CAR031, more than 50% of patients failed to achieve PR. High-throughput analyses and experimental validation indicate several potential mechanisms that could contribute to tumour resistance to C-CAR031 (ref. 29). We identified GPC3 loss or downregulation as a potential mechanism of resistance to C-CAR031. Resistance may also arise from reduced dnTGFβRII expression in CAR T cells or increased TGFβ levels in the TME, which would imply a TGFβ concentration threshold for dnTGFβRII-mediated protection on CAR T cells. Another possibility is that decreased GPC3 levels and enhanced TGFβ levels may disrupt the feed-forward effect of CAR T cell cytotoxicity and recruitment through a potential IFNγ–CCL3/CCL5 axis. We therefore propose a model wherein excessive TGFβ in the TME drives CAR T cell resistance by overcoming dnTGFβRII protection, directly causing CAR T cell dysfunction and indirectly disrupting immune recruitment through suppression of the IFNγ–CCL3/CCL5 axis. Optimizing CAR T cell design or combining therapies on the basis of these findings may further improve the efficacy of C-CAR031.

The main limitations of the study were as follows. First, we used a single-centre, single-arm design with a small, predominantly HBV-infected Asian cohort; the safety and efficacy of C-CAR031 in a broader, global population of patients with HCC thus remain unknown. Second, GPC3 expression was assessed from single lesions, which may not have captured overall tumour GPC3 expression in the patients

owing to inter- and/or intratumour heterogeneity. Indeed, nine patients showed notable shrinkage of target lesions but enlargement of non-target lesions, indicating heterogeneous responses of these lesions to C-CAR031. In addition, some important factors that may distinguish responders and non-responders, such as GPC3 isoforms and GPC3 signalling dependence of tumour cells, remain unclear. Third, patients were not screened for TGFβ levels in the TME as an inclusion criterion; this could be used to facilitate patient selection in future studies of C-CAR031. The exploratory association findings also need to be confirmed in larger studies controlling for potential confounding factors, such as variations in previous therapies and use of non-standard experimental treatments. Notably, although a 6-week evaluation time frame is commonly used in HCC trials^{7,25,26,30}, this could lead to slight overestimation of the DCR through potential misclassification of cases involving very slow-growing tumours as ‘disease controlled’ in certain situations. Nevertheless, the significant drop in AFP levels observed in some patients who did not achieve PR imply possible biological activity of C-CAR031, even in the absence of clinically meaningful benefit. Finally, the overall survival analysis is immature at present; continuing follow-up is needed to provide more accurate estimates.

In conclusion, C-CAR031 showed a manageable safety profile with promising antitumour activity in heavily pretreated patients with advanced HCC. Given the potential resistance mechanisms against C-CAR031, studies using combinations with other therapies such as antiangiogenesis tyrosine kinase inhibitor(s) and/or immune checkpoint inhibitor(s) are planned to determine whether these agents may enhance efficacy. Use of customized probes to identify CAR T cells in the TME may further deepen our understanding of the mechanisms of resistance to C-CAR031.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-026-10786-z>.

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Methods

Methods for preclinical and translational experiments are presented in the Supplementary Information.

Clinical development and trial of C-CAR031

Study design. This open-label, phase I clinical trial was conducted at The First Affiliated Hospital, Zhejiang University School of Medicine (FAHZU) and was designed in two stages. The first involved a combination of standard accelerated titration and i3+3 dose escalation to establish the maximum tolerated dose and recommended phase 2 dose (RP2D). The second stage involved an expansion cohort of the selected RP2D.

Inclusion and exclusion criteria. Key inclusion criteria were: age 18–75 years; diagnosis of unresectable HCC with at least one previous systematic therapy; GPC3 positivity in tumour cells; measurable disease per RECIST v.1.1; Barcelona Clinic Liver Cancer stage B or C; Eastern Cooperative Oncology Group performance status of 0 or 1; adequate organ function, including liver function with Child–Pugh score of 6 or lower; being HBV-Ag negative or having HBV DNA less than 2,000 IU; and estimated survival of 12 weeks or longer. Main exclusion criteria included history of liver transplantation or previous treatment with cell therapy, with a tumour volume greater than 50% liver tissue, or portal vein trunk cancer thrombus or at least moderate ascites. Specifically, the screening criterion for GPC3 were: positive expression of GPC3 in at least 20% of tumour cells in tumour tissues (either cytoplasmic or membranous), with at least 5% positive membranous staining. Patients with any central nervous system metastases or neurological conditions including hepatic encephalopathy, epilepsy and cerebrovascular accident were also excluded.

Study procedures. After signing the informed consent form, eligible participants underwent peripheral blood mononuclear cell collection for manufacture of C-CAR031. Participants had leukapheresis on day –28, followed by baseline assessment on day –7, and then proceeded to lymphodepletion conditioning chemotherapy on days –5 to –3 with the following regimen: fludarabine (25 mg m^{–2} per day) and cyclophosphamide (500 mg m^{–2} per day), by intravenous injection, once per day, for 3 consecutive days (bridging therapy was allowed after leukapheresis if necessary). After 2 days (day 0), participants proceeded to C-CAR031 infusion. C-CAR031 was administered according to an accelerated titration and i3+3 dose escalation design using four dose levels: 0.75 × 10⁶ (DL1), 1.5 × 10⁶ (DL2), 2.5 × 10⁶ (DL3) and 4.0 × 10⁶ (DL4) CAR T cells kg^{–1} (Extended Data Fig. 3c). Dose expansion was performed at the two highest dose levels. Patients were monitored until death or withdrawal of consent; follow-up of 15 years was planned for all patients.

End points. The primary end point of the dose escalation phase was safety and tolerability, as assessed by monitoring of adverse events and SAEs, clinical laboratory measurements and physical examinations. Adverse events were categorized according to severity (National Cancer Institute Common Terminology Criteria for Adverse Events v.5.0) and the relationship with the investigated product. CRS, ICANS and IEC-HS were evaluated according to the American Society for Transplantation and Cellular Therapy guidelines. Secondary end points included tumour response, as evaluated using RECIST v.1.1 and modified RECIST, as well as ORR, DCR, DOR, PFS and overall survival. Exploratory end points included but were not limited to pharmacokinetics and pharmacodynamics, cytokine levels, GPC3 levels, and biomarkers of efficacy and CRS in peripheral blood and tumour samples at baseline and during response and disease progression.

Determination of GPC3 expression

Fresh or archived tumour formalin-fixed, paraffin-embedded specimens were tested for GPC3 expression by immunohistochemistry at

a central laboratory (Guangzhou KingMed Centre for Clinical Laboratory, Guangzhou, Guangdong, China) using glypican-3 (1G12) Mouse Monoclonal Antibody (catalogue no. 05973864001, Roche Diagnostics) on the Ventana Benchmark Ultra platform. Overall and membrane expression of GPC3 was reported in terms of percentages of cells and corresponding staining intensity. GPC3 positivity was defined as a staining intensity greater than 1. *H*-scores were calculated according to the following formula: $H\text{-score} = \sum i \times p_i$, where *i* represents the grade of staining intensity (typically 0–3, with 0 indicating no staining, 1 weak staining, 2 moderate staining and 3 strong staining), and *p_i* represents the percentage of cells with staining intensity *i*.

Manufacture and infusion of C-CAR031

C-CAR031 was manufactured with a third-generation lentiviral vector, a self-inactivated vector with deletion of the *tat* gene, in a serum-free and semiautomated and functionally closed system with the ClinMACS Prodigy system (Miltenyi Biotec). In detail, CD4⁺ and CD8⁺ T cells from patients were stimulated using Dynabeads CD3/CD28 (Invitrogen) at a 1:1 bead-to-cell ratio. Approximately 18–26 h after stimulation, T cells were transduced with lentiviral vectors encoding GPC3-targeted CAR and subsequently expanded in medium containing IL-2 (PeproTech). At the end of the production run, the Dynabeads were removed with a magnetic bead concentrator. CAR T cells were collected, washed and concentrated. Cryopreservation medium was then added, and the resulting cell suspensions were aseptically transferred into cryopreservation bags at the designated filling volume. After filling, the bags were frozen to –80 °C to –90 °C in a controlled-rate freezer. Once frozen, the cryopreserved bags were transferred to a liquid nitrogen storage tank maintained at a temperature of –135 °C or lower for long-term preservation. The CAR T cells were tested for viability before infusion.

Quality control of C-CAR031 before dosing

The median frequency of CAR-expressing T cells in infusion products was 57% (range: 26–82%; *n* = 36), with a median vector copy number per transduction cell of 6.0 (range: 1.9–11.0; *n* = 36). C-CAR031 products showed *in vitro* cytotoxic activity in all batches before dosing. The median value for AD293-GPC3 tumour cell killing by C-CAR031 (effector cell/target cell ratio = 6:1) was 57.1% (range: 25.1–90.0%; *n* = 33) at 6 h, and no association was observed between killing activity and clinical response (*n* = 31) (Supplementary Fig. 10). Evaluation of IFN γ production by the C-CAR031 cells showed a median value of 0.0161 pg per transduction cell (range: 0.0029–0.0410 pg per transduction cell; *n* = 36).

Assessment of cytokines in patients

Peripheral blood samples were collected on days 0, 1, 4, 7, 10, 14, 21 and 28 following C-CAR031 infusion and on the day of tumour response evaluation per the study protocol. Cytokines were detected by multiple microsphere flow immunofluorescence using a DxFLEx flow cytometer (Beckman Coulter) according to the manufacturer's instructions. Plasma cytokines were detected with a Cytometric Bead Array cytokine kit (Jiangxi Cellgene Biotechnology).

Detection of blood transgene levels by quantitative PCR

gDNA was extracted from patients' peripheral blood samples using a QIAamp DNA Blood Mini kit (Qiagen) per the manufacturer's instruction. Primers and TaqMan probes specific to the C-CAR031 CAR transgene sequence were labelled with a fluorescent reporter dye (FAM) at the 5' end and a quencher dye (TAMRA) at the 3' end. TaqMan copy number reference assay RNase P (Thermo Fisher Scientific) was used for normalization. The negative control was gDNA isolated from peripheral blood mononuclear cells of healthy donors, and the positive control was gDNA isolated from C-CAR031 cells (provided by the Production Department of AbelZeta Pharma). Quantitative PCR was performed on a Quant Studio Dx System (Thermo Fisher Scientific), with the CAR

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gene and reference gene thresholds set to 0.04 and 'auto', respectively. The copy number of CAR genes contained in 1 µg gDNA was calculated according to the detection protocol (lower limit of quantification = 200 copies per µg gDNA).

Correlative analyses

Variables for correlative analyses were selected on the basis of literature review, clinical relevance and statistical prescreening. For logistic regression models, assessments of potential collinearity among variables were performed, and stepwise selection methods were used to refine the models. Key selected variables included age, sex, cirrhosis status and cytokine levels (for instance, IFN γ and IL-2), given their relevance to HCC prognosis and CRS pathogenesis. CAR T cell infusion dose and GPC3 expression were included as treatment-specific and target-specific variables. The final model was intended to prioritize clinically relevant variables while maintaining model simplicity. Owing to the small sample size and exploratory nature of the study, no adjustments for multiplicity were performed, and results should be interpreted with caution.

Statistical analysis

An efficacy analysis was conducted on the full analysis set, defined as all participants who underwent leukapheresis; safety analysis was performed for all participants who received any dose of C-CAR031 infusion; and pharmacokinetic analysis was performed on the pharmacokinetic parameter set, which included all patients who received C-CAR031 infusion and had valid data for at least one pharmacokinetic parameter after infusion.

Descriptive statistics were used: continuous variables were reported as median (range), mean (s.d.) or mean (s.e.m.) as indicated; categorical variables were reported as counts (percentages). The 95% confidence intervals for ORR and DCR were estimated using the Clopper–Pearson method. DOR, PFS and overall survival were analysed using the standard Kaplan–Meier method, and median follow-up times for DOR, PFS and overall survival were based on the reverse Kaplan–Meier method. Paired or unpaired *t*-tests, Mann–Whitney U tests, or two-way or one-way analyses of variance (followed by Tukey's honest significant difference test, Dunnett's test, or Šidák's multiple comparisons test) were used for group comparison of continuous variables, as appropriate. *P* < 0.05 was considered to indicate statistical significance. Pharmacokinetic parameters were calculated with Phoenix WinNonlin (v.8.3 or later; Certara), and other analyses were performed with SAS (v.9.4 or later; SAS Institute) or GraphPad Prism (v.10.5.0).

Ethics statement

This study was approved by the Ethics Committee at FAHZU (approval no. 2021-86) and was conducted in accordance with the principles of the Declaration of Helsinki and the International Council for Harmonisation Guidelines for Good Clinical Practice (registration no. NCT05155189). Written informed consent was obtained from all participants. The efficacy and CRS biomarker and high-throughput studies were approved by the Ethics Committee at FAHZU (approval nos. 2024-1206 and 2025-0548). Patient confidentiality was maintained throughout the study, and all procedures were performed with appropriate ethical considerations. The animal studies were approved by the Animal Care and Medical Ethics Committee at FAHZU (approval no. 2025-094) and AstraZeneca's Institutional Animal Care and Use Committee. All animal experiments were conducted in accordance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal

Care International. All study protocols were reviewed and approved in advance by the relevant Institutional Animal Care and Use Committee.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The clinical study protocol and statistical analysis plan are available in the Supplementary Information. Deidentified individual patient data have been deposited in Science Data Bank (<https://doi.org/10.57760/sciencedb.39391>) under controlled access. Researchers wishing to access these data should clearly state their research purpose. The principal investigators will review requests to assess the risk of patient reidentification and scientific values and will respond within 2 months. The Digital Spatial Profiler data are available from the Genome Sequence Archive at HRA010444, and the Xenium data are available from the Gene Expression Omnibus at GSE332850. Source data are provided with this paper.

Acknowledgements We thank Applied Protein Technology (Shanghai, China) and Cosmos Wisdom Biotech (Hangzhou, Zhejiang, China) for technical support with Digital Spatial Profiler and Xenium analyses, respectively; and J. Cui from China Clinical Analytics, Caidya, for professional suggestions and support from a biostatistical perspective throughout this research. AstraZeneca designed the anti-GPC3 CAR single-chain variable fragment and dnTGFR β II α armour molecules, and Shanghai AbelZeta contributed to C-CAR031 product engineering and manufacturing. Both sponsors contributed to assay development and translational research, and provided scientific and statistical input into study design, analysis, data interpretation and reporting.

Author contributions Q.Z., Q.F., T.L., Y.S., W.C., G.J., Y.Z., J.W., C.C., H.W., X. Xu, K.S., X.B. and X. Xue were responsible for scientific input into the study design, set-up of assays to measure key biomarkers, patient recruitment, execution of the clinical trial, study oversight, patient care and safety, clinical data analysis and report writing. E.T., M.L.G.T., G.M., N.C., N.D. and M.C. were responsible for leading and executing preclinical development, design of the lentiviral manufacturing strategy, design of the CAR molecule, execution and analysis of experiments, and translational data analysis. J.S., A.B. and B.F. provided scientific input into study design, conduct of the study, and analysis and reporting. As a statistician, Y. Yang contributed to the study design, statistical analysis plan, data interpretation and exploratory analyses. Q.Z., W.C., G.J., D.C., Y. Hong, J.H., Q.J., L.S., Fei Wang and Y. Yao were responsible for the basic and translational research work, including translational study design, in vitro assay development, clinical pharmacokinetics and pharmacodynamics, translational study analysis, and the CAR T cell process development. Y. Hao, M.H., Fuzhe Wang and A.Z. (project lead) wrote the synopsis and supervised the study, including medical monitoring and safety reporting, analysis, interpretation and report writing. Q.Z., Q.F., W.C. and Q.J. wrote the manuscript. All authors read and approved the final draft of the paper.

Funding The clinical trial was funded by Shanghai AbelZeta and AstraZeneca. This work was also supported by the Noncommunicable Chronic Diseases-National Science and Technology Major Project (grant no. 2024ZD0525100/2024ZD0525101 to Q.Z.), the National Natural Science Foundation of China (grant nos. 82188102 to T.L., 92359304 to Q.Z., 32321002 to Q.Z. and 82473461 to Q.Z.), the National Key Research and Development Program of China (grant no. 2020YFA0804300/2020YFA0804301 to Q.Z.) and the K.C. Wong Education Foundation.

Competing interests D.C., Y. Hao, Y. Hong, M.H., Q.J., L.S., Fei Wang, Fuzhe Wang and A.Z. are employees of Shanghai AbelZeta, Shanghai, China. Y. Yao and J.H. are employees of AbelZeta Pharma, Gaithersburg, MD, USA. A.B., M.L.G.T. and J.S. are employees of AstraZeneca, Cambridge, UK. N.D., B.F., N.C., G.M., E.T., Y. Yang and M.C. are employees of AstraZeneca, Gaithersburg, MD, USA. J.S. and M.C. hold shares in AstraZeneca. G.M. and M.L.G.T. are listed as inventors on a patent (PCT/EP2021/060622) filed by AstraZeneca AB, which pertains to compositions and methods for treating cancer using armoured CAR T cells. The other authors declare no competing interests.

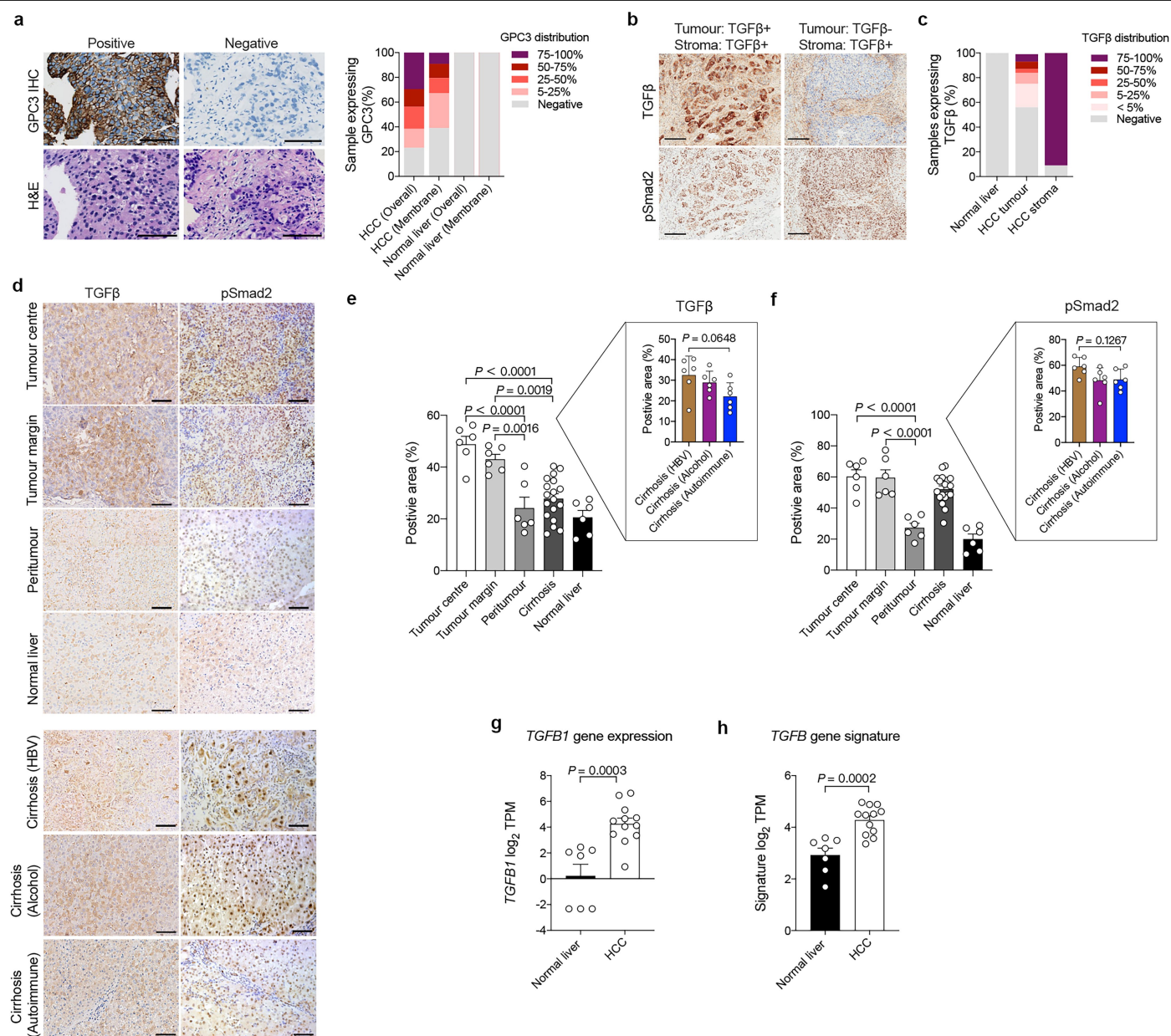
Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-026-10786-z>.

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Peer review information Nature thanks Tim Greden and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

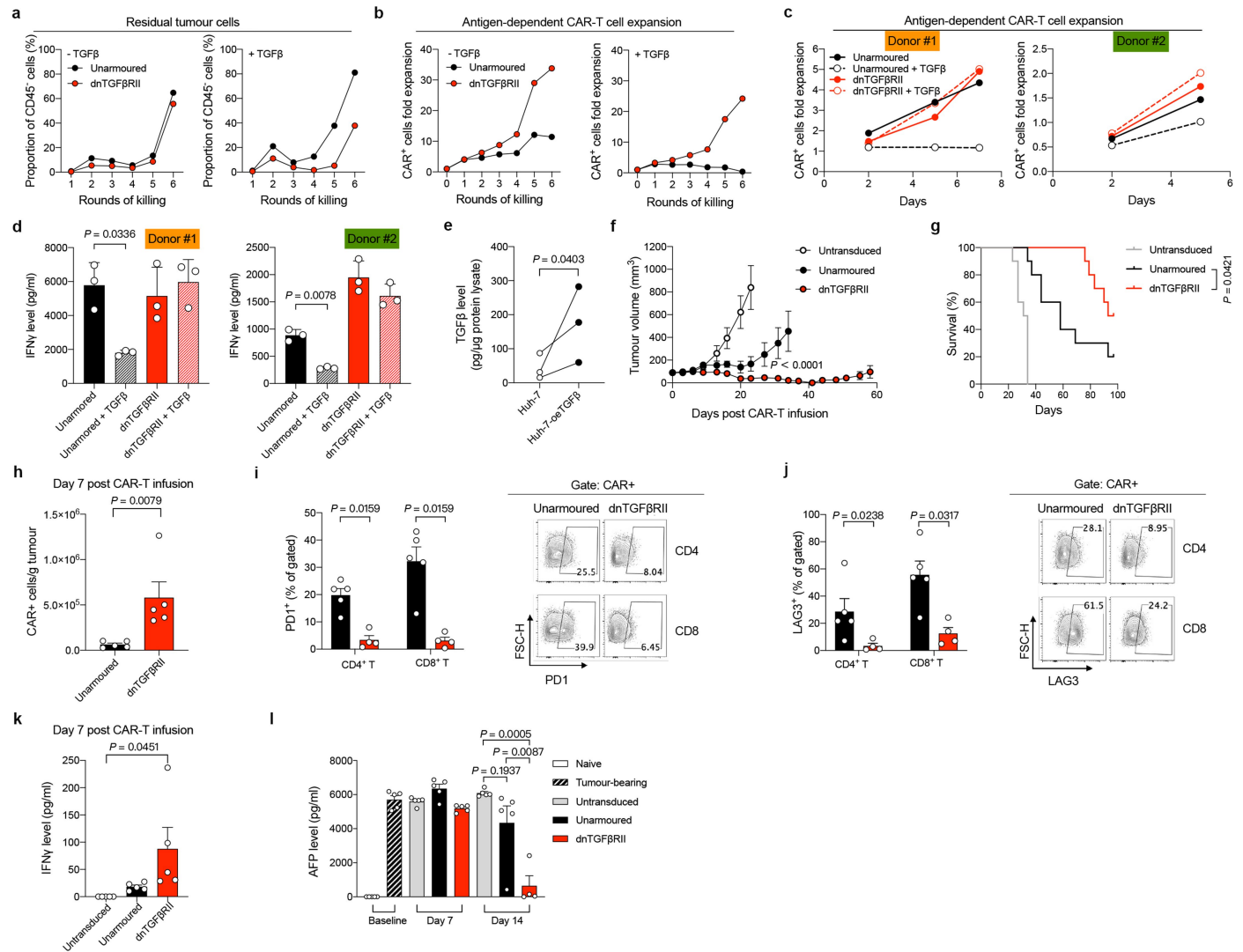
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Extended Data Fig. 1 | GPC3 and TGFβ expression in hepatocellular carcinomas and liver tissues. **a**, Representative IHC images showing positive and negative expression of GPC3 in HCC samples using the antibodies for patient screening in this trial. Overall expression and membrane expression of GPC3 were calculated. $n = 156$ for HCC, and $n = 10$ for normal liver.

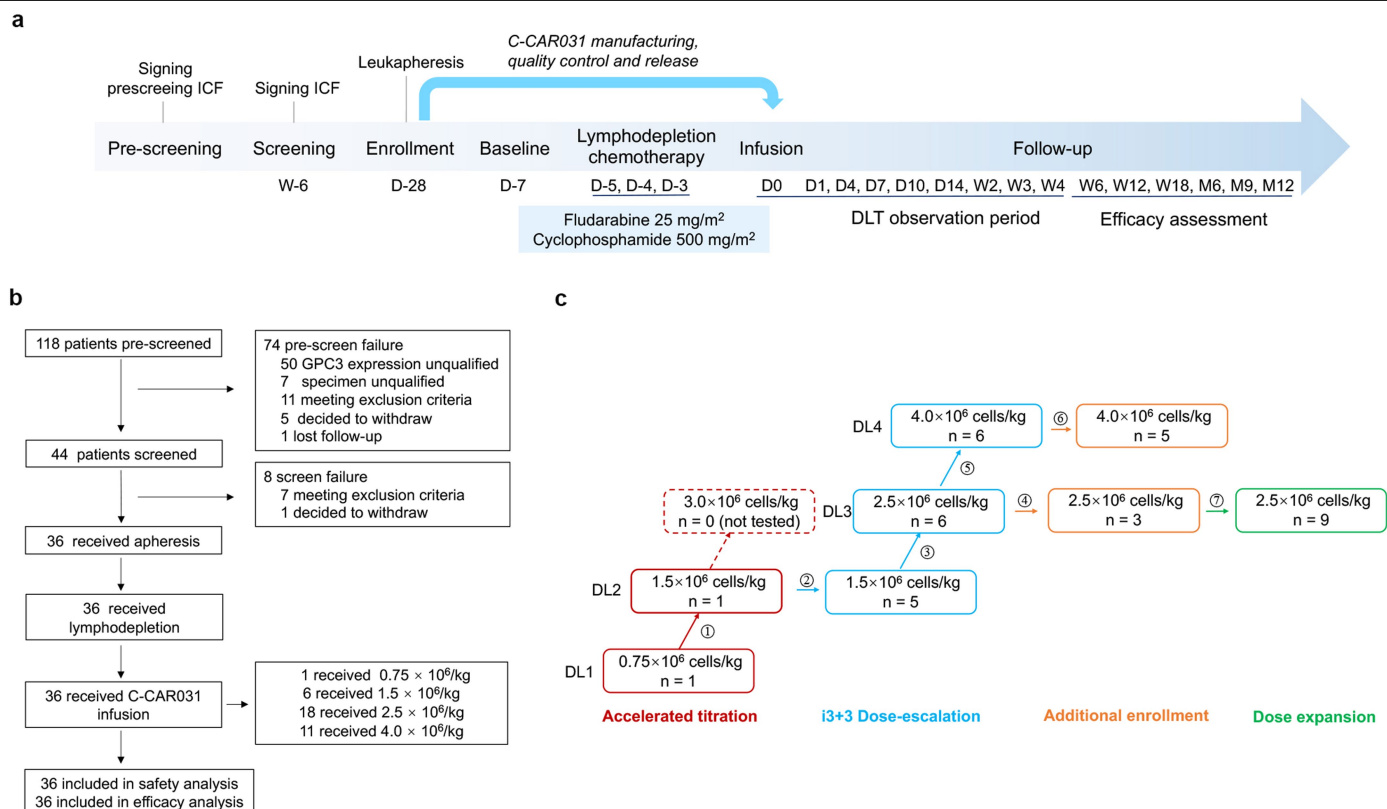
b, Representative IHC images of TGFβ and pSmad2 showing distinct staining patterns in tumour versus stromal compartments. A representative case of 32 HCC tumours is shown. **c**, TGFβ expression levels assessed by IHC on HCC tumour and stroma, and normal liver sections. $n = 5$ for normal liver, $n = 32$ for tumour and stroma. **d-f**, Representative IHC images and statistical results of TGFβ and pSmad2 in tumour centre, tumour margin, peritumour tissue,

normal liver, and cirrhotic tissues of different aetiology (e.g., HBV, alcohol, and autoimmune diseases). $n = 6$ samples per group. **g,h**, RNA-seq analysis showing (**g**) TGFβ gene expression and (**h**) TGFβ gene signature in normal liver and HCC samples. $n = 7$ and 12 independent samples for normal liver and HCC, respectively. Scale bars, 100 μm (**a,d**) and 200 μm (**b**). Data are mean $\pm$ s.e.m for **e-h**. One-way ANOVA with Tukey's honest significant difference test for **e,f**; two-sided unpaired Student's *t*-test for **g,h**. GPC3, glypican-3; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IHC, immunohistochemistry; pSmad2, phosphorylated Smad2; TGFβ, transforming growth factor β; TPM, transcripts per million.



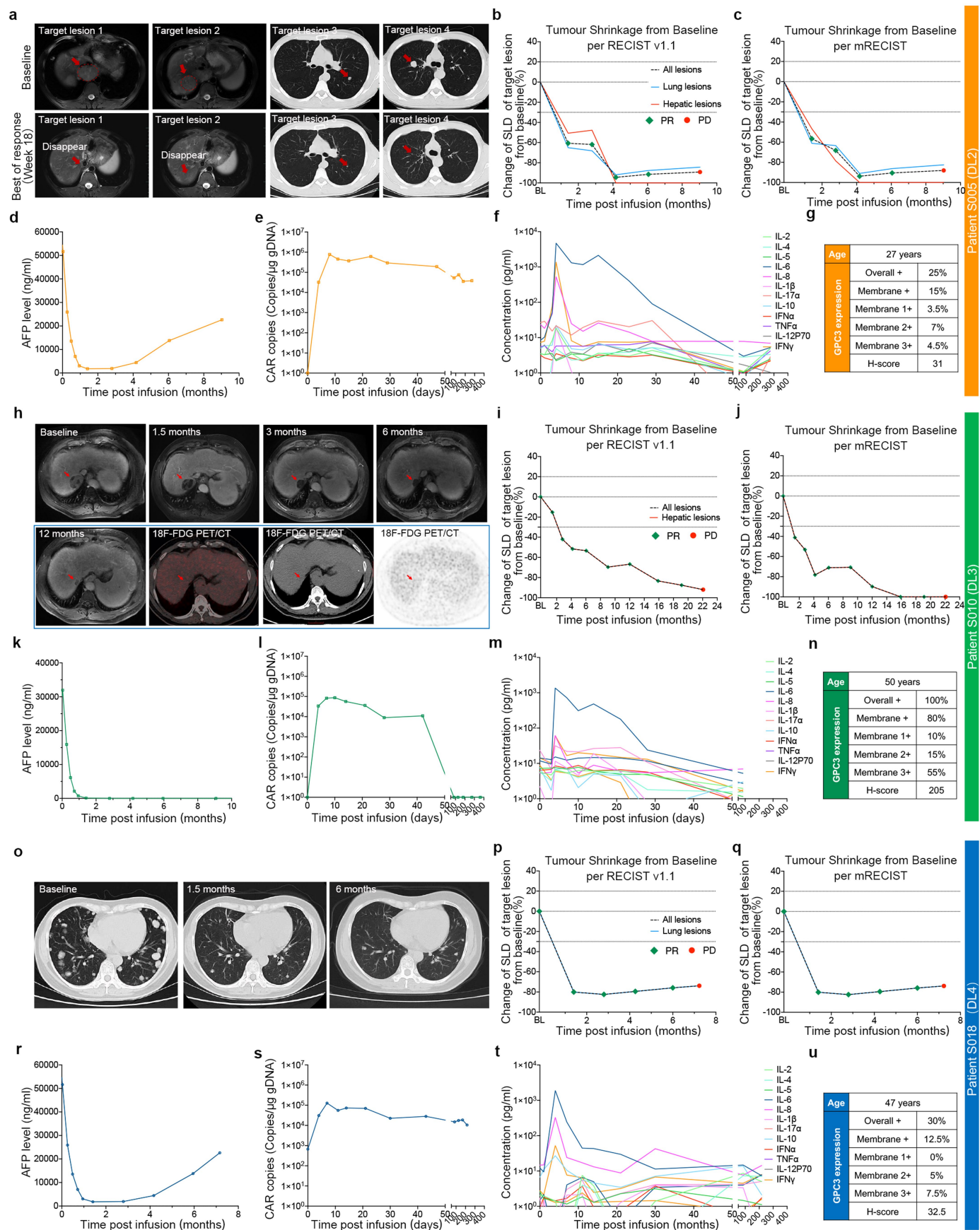
Extended Data Fig. 2 | Functional characteristics of dnTGFβRII-armoured CAR-T cells. **a, b**, CAR-T cells were repetitively incubated with PLC/PRF/5 cells with or without TGFβ (10 ng/ml). One of two independent experiments is shown. **(a)** CD45⁺ tumour cells were quantified by flow cytometry 3-4 days after each killing round. **(b)** CAR-T expansion was determined as fold increase of CAR⁺ cells over initial seeding. **c, d**, CAR-T cells were cultured for the indicated time on plates coated with recombinant human GPC3 (10 μg/ml), with or without TGFβ (10 ng/ml). **(c)** CAR-T expansion was determined as in **b**. **(d)** Supernatant IFNγ levels were evaluated at day 7 (Donor #1) or day 5 (Donor #2). **e**, TGFβ levels were evaluated in the lysate of wild-type Huh-7 cells and TGFβ-overexpressing Huh-7 (Huh-7-oeTGFβ) cells by ELISA. *n* = 3 independent assays. **f, g**, Huh-7-oeTGFβ tumour-bearing mice received 7 × 10⁶ untransduced T or CAR-T cells. *n* = 10 mice. **h**, CAR⁺ cells in tumours were quantified by flow cytometry 7 days postinfusion. *n* = 5 mice. **i, j**, PD1 and LAG3 expression was evaluated by flow cytometry in the

tumours of mice treated with unarmoured or dnTGFβRII-armoured CAR-T cells 14 days postinfusion. Unarmoured: *n* = 5 mice; armoured: *n* = 4 mice (one was excluded for not receiving the intended CAR-T dose). A representative dot plot is shown. **k**, Serum IFNγ levels were evaluated by Meso Scale Discovery assays 7 days postinfusion (*n* = 5). **l**, Serum AFP levels were evaluated by ELISA before, 7 days, and 14 days postinfusion. *n* = 5 mice (an infusion error existed for one mouse on day 14). Data are mean ± s.d. for **d**, and mean ± s.e.m. for **f, h-l**. Two-sided unpaired Student's *t*-test for **d**; one-sided ratio paired *t*-test for **e**; two-way ANOVA with Šidák's multiple comparisons test for **f**; Kaplan-Meier method with log-rank test for **g**; one-way ANOVA with Tukey's honest significant difference test for **k, l**; two-sided Mann-Whitney U test for **h-j**. AFP, alpha-fetoprotein; ELISA, enzyme-linked immunosorbent assay; LAG3, lymphocyte-activation gene 3; PD1, programmed cell death protein 1.



Extended Data Fig. 3 | Study procedure and patient inclusion. a, Study procedures. **b**, Flow chart showing patient disposition. A total of 74 patients were screen failures, primarily due to unqualified GPC3 expression. **c**, Trial flow diagram. One patient each in the DL2 and DL3 groups received a repeated infusion at the same dose. (1) The safety review committee (SRC) decided to escalate the dose to 1.5×10^6 CAR-T cells/kg. (2) Shift to i3 + 3, and an additional 5 patients were enrolled. (3) The SRC decided to escalate dose to 2.5×10^6 CAR-T

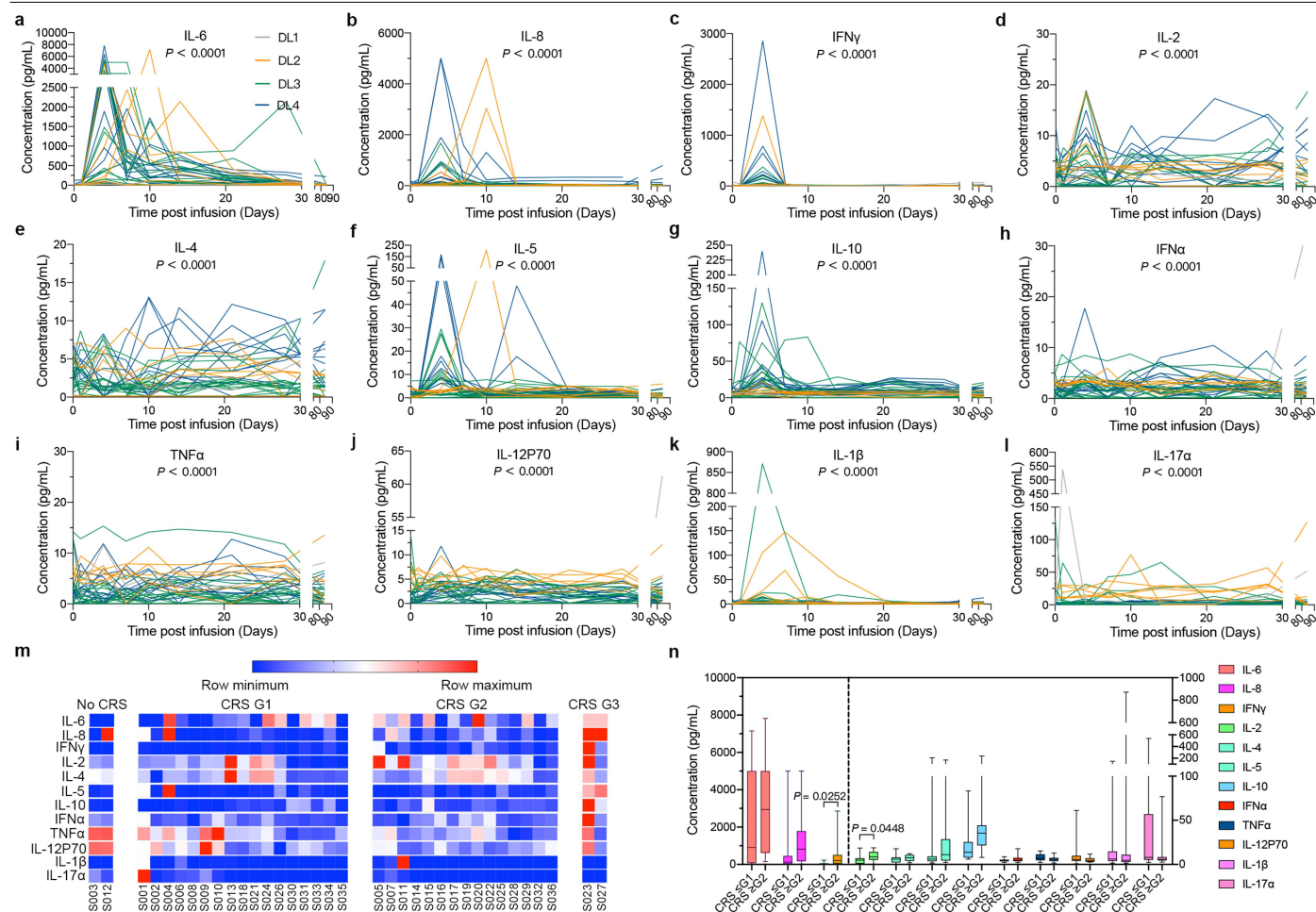
cells/kg rather than 3.0×10^6 CAR-T cells/kg. (4) According to the study protocol, in dose levels that showed potential anti-tumour efficacy, another 3 additional patients could be included. (5) The SRC decided to further escalate dose to 4.0×10^6 CAR-T cells/kg. (6) The SRC decided to recruit an additional 5 patients at DL4. (7) The SRC decided to have dose expansion at DL3 with an additional 9 patients. DL, dose level; DLT, dose-limiting toxicity; ICF, informed consent form.



Extended Data Fig. 4 | See next page for caption.

Extended Data Fig. 4 | Clinical response and cellular expansion in three representative patients. a-g, Patient S005 (DL2) with three lines of prior treatment, an example of early response. **a**, Radiological images of the patient. The sum of longest diameters (SLD) of targeted lesions at baseline was 121.7 mm. The intra-hepatic lesions and metastatic lung lesions shrank substantially. Red dash circle shows hepatic lesions. **b,c**, Change in SLD of targeted lesions evaluated per **(b)** RECIST v.1.1 and **(c)** mRECIST. **d**, Change in serum AFP levels. **e**, Expansion levels of C-CAR031 in peripheral blood. **f**, Change in twelve cytokine levels in peripheral blood. **g**, Summary of tumour GPC3 expression during screening. **h-n**, Patient S010 (DL3) with two lines of prior treatment, an example of durable response. **h**, Abdominal MRI and PET-CT images of the patient and SLD of targeted lesion at baseline was 52.3 mm. The hepatic lesions shrank substantially. **i,j**, Change in SLD of targeted lesion evaluated per **(i)** RECIST v.1.1

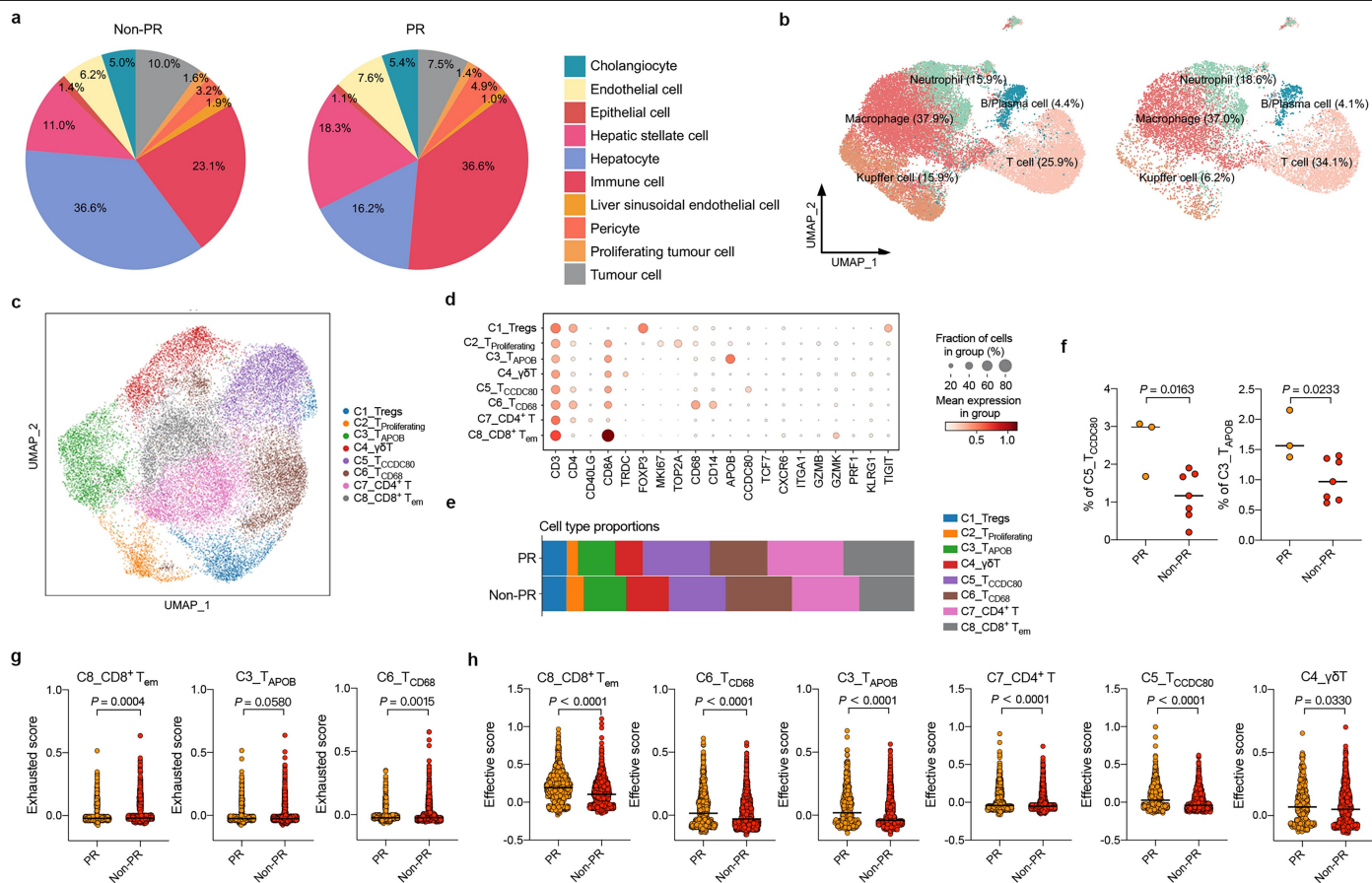
and **(j)** mRECIST. **k**, Change in serum AFP levels. **l**, Expansion levels of C-CAR031 in peripheral blood. **m**, Change in twelve cytokine levels in peripheral blood. **n**, Summary of tumour GPC3 expression during screening. **o-u**, Patient S018 (DL4) with six lines of prior treatment, an example of resolution of extensive metastases. **o**, Chest CT images of the patient and SLD of targeted lesions at baseline was 33.6 mm. The metastatic lung lesions shrank substantially. **p,q**, Change in SLD of targeted lesions evaluated per **(p)** RECIST v.1.1 and **(q)** mRECIST. **r**, Change in serum AFP levels. **s**, Expansion levels of C-CAR031 in peripheral blood. **t**, Change in twelve cytokine levels in peripheral blood. **u**, Summary of tumour GPC3 expression during screening. AFP, alpha-fetoprotein; CT, computed tomography; DL, dose level; MRI, magnetic resonance imaging; PET-CT, positron emission tomography-computed tomography; RECIST, Response Evaluation Criteria in Solid Tumours.



Extended Data Fig. 5 | Cytokines dynamic analysis post C-CAR031 infusion.

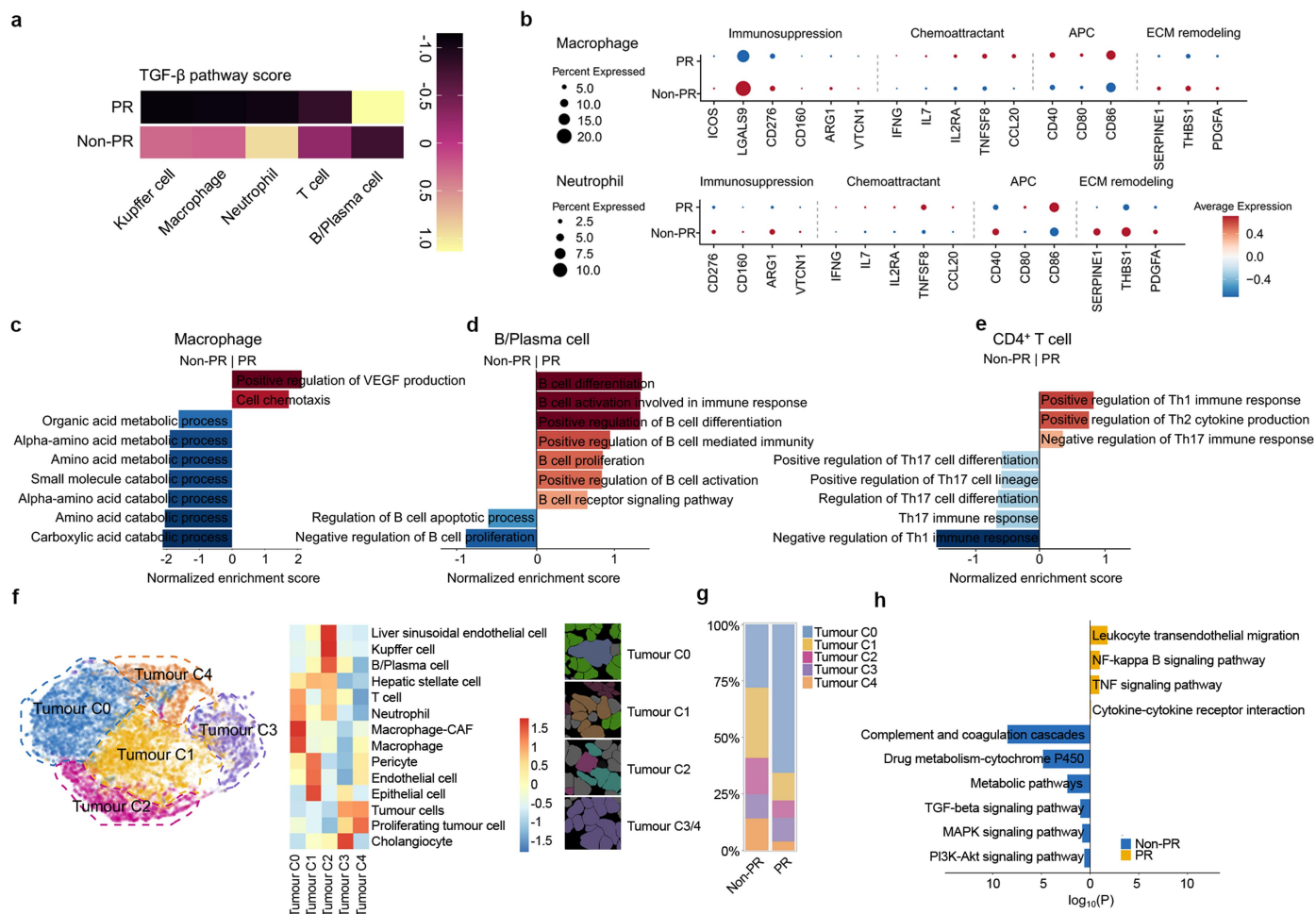
a-l, The serum concentration of twelve cytokines as indicated at different time points post C-CAR031 infusion were evaluated. Peak and baseline levels of each cytokine were compared; two-sided Wilcoxon matched-paired signed rank test. **m**, Heatmaps (grouped by individual patients and CRS grade) showing the peak levels of cytokine production post C-CAR031 infusion. **n**, Boxplots showing

differences between groups of different CRS grades. CRS $\leq$ G1, $n = 19$; CRS $\geq$ G2, $n = 17$. Box plots indicate median and the range of the central 50% of the data. Whiskers extend from each box to show the range of the remaining data, with dots placed past the line edges to indicate outliers; two-sided unpaired Student's t -test was used on natural logarithm-transformed values. IFN γ , interleukin; IL, interleukin; TNF α , tumour necrosis factor- α .



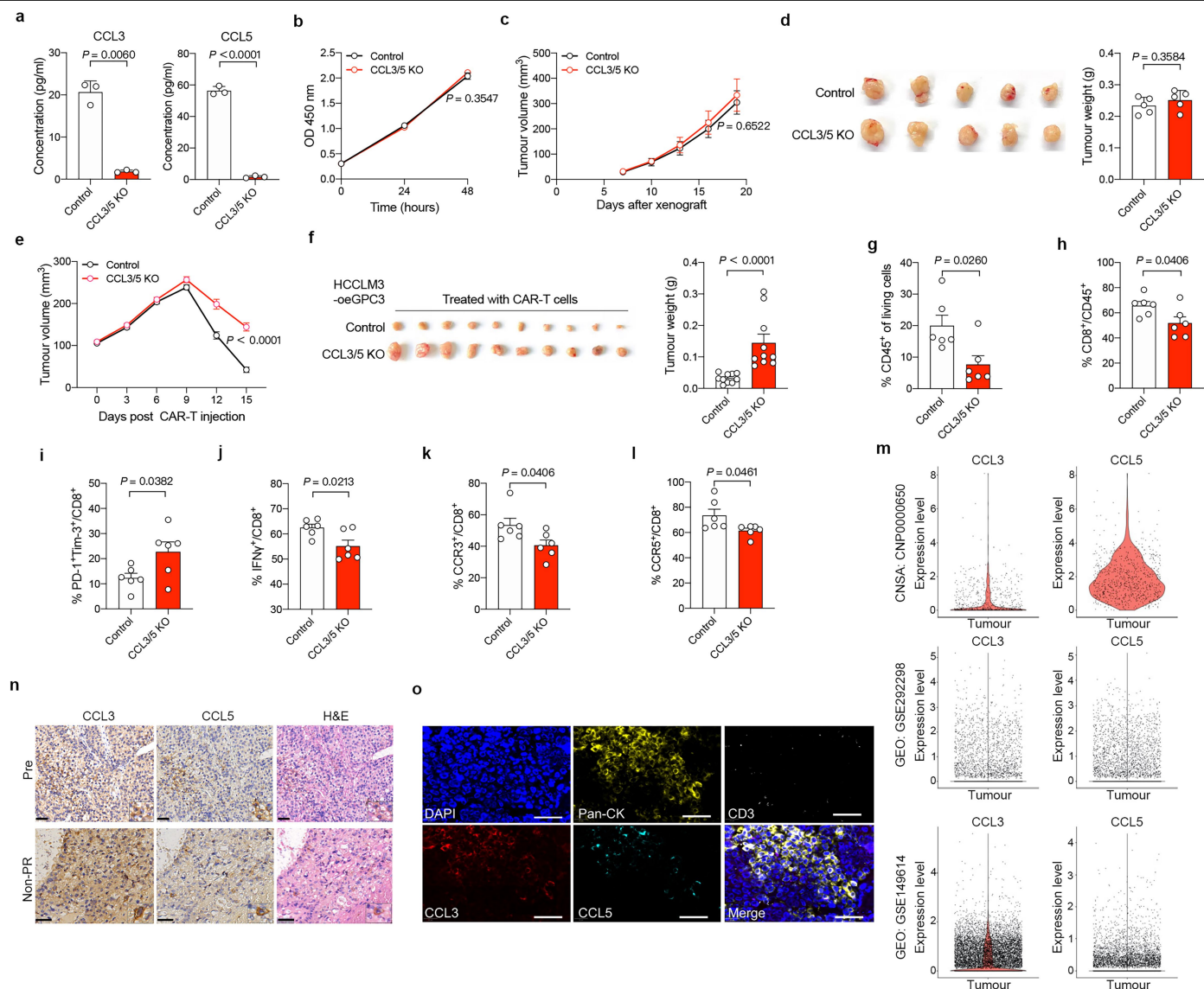
Extended Data Fig. 6 | Cell compositions and different types of tumour-infiltrating T cells in PR samples and non-PR samples. a, Pie chart representative of the cell compositions in PR and non-PR groups. **b**, UMAP visualization showing the proportions of different immune cells within the CD45⁺ compartment. **c**, UMAP plot depicting fine-grained clusters of T cells. **d**, Expression patterns of canonical marker genes for each identified cluster of T cells. **e**, Stacked bar plot showing the distribution and relative abundance of identified clusters of T cells. **f**, The proportions of C5_T_{CCDC80} and C3_T_{APOB}

among all non-tumour cells are compared between PR and Non-PR groups. **g,h**, Comparison of exhausted and effective signature scores between PR and Non-PR groups across various clusters of T cells. Median values are shown for **f-h**, and T cell clusters which are not presented had no significant difference between PR and Non-PR groups. Two-sided unpaired Student's *t*-test for **f**; two-sided Mann-Whitney U test for **g,h**. PR, partial response; UMAP, uniform manifold approximation and projection.



Extended Data Fig. 7 | TGF β signalling analysis in tumours from responders and non-responders. **a, TGF β signalling pathway scores derived from the MSigDB Hallmark gene set are visualised. **b**, Dot plots showing canonical marker gene expression in macrophage and neutrophil subsets between PR and Non-PR groups. **c-e**, Pathways enriched in macrophages, B/plasma cells, and CD4⁺ T cells from PR and Non-PR patients. **f**, Clustering analysis was conducted using the 100 nearest neighbouring cells surrounding each tumour cell, following a heatmap showing their respective frequencies of neighbouring cell types within each**

tumour cluster, with representative Xenium images illustrating distinct spatial cellular architectures. **g**, Stacked barplots showing the neighbourhood compositions of tumour cells from different tumour sites. **h**, Functional analysis of differentially expressed genes in immune cells within the Tumour C0 neighbourhood, primarily comprising macrophages and neutrophils. APC, antigen-presenting cell; CAF, carcinoma-associated fibroblast; ECM, extracellular matrix; MAPK, mitogen-activated protein kinase; PR, partial response; TGF, transforming growth factor; TNF, tumour necrosis factor.



Extended Data Fig. 8 | Knockout of CCL3 and CCL5 dampens secondary immune response following CAR-T cell infusion. **a**, CCL3 and CCL5 levels in the culture supernatant of CCL3/CCL5 double-knockout HCCLM3-oeGPC3 cells (CCL3/5 KO) or HCCLM3-oeGPC3 cells (control) after 36-hour exposure to IFN γ (50 ng/ml). **b**, Cell proliferation of HCCLM3-oeGPC3 cells with or without CCL3/5 knockout was assessed by CCK-8 assays. **c**, Tumour growth curves of mice bearing HCCLM3-oeGPC3 cells with or without CCL3/5 knockout. $n = 5$ mice. **d**, Tumour images and tumour weight on day 19 after inoculation. **e**, Tumour growth curves after GPC3 CAR-T cell infusion. $n = 10$ mice. **f**, Tumour images and tumour weight on day 21 postinfusion. $n = 10$ mice. **g-l**, Spectral flow cytometry analysis of the **(g)** CD45⁺ cells, **(h)** CD8⁺ T cells, **(i)** exhausted CD8⁺ T cells, **(j)** cytotoxic T cells, **(k)** CCR3⁺CD8⁺ T cells, and **(l)** CCR5⁺CD8⁺ T cells in tumours. $n = 6$ mice. **m**, Analysis of public single-cell transcriptomics datasets from China National GeneBank Sequence Archive (CNP0000650) and Gene

Expression Omnibus (GSE292298 and GSE149614) showed CCL3 and CCL5 expression in tumour cells. **n**, Immunohistochemistry staining of CCL3 and CCL5 performed on serial sections from pre-screened and Non-PR samples used for Digital Spatial Profiler. H&E staining was conducted on the same section for CCL5 staining. Representative samples from 7 pre-screened and 6 Non-PR samples are shown. Scale bars, 50 μ m. **o**, Multiplex immunofluorescence staining of subcutaneous tumours derived from HCCLM3-oeGPC3 cells in NSG mice. One of 4 cases is shown. Blue, DAPI for nuclei; yellow, Pan-CK for epithelial tumour cells; white, CD3 for T cells; red, CCL3; cyan, CCL5. DAPI, 4',6-diamidino-2-phenylindole. Scale bars, 40 μ m. Data are mean $\pm$ s.d. for **a,b**, and mean $\pm$ s.e.m. for **d-l**. Two-sided unpaired t -test for **a,d,g-l**; two-way ANOVA for **b,c,e** (with Šidák's multiple comparisons test for **c,e**); Mann-Whitney U test for **f**. CCL, C-C motif chemokine ligand; CCK-8, Cell Counting Kit-8; GPC3, glypican-3; H&E, hematoxylin and eosin; KO, knockout; OD, optical density; PR, partial response.

Extended Data Table 1 | Cytokine release syndrome across C-CAR031 dose levels

CRS	0.75×10 ⁶ cells/kg (DL1) n = 1	1.5×10 ⁶ cells/kg (DL2) n= 6	2.5×10 ⁶ cells/kg (DL3) n = 18	4.0×10 ⁶ cells/kg (DL4) n = 11	Total n = 36
CRS, n (%)	1 (100.0)	5 (83.3)	17 (94.4)	11 (100.0)	34 (94.4)
Grade 1	1 (100.0)	3 (50.0)	9 (50.0)	4 (36.4)	17 (47.2)
Grade 2	0	2 (33.3)	8 (44.4)	5 (45.4)	15 (41.7)
Grade 3	0	0	0	2 (18.2)	2 (5.6)
Grade 4	0	0	0	0	0
Median CRS onset time, days (range)	7 (7–7)	3 (2–3)	2 (2–4)	2 (1–3)	2 (1–7)
Median CRS duration time, days (range)	4 (4–4)	6 (4–8)	4 (2–7)	4 (3–8)	4 (2–8)
Treatment with vasopressor drugs, n (%)	0	0	0	1 (9.1)	1 (2.8)
Treatment with tocilizumab, n (%)	0	4 (66.7)	12 (66.7)	10 (90.9)	26 (72.2)
Treatment with steroids, n (%)	0	2 (33.3)	1 (5.6)	2 (18.2)	5 (13.9)

Footnote: CRS, cytokine release syndrome; DL, dose level.

Extended Data Table 2 | Efficacy per mRECIST across C-CAR031 dose levels in full analysis set

Variables	0.75×10 ⁶ cells/kg (DL1) n = 1	1.5×10 ⁶ cells/kg (DL2) n = 6	2.5×10 ⁶ cells/kg (DL3) n = 18	4.0×10 ⁶ cells/kg (DL4) n = 11	Total n = 36
Best overall response* — n (%)					
Complete response	0	0	0	0	0
Partial response	0	3 (50.0)	7 (38.9)	7 (63.6)	17 (47.2)
Stable disease	1 (100.0)	2 (33.3)	11 (61.1)	2 (18.2)	16 (44.4)
Progression disease	0	1 (16.7)	0	1 (9.1)	2 (5.6)
NE	0	0	0	1 (9.1)	1 (2.8)
Objective response rate — n (%)	0	3 (50.0)	7 (38.9)	7 (63.6)	17 (47.2)
Disease control rate — n (%)	1 (100.0)	5 (83.3)	18 (100.0)	9 (81.8)	33 (91.7)
Duration of response — months					
Median	NE	7.4	3.4	4.4	4.2
95% CI	NE	2.9–NE	2.8–4.4	2.8–5.8	2.9–5.8
PFS— months					
Median	NE	4.3	4.3	4.3	4.3
95% CI	NE	1.5–NE	2.8–5.6	1.7–5.8	2.9–5.6
PFS follow-up time — months					
Median	2.8	NE	NE	NE	NE
95% CI	NE	2.1–NE	4.2–NE	NE	NE

Footnote: *Best overall response is defined as the best response outcome from the first cell infusion to disease progression, death, or new anti-tumour therapy, whichever comes first. DL, dose level; NE, not evaluable; PFS, progression-free survival.