nature medicine

Article

https://doi.org/10.1038/s41591-026-04380-z

Cibisatamab and FAP-4-1BBL in microsatellite-stable colorectal cancer: a phase 1b trial

Received: 17 January 2026

Accepted: 30 March 2026

Published online: 20 April 2026

Check for updates

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We evaluated cibisatamab, a carcinoembryonic antigen (CEA)-directed CD3 T cell-engaging bispecific antibody, in combination with FAP-4-1BBL, a fibroblast activation protein (FAP)-targeted 4-1BB ligand providing tumor-localized co-stimulation, in an open-label phase 1b dose-escalation study in patients with microsatellite-stable (MSS) metastatic colorectal cancer (mCRC) progressing after two or more prior therapies. Patients received cibisatamab with escalating doses of FAP-4-1BBL weekly or every 3 weeks after obinutuzumab pretreatment to mitigate anti-drug antibody formation. The primary endpoint was safety; secondary endpoints included antitumor activity, pharmacokinetics and biomarker analyses. Among 52 treated patients, the combination showed a manageable safety profile. Dose-limiting toxicities occurred in 2 out of 52 patients (3.8%). Cytokine release syndrome (CRS) occurred in 30 out of 52 patients (57.7%; grade ≥3: 2 out of 52, 3.8%) and was manageable; after a cycle 1 cibisatamab dose reduction to 60 mg, serious CRS occurred in 4 out of 27 patients (14.8%; grade ≥3: 0 out of 27). Gastrointestinal toxicities consistent with CEA-directed T cell engagement were observed. Colitis occurred in 7 out of 52 patients (13.5%), including immune-mediated enterocolitis and one fatal cytomegalovirus colitis. No maximum tolerated dose of FAP-4-1BBL was established. Confirmed partial responses were observed in 7 out of 52 patients (13.5%). Pharmacodynamic analyses demonstrated systemic immune activation, including increased IFNγ, soluble CD25, soluble 4-1BB (CD137) and activated, proliferating $CD8^+$ T cells. Paired tumor biopsies showed increased intratumoral $CD8^+$ and $CD8^+Ki67^+$ T cell infiltration. These findings demonstrate the feasibility of combining tumor antigen-directed T cell engagement with localized co-stimulation, with evidence of immune activation and preliminary antitumor activity supporting further clinical development. ClinicalTrials.gov identifier: NCT04826003.

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Immune checkpoint inhibitors have substantially improved outcomes in CRC with mismatch repair deficiency or high microsatellite instability (dMMR/MSI-H)[1]. However, more than 85% of patients have mismatch repair proficient (pMMR)/MSS disease[2], which is typically characterized by limited immune infiltration and poor responsiveness to current immunotherapies[3,4], resulting in a persistently poor prognosis in the metastatic setting. Effective therapeutic strategies for this population remain an unmet need.

T cell receptor (TCR)-engaging therapeutics can activate cytotoxic T cells and have demonstrated clinical benefit in hematologic malignancies[5,6] and, more recently, in selected solid tumors[7,8]. Nonetheless, extending these approaches to non-inflamed tumors such as pMMR/MSS CRC remains challenging.

Effective activation of T lymphocytes requires integration of multiple signals. In the classical two-signal model of T cell activation[9], signal 1 is provided by recognition of peptide–MHC complexes by the TCR, whereas signal 2 is delivered through co-stimulatory receptors that amplify TCR signaling[10]. In the absence of adequate co-stimulation, TCR engagement alone can result in incomplete activation or functional tolerance[11].

Among inducible co-stimulatory receptors, 4-1BB (also known as CD137 or TNFRSF9) is upregulated after T cell activation and promotes proliferation, survival, memory differentiation and cytotoxic function upon engagement with its ligand or agonistic antibodies[10,12–14]. Combining targeted TCR engagement with complementary co-stimulatory signals may, therefore, enhance the magnitude and durability of antitumor T cell responses.

Cibisatamab is a 2:1 T cell bispecific antibody that targets CEA, expressed in more than 80% of CRCs, and CD3ε on T cells[15,16]. By simultaneously binding CEA and CD3ε, cibisatamab induces T cell activation independently of native TCR specificity, resulting in lymphocyte-mediated tumor cell killing[16,17]. In a previous phase 1 study of cibisatamab monotherapy in CEA-positive solid tumors, preliminary antitumor activity was observed, with confirmed partial responses in 4.0% of evaluable patients and a median duration of response of 6.5 months[18].

We hypothesized that the antitumor activity of a T cell engager such as cibisatamab could be enhanced by spatially restricted co-stimulation mediated by a FAP-targeted 4-1BB ligand (FAP-4-1BBL). This concept is supported by preclinical co-culture models of tumor organoids and FAP-expressing cancer-associated fibroblasts (CAFs)[19]. FAP-4-1BBL enables selective 4-1BB engagement within FAP-expressing tissues, including the tumor microenvironment and tumor-draining lymph nodes[13,20]. In a first-in-human dose-escalation study, FAP-4-1BBL (RO7122290) monotherapy showed modest clinical activity but demonstrated an acceptable safety profile and evidence of increased intratumoral T cell infiltration[13].

Here we report the results of the phase 1b dose-escalation study evaluating the combination of cibisatamab and FAP-4-1BBL after obinutuzumab pretreatment in patients with heavily pretreated MSS mCRC. Obinutuzumab pretreatment to deplete B cells was required because of induction of anti-drug antibodies (ADAs) by cibisatamab[21]. We further contextualize these findings in the combination setting by comparison with previously reported cibisatamab monotherapy data and associated pharmacodynamic analyses[18]. Our study assessed the impact of tumor-localized 4-1BB co-stimulation on safety, immune activation and clinical activity when combined with cibisatamab.

Results

Patient disposition

Between 19 April 2021 and 11 November 2024, patients with pMMR/MSS mCRC who had progressed after at least two prior lines of therapy were screened for this open-label, multicenter, phase 1b dose-escalation study. Fifty-four patients with measurable disease at baseline were enrolled. Fifty-two patients received at least one administration of study drug and were analyzed for safety and efficacy. Baseline characteristics and demographics are summarized in Table 1, and patient disposition is shown in the CONSORT diagram (Fig. 1a,b). The safety and efficacy analyses were conducted in all patients who received at least one dose of study treatment ($n = 52$).

The study comprised two parts. Part 1 evaluated safety, pharmacokinetics and pharmacodynamics during weekly (QW) escalation of FAP-4-1BBL. Part 2 explored doses deemed safe in part 1 using every-3-week (Q3W) dosing (Extended Data Fig. 1a,b). Cibisatamab was administered Q3W in both parts.

Initially, cibisatamab was given at 100 mg from cycle 1 (part 1 cohorts 1 and 2; part 2 cohort 1). After a grade 3 CRS event, the cycle 1 dose was reduced to 60 mg from part 1 cohort 3 and part 2 cohort 2 onward, with escalation to 100 mg from cycle 2 (60 → 100 mg).

Study design and dosing schedules are shown in Extended Data Fig. 1a,b, with baseline characteristics and cohort dose levels summarized in Table 1 and in the CONSORT diagrams (Fig. 1). Dose escalation of FAP-4-1BBL in combination with cibisatamab was discontinued for strategic reasons before a maximum tolerated dose (MTD) was defined.

Primary outcomes

Safety and tolerability. Treatment with cibisatamab plus FAP-4-1BBL was associated with immune-mediated adverse events consistent with the known safety profile of T cell-engaging therapies and prior clinical experience with cibisatamab, with most events occurring during the early treatment cycles. Patients routinely received low-dose corticosteroid and non-steroidal anti-inflammatory premedication prior to infusions. The highest FAP-4-1BBL doses administered were 130 mg QW and 90 mg Q3W. Two dose-limiting toxicities (DLTs) were observed: presyncope (grade 3 serious adverse event (SAE), related to FAP-4-1BBL) in the cibisatamab 100 mg plus FAP-4-1BBL 35 mg QW cohort and CRS (grade 3 SAE, related to both cibisatamab and FAP-4-1BBL) in the cibisatamab plus FAP-4-1BBL 50 mg QW plus cohort. A summary of treatment-emergent adverse events is presented in Table 2, and details by cohort are provided in Extended Data Tables 1 and 2.

All patients (52 out of 52) experienced at least one adverse event. The most common (≥30%) were fatigue (63.5%), CRS (57.7%), diarrhea (55.8%), pyrexia (50.0%), decreased appetite (44.2%), cough (32.7%), anemia (32.7%), nausea (30.8%) and arthralgia (30.8%) (Table 2 and Extended Data Tables 1 and 2). Grade 3 or higher adverse events occurred in 38 patients (73.1%), and SAEs occurred in 34 patients (65.4%). Four patients (7.7%) experienced grade 5 events (sepsis, sudden death, general physical health deterioration and cytomegalovirus (CMV)-related colitis); the former two were not considered treatment related, whereas general physical health deterioration and CMV-related colitis were. The event coded as 'general physical health deterioration' reflects progressive clinical decline in the context of advanced disease without a clearly identifiable alternative cause.

Dose interruptions or modifications of FAP-4-1BBL occurred in 21 patients (40.4%), and adverse events resulted in treatment discontinuation in eight patients (15.4%). Adverse events considered related to FAP-4-1BBL led to dose modification or interruption in 17 patients (32.7%) and to treatment discontinuation in one patient (1.9%) (Table 2 and Extended Data Table 1). Adverse events by dose level showed no clear dose-dependent increase in grade 3 or higher toxicity, although higher-dose weekly cohorts demonstrated a trend toward increased incidence of gastrointestinal and immune-mediated events (Extended Data Table 1)

CRS occurred predominantly during cycle 1 (median onset, day 2) and was grade 1 in 25 patients (48.1%), grade 2 in 4 patients (7.7%) and grade 3 in 2 patients (3.8%). Serious CRS events were reported in 13 patients (25.0%) overall, including 4 out of 27 patients (14.8%) treated with cibisatamab 60 mg in cycle 1 followed by 100 mg from cycle 2 (Table 2 and Extended Data Table 3). CRS was managed per protocol and institutional guidelines with supportive care (antipyretics,

https://doi.org/10.1038/s41591-026-04380-z

Table 1 | Baseline demographic and clinical characteristics

Characteristic	QW regimen (*n*=30)[a]	Q3W regimen (*n*=22)	Total (*N*=52)
Age, median (range), years	56.0 (38–74)	54.5 (34–75)	57.0 (34–75)
Male sex, *n* (%)	18 (60.0)	15 (68.2)	33 (63.5)
Female sex, *n* (%)	12 (40.0)	7 (31.8)	19 (36.5)
White European, *n* (%)	9 (30.0)	6 (27.3)	15 (28.8)
Weight, median (range), kg	79.20 (50.9–121.8)	76.15 (37.1–116.1)	77.35 (37.1–121.8)
ECOG performance status 0, *n* (%)	27 (90.0)	16 (72.7)	43 (82.7)
ECOG performance status 1, *n* (%)	3 (10.0)	6 (27.3)	9 (17.3)
Colon disease by location[b], *n* (%)			
Left	18 (60.0)	8 (36.3)	26 (50.0)
Right	5 (16.6)	9 (40.9)	14 (26.9)
No data	7[b] (23.3)	5 (22.7)	12[b] (23.1)
Primary tumor site: colon, *n* (%)	21 (70.0)	16 (72.7)	37 (71.1)
Primary tumor site: rectum, *n* (%)	6 (20.0)	3 (13.6)	9 (17.3)
RAS mutant (*NRAS*+*KRAS*), *n* (%)	16 (53.3)	16 (72.7)	32 (61.5)
BRAF mutant, *n* (%)	2 (6.6)	1 (3.3)	3 (5.8)
Liver metastases present, *n* (%)	20 (66.6)	18 (81.8)	38 (73.1)
Lung metastases present, *n* (%)	14 (46.6)	12 (54.5)	26 (50.0)
Peritoneum metastases present, *n* (%)	4 (13.3)	4 (18.2)	8 (15.4)
Prior lines of therapy, median (range)	3 (2–6)	3 (2–5)	3 (2–6)
CEA expression H-score, median (range)[c]	190.0 (13.0–290.0)	190.0 (0.0–290.0)	190.0 (0.0–290.0)
Prior immunotherapy, *n* (%)	28 (93.3)	21 (95.4)	49 (94.2)
Prior anti-VEGF therapy, *n* (%)	26 (86.6)	20 (90.9)	46 (88.5)
Prior anti-EGFR therapy, *n* (%)	14 (46.6)	5 (22.7)	19 (36.5)
Prior CPI, *n* (%)	2 (6.6)	0	2 (3.8)

[a]Counts derived from cohort-level data in part 1 (QW cohorts) and part 2 (Q3W cohorts). Some demographic values were approximated from cohort summaries where pooled values were not explicitly reported. [b]One patient was reported with transverse colon disease. [c]A valid test result from available archival or fresh tissue was obtained for only 27 patients in the QW regimen and from only 21 patients in the Q3W regimen. CPI, checkpoint inhibitor; ECOG, Eastern Cooperative Oncology Group.

intravenous fluids and corticosteroids as indicated); some patients required hospitalization, and tocilizumab was used in selected cases. Additional details on management of CRS are provided in the Methods.

Gastrointestinal toxicity, a known on-target/off-tumor effect of cibisatamab[18], was also observed with combination therapy, including nausea (30.5%), vomiting (23.1%) and diarrhea (55.8%). Diarrhea occurred throughout treatment (median onset, 36 days; range, −2 to 393 days relative to first dose). The negative onset reflects the study design, in which treatment was initiated on study day 1, and patients received obinutuzumab lead-in dosing on study day −13 or −8 depending on the protocol version. Colitis was reported in seven patients (13.5%), including cases of immune-mediated enterocolitis and one case of CMV colitis.

Obinutuzumab was administered as B cell-depleting pretreatment to mitigate ADA formation; attribution of individual adverse events is limited. Immune-related adverse events were not prospectively categorized as a separate safety analysis group in this early-phase study. Details regarding the management of adverse events are provided in the Methods.

Secondary outcomes

Pharmacokinetics and immunogenicity outcomes. FAP-4-1BBL exposure increased with dose. Geometric mean pharmacokinetic parameters, including area under the curve (AUC) and maximum plasma concentration (C_{max}), rose with escalating doses. Elimination was nonlinear, consistent with target-mediated drug disposition (TMDD)[22]. Pharmacokinetic parameters are summarized in Supplementary Table 1.

Cibisatamab exposure was consistent with previous reports in monotherapy and in combination with atezolizumab, with cycle 1 C_{max} and AUC within established ranges[18], suggesting no meaningful effect of FAP-4-1BBL co-administration on cibisatamab pharmacokinetics. Cycle 1 pharmacokinetic parameters are summarized in Supplementary Table 2.

Two patients pretreated with obinutuzumab developed ADAs to FAP-4-1BBL; no patients had treatment-induced ADAs to cibisatamab.

Antitumor activity. All 52 patients receiving at least one dose of study drug were included in the efficacy analysis. One patient discontinued treatment owing to clinical deterioration before the first radiographic assessment and was, therefore, classified as having progressive disease. Confirmed partial responses were observed in seven patients (13% (7 out 52); 95% confidence interval: 6–26), and two patients had unconfirmed responses. No complete responses were observed. The disease control rate was 50.0% (26 out of 52). Individual tumor burden dynamics over time are shown in the spider plot (Fig. 2a).

Partial responses were observed across multiple dosing cohorts. By cibisatamab dosing strategy, partial responses occurred in three of 25 patients (12.0%) treated with 100 mg from cycle 1 and in four of 27 patients (14.8%) treated with cibisatamab 60 → 100 mg. Partial responses were observed across both QW and Q3W dosing schedules. Objective response rates (ORRs) with corresponding 95% confidence intervals (CI) across dosing cohorts are summarized in Table 3.

The disease control rate (DCR; confirmed partial response + stable disease) was 50.0% (26 out of 52), including 56.6% (17 out of

https://doi.org/10.1038/s41591-026-04380-z

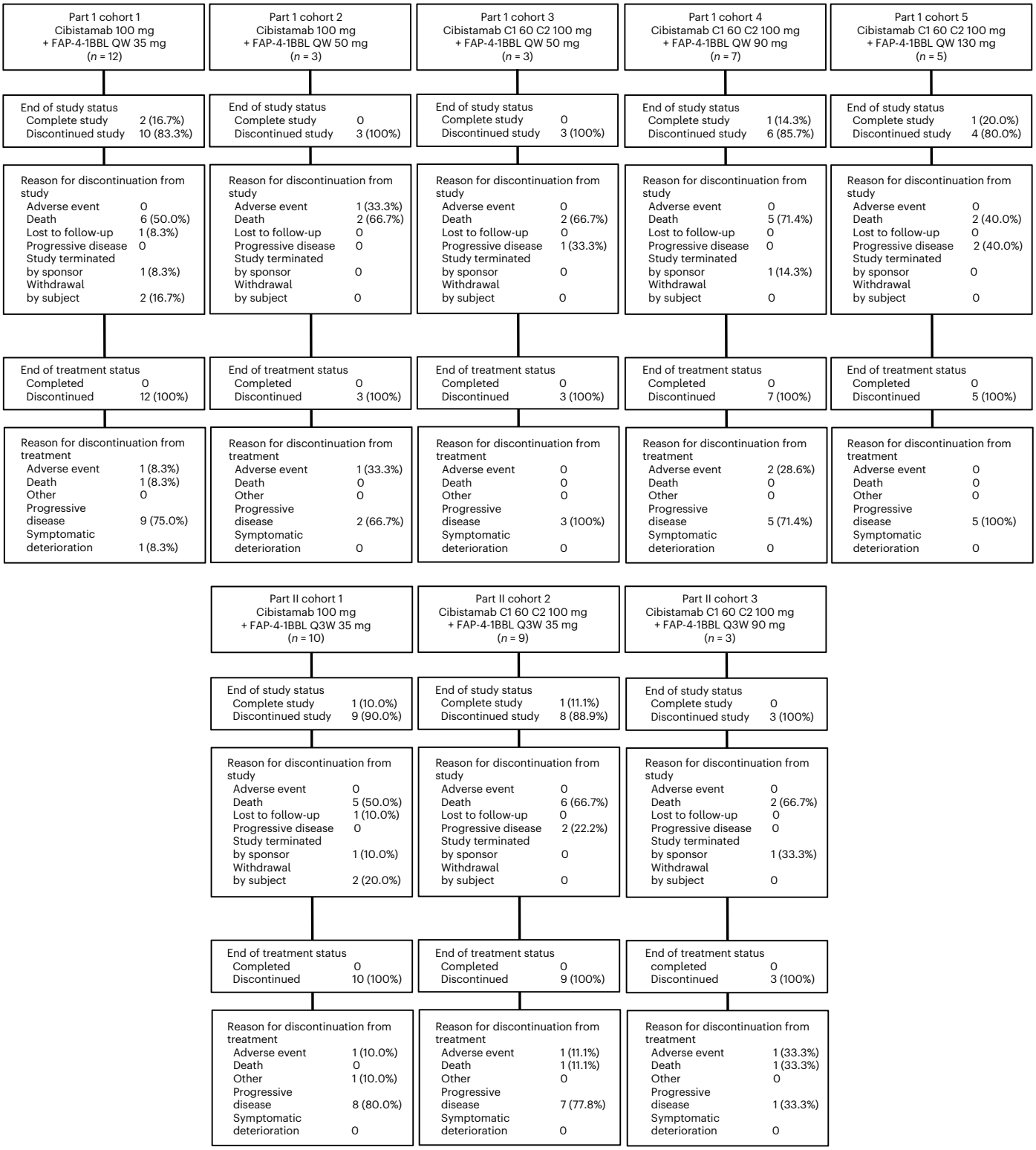

Fig. 1 | Study design and participant disposition. CONSORT diagrams summarizing the design of the phase 1b study evaluating the CEA-targeted T cell engager cibisatamab in combination with the tumor-targeted 4-1BB agonist FAP-4-1BBL in patients with advanced solid tumors and the disposition of patients included in the present analysis. The diagrams illustrate the dose-escalation cohorts evaluating different FAP-4-1BBL dose levels and schedules administered with cibisatamab, including cohorts receiving a flat cibisatamab dose from cycle 1 and cohorts evaluating a refined step-up regimen (60 mg at cycle 1 followed by 100 mg from cycle 2). C1, cycle 1; C2, cycle 2.

30) among patients treated with FAP-4-1BBL QW and 40.9% (9 out of 22) among patients treated Q3W. DCR was 56.0% (14 out of 25) in patients treated with cibisatamab 100 mg from cycle 1 and 44.4% (12 out of 27) in patients treated with the reduced cibisatamab dose at the first treatment cycle. Responses were observed across multiple dose levels, including higher weekly dose cohorts (Table 3). Notably, higher response rates were observed in later part 1 cohorts that evaluated higher FAP-4-1BBL dose levels in combination with the reduced

https://doi.org/10.1038/s41591-026-04380-z

Table 2 | Summary of adverse events

Adverse event	Any grade *n* (%)	Grade≥3 *n* (%)
Overall safety outcomes		
Any adverse event	52 (100)	38 (73.1)
SAEs	34 (65.4)	—
Adverse events leading to treatment withdrawal	8 (15.4)	—
Adverse events leading to dose modification/interruption	21 (40.4)	—
DLTs	2 (3.8)	2 (3.8)
Grade 5 adverse events	4 (7.7)	4 (7.7)
Treatment-related grade 5 adverse events	2 (3.8)	2 (3.8)
Most common adverse events (≥30% of patients)		
Fatigue	33 (63.5)	—
CRS	30 (57.7)	2 (3.8)
Diarrhea	29 (55.8)	—
Pyrexia	26 (50.0)	—
Decreased appetite	23 (44.2)	—
Cough	17 (32.7)	—
Anemia	17 (32.7)	—
Nausea	16 (30.8)	—
Arthralgia	16 (30.8)	—

Data represent treatment-emergent adverse events among patients who received at least one dose of cibisatamab plus FAP-4-1BBL (n=52). Values are the number of patients with events and corresponding percentages. Adverse events were graded according to CTCAE version 5.0. CRS grading followed ASTCT consensus criteria.

cibisatamab dose at cycle 1, compared to earlier cohorts receiving the full cibisatamab dose of 100 mg also at the first cycle. Cohort-level efficacy is summarized in Table 3, categorized by FAP-4-1BBL dose and schedule and by cibisatamab dosing strategy (cibisatamab 100 mg versus cibisatamab 60 → 100 mg). Progression-free survival (PFS) by investigator assessment suggested a trend toward longer disease control in patients treated in the later dose-escalation cohorts part 1 cohort 4 (P1C4) and part 1 cohort 5 (P1C5) (n = 12) compared to earlier part 1 cohorts 1–3 (P1C1–P1C3) (n = 18) (Extended Data Fig. 2). Although progression events occurred in both groups, separation of the PFS curves became apparent at later timepoints, with a higher proportion of patients in part 1 cohorts 4–5 (P1C4–P1C5) remaining progression free beyond approximately 150 days. Median PFS was not reached in the P1C4–P1C5 group at the time of analysis, whereas patients in earlier part 1 cohorts experienced progression earlier. These findings are exploratory and should be interpreted cautiously given the limited sample size and non-randomized cohort structure.

Treatment duration suggested longer exposure with QW versus Q3W FAP-4-1BBL (Fig. 2a,b). For example, median FAP-4-1BBL treatment duration was 123 days (range, 1–435) in the 35 mg QW cohort plus cibisatamab 100 mg (n = 12) versus 64 days (range, 1–260) in the corresponding 35 mg Q3W cohort (n = 10). Similarly, in the 90 mg cohorts, median duration was 99 days (range, 1–232) for QW (n = 6) versus 43 days (range, 22–57) for Q3W (n = 3). In cohorts treated with the same FAP-4-1BBL dose and schedule, longer treatment duration was observed in patients who received cibisatamab 60 → 100 mg versus 100 mg from cycle 1 (Extended Data Table 4).

Exposure–safety analysis. We evaluated associations between FAP-4-1BBL exposure and adverse events of interest (CRS, infusion-related reaction (IRR), rash, diarrhea and colitis). For acute events (CRS and IRR), analyses used first-dose C_{max} (evaluable: QW n = 28 and Q3W n = 22). For delayed events (rash, diarrhea and colitis), analyses used cumulative exposure over the first 63 days (AUC_{0-63d}) to approximate exposure through approach to steady state.

No consistent exposure–adverse event relationships were observed for CRS, IRR, rash, or colitis across regimens. A possible trend between higher exposure and diarrhea was noted in the QW regimen but not in Q3W. Owing to low event counts, logistic regression analyses were underpowered for definitive inference. Additional details are provided in Supplementary Fig. 1.

Analysis of baseline markers in association with clinical response. To explore predictors of response, we assessed changes in tumors from baseline across patients (Fig. 2b). Partial responses occurred across a range of baseline biomarker profiles, with clinical benefit not restricted to a single subgroup. Heatmap overlays of CEA and FAP expression, immune phenotype, prior therapy and baseline tumor $CD8^+$ infiltration suggested that responses also occurred in patients with adverse features, including liver metastases and excluded/desert immune phenotypes.

Baseline profiling identified tumor-intrinsic and clinical features associated with outcome. Tumor CEA by immunohistochemistry (IHC) was associated with DCR (Extended Data Fig. 3a): patients with low CEA (histochemistry score (H-score) ≤100) had shorter PFS than those with medium (101–200) or high (201–300) scores. No association was observed when CEACAM5 was assessed by RNA sequencing (RNA-seq) (Supplementary Fig. 2b). Baseline FAP expression did not correlate with DCR or ORR; tertile stratification suggested a favorable PFS trend in the intermediate-expression group (Extended Data Fig. 3b), which may reflect two opposing effects of FAP expression in this combination setting: sufficient target availability for FAP-4-1BBL activity but less stromal immunosuppression than in highly FAP-rich tumors.

Consensus molecular subtype (CMS) analysis[23] suggested enrichment of responders in CMS2, an epithelial, WNT/MYC-driven transcriptional subtype (Extended Data Fig. 3c). Although the overall association between CMS category and clinical outcome did not reach statistical significance (P = 0.069), patients with CMS2 tumors showed numerical improvements in both PFS and overall survival. No partial responses were observed in CMS1 and CMS4 tumors, characterized by epithelial–mesenchymal transition and a TGFβ-driven immunosuppressive phenotype, were likewise non-responsive[24].

Liver metastases, an adverse prognostic factor[25,26], did not influence response, PFS or overall survival; four of seven of confirmed partial responses (57%) occurred in patients with at least one liver target lesion (Extended Data Fig. 3d). No clear pattern of early response followed by rapid progression was observed among patients with liver metastases, although the limited sample size precludes firm conclusions regarding durability.

Pretreatment immune phenotyping showed that most partial responses occurred in tumors with an excluded immune phenotype, whereas progressive disease was more frequently observed in immune-desert tumors[27]. The T cell-excluded phenotype was associated with longer PFS (Extended Data Fig. 3e). By contrast, neither baseline tumor $4\text{-}1BB^+$ or $CD8^+$ density nor circulating cytokines/immune cell subsets (including IL-8, lymphocyte-to-neutrophil ratio, circulating $CD8^+Ki67^+$ T cells, IFNγ, CXCL10, soluble IL-2 receptor and soluble target proteins) were associated with clinical outcomes (Supplementary Fig. 2).

Pharmacodynamics. Pharmacodynamic effects were assessed in peripheral blood and paired tumor biopsies.

Pharmacodynamic changes in peripheral blood. Peripheral blood cytokines and immune cell populations were analyzed to characterize treatment-associated pharmacodynamic changes[18] and further extended by additional pharmacodynamic analyses performed on previously unpublished data from the cibisatamab monotherapy study.

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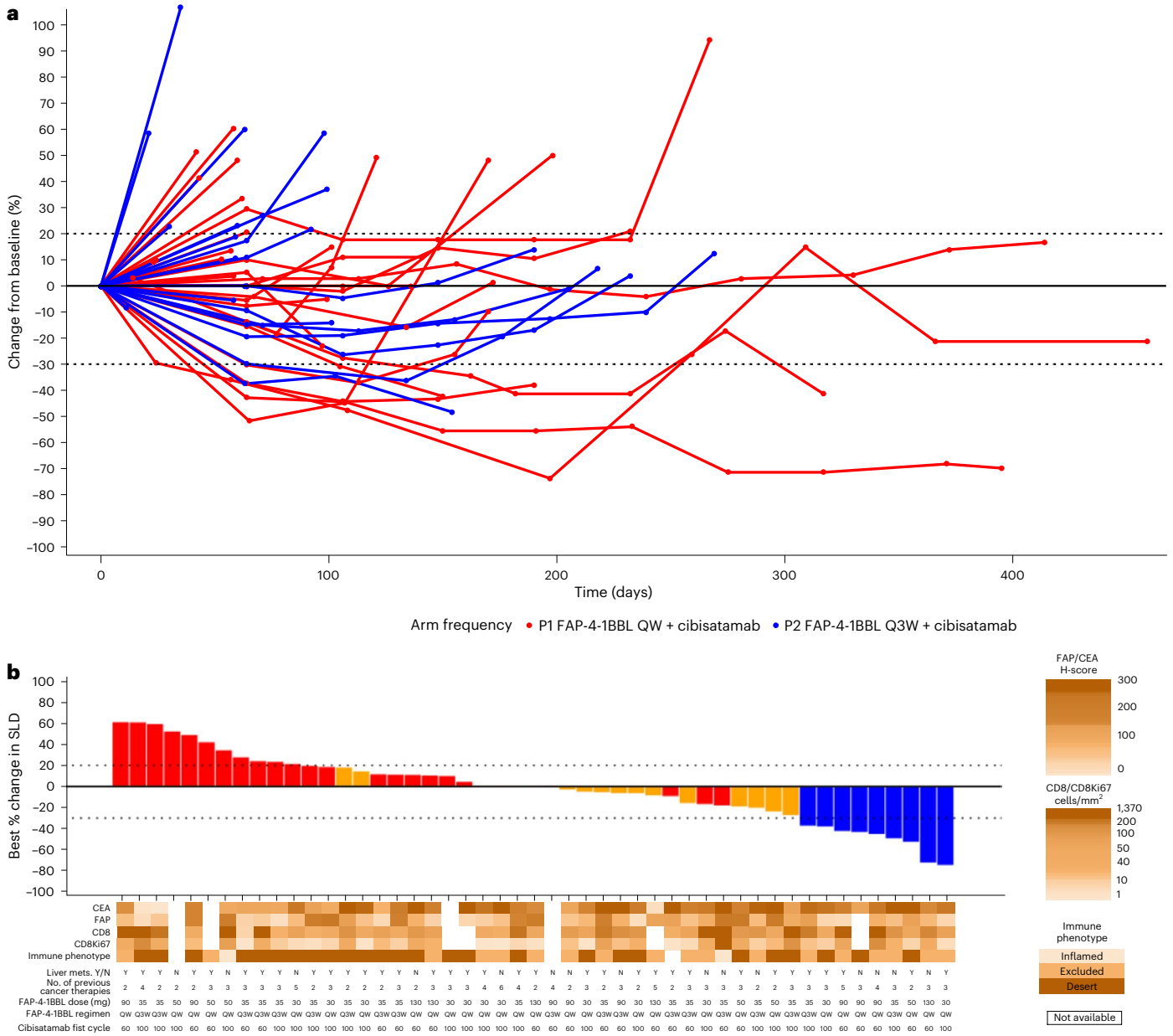

Fig. 2 | Tumor response dynamics and best change in tumor burden. **a**, Spider plot showing the longitudinal percentage change from baseline over time according to RECIST v1.1 for individual patients ($n = 52$). Lines represent individual patients and are color coded by FAP-4-1BBL dosing regimen (QW, red; Q3W, blue). **b**, Waterfall plot showing the best percentage change from baseline in sum of longest diameters (SLD) by RECIST v1.1. Bars are color coded by best overall response (BOR): progressive disease (PD, red), stable disease (SD, orange) and partial response (PR, blue). Heatmaps indicate baseline tumor and clinical features, including CEA and FAP expression, immunephenotype, liver metastases, prior lines of therapy and dosing regimen. Color gradients represent quantitative assessments of CEA and FAP H-score and CD8$^+$Ki67$^+$ cell density and the immunephenotype. mets., metastases; P1, part 1; P2, part 2; Y/N, yes/no.

Cibisatamab plus FAP-4-1BBL induced increases in circulating IFNγ, soluble CD25 (also known as IL-2Rα) and IL-6 (Extended Data Fig. 4). IFNγ rose by day 15 and remained elevated. Soluble CD25 showed a similar sustained pattern. IL-6 increased with kinetics similar to monotherapy. TNF and IL-10 were also higher on day 15, consistent with an enhanced immunostimulatory profile.

Flow cytometry (Extended Data Fig. 4b) showed an early, transient circulating lymphocyte reduction (8 h and cycle 1 day 2), consistent with margination and/or trafficking, followed by return to baseline.

The combination increased proliferating CD8$^+$Ki67$^+$ T cells and activated CD8$^+$ subsets (TIM3, 4-1BB and HLA-DR), along with expansion of central and effector memory compartments. By day 22 (cycle 2 day 1), signs of immune activation were more pronounced, including higher CD8$^+$4-1BB$^+$ and CD8$^+$HLA-DR$^+$ frequencies and increased expression of PD-1 (also known asCD279) on central memory and effector CD8$^+$ T cells, consistent with more pronounced CD8$^+$ activation.

Soluble 4-1BB increased across response groups, consistent with target engagement[28]. Patients with partial response showed more sustained induction during cycles 1–2, whereas stable disease/progressive disease showed lower and more variable changes.

Serum CEA declined in patients with partial response from cycle 2 onward (median, approximately 0.5–1.0 log reductions by cycles 6–8), with smaller changes in stable disease and little change in progressive disease.

Pharmacodynamic changes in fresh paired tumor biopsies. Paired fresh biopsies were collected at baseline and on day 23 after the first combination dose to assess intratumoral immune pharmacodynamics.

https://doi.org/10.1038/s41591-026-04380-z

Table 3 | Best overall response by investigator assessment (RECIST v1.1)

Combination schedule	FAP-4-1BBL dose (mg)	Cibisatamab dose (mg)	*n* evaluable	Confirmed PR *n* (%) (95% CI)	Confirmed SD *n* (%) (95% CI)	Confirmed ORR *n* (%) (95% CI)	Confirmed DCR *n* (%)
Cibisatamab + FAP-4-1BBL QW	35	100	12[a]	2 (17) (2–48)	5 (42) (15–72)	2 (17) (2–48)	7 (58) (28–85)
	50	100	3	0 (0–71)	1 (33) (1–91)	0 (0–71)	1 (33) (1–91)
	50	60→100	3	1 (33) (1–91)	1 (33) (1–91)	1 (33) (1–91)	2 (67) (9–99)
Cibisatamab + FAP-4-1BBL QW	90	60→100	7	2 (29) (4–71)	2 (29) (4–71)	2 (29) (4–71)	4 (57) (18–90)
	130	60→100	5	1 (20) (1–72)	2 (40) (5–85)	1 (20) (1–72)	3 (60) (15–95)
Cibisatamab + FAP-4-1BBL Q3W	35	100	10	1 (10) (0–45)	5 (50) (19–81)	1 (10) (0–45)	6 (60) (26–88)
	35	60→100	9	0 (0–34)	2 (22) (3–60)	0 (0–34)	2 (22) (3–60)
	90	60→100	3	0 (0–71)	1 (33) (1–91)	0 (0–71)	1 (33) (1–91)
All	All	All	52	7 (13) (6–26)	19 (37) (24–51)	7 (13) (6–26)	26 (50) (36–64)

Cibisatamab + FAP-4-1BBL, confirmed responses only Cibisatamab 60→100 indicates 60 mg at cycle 1 followed by 100 mg from cycle 2 onward. [a]One participant withdrew after a single study dose (cibisatamab + FAP-4-1BBL) but was later reported as a confirmed PR without receiving any anticancer therapy, per investigator assessment.

Duplex IHC (CD8 and Ki67) showed more than two-fold increases in intratumoral $CD8^+$ T cells in 12 out of 14 evaluable pairs and more than two-fold increases in $CD8^+Ki67^+$ cells in 10 out of 14 pairs (Fig. 3a and Extended Data Fig. 5a). Immune phenotype profiling[27] classified baseline lesions as desert (79%), excluded (14%) or inflamed (7%). On treatment, desert lesions decreased to 21%, with increases in excluded (50%) and inflamed (29%) phenotypes (Fig. 3b).

Quantitative image analysis demonstrated increases in intratumoral $CD8^+$ and $CD8^+Ki67^+$ T cell density after treatment. Substantial increases in intratumoral $CD8^+$ and $CD8^+Ki67^+$ T cell density were observed after combination therapy ($P = 0.056$), with a corresponding five-fold increase in $CD8^+Ki67^+$ cell density ($P = 0.019$) (Fig. 3c and Extended Data Fig. 5a). Such increases were also observed in 10 out of 11 patients with liver metastases (Extended Data Fig. 5a).

RNA-seq of paired biopsies passing quality control ($n = 6$) showed induction of immune effector genes (*CXCL9*, *CXCL10*, *IFNG* and *TNFRSF9*) (Fig. 3d and Extended Data Fig. 5b,c). IFNγ response, cytotoxic T cell, T effector and antigen processing machinery (APM) signatures increased (all $P < 0.01$). Induction was greater in partial response/stable disease than in progressive disease despite higher baseline expression in progressive disease, consistent with a less robust transcriptional response in progressive disease tumors.

A modest but significant increase in the M1/M2 macrophage ratio was estimated based on gene signatures (Extended Data Fig. 5b,c). Adaptive immune-regulatory programs were also induced, including PD-L1 (also known as CD274), LAG3, EOMES[29,30] and IDO1 (refs. 31,32) (Extended Data Fig. 5c). FAP and CEA expression showed no consistent treatment-induced change (Extended Data Fig. 5d).

Exploratory outcomes

Circulating tumor DNA. In an exploratory, tissue-informed patient-specific assay, baseline circulating tumor DNA (ctDNA) levels varied widely (median, 1,604 mean tumor molecules per milliliter (MTM/ml)) and tended to be lower in patients with partial response than in those with stable disease or progressive disease (Extended Data Fig. 6a). ctDNA was not clearly associated with liver metastases, suggesting that it reflects overall tumor burden rather than site-specific disease. Lower baseline ctDNA showed a non-significant trend toward improved PFS and overall survival (Extended Data Fig. 6b), although these findings are exploratory and limited by small sample size. Lower baseline ctDNA showed a non-significant trend toward improved PFS ($P = 0.061$) and overall survival ($P = 0.2$) (Extended Data Fig. 6b). By cycle 3 day 1 (C3D1), lower residual ctDNA (<364.42 MTM/ml) was associated with longer PFS ($P = 0.008$) and overall survival ($P = 0.033$) (Extended Data Fig. 6c,d). From baseline to C3D1, ctDNA decreased more than three-fold in five of five patients with partial response and in six of eight patients with stable disease, whereas patients with progressive disease showed minimal change or increases (Extended Data Fig. 6e). A greater ctDNA decline was associated with longer PFS ($P = 0.009$) (Extended Data Fig. 6f).

Sensitivity analyses

Sensitivity analyses were limited by the non-randomized, dose-escalation design, small cohort sizes and heterogeneity across dose levels and schedules. Stratified analyses by FAP-4-1BBL dosing schedule (QW versus Q3W) and cibisatamab dosing strategy (100 mg all cycles versus reduced dose of 60 mg at cycle 1) showed generally consistent directional trends in safety and efficacy outcomes but were not powered for formal statistical comparisons. Exposure–safety analyses did not demonstrate consistent relationships between FAP-4-1BBL exposure and adverse events of interest. A potential association between higher exposure and diarrhea was observed in the QW cohort but not in the Q3W cohort. Comparisons of PFS across dose-escalation cohorts suggested longer disease control in later cohorts; however, these findings were sensitive to cohort composition and dosing modifications and should be interpreted with caution.

Discussion

Our findings demonstrate that coordinated signal 1 and signal 2 immunostimulation can be achieved using a tumor-directed T cell engager with spatially restricted co-stimulation[33,34]. As a signal 1 provider, the CEA-directed T cell engager cibisatamab induces antigen-dependent T cell activation and tumor infiltration[16] and previously demonstrated modest but reproducible clinical activity in heavily pretreated MSS CRC[18].

Cibisatamab provides antigen-dependent T cell activation, and FAP-4-1BBL delivers tumor-localized co-stimulation. Together, this approach aims to enhance T cell activation and durability while limiting systemic toxicity[35]. 4-1BB is selectively upregulated after T cell priming and largely absent on resting lymphocytes[13,20]. Based on this biology, we hypothesized that cibisatamab-mediated T cell engagement combined with FAP-4-1BBL co-stimulation would enhance antitumor immunity while restricting signal 2 delivery to the tumor microenvironment[36,37].

Obinutuzumab was incorporated to mitigate ADA formation previously observed with cibisatamab monotherapy and to enable sustained drug exposure[18]. Although B cell depletion raises theoretical concerns regarding disruption of tertiary lymphoid structures and humoral support of antitumor immunity[38–40], no clear detrimental effects on clinical or immunologic outcomes were observed. However, the absence of a non-B-cell-depleted comparator cohort remains a limitation, particularly given that there is preclinical evidence that B cells can also promote tumor progression[41].

Dose and schedule selection for cibisatamab was informed by clinical and preclinical evidence of prolonged intratumoral retention despite a short systemic half-life[17]. For FAP-4-1BBL, both QW and Q3W

https://doi.org/10.1038/s41591-026-04380-z

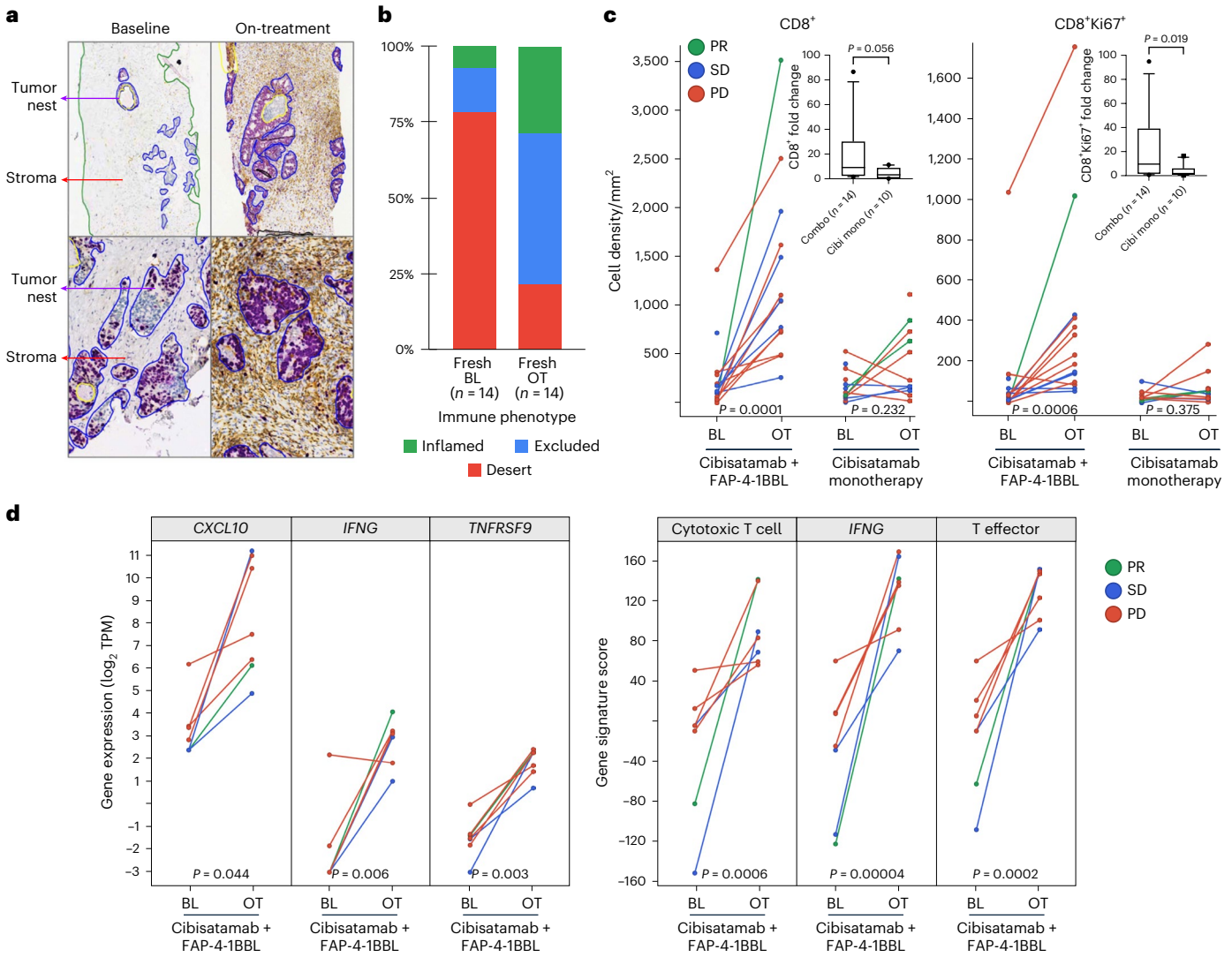

Fig. 3 | Intratumoral immune remodeling after cibisatamab + FAP-4-1BBL treatment in MSS mCRC. a, Representative duplex IHC of paired baseline (BL) and on-treatment (OT) tumor biopsies showing increased infiltration of $CD8^+$ T cells (yellow) and proliferating $CD8^+Ki67^+$ T cells (purple) within tumor nests and stroma after combination therapy. **b**, Distribution of tumor immune phenotypes at BL and OT ($n = 14$ paired biopsies), demonstrating a shift from immune-desert toward inflamed and immune-excluded phenotypes after treatment. **c**, Quantification of intratumoral $CD8^+$ and $CD8^+Ki67^+$ T cell densities (cells per mm^2) across paired biopsies. Lines connect individual participants from BL to OT, stratified by best overall response (partial response, stable disease and progressive disease). Insets show corresponding fold change distributions. A significant increase in $CD8^+$ and $CD8^+Ki67^+$ T cells was observed with cibisatamab + FAP-4-1BBL (Combo) but not with cibisatamab alone (Cibi mono). **d**, RNA-seq analysis of paired biopsies ($n = 6$) demonstrating induction of immune activation markers (*CXCL10*, *IFNG* and *TNFRSF9* (encoding 4-1BB)) and enrichment of cytotoxic T cell, *IFNG* and T effector gene signatures after treatment. Lines represent individual participants, colored by best overall response (PR, SD and PD).

regimens were explored to evaluate sustained versus intermittent co-stimulatory signaling[42]. Weekly dosing showed numerically higher response rates, greater tumor shrinkage and longer treatment duration; however, interpretation is limited because the Q3W cohort was predominantly treated at the lowest (35 mg) dose.

Clinical activity with cibisatamab plus FAP-4-1BBL was encouraging in this heavily pretreated MSS mCRC population. Although cross-study comparisons should be interpreted cautiously, prior cibisatamab monotherapy provides context for these findings[18]. These findings are consistent with preclinical evidence demonstrating synergistic antitumor effects of T cell engagement combined with 4-1BB co-stimulation[20,43].

The safety profile was consistent with known toxicities of cibisatamab and prior experience with FAP-4-1BBL. Gastrointestinal toxicity, an on-target/off-tumor effect of CEA-directed T cell engagement[18], was common but manageable and not dose limiting. Notably, tumor-targeted 4-1BB co-stimulation did not increase systemic immune toxicity, in contrast to untargeted 4-1BB agonists[44].

Biomarker analyses supported treatment activity, showing induction of peripheral immune activation markers and increased intratumoral $CD8^+$ and proliferating $CD8^+Ki67^+$ T cells, consistent with coordinated T cell engagement and co-stimulation[45]. These effects were also observed in liver metastases, suggesting partial mitigation of liver-associated immune suppression.

Baseline analyses identified tumor CEA expression by IHC as a potential predictor of disease control and PFS, whereas intermediate FAP expression was associated with more favorable outcomes, suggesting that optimal co-stimulation may require sufficient but not excessive FAP targeting[46]. Partial responses in CMS2 tumors and immune-excluded lesions support redirection of stromal T cells by CD3-engaging bispecific antibodies enhanced by tumor-localized 4-1BB co-stimulation[47,48].

On-treatment biomarkers confirmed robust immune activation, including sustained induction of IFNγ, soluble CD25, activated CD8⁺ T cell subsets and soluble 4-1BB, consistent with 4-1BB pathway engagement[28]. A transient decrease in circulating lymphocytes after dosing was consistent with redistribution observed with T cell engagers[7,18,49,50], and early reductions in serum CEA and ctDNA were associated with improved outcomes[51–53]. Baseline ctDNA did not clearly associate with liver metastatic burden, suggesting that it reflects overall tumor burden, although interpretation is limited by sample size.

Treatment also induced adaptive immune regulatory programs, including PD-L1, LAG3, EOMES and IDO1 (ref. 54), alongside a more proinflammatory myeloid milieu and increased antigen presentation not seen with cibisatamab monotherapy[18]. These findings support potential benefit from PD-(L)1 blockade combinations[55], although cross-study comparisons remain exploratory.

This study has several limitations. As a phase 1, non-randomized, dose-escalation trial, the sample size was limited, and biomarker analyses were performed in relatively small subsets based on sample availability. Nevertheless, the consistent clinical, pharmacodynamic and biomarker findings support the biological rationale for combining T cell-engaging bispecific antibodies with tumor-localized 4-1BB co-stimulation and warrant further investigation of this strategy in solid tumors.

In conclusion, cibisatamab plus FAP-4-1BBL demonstrates that spatially restricted co-stimulation can enhance intratumoral T cell activation and antitumor activity while maintaining an acceptable safety profile in MSS CRC. These findings provide proof of principle that localized co-stimulatory signaling can augment the efficacy of T cell engagers without recapitulating the systemic toxicities associated with untargeted agonists and support further development of T cell engager combinations incorporating tumor-restricted co-stimulation.

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/s41591-026-04380-z.

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Methods

Inclusion and ethics

This study was an open-label, multicenter, phase 1b dose-escalation trial conducted across 15 globally approved clinical sites, of which 12 actively recruited patients. The trial was performed in accordance with Good Clinical Practice guidelines and applicable regulatory requirements. Approval was obtained from institutional review boards or independent ethics committees at each participating site. All patients provided written informed consent prior to enrollment and before the initiation of any study-related procedures.

Ethics approval and consent

Details of the boards/committees and approvals are provided below:

Netherlands: Sekretariatet for De Videnskabsetiske Komiteer for Region Hovedstaden. Date of approval: 30 November 2021

Canada: Ontario Cancer Research Ethics Board. Date of approval: 3 June 2022

Denmark: Medisch-Ethische Toetsingscommissie. Date of approval: 15 March 2022

UK: NHS Health Research Authority. Date of approval: 7 March 2022

Spain: Comité de Ética de la Investigación con Medicamentos. Date of approval: 8 April 2022

Korea: Korean Institutional Review Board, Seoul. Date of approval: 16 April 2022

Trial design, patients and treatments

The study followed a standard 3 + 3-style dose-escalation framework designed to explore different FAP-4-1BBL exposure profiles. The two study parts were conducted sequentially rather than in parallel, with the weekly FAP-4-1BBL dose-escalation cohorts in part 1 informing the dose levels and schedules subsequently explored in part 2. Part 1 evaluated continuous exposure to FAP-4-1BBL administered QW, whereas part 2 investigated a pulsatile exposure strategy with FAP-4-1BBL administered Q3W. In both study parts, cibisatamab was administered intravenously Q3W, with dosing consisting of either 100 mg in all cycles or a step-up regimen of 60 mg in cycle 1 followed by 100 mg thereafter (Extended Data Fig. 1).

At study initiation, participant eligibility required high tumor CEACAM5 expression determined by quantitative PCR analysis of formalin-fixed paraffin-embedded (FFPE) tumor tissue, consistent with previous cibisatamab studies[18]. After enrollment of the first 11 patients, this requirement was removed to enable assessment of the cibisatamab and FAP-4-1BBL combination in an unselected CRC population, reflecting the high prevalence of CEA expression in CRC[18].

Key inclusion criteria included histologically confirmed mCRC with pMMR/MSS status, progression after at least two prior lines of therapy, measurable disease per Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) and adequate organ function. Key exclusion criteria included prior treatment with CEA-targeting T cell engagers, active autoimmune disease requiring systemic therapy and uncontrolled comorbid conditions.

The primary endpoints were safety, tolerability and DLTs. Secondary endpoints included preliminary antitumor activity assessed by RECIST v1.1, pharmacokinetics, immunogenicity and pharmacodynamic effects. Exploratory endpoints included biomarker analyses, including tumor and circulating immune parameters and ctDNA dynamics.

ADA mitigation strategy

To mitigate the development of ADAs against cibisatamab, as previously reported[18], patients received B cell-depleting obinutuzumab as pretreatment 1–2 weeks prior to cycle 1 day 1. Repeat dosing of obinutuzumab was planned after approximately 6 months of treatment in patients who remained on study therapy. This approach was intended to suppress de novo ADA formation while preserving preexisting humoral immunity mediated by long-lived plasma cells.

Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. CRS was graded according to American Society for Transplantation and Cellular Therapy (ASTCT) consensus criteria. Study objectives and corresponding endpoints for BP42675 are summarized in the study protocol provided as Supplementary Information.

Statistical analysis

Safety and efficacy analyses were conducted in the intention-to-treat population unless otherwise specified. The safety population included all patients who received at least one dose of study treatment. One participant discontinued treatment before the first post-baseline tumor assessment and was classified as having progressive disease in efficacy analyses, in accordance with intention-to-treat principles.

No formal sample size calculation was performed. The sample size was based on standard considerations for phase 1 dose-escalation studies to evaluate safety, tolerability and pharmacokinetics.

Pharmacokinetic sampling and parameter calculation

Serial pharmacokinetic samples were collected from all patients to characterize the pharmacokinetic profiles of FAP-4-1BBL and cibisatamab after initial administration. Based on these data, key pharmacokinetic exposure parameters were calculated for each participant using standard non-compartmental analysis. Only patients with a sufficient number of pharmacokinetic samples to allow robust non-compartmental analysis were included in exposure–response analyses.

Exposure–safety analysis

Potential associations between FAP-4-1BBL exposure and the incidence and severity of adverse events of particular interest were evaluated in all safety-evaluable patients. Adverse events assessed included CRS, IRR, rash, diarrhea and colitis.

The pharmacokinetic exposure metric selected for analysis was tailored to the anticipated timing and mechanism of each adverse event. For acute events such as CRS and IRR, which typically occur shortly after infusion, exposure was evaluated using C_{max} after the first dose. For adverse events with potentially delayed onset, including rash, diarrhea and colitis, cumulative exposure over the first 63 days (AUC_{0-63d}) was used, corresponding to the period during which drug concentrations were expected to approach steady state. Exposure–safety analyses were stratified by QW and Q3W dosing regimens. Exploratory box plots were generated to visualize the distribution of exposure metrics across adverse event grades.

Baseline and pharmacodynamic assessments

Pharmacodynamic assessments were conducted using whole blood, plasma and tumor tissue samples, all of which were analyzed centrally. Whole blood and plasma samples were collected to evaluate changes in soluble biomarkers, cytokines and immune cell subsets using validated flow cytometry and immunoassay methods. Tumor tissue samples were analyzed by IHC, immunofluorescence and whole-transcriptome RNA-seq to assess biomarkers of tumor inflammation and immune infiltration, including $CD8^+$ tumor-infiltrating lymphocytes and immune-related gene signatures.

On-treatment tumor biopsies for the combination study were obtained on cycle 2 day 2 (day 23). In the cibisatamab monotherapy study, on-treatment biopsies were collected at either cycle 2 day 1 (day 21; Q3W dosing) or cycle 7 day 1 (day 43; QW dosing), reflecting differences in treatment schedules.

Cytokines, soluble 4-1BB and CEA assessment

Blood samples were collected at baseline and at 1, 8, 15, 22, 29, 36 and 43 days after the first administration of FAP-4-1BBL. Peripheral

inflammatory cytokines, soluble CD25, soluble 4-1BB and serum CEA were quantified using validated ELISA-based platforms, including ProteinSimple Ella cartridges for IFNγ, IL-6, IL-2 receptor and TNF; PeproTech ELISA for soluble 4-1BB; and the Elecsys CEA assay (cobas E170).

For cytokine analyses, values below the lower limit of quantification (LLOQ) were imputed as 0.5× LLOQ with the addition of a small random noise term to facilitate model convergence, followed by $\log_2$ transformation ($\log_2$(value + 1)). Soluble CEA values were analyzed without imputation and were $\log_{10}$ transformed. Soluble 4-1BB values below the LLOQ were handled similarly to cytokines and $\log_{10}$ transformed. All analyses were performed on changes from baseline.

Random-effects models were used for longitudinal analyses, with participant specified as a random effect and visit timepoint as a fixed effect. Correction for multiple testing was performed using the false discovery rate (FDR), with statistical significance defined as FDR < 0.05.

Flow cytometry

Peripheral blood samples were collected in sodium heparin tubes and processed according to validated assay protocols. All staining and incubation steps were performed at room temperature in the dark. Data acquisition was performed using FACSCanto II instruments (BD Biosciences), with analysis conducted using FACSDiva software and assay-specific acquisition templates. Cell preparation was automated using a FACS Lyse Wash Assistant (BD Biosciences).

Surface staining was performed using antibody cocktails applied to whole blood samples, followed by red blood cell lysis, washing and resuspension in PBS. For intracellular staining, samples were additionally permeabilized using Perm Buffer II prior to incubation with intracellular antibody cocktails. A complete list of antibody reagents is provided as supplementary data.

Immunohistochemistry

Tumor tissue was collected at baseline and on cycle 2 day 2 (±2 days where feasible), preferentially from the same lesion. All samples were processed and paraffin embedded according to standardized histopathology protocols. FFPE tissue sections were stained for hematoxylin and eosin, IHC and immunofluorescence at Discovery Life Sciences or Roche Tissue Diagnostics. Only samples meeting predefined quality control criteria for tumor content and tissue integrity were included. Details of staining assays are provided as supplementary data.

CD3/CD8/Ki67/4-1BB/OX40 multiplex immunofluorescence

Multiplex immunofluorescence staining using five primary antibodies and tyramide signal amplification fluorophores was performed on a Ventana DISCOVERY ULTRA IHC/ISH platform, as previously described[18]. Whole-slide imaging was performed using a Zeiss Axio Scan.Z1 scanner. Quantitative image analysis was conducted using a custom-developed algorithm implemented in HALO (Indica Labs). Antibody and fluorophore details are provided as supplementary data.

Management of adverse events

Adverse events were managed according to protocol-defined guidelines and institutional standards of care. Premedication with low-dose corticosteroids and non-steroidal anti-inflammatory drugs was routinely administered prior to study drug infusions to mitigate infusion-related reactions and CRS. Treatment interruption, dose delay or discontinuation was implemented according to protocol-defined toxicity management guidelines. CRS was graded according to ASTCT criteria. Patients were closely monitored during early treatment cycles when CRS events were most frequently observed. Management strategies included symptomatic treatment with antipyretics and intravenous fluids, corticosteroids for more severe symptoms and temporary interruption of study treatment when clinically indicated. Patients with more pronounced CRS manifestations were hospitalized for observation and supportive care as needed. Tocilizumab, an IL-6 receptor antagonist, was administered in selected cases of clinically manifested CRS in accordance with institutional practice and established CRS management guidelines. Most CRS events occurred early during treatment and resolved with supportive management.

Gastrointestinal adverse events associated with CEA-directed T cell engagement, including diarrhea and colitis, were managed using standard supportive care measures, such as antidiarrheal agents, hydration and corticosteroids in cases of suspected immune-mediated enterocolitis. Diagnostic evaluation for infectious etiologies was performed when clinically indicated. Participants with severe gastrointestinal toxicity underwent treatment interruption and additional clinical assessment to guide management.

Participants received obinutuzumab pretreatment for B cell depletion to mitigate ADA formation against cibisatamab. Monitoring for infectious complications was performed throughout the study. Routine antimicrobial or antiviral prophylaxis was not mandated by the study protocol and, therefore, was not systematically administered. Suspected infections were managed according to institutional standards of care, including targeted antimicrobial therapy where appropriate. In the case of CMV colitis observed during the study, diagnostic confirmation and antiviral treatment were initiated according to institutional practice.

Overall, adverse event management was guided by protocol-defined safety procedures, investigator clinical judgment and established institutional practices to ensure participant safety during treatment.

Statistical considerations

The safety, efficacy, pharmacokinetic and pharmacodynamic analysis populations comprised all participants who received at least one dose of the combination of FAP-4-1BBL and cibisatamab. For efficacy analyses, participants were required to have at least one evaluable post-baseline assessment, such as a tumor response evaluation.

Dose escalation and dose selection were guided by a modified continual reassessment method (mCRM) incorporating overdose control through the escalation with overdose control (EWOC) framework, in conjunction with clinical judgment. Multiple participants were enrolled at each dose level, with a minimum of three participants required per level to inform mCRM-based decisions. Escalation decisions were made jointly by the sponsor and participating investigators after a comprehensive review of available safety data and in accordance with the clinical trial protocol.

Statistics for IHC and immunofluorescence analyses

Pharmacodynamic analyses were restricted to patients with matched baseline and on-treatment tumor samples. Paired changes were assessed using Wilcoxon matched-pairs signed-rank tests. Comparisons between pooled complete response/partial response patients and stable disease/progressive disease patients were conducted using unpaired Mann–Whitney tests. Additional comparisons were performed between patients with progressive disease and all remaining patients. All analyses were performed using GraphPad Prism version 10.0.0 software.

Statistics for baseline associations with response

Associations between baseline biomarkers and clinical response were evaluated using unpaired Mann–Whitney tests for continuous variables. Fisher's exact test was used to assess associations among liver metastases, consensus molecular subtype (CMS) and objective response. PFS and overall survival were estimated using Kaplan–Meier methods with 95% confidence intervals, and group comparisons were performed using log-rank tests.

Longitudinal analyses of circulating cytokines and flow-cytometry-derived immune cell populations were performed using random-effects models, with participant specified as a random effect

and visit timepoint as a fixed effect. Correction for multiple testing was applied using the FDR, with statistical significance defined as FDR < 0.05. All analyses were conducted using the lme4 and multcomp packages in R.

In addition to longitudinal model-based analyses, non-parametric statistical tests were used for paired and groupwise comparisons. Paired longitudinal changes were assessed using two-tailed Wilcoxon matched-pairs signed-rank tests. Comparisons between pooled responders (complete or partial response) and non-responders (stable or progressive disease) were performed using two-tailed unpaired Mann–Whitney *U*-tests. Associations between baseline biomarker values and clinical response categories were evaluated using two-tailed unpaired Mann–Whitney *U*-tests for continuous variables.

Gene expression analyses

RNA sequencing and processing. RNA was isolated from FFPE tumor tissue using the Qiagen AllPrep DNA/RNA FFPE kit. Library preparation was performed using the Illumina TruSeq RNA Exome kit. Basecalling was conducted using bcl2fastq2, and sequence quality was assessed using FastQC[56]. Paired-end reads were aligned to the human genome (hg38) using STAR. Quality control was performed using MultiQC[18]. Gene-level read counts were generated using featureCounts[57] and normalized to transcripts per million (TPM).

Differential gene expression and signature analyses. Differential gene expression analyses were conducted using the limma package with log-transformed TPM values. Single-sample gene signature scores were calculated as the mean *z*-score of constituent genes. For signatures with upregulated and downregulated components, *z*-scores for downregulated genes were sign inverted. Group comparisons were performed using limma.

Adjustment of *P* values. Raw *P* values were adjusted for multiple testing using the Benjamini–Hochberg procedure[58].

ctDNA analysis. ctDNA was analyzed using a personalized, tumor-informed, 16-plex PCR next-generation sequencing assay (Signatera RUO; Natera)[59]. Patient-specific somatic variants were identified from whole-exome sequencing data and used to design multiplex PCR assays. Plasma samples with two or more detected variants were classified as ctDNA positive, and ctDNA levels were reported as MTM/ml.

Whole-exome sequencing. Whole-exome sequencing was performed on DNA extracted from macrodissected FFPE tumor tissue and matched germline DNA from peripheral blood, as previously described[59]. Reads were aligned to hg38 using BWA; duplicates were removed; and base quality score recalibration was performed using GATK[60]. Somatic variants were identified using Mutect2 and annotated with Variant Effect Predictor (VEP)[61]. Tumor mutational burden was calculated after standard filtering and normalized per megabase[62,63].

ctDNA data analysis and statistics. Kaplan–Meier analyses for PFS and overall survival were performed using survfit2 and visualized using ggsurvfit[64]. Patients were stratified into high and low ctDNA groups based on median baseline values. Landmark analyses at cycle 3 day 1 were applied to minimize immortal time bias, with follow-up reanchored at the landmark timepoint.

Reporting summary

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

Data availability

Qualified researchers may request access to individual participant-level data and supporting documents for Roche-sponsored studies via the Vivli platform (https://vivli.org/ourmember/roche/). Access is granted to researchers with appropriate qualifications who submit a scientifically sound proposal and analysis plan, subject to independent review for scientific merit and compliance with patient consent and data-sharing policies. Eligible studies typically include phase 2–4 trials after regulatory review and a defined waiting period that varies depending on the nature of the trial. Approved requesters must sign a data use agreement and access data within a secure environment. Initial review may occur within days; however, full review, contracting and data access typically take up to 3 months.

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Acknowledgements

We thank the patients for their participation in the study; the study nurses and site coordinators for their professionalism and dedication in supporting the conduct of the study; C. Claus, C. Klein, V. Karanikas and M.-H. Wasmer for continuous support and valuable discussions; A. Geiger for ensuring drug availability; M. Wilde, N. Kumpesa and M. Cordonnier for their contributions to study setup; and C. Oii for providing the initial RNA-seq analysis and D. Dejardin and V. Vasylenko for assistance during the revision of the manuscript. We also thank S. Heyn for editorial assistance with the preparation of this article as well as the numerous anonymous individuals at the research sites and at Roche who have been essential in supporting this project. Medical writing and editorial assistance were provided by A. R. Choudhury in accordance with Good Publication Practice (GPP3) guidelines and were funded by F. Hoffmann-La Roche Ltd. This work was supported by F. Hoffmann-La Roche Ltd., which substantially contributed to the study design in collaboration with study investigators; contributed to the collection of data; substantially contributed to the analysis and interpretation of the data in collaboration with study investigators; contributed to the writing of the report with study collaborators; and agreed with the study investigators in the decision to submit the manuscript for publication. No grant number is applicable.

Author contributions

I.M., E.C.A., C.Q., M.D., I.B., S.B., F.T., M.C.R., M.M.G., V.W., T.W.K. and V.M. were clinical investigators on the study. A.B., E.G., C.H., T.T., H.H., C.M., P.U. and I.M. planned, designed and refined the study. A.B. led

the Roche study team. T.T., E.G. and A.B. wrote the initial draft of the manuscript, and all authors reviewed and edited the manuscript. C.H., I.D. and T.T. conducted biomarker analyses. C.M. conducted pharmacokinetics analysis. L.C. developed the clinical analysis plan and analyzed data. H.H. conducted the safety analysis. E.G., E.C. and O.K. provided medical support and oversight. T.S. performed operations oversight.

Competing interests

T.T., P.U., C.M., L.C., C.H., H.H., T.S., I.I.D., E.G. and A.B. are employees of F. Hoffmann-La Roche Ltd. and/or Genentech Inc. and may hold Roche stock or stock options as part of their employment compensation. I.B. reports accommodation and travel expenses from Amgen, Merck, Sanofi and Servier and personal speaker honoraria from AstraZeneca and Amgen. V.M. declares financial and non-financial interests with AbbVie, Achilles, Adaptimmune, ADC Therapeutics, Ascendis Pharma, AstraZeneca, Bayer, BeiGene, Bicycle Therapeutics, BioInvent, Biomea Fusion, BioNTech, Bristol Myers Squibb, Boehringer Ingelheim, C4 Therapeutics, Calico Life Sciences, Celgene, Constellation Pharmaceuticals, Crescendo Biologics, Cullinan Therapeutics, Daiichi Sankyo, Debiopharm, Dragonfly Therapeutics, Enliven Therapeutics, Epizyme, Exelixis, FameWave, F-Star Beta Limited, Genentech, Genmab, Gilead, Grey Wolf Therapeutics, GlaxoSmithKline, Hexal Ag & Sandoz, HiFiBiO, Hookipa Biotech, HUTCHMED, IGM Biosciences, ImCheck Therapeutics, Immunocore, Immutep, Incyte, iOmx Therapeutics, Iovance, Italfarmaco, Iteos, Janssen, Light Chain Bioscience, Lilly, Loxo Oncology, Merck, Merus, Miltenyi Biomedicine, Monta Biosciences, Merck Sharp & Dohme, Mythic Therapeutics, Ningbo Newbay, Novartis, Oxford Biotherapeutics, Pfizer, PharmaMar, PMV Pharma, Prelude Therapeutics, Pyxis Oncology, Regeneron, Relay Therapeutics, Repare Therapeutics, Revolution, Roche, Schrödinger, Scorpion Therapeutics, Seagen, Shattuck Labs, Synthorx, Takeda, Tango Therapeutics, Tesaro, Totus Medicines, Turning Point Therapeutics and Vividion Therapeutics. F.T. declares institutional funding from Achilles, Adaptimmune, Amgen, Bicycle Therapeutics, BioNTech, Bristol Myers Squibb, Byondis, Chugai, Corbus, Crescendo Biologics, Epkin, Grey Wolf Therapeutics, GlaxoSmithKline, Immunocore, Iovance, Janssen, Kymab/Sanofi, Leucid, Moderna, Novalgen, Nucana, Oxford Vacmedix, Roche, RS Oncology, Seagen, Takeda, T-Knife Therapeutics, UCB, Zelluna and Zymeworks; advisory board/consultancy/honoraria from AstraZeneca, Grey Wolf Therapeutics, OncoBayes, Pan Cancer T, T-Knife Therapeutics and Waypoint; and data and safety monitoring board for Immatics. I.M. declares the following interests: grants from Roche, AstraZenca, Bristol Myers Squibb and Genmab and consultancy for Roche, Genmab, Regeneron, Pioneer Medicines, Bright Peak Therapeutics, PharmaMar, Curon, Mestag Therapeutics and Light Chain Bioscience. E.C. reports the following interests: ownership interests in Oncoart Associated; honoraria from HM Hospitales Group; consulting/advisory roles at Nanobiotix, Janssen-Cilag, Roche/Genentech, TargImmune Therapeutics, Servier, Bristol Myers Squibb, Amunix, Adcendo, Anaveon, AstraZeneca/MedImmune, Chugai, MonTa, MSD Oncology, Nouscom, Novartis, OncoDNA, T-Knife Therapeutics, Elevation Oncology, PharmaMar, Ellipses Pharma, Syneos Health, Genmab and Diaccurate; and president and founder of Foundation INTHEOS. T.W.K. declares institutional funding from Genentech and Inocras. S.H.B. declares the following interests: researcher/grant/contract (clinical trial principal investigator): Roche, Merck Sharp & Dohme, Bayer, Boryung, Takeda, Jeil Pharmaceutical, Merck, Janssen, Pfizer, Amgen, Revolution Medicines, IGM Biosciences and STCube. All other authors not listed above declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-026-04380-z.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-026-04380-z.

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Peer review information *Nature Medicine* thanks Andrew Ko, Benjamin Schlechter, and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available. Primary Handling Editor: Ulrike Harjes, in collaboration with the *Nature Medicine* team.

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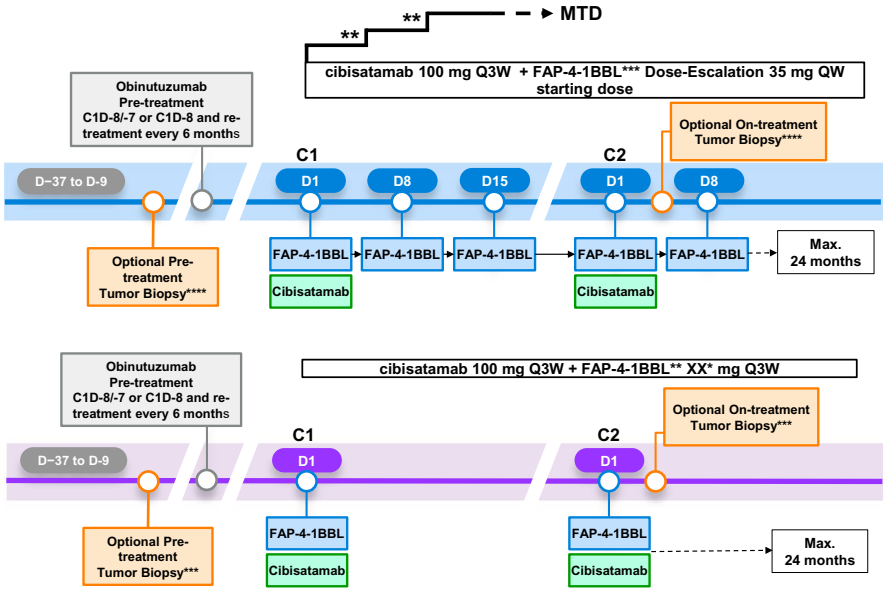
https://doi.org/10.1038/s41591-026-04380-z



Extended Data Fig. 1 | Dose escalation schema of FAP-4-1BBL in combination with cibisatamab across weekly and 3-weekly dosing schedules. a. Study design Part 1: Q3W cibisatamab in combination with QW FAP-4-1BBL dose-escalation*. **b**. Study design Part 2: Q3W cibisatamab in combination with Q3W FAP-4-1BBL*. C = Cycle; D = Day; MTD = Maximum tolerated dose; QW = Once a week; Q3W = Every 3 weeks. Modified continual reassessment method (mCRM) with escalation with overdose control (EWOC) design; n ≥ 3 per cohort; N: up to approximately 60 evaluable participants (Part 1 and Part 2 combined not to exceed approximately 80 evaluable participants). For a FAP-4-1BBL QW schedule in combination with cibisatamab in a Q3W schedule, 1 cycle 21 days, which corresponds to 3 doses of FAP-4-1BBL and 1 dose of cibisatamab. * Depending on the nature and the time course of emerging safety events, the dosing frequency for FAP-4-1BBL during Part 1 can be modified as described in Protocol Section 4.1. ** If deemed necessary to further characterize the safety, PK, and/or pharmacodynamic profile of FAP-4-1BBL, additional participants may be enrolled at specific dose levels. *** Based on emerging safety data, FAP-4-1BBL may be initiated only at C1D8,15 or C2D1. **** For the first 3 evaluable participants at every dose level, optional tumor biopsies at baseline and on treatment can be collected. For each additional evaluable participant at each dose level (up to a maximum of 9 participants), mandatory tumor biopsies at baseline and on treatment will be collected. C = Cycle; D = Day; Q3W = Every 3 weeks. A maximum of 5 specific dose levels will be tested; N: Up to approximately 40 evaluable participants (Part 1 and Part 2 combined not to exceed approximately 80 evaluable participants). For the FAP-4-1BBL Q3W schedule in combination with cibisatamab in a Q3W schedule, 1 cycle 21 days, which corresponds to 1 dose of FAP-4-1BBL and 1 dose of cibisatamab. * The selection of FAP-4-1BBL dose levels will be based on emerging clinical data, including safety, PK, and/or pharmacodynamic from Part 1 and Part 2. ** Based on emerging safety data, FAP-4-1BBL may be initiated only at Cycle 2. *** For the first 3 evaluable participants at every dose level, optional tumor biopsies at baseline and on treatment can be collected. For each additional evaluable participant at each dose level (up to a maximum of 9 participants), mandatory tumor biopsies at baseline and on treatment will be collected.

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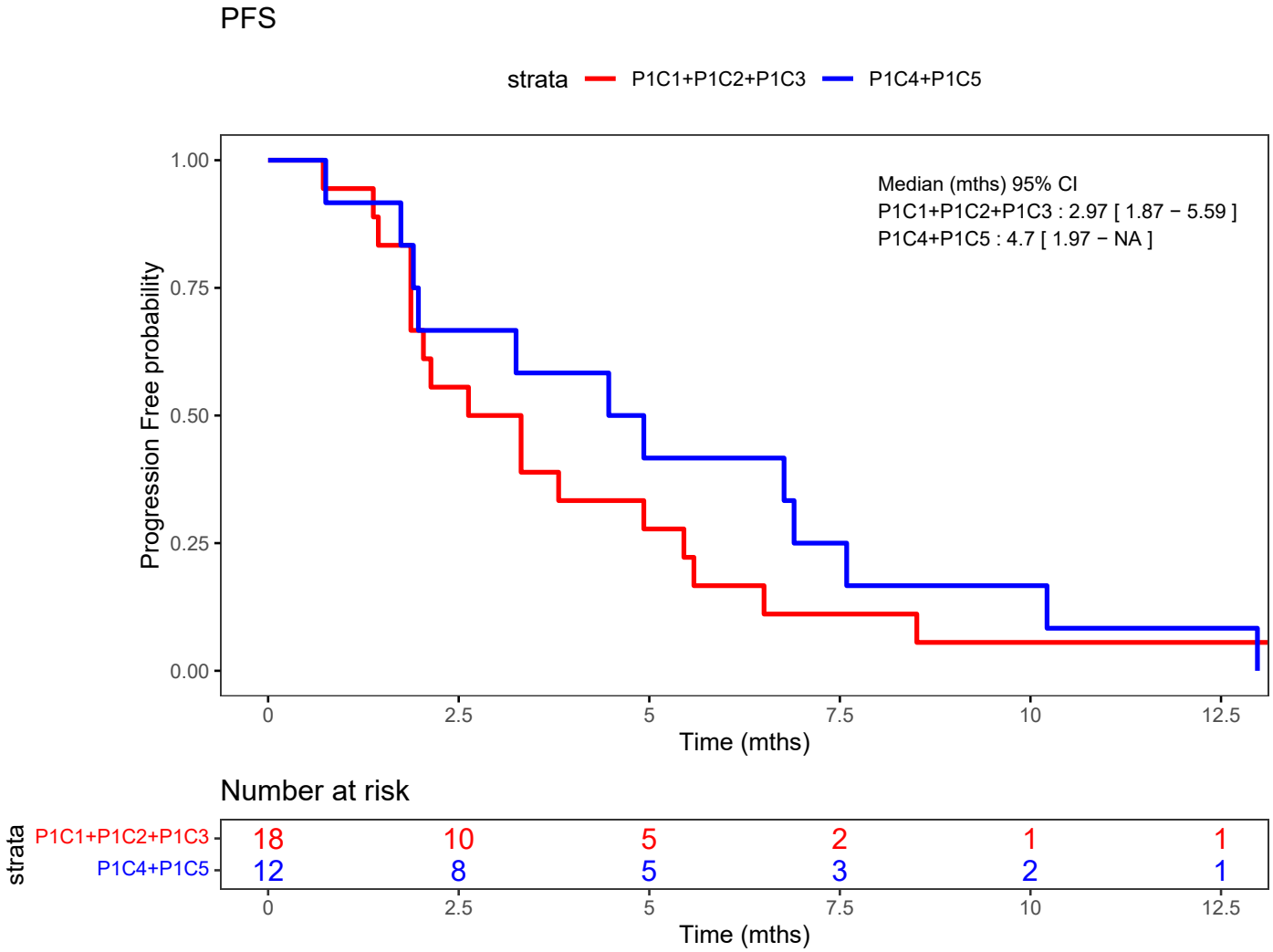

Extended Data Fig. 2 | KM Plot cibisatamab + FAP-4-1BBL QW low dose (35 + 50) vs high dose (90 + 130) - progression free survival. PFS was evaluable in 18 participants receiving low-dose FAP-4-1BBL QW (35 or 50 mg; blue line) and 12 participants receiving high-dose FAP-4-1BBL QW (90 or 130 mg; red dotted line). Both groups received cibisatamab Q3W. Small sample sizes limit the interpretation of the PFS data; however, consistent with PD data and responses, PFS appears to be longer in the FAP-4-1BBL higher dose cohorts compared with the lower dose cohorts. (See figure 4b KM plot). In the group of lower doses (n = 18), time to event median was 90.5 days (95% CI 57-150), while in the group of higher doses (n = 12), time to event median was 143 days (95% CI 60-210). Of note, all participants in the higher FAP-4-1BBL dose cohorts received a reduced cibisatamab dose of 60 mg at the first cycle, while 15 out of 18 participants received 100 mg cibisatamab also at the first cycle.

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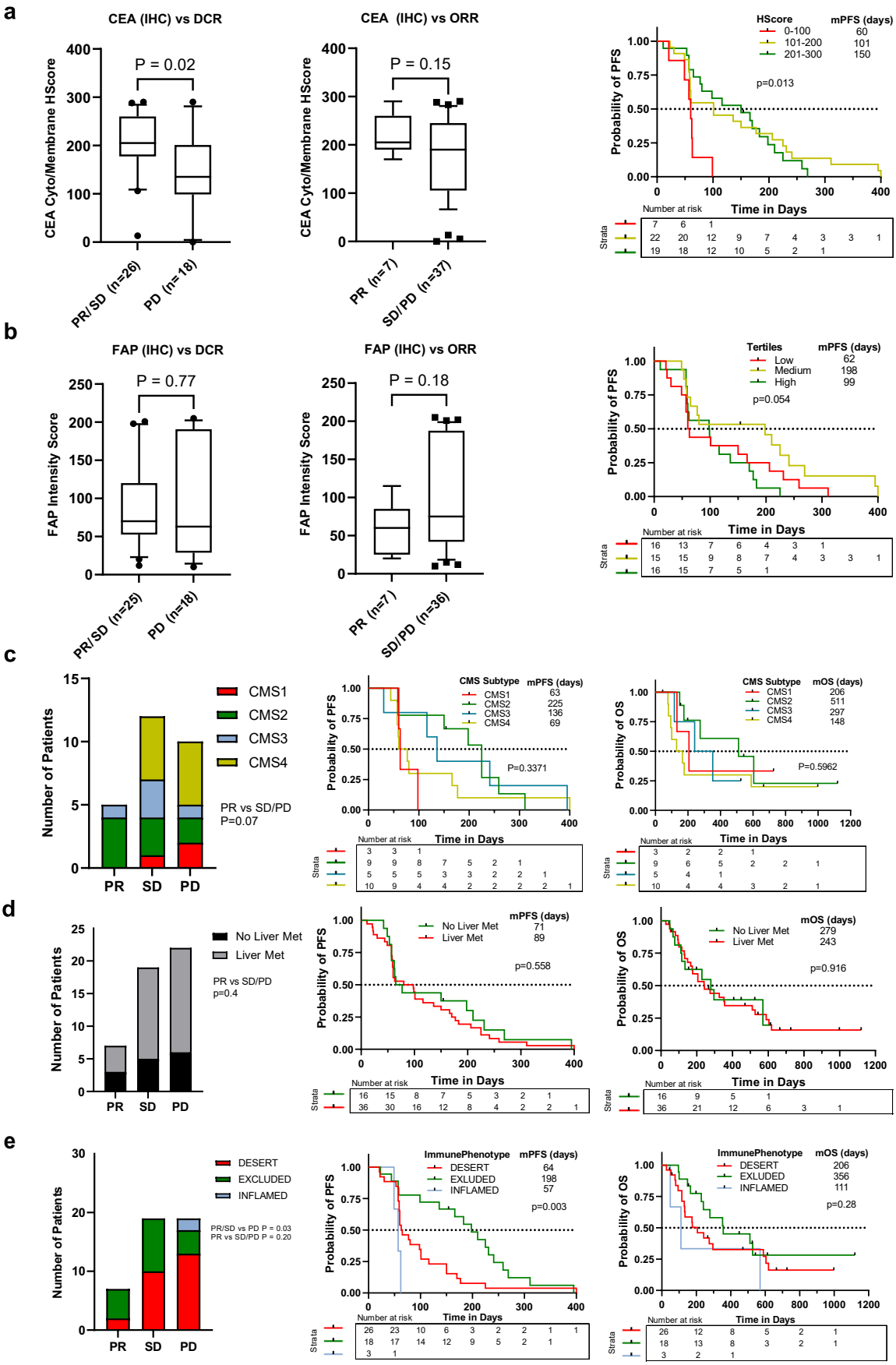

Extended Data Fig. 3 | See next page for caption.

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Extended Data Fig. 3 | Baseline biomarker associations with clinical outcomes. (**a**) Baseline tumor CEA expression by immunohistochemistry (IHC) H-score in relation to disease control rate (DCR; PR/SD vs PD) and objective response rate (ORR; PR vs SD/PD), with progression-free survival (PFS) stratified by CEA H-score categories (< 100, 101-200, and ≥201-300). Analyses included n = 44 for ORR/DCR and n = 48 for PFS. (**b**) Baseline FAP expression by IHC median intensity score in relation to DCR and ORR, with PFS stratified by FAP tertiles. Analyses included n = 43 for ORR/DCR and n = 47 for PFS. (**c**) Distribution of consensus molecular subtypes (CMS1-4) across best overall response categories (PR, SD, PD), with PFS and overall survival (OS) shown according to CMS subtype. Analyses included n = 27. (**d**) Best overall response according to the presence or absence of liver metastases, with corresponding PFS and OS analyses stratified by liver metastasis status. Analyses included n = 48 for best overall response and n = 52 for PFS/OS. (**e**) Baseline immune phenotype distribution (desert, excluded, inflamed) across best overall response categories, with corresponding PFS and OS analyses according to immune phenotype. Analyses included n = 45 for best overall response and n = 47 for PFS/OS.

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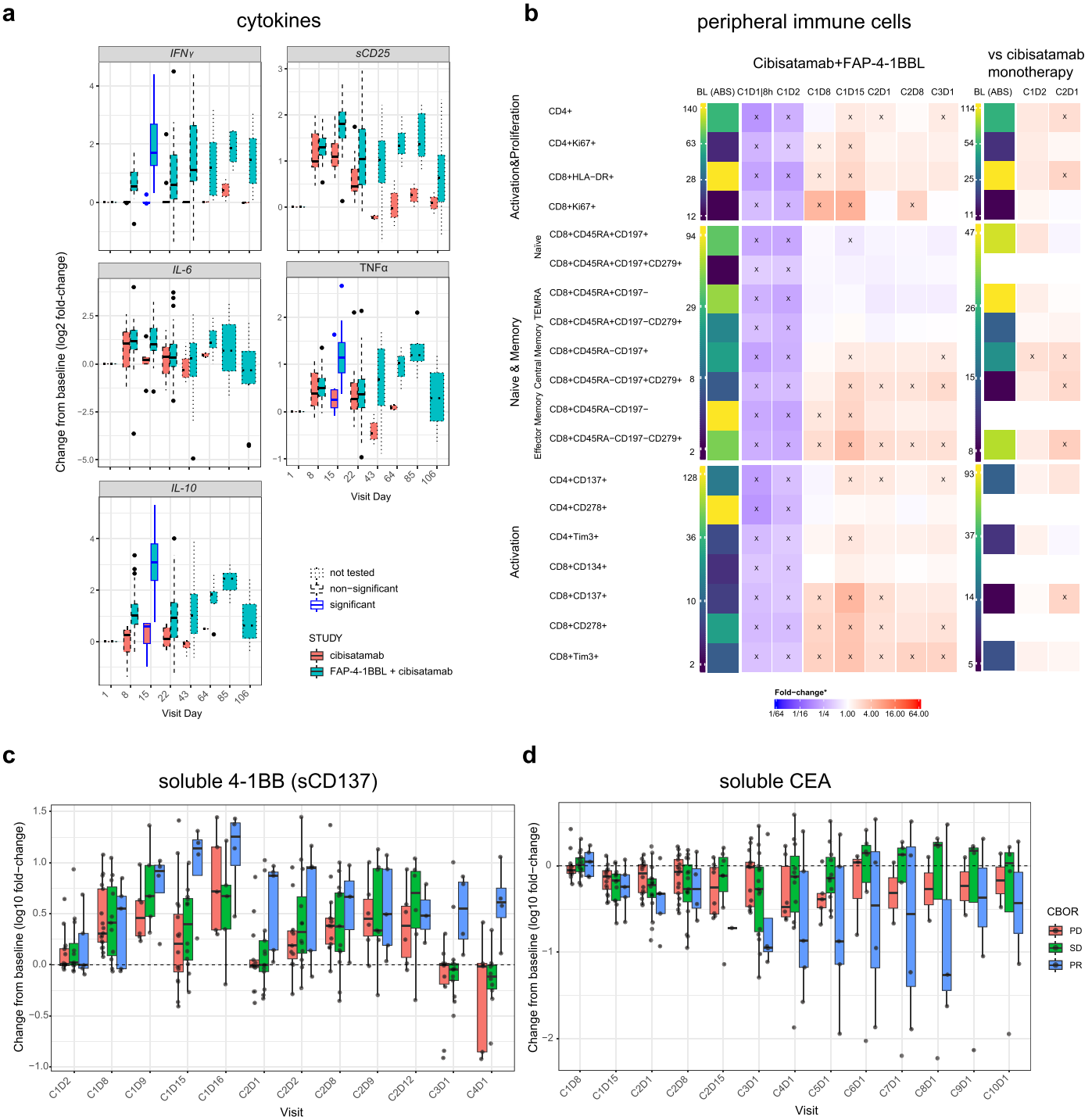

Extended Data Fig. 4 | Peripheral pharmacodynamic effects of cibisatamab plus FAP-4-1BBL. (**a**) Longitudinal changes in circulating cytokines following treatment with cibisatamab plus FAP-4-1BBL in comparison with cibisatamab monotherapy, including IFNγ, soluble CD25 (sCD25), IL-6, TNFα and IL-10, shown relative to baseline (cibisatamab + FAP-4-1BBL n = 44, cibisatamab monotherapy n=19). (**b**) Changes in peripheral immune-cell populations assessed by flow cytometry. The left panel includes n = 47 patients, and the right panel includes n = 23 patients, reflecting analyte-specific availability across visits. (**c**) Longitudinal changes in soluble 4-1BB (sCD137). Analyses include n = 39 patients in total, with per-timepoint sample sizes ranging from n = 14 to n = 33 across visits. (**d**) Longitudinal changes in serum CEA (sCEA). Analyses include n = 41 patients in total, with per-timepoint sample sizes ranging from n = 8 to n = 38. Boxplots show median and interquartile range; whiskers are based on 1.5 IQR. Longitudinal measurements were obtained from repeated samples from the same participants. Statistical analyses are described in the Methods. Differences in sample size across panels and timepoints reflect variability in biological sample and assay readout availability.

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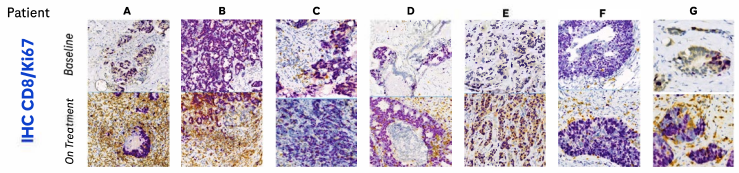
a Qualitative PD changes of CD8+ T cell infiltration

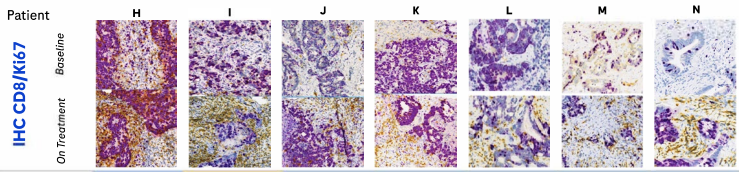
Flat regimen patients, first cibisatamab dose of 100mg



	A	B	C	D	E	F	G
BOR (best SLD)	PR (-37.1)	SD (-5.5)	SD (+17.2)	PD (+18.7 & NL*)	PD (+58.3)	PD (+60)	PR (-36.4)
Schedule	QW	QW	Q3W	Q3W	Q3W	Q3W	Q3W
Dose	35	35	35	35	35	35	35
C1D1 Dose	100	100	100	100	100	100	100
CD8+ FC	70.4	14.3	2.3	48.4	2.4	1.5	4.3
CD8+Ki67+ FC	94.9	14.2	1.2	74.5	5.4	0.6	9.1
CD8-Ki67+ FC	0.3	0.1	0.8	10	2.4	0.6	5
Immunephenoype Bsl	Desert	Desert	Desert	Desert	Desert	Desert	Excluded
Immunephneotype On-Tx	Excluded	Inflamed	Desert	Inflamed	Desert	Excluded	Excluded
biopsy site	Liver	Liver	Liver	Soft tissue	Liver	Liver	Liver
Bsl FAP HScore (Ventana)	25	12	120	195	48	-	120
Bsl CEA HScore	215	195	261	115	0	5	230

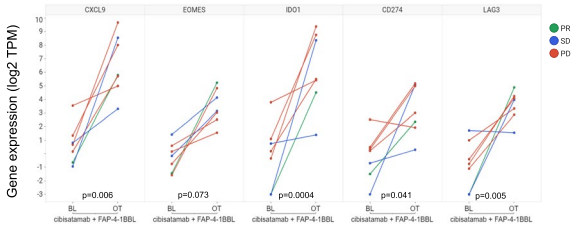
Step up dose patients. First cibisatamab dose of 60mg

*NL=New lesion

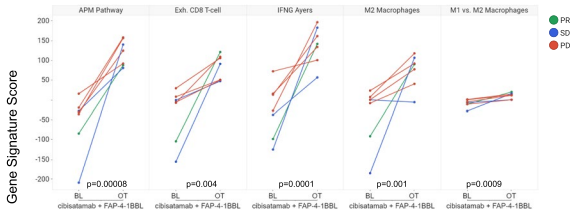


	H	I	J	K	L	M	N
BOR (best SLD)	PD (**-17.2**&NL)	SD (-4.7)	PD (+23.2 & NL*)	PD (+10.8)	PD (26.9)	PD (10.5 & NL)	SD (-6)
Schedule	Q3W	Q3W	Q3W	Q3W	Q3W	Q3W	Q3W
Dose	35	35	35	35	35	35	90
C1D1 Dose	60	60	60	60	60	60	60
CD8+ FC	1.8	10.5	3.5	9.8	86.5	24	8.4
CD8+Ki67+ FC	1.7	1	10.3	10.1	48.2	36.1	9.1
CD8-Ki67+ FC	0.9	0.1	1.9	0.6	0.5	0.5	2.4
Immunephenoype Bsl	Inflamed	Excluded	Desert	Desert	Desert	Desert	Desert
Immunephneotype On-Tx	Inflamed	Excluded	Inflamed	Desert	Excluded	Excluded	Excluded
biopsy site	Lymph Node	Liver	Liver	Liver	Liver	Liver	Peritoneum
Bsl FAP HScore (Ventana)	202	190	40	20	30	190	80
Bsl CEA HScore	290	288	105	80	105	195	275

b



c



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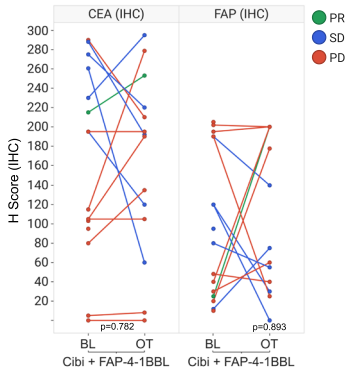
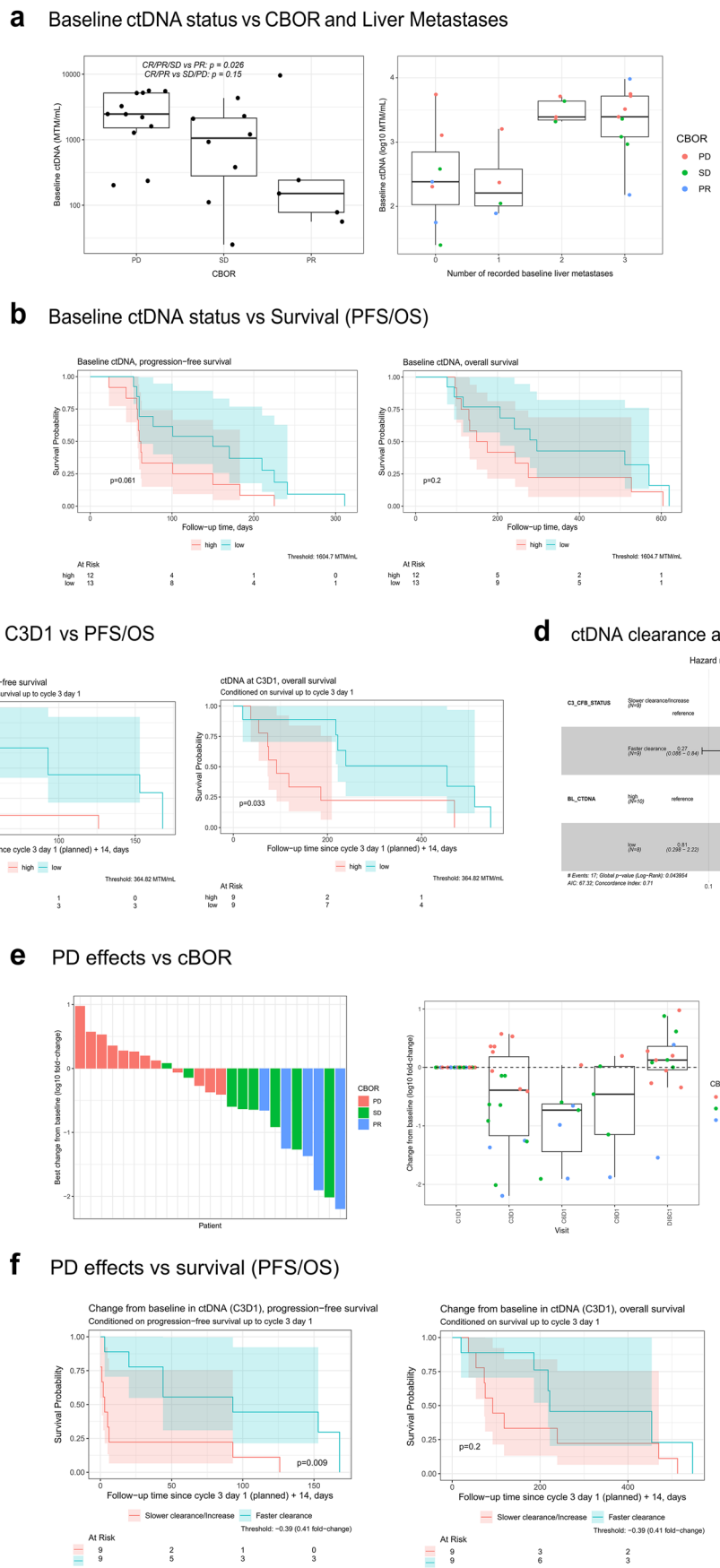


Extended Data Fig. 5 | Tumor pharmacodynamics. (**a**) Representative duplex CD8/Ki67 IHC of paired baseline and on-treatment biopsies from all participants providing fresh paired biopsies. Participants receiving cibisatamab 100 mg all cycles (top) or cibistamab 60 mg at cycle 1 followed by 100 mg for subsequent treatment cycles (bottom). Tables summarize BOR, treatment regimen, fold-change in CD8+ and CD8 + Ki67+ cells, immune-phenotype shifts, biopsy site, and baseline FAP/CEA scores. (**b**/**c**) RNA-seq of paired biopsies (n = 6) after combination treatment showing induction of APM, IFNγ response, cytotoxic T-cell, T-effector, and M2-macrophage signatures (**b**), with a modest increase in the M1/M2 index. Single-gene analysis demonstrated upregulation of CD274, CXCL9, EOMES, IDO1, and LAG3 (**c**). Lines denote BOR (PR, SD, PD). (**d**) Paired IHC showed no consistent change in CEA H-score and heterogeneous, non-directional changes in FAP intensity, suggesting continued target availability for cibisatamab and FAP-4-1BBL.

https://doi.org/10.1038/s41591-026-04380-z



Extended Data Fig. 6 | See next page for caption.

https://doi.org/10.1038/s41591-026-04380-z

Extended Data Fig. 6 | Circulating tumor DNA (ctDNA) dynamics, pharmacodynamic effects, and clinical outcomes. (a) Association of baseline ctDNA with best overall response (cBOR) and liver metastases. Left, baseline ctDNA levels (MTM/mL, log scale) stratified by cBOR (PD, SD, PR). Right, baseline ctDNA levels according to the number of baseline liver metastases. Individual patients are shown with overlaid boxplots; p-values from group comparisons are indicated. **(b)** Association of baseline ctDNA with survival outcomes. Kaplan–Meier curves for progression-free survival (PFS; left) and overall survival (OS; right) stratified by baseline ctDNA (high vs low, threshold as indicated). Shaded areas represent 95% confidence intervals; p-values from log-rank tests are shown. **(c)** Association of ctDNA status at Cycle 3 Day 1 (C3D1) with survival outcomes. Kaplan–Meier curves for PFS (left) and OS (right), conditioned on patients remaining event-free up to C3D1. Patients are stratified by ctDNA levels at C3D1 (high vs low, threshold indicated). **(d)** Forest plot of hazard ratios for PFS according to ctDNA dynamics and baseline ctDNA. Faster ctDNA clearance at C3D1 is associated with improved PFS compared with slower clearance/increase (reference). Hazard ratios with 95% confidence intervals and p-values are shown. Baseline ctDNA (low vs high) is included as a comparator. **(e)** Association of pharmacodynamic (PD) effects with cBOR. Left, waterfall plot showing best percentage change from baseline in ctDNA (log10 fold-change) per patient, colored by cBOR. Right, distribution of ctDNA changes across visits (for example, C1D1, C3D1, etc.) with individual patient data overlaid and stratified by cBOR. **(f)** Association of ctDNA dynamics at C3D1 with survival outcomes. Kaplan–Meier curves for PFS (left) and OS (right), conditioned on patients event-free at C3D1, comparing patients with faster ctDNA clearance versus slower clearance or increase (threshold indicated). Shaded areas represent 95% confidence intervals; p-values from log-rank tests are shown. Abbreviations: ctDNA, circulating tumor DNA; MTM, mean tumor molecules; cBOR, confirmed best overall response; PD, progressive disease; SD, stable disease; PR, partial response; PFS, progression-free survival; OS, overall survival.

https://doi.org/10.1038/s41591-026-04380-z

Extended Data Table 1 | Summary of adverse events

	Part I					Part II			All (N=52)
	Cohort 1 (N=12)	Cohort 2 (N=3)	Cohort 3 (N=3)	Cohort 4 (N=7)	Cohort 5 (N=5)	Cohort 1 (N=10)	Cohort 2 (N=9)	Cohort 3 (N=3)	
Total No. of pts with at least one AE, n (%)	12 (100%)	3 (100%)	3 (100%)	7 (100%)	5 (100%)	10 (100%)	9 (100%)	3 (100%)	52 (100%)
Total No. of Aes	250	36	44	153	92	159	222	18	974
Total No. of patients with at least one, n (%)									
AE with fatal outcome	1 (8.3%)	1 (33.3%)	0	0	0	0	1 (11.1%)	1 (33.3%)	4 (7.7%)
Serious AE	10 (83.3%)	3 (100%)	2 (66.7%)	4 (57.1%)	4 (80.0%)	5 (50.0%)	5 (55.6%)	1 (33.3%)	34 (65.4%)
AE leading to withdrawal from FAP-4-1BBL	1 (8.3%)	1 (33.3%)	0	2 (28.6%)	0	2 (20.0%)	1 (11.1%)	1 (33.3%)	8 (15.4%)
AE leading to FAP-4-1BBL dose modification/ dose interruption	6 (50.0%)	3 (100%)	2 (66.7%)	3 (42.9%)	4 (80.0%)	1 (10.0%)	2 (22.2%)	0	21 (40.4%)
AE related to FAP-4-1BBL leading to withdrawal from treatment	0	0	0	0	0	0	0	1 (33.3%)	1 (1.9%)
AE related to FAP-4-1BBL leading to dose modification/interruption	6 (50.0%)	3 (100%)	2 (66.7%)	3 (42.9%)	2 (40.0%)	1 (10.0%)	0	0	17 (32.7%)
Grade 3-5 AE	8 (66.7%)	3 (100%)	3 (100%)	6 (85.7%)	4 (80.0%)	6 (60.0%)	7 (77.8%)	1 (33.3%)	38 (73.1%)
No. of patients with a DLT	1 (8.3%)	1 (33.3%)	0	0	0	0	0	0	2 (3.8%)

https://doi.org/10.1038/s41591-026-04380-z

Extended Data Table 2 | Adverse events in ≥ 30% patients overall regardless of relationship to study treatment

	Part I					Part II			
MedDRA Preferred Term	**Cohort 1 (N=12)**	**Cohort 2 (N=3)**	**Cohort 3 (N=3)**	**Cohort 4 (N=7)**	**Cohort 5 (N=5)**	**Cohort 1 (N=10)**	**Cohort 2 (N=9)**	**Cohort 3 (N=3)**	**ALL**
Fatigue	8 (66.7%	2 (66.7%)	3 (100%)	5 (71.4%)	3 (60.0%)	6 (60.0%)	5 (55.6%)	1 (33.3%)	33 (63.5%)
Cytokine release syndrome	6 (50.0%)	1 (33.3%)	3 (100%)	6 (85.7%)	2 (40.0%)	6 (60.0%)	4 (44.4%)	2 (66.7%)	30 (57.7%)
Diarrhea	6 (50.0%)	1 (33.3%)	2 (66.7%)	5 (71.4%)	3 (60.0%)	5 (50.0%)	6 (66.7%)	1 (33.3%)	29 (55.8%)
Pyrexia	7 (58.3%)	1 (33.3%)	2 (66.7%)	2 (28.6%)	3 (60.0%)	5 (50.0%)	5 (55.6%)	1 (33.3%)	26 (50.0%)
Decreased appetite	7 (58.3%)	1 (33.3%)	1 (33.3%)	1 (14.3%)	4 (80.0%)	4 (40.0%)	4 (44.4%)	1 (33.3%)	23 (44.2%)
Cough	4 (33.3%)	0	0	3 (42.9%)	2 (40.0%)	3 (30.0%)	4 (44.4%)	1 (33.3%)	17 (32.7%)
Anemia	5 (41.7%)	3 (100%)	1 (33.3%)	3 (42.9%)	1 (20.0%)	1 (10.0%)	3 (33.3%)	0	17 (32.7%)
Nausea	4 (33.3%)	0	1 (33.3%)	3 (42.9%)	2 (40.0%)	2 (20.0%)	4 (44.4%)	0	16 (30.8%)
Arthralgia	5 (41.7%)	2 (66.7%)	1 (33.3%)	2 (28.6%)	2 (40.0%)	1 (10.0%)	3 (33.3%)	0	16 (30.8%)

https://doi.org/10.1038/s41591-026-04380-z

Extended Data Table 3 | Cytokine release syndrome

	Cibisatamab 60→100 mg dose (n=27)	Cibisatamab flat dose (n=25)	All Participants (n=52)
All grades	17 (63.0%)	13 (52.0%)	30 (57.7%)
Grade 1	14 (51.9%)	11 (44.0%)	25 (48.1%)
Grade 2	3 (11.1%)	0	4 (7.7%)
Grade 3	0	2 (8.0%)	2 (3.8%)
Grade 4	0	0	0
Grade 5	0	0	0
SAE	4 (14.8%)	9 (36.0%)	13 (25.0%)

https://doi.org/10.1038/s41591-026-04380-z

Extended Data Table 4 | Cibisatamab + FAP-4-1BBL treatment exposure (days)

Combination Schedule	FAP-4-1BBL Dose (mg)	Cibisatamab (mg)	# of participants evaluable for efficacy	FAP-4-1BBL Median	Cibisatamab Median	FAP-4-1BBL Mean	Cibisatamab mean
FAP-4-1BBL *QW* + Cibisatamab Q3W	35	100	12*	123	116	153.9	147.4
	50	100	3	57	50	59.3	52.3
		60→100	3	114	98	97.7	83.0
	90	60→100	7	99	85	121.7	133.9
	130		5	85	85	131.6	125.6
FAP-4-1BBL *Q3W* + Cibisatamab Q3W	35	100	10	64	64	108.1	108.1
		60→100	9	85	85	87.6	87.6
	90		3	43	43	40.7	40.7
All	All	All	52	85	85	111.9	110.2

60→100: cibisatamab 60 mg at C1, 100 mg from C2. *1 participant withdrew after a single study dose (FAP-4-1BBL +cibisatamab). However, the participant was reported with cPR later on without receiving any anticancer treatment after study withdrawal according to the main site investigator.

60→100: cibisatamab 60 mg at C1, 100 mg from C2. *1 participant withdrew after a single study dose (FAP-4-1BBL +cibisatamab). However, the participant was reported with cPR later on without receiving any anticancer treatment after study withdrawal according to the main site investigator.

nature portfolio

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Last updated by author(s): Jan 22, 2026

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	This study did not utilize custom algorithms or bespoke software that are central to the research and not previously described in the published literature. Therefore, code deposition in a public repository is not applicable.
Data analysis	Data analysis was conducted using standard, validated software packages and established workflows. Statistical analyses were performed using SAS and R. Graphical analyses and summary statistics were generated using GraphPad Prism. Flow cytometry data were analyzed using FlowJo. Digital pathology and image quantification were performed using HALO (Indica Labs). RNA sequencing analyses used established bioinformatics pipelines and widely used software tools as described in the Methods. No custom code was developed.

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Reporting on sex and gender	Sex was recorded as a biological variable for all participants and is reported in the baseline demographic characteristics. Gender identity was not specifically collected or analyzed. No sex- or gender-specific analyses were prespecified or conducted, as this was an exploratory phase 1 study not powered to detect such differences.
Reporting on race, ethnicity, or other socially relevant groupings	Race and ethnicity were recorded according to local regulatory and institutional practices at participating study sites and are summarized in the baseline characteristics where available. No analyses stratified by race or ethnicity were prespecified or performed, as the study was not designed or powered to assess outcome differences across these groupings.
Population characteristics	The study population comprised adult participants with microsatellite-stable metastatic colorectal cancer who had progressed after at least two prior lines of systemic therapy. Key demographic and clinical characteristics, including age, sex, disease status, prior treatments, and metastatic sites, are reported in the manuscript and Supplementary Information.
Recruitment	Participants were recruited at multiple international clinical sites in accordance with the study protocol. Recruitment occurred sequentially by dose-escalation cohort. Enrollment was based on predefined inclusion and exclusion criteria and was not influenced by sex, race, or ethnicity. Recruitment timelines and enrollment numbers are reported in the manuscript.
Ethics oversight	The trial was performed in accordance with Good Clinical Practice guidelines and applicable regulatory requirements. Approval was obtained from institutional review boards or independent ethics committees at each participating site. All patients provided written informed consent prior to enrolment and before the initiation of any study-related procedures. Ethical Approval and Consent Details of the IRB committees and approvals are provided below: Netherlands: Sekretariatet for De Videnskabsetiske Komiteer for Region Hovedstaden. Date of Approval. 30/November/2021 Canada: Ontario Cancer Research Ethics Board, Date Approval Issued: 03/June/2022 Denmark: Medisch-Ethische Toetsingscommissie, Date Approval Issued: 15/March/2022 UK: NHS Health Research Authority, Date Approval Issued: 07/March/ 2022 Spain: COMITÉ DE ÉTICA DE LA INVESTIGACIÓN CON MEDICAMENTOS. Date Approval Issued: 08/April/ 2022 Korea: Korean Institutional Review Board, Seoul, Date Approval Issued: 16/ April/ 2022

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*
Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
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Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

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Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
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Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☐	☒ Dual use research of concern
☐	☒ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Antibodies were used for flow cytometry, immunohistochemistry (IHC), and multiplex immunofluorescence (mIF). For flow cytometry, antibodies included CD3 (clone SK7, BD Biosciences, catalog numbers 563798 and 345764; clone UCHT1, BD Biosciences, catalog number 563109), CD4 (clone SK3/SK4, BD Biosciences, catalog number 347413; clone SK3, BD Biosciences, catalog number 562970), CD8 (clone SK1/RPA-T4, BD Biosciences, catalog number 341050; clone SK1, BD Biosciences, catalog number 641400), CD16 (clone 3G8, BD Biosciences, catalog number 563172), CD19 (clone HIB19, BD Biosciences, catalog number 557921), CD45RA (clone HI100, BD Biosciences, catalog number 550855), CD56 (clone NCAM16.2, BD Biosciences, catalog number 562780), CD137/4-1BB (clone 4B4, BD Biosciences, catalog number 550890), CD152/CTLA-4 (clone L3D10, BioLegend, catalog number 349914), CCR7/CD197 (clone 150503, BD Biosciences, catalog number 560765), ICOS/CD278 (clone DX29, BD Biosciences, catalog number 562970), HLA-DR (clone G46-6, BD Biosciences, catalog number 555812), Ki67 (clone B56, BD Biosciences, catalog number 558615), TIM-3 (clone F38-2E2, BioLegend, catalog number 345006), OX40/CD134 (clone Ber-ACT35, BioLegend, catalog number 350010), and an IgG1 isotype control (clone MOPC-21, BD Biosciences, catalog number 557714). For immunohistochemistry, antibodies included CEA (clone T84.66, Ventana Medical Systems, catalog number 790-4795), FAP (clone SP325, Spring Biosciences, catalog number M6250), CD8 (clone SP239, Abcam, catalog number ab178089), and Ki67 (clone 30-9, Ventana Medical Systems, catalog number 790-4286). For multiplex immunofluorescence, antibodies included 4-1BB (clone D2Z4Y, Cell Signaling Technology, catalog number 34594), CD3 (clone SP162, Abcam, catalog number ab135372), CD8 (clone SP239, Abcam, catalog number ab178089), Ki67 (clone 30-9, Ventana Medical Systems, catalog number 790-4286), and OX40 (clone ACT35, Invitrogen, catalog number 14-1347-82).

Validation

All antibodies used in this study were commercially available and validated by the manufacturers for their intended applications. Where required, antibodies were further validated internally using established positive and negative controls, isotype controls, and staining controls, and were applied according to validated or standardized protocols. Immunohistochemistry and multiplex immunofluorescence assays were performed in centralized laboratories using optimized and quality-controlled procedures. Flow cytometry panels were validated prior to use, including assessment of specificity, compensation, and reproducibility. No novel antibodies were generated for this study.

Clinical data

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Clinical trial registration

The study was registered at ClinicalTrials.gov prior to patient enrollment (ClinicalTrials.gov identifier: NCT04826003).

Study protocol

Study protocol has been provided as Supplementary Information document.

Data collection

Clinical data were collected prospectively at participating sites using sponsor-approved electronic data capture systems in accordance with Good Clinical Practice guidelines. Data collected included baseline demographic and disease characteristics, treatment exposure, safety assessments, imaging-based tumor evaluations, pharmacokinetic and pharmacodynamic measurements, and biomarker analyses. Data quality was ensured through predefined monitoring and validation procedures.

Outcomes

Primary outcomes were safety and tolerability, including the incidence and severity of adverse events. Secondary and exploratory outcomes included pharmacokinetics, pharmacodynamic biomarker modulation, and antitumor activity as assessed by objective response rate, disease control rate, duration of response, progression-free survival, and overall survival. Outcome definitions and assessment methods are described in the Methods section.

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Confirm that:

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☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

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Methodology

Sample preparation	Peripheral blood samples were collected in sodium heparin tubes and processed according to validated assay protocols. All staining and incubation steps were performed at room temperature in the dark. Data acquisition was performed using BD FACSCanto II instruments, with analysis conducted using FACSDiva software and assay-specific acquisition templates. Cell preparation was automated using a BD FACS Lyse Wash Assistant.

Surface staining was performed using antibody cocktails applied to whole blood samples, followed by red blood cell lysis, washing, and resuspension in phosphate-buffered saline. For intracellular staining, samples were additionally permeabilized using Perm Buffer II prior to incubation with intracellular antibody cocktails. A complete list of antibody reagents is provided in Supplementary Table 9.
Peripheral blood samples were collected in sodium heparin tubes and processed according to validated assay protocols. All staining and incubation steps were performed at room temperature in the dark. Data acquisition was performed using BD FACSCanto II instruments, with analysis conducted using FACSDiva software and assay-specific acquisition templates. Cell preparation was automated using a BD FACS Lyse Wash Assistant.
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Surface staining was performed using antibody cocktails applied to whole blood samples, followed by red blood cell lysis, washing, and resuspension in phosphate-buffered saline. For intracellular staining, samples were additionally permeabilized using Perm Buffer II prior to incubation with intracellular antibody cocktails. A complete list of antibody reagents is provided in Supplementary Table 9.
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Instrument: BD FACS CANTO II

Software: FACSDiva, FlowJo

Cell population abundance: Described in Methods and Supplementary data

Gating strategy: Described in methods and Supplementary data

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.