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Reporting Summary

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Statistics

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	No custom software was used for data collection. Flow cytometry data were acquired on a BD FACSCanto using BD FACSDiva software version 9.0 (with CS&T bead-based MFI tracking); immunophenotyping of memory-like T cells was acquired on a BD Biosciences LSR Fortessa using DIVA version 8. NanoString data were collected on the nCounter Digital Analyzer 5s / nCounter Prep Station 5s; RNA quality was assessed using an Agilent 2100 Bioanalyzer. Electrochemiluminescence assays (PK, sCD25, cytokines) were read on MSD QuickPlex SQ120 or MSD Sector S600 instruments. Multiplex immunofluorescence slides were stained on a Leica Bond RX and scanned using an Akoya Vectra Polaris.
Data analysis	No custom code was generated for data analysis in this study. Flow cytometry data were analyzed with FlowJo version 10.8 (validation panel) and FlowJo v10 (Tree Star, Inc.; memory-phenotype panel). NanoString data were processed using nSolver with additional analysis per cartridge map design. Multiplex immunofluorescence images were analyzed with HALO image analysis software. sCD25 ELISA/MSD curve fitting and interpolation were performed in MSD Discovery Workbench 4.0.12 (4-PL regression, $1/y^2$ weighting).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The sponsor, Marengo Therapeutics, is committed to responsible clinical trial data sharing. Individual de-identified participant data underlying the results reported in this article may be made available to qualified researchers whose proposed use of the data has been approved. Because START-001 is an ongoing trial (NCT05592626), data will be available after completion of the study and its primary analysis. Requests should be directed to K.L. Proposals will be reviewed by the sponsor and, where appropriate, access will be granted under the terms of a data-sharing agreement to protect participant privacy and confidentiality. Study-level results will be posted on ClinicalTrials.gov (NCT05592626) after the end of the study.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Sex was recorded as part of baseline demographics; 25 of 52 participants (48.1%) were female. Sex was not a factor in study design, and no sex- or gender-based analyses of safety, pharmacokinetic, pharmacodynamic, or efficacy endpoints were performed, as the study was not powered for such comparisons given the small sample size and heterogeneity across 21 differerent tumor types. Gender identity data were not collected. Individual-level sex data disaggregated in source data are not reported here. Numbers are provided above and in Table 1.
Reporting on race, ethnicity, or other socially relevant groupings	Race was collected as a baseline demographic variable and reported descriptively in Table 1: Asian 2 (3.8%), Black or African American 3 (5.8%), White 37 (71.2%), Other/missing/not reported 10 (19.2%) due to EU GDPR limiting on reporting personal data. Race categories were as recorded at participating sites. No analyses were stratified by or controlled for race or ethnicity. The predominantly White study population is acknowledged in the Discussion as a limitation that may affect generalizability.
Population characteristics	Adults ≥18 years with unresectable locally advanced or metastatic solid tumors were enrolled. Median age was 59.0 years (Q1, Q3: 50.5, 65.5); 48.1% female. ECOG performance status was 0 in 17 (32.7%) and 1 in 35 (67.3%). Prior lines of therapy: 1–3 in 23 (44.2%), ≥4 in 28 (53.8%), missing in 1 (1.9%). Prior immune checkpoint blockade therapy (ICB) was reported in 37 (71.2%). Best response to prior ICB was partial response in 6 (11.5%), stable disease in 10 (19.2%), progressive disease in 14 (26.9%), unknown in 7 (13.5%). Primary ICB resistance in 18 (34.6%) and secondary resistance in 12 (23.1%). Biomarkers: TMB-H in 32 (61.5%), MSI-H in 6 (11.5%), virally associated in 16 (30.8%). Twenty-one tumor types were represented, most commonly colorectal (26.9%), head and neck squamous cell carcinoma (15.4%), and cervical, NSCLC, and anal cancer (7.7% each).
Recruitment	Participants were recruited at 11 investigational sites in four countries (Canada, France, Spain, United States) between 4 January 2023 and 12 March 2025 with the data cutoff of 31 March 2025. Enrollment required histologically confirmed unresectable locally advanced or metastatic solid tumors with TMB-H, MSI-H/dMMR status, or viral association, for which prior therapy was ineffective or intolerable or no standard curative therapy was available. As an open-label, non-randomized, single-arm trial conducted at specialist academic and cancer centers, the study participants enrolled by referral and self-selection bias. Participants were generally fit (ECOG 0–1, life expectancy ≥12 weeks), heavily pretreated, and able to travel to clinical sites, which may bias response and tolerability estimates relative to an unselected population. The predominantly White population may further limit generalizability.
Ethics oversight	The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, and applicable local regulatory requirements. The protocol and all written materials provided to participants were approved by the appropriate institutional review board or independent ethics committee at each participating site. Specifically, the following ethics committees/IRBs have approved START-001 trial for the results reported here: Mass General Brigham Cancer Institute, Boston, MA, USA; National Cancer Institute, National Institutes of Health, Bethesda, MD, USA. Memorial Sloan Kettering Cancer Center, New York, NY, USA; Princess Margaret Cancer Centre, Toronto, Ontario, Canada; AdventHealth Cancer Institute, Orlando, FL, USA; The Ohio State University Comprehensive Cancer Center, Columbus, OH, USA; Institut Bergonié, Bordeaux, France; University of Paris Saclay, Gustave Roussy Cancer Center, Villejuif, France; Vall d'Hebron Hospital Campus and Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain; and START-FJD, Madrid, Spain.

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

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No formal statistical hypothesis was tested in Phase 1; sample size was determined by the standard 3+3 dose-escalation design across six dose levels (0.01–0.16 mg/kg), with additional backfill participants (n=9) enrolled at the 0.08 and 0.12 mg/kg dose levels to further characterize pharmacology and safety, and 6 participants in Group B (step-up dosing). For the ongoing Phase 2 dose expansion, sample size for each cohort was estimated using an optimal Simon's two-stage design, with each cohort assessed independently; the null hypothesis of a true response rate of 0.05 was tested against the one-sided alternative pA > p0. Planned sample sizes were 23–56 for Cohort 1 (TMB-H tissue-agnostic, non-CRC), 10–29 for Cohort 2 (MSI-H, or MSI-H and TMB-H, tissue-agnostic, non-CRC), and 23–56 for Cohort 3 (CRC TMB-H, CRC MSI-H, or both).
Data exclusions	No data were excluded from the analyses. Of 58 treated participants, results are presented for the 52 participants comprising Phase 1 Group A (n=34) and the initial Phase 2 cohort (n=18); the 6 participants in Phase 1 Group B (step-up dosing) are reported separately and were not pooled with Group A, as pre-specified by the differing dosing regimen. Response analyses were restricted to participants at the optimal biological doses with ≥1 post-treatment tumor assessment (n=27), consistent with pre-specified response evaluability criteria.
Replication	This is a first-in-human clinical trial; the clinical findings are not replicated within the study. Analytical reproducibility of the laboratory assays was addressed through formal validation and qualification. The 10-color T/B/NK and TCRvβ6/10 flow cytometry panel was validated using cryopreserved PBMCs from three healthy donors and included assessment of intra-assay precision, inter-assay precision, inter-operator precision, and post-staining stability; instrument performance was monitored with CS&T beads and fixed FACSDiva application settings. The NanoString nCounter Human CAR-T panel was qualified against manufacturer specifications using PBMC, whole blood, and FFPE tumor tissue, with pre-specified RNA concentration, RIN, and DV200 acceptance criteria. The multiplex immunofluorescence panel was optimized on normal tonsil, pancreas, and PBMC FFPE sections and further qualified in melanoma, MSI-H CRC, and cervical cancer tissue, with each antibody titrated at three concentrations against matched isotype controls. Tumor responses were confirmed by repeat imaging after 4 weeks (no later than 5 weeks) per RECIST/iRECIST. All attempted validation and qualification exercises met their acceptance criteria.
Randomization	This was an open-label, non-randomized, single-arm study; participants were not randomized. Allocation to dose level in Phase 1 was sequential according to the 3+3 dose-escalation schema, with backfill assignment to the 0.08 and 0.12 mg/kg levels. All Phase 2 participants received the RP2D of 0.08 mg/kg.
Blinding	Blinding was not performed. START-001 is an open-label, single-arm, dose-escalation and dose-expansion trial in which dose assignment must be known to investigators for safety monitoring,

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

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

Flow cytometry (10-color T/B/NK and TCRvβ6/10 validation panel): CD25 BV421 (BioLegend, 356114, clone M-A251, 5.00 µL/test); Zombie Aqua viability dye (BioLegend, 423104, 1:200); CD8 BV605 (BioLegend, 301040, clone RPA-T8, 5.00 µL); CD3 Alexa Fluor 488 (BioLegend, 300415, clone UCHT1, 2.00 µL); CD56 PE (BD Biosciences, 340363, clone NCAM16.2, 2.50 µL); CD4 PerCP-Cy5.5 (BD Biosciences, 552838, clone L200, 2.50 µL); CD45 PE-Cy7 (BioLegend, 304016, clone HI30, 2.00 µL); TCRvβ6/10 APC (Marengo Therapeutics, custom, 100 nM); CD14 Alexa Fluor 700 (BioLegend, 301822, clone M5E2, 2.50 µL); CD20 APC-Fire750 (BioLegend, 302358, clone 2H7, 2.50 µL). Human TruStain FcX was used to block non-specific binding.

Flow cytometry (memory-like T-cell immunophenotyping, NCI): anti-Vβ6/Vβ10 antibodies provided by Marengo Therapeutics, used either biotinylated with an AF488-conjugated streptavidin secondary or directly conjugated with AF657; anti-human CD8 BV785 (BioLegend, clone RPA-T8); anti-human CD4 BV605 (BioLegend, clone OKT4); anti-human CCR7 BV510 (BioLegend, clone G043H7); anti-human CD45RA AF700 (BioLegend, clone HI100); anti-human Granzyme B FITC (BioLegend, clone GB11).

Multiplex immunofluorescence (8-color panel; CD8, CD25, CD3, CD4, CD56, CD20, CD14, DAPI): anti-CD20 [EP459Y] (Abcam, ab78237, lot 1006245-2, 0.056 mg/mL); CD8α (C8/144B) mouse mAb, IHC-specific (Cell Signaling, 70306S, lot 4, 25 µg/mL); CD3ε (D7A6E) XP rabbit mAb (Cell Signaling, 85061S, lot 5, 76 µg/mL); NCAM/CD56 (123C3) mouse mAb (Cell Signaling, 3576S, lot 9, 559 µg/mL); CD14 (D7A2T) rabbit mAb, IHC formulated (Cell Signaling, 75181S, lot 1, 5 µg/mL); recombinant anti-CD4 [EPR6855] (Abcam,

ab133616, lot 1008803-3, 0.143 mg/mL); recombinant anti-IL-2 receptor alpha [EPR6452] (Abcam, ab128955, lot GR280865-14, 1.999 mg/mL); CD45 monoclonal antibody (CD45-2B11), biotin, eBioscience (Thermo Fisher, 13-9457-82, lot 2527221, 0.5 mg/mL); CD3ε (C3e/1931), biotin (Novus Biologicals, NBP2-79840B, lot D123361, 1.28 mg/mL). Isotype controls: rabbit (DA1E) mAb IgG XP (Cell Signaling, 3900, lot 48, 2.5 mg/mL); mouse (G3A1) mAb IgG1 (Cell Signaling, 5415, lot 13, 2.5 mg/mL); human IgG isotype control, biotin (Novus Biologicals, NBP1-96855, lot SEP2022, 1 mg/mL).

Immunoassays: Human CD25/IL-2 Rα DuoSet ELISA capture and detection antibodies (R&D Systems, DY223) adapted to MSD format, with Sulfo-Tag streptavidin (MSD, R32AD-1). PK assay: TCR antigen coating reagent (Marengo), anti-IL-2 and biotinylated anti-IL-2 detection antibodies (Marengo; biotin conjugation performed at Marengo), with Sulfo-Tag streptavidin (MSD, R32AD-1). Cytokines were measured with the MSD V-Plex custom panel (catalog K15049G) for IFN-γ, IL-1β, IL-6, and TNF-α.

Validation

Flow cytometry panel: Panel sensitivity was optimized by titrating each antibody on stimulated and unstimulated cryopreserved healthy-donor PBMCs (1 million PBMCs per test); PMT voltages were determined using single-color-stained beads and PBMCs, and fixed BD FACSDiva v9.0 application settings were created to ensure consistency across the study. Gating was established using fluorescence-minus-one controls (FMO CD25 and FMO TCRvβ6/10) run on unstimulated cells from each donor, with CD3/CD28-stimulated cells serving as a positive assay control for detection of activated TCRvβ6/10 populations. Formal validation across three donor PBMC lots assessed intra-assay, inter-assay, and inter-operator precision and post-staining stability. For the memory-phenotype panel, single-color compensation controls were included, instrument performance was verified with CS&T beads before acquisition, and all gates were set using FMO controls.

Multiplex immunofluorescence panel: One antibody per biomarker was titrated at three concentrations on positive-control FFPE tissues (normal tonsil, normal pancreas, PBMC) alongside a matched isotype negative control. The optimal working concentration was selected based on appropriate staining pattern, specificity, adequate intensity, and display of a full dynamic range of expression with low background. The optimized assay was further qualified in three disease indications: melanoma, MSI-H colorectal cancer, and cervical cancer FFPE tissue.

Immunoassays: The CD25/IL-2 Rα ELISA was adapted to MSD format with an 11-point standard curve (0–40,000 pg/mL) and 4-PL, $1/y^2$ weighted regression. The PK assay was a quantitative electrochemiluminescence assay using a TCR-antigen capture format with a 10-point human serum standard curve (5.0–5000 ng/mL) at a minimum required dilution of 1:50 with LowCross Buffer-Strong. Cytokine measurements followed the manufacturer's kit instructions for the MSD V-Plex panel.

Antibodies used are commercially available and validated by the manufacturers for the stated species and application per their datasheets, except the anti-Vβ6/Vβ10 reagents, which were custom-generated by Marengo Therapeutics and validated in-house as described above and in previously published work (Hsu et al., Sci Transl Med 2023;15:eadi0258).

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

ClinicalTrials.gov NCT05592626 (START-001)

Study protocol

A redacted version of the full trial protocol is provided with this submission.

Data collection

START-001 is a two-part, open-label, first-in-human Phase 1/2 study conducted at 11 centers in four countries (Canada, France, Spain, and the United States). Participant enrollment began in January 2023. The Phase 1 dose-escalation portion completed in June 2024, and Phase 2 dose expansion was initiated in October 2024 once the RP2D was established. Data reported here reflect an enrollment of 12 March 2025 with data cutoff of 31 March 2025. Invikafusp alfa was administered intravenously every two weeks (first infusion over 60 ± 5 minutes, subsequent infusions over 30 ± 5 minutes if deemed safe), with 28 days (two doses) defined as one cycle. Tumor imaging (CT and/or MRI) was performed every 8 weeks, with confirmatory imaging 4 weeks (no later than 5 weeks) after a first PR or CR using the same modality. Blood samples for pharmacokinetics, cytokines, sCD25, and PBMC pharmacodynamic analyses were collected at protocol-specified timepoints; paired tumor biopsies were obtained at baseline and Cycle 1 Day 21.

Outcomes

For Phase 1, the primary endpoint was safety, comprising dose-limiting toxicities (DLTs; defined in Supplementary Table 9), treatment-emergent adverse events, serious adverse events, deaths, and clinical laboratory abnormalities graded per NCI CTCAE version 5.0, with cytokine release syndrome graded per ASTCT (Lee) criteria. Adverse events were coded by system organ class and preferred term using MedDRA version 25.1. The MTD was pre-defined as the highest dose level at which <33% of participants experienced a DLT during the DLT evaluation period. Secondary Phase 1 endpoints were pre-defined measures of anti-tumor activity — objective response rate (proportion with complete or partial response per iRECIST), duration of response (time from first documented PR/CR to first documentation of confirmed disease progression per iRECIST and RECIST v1.1), and disease control rate (proportion with CR + PR + stable disease per iRECIST and RECIST v1.1) — and pharmacokinetic parameters (AUC(0–t) and AUC(0–inf), Cmax, Tmax, clearance, volume of distribution at steady state, single-dose half-life, and repeat-dose Cmax and Ctrough). The primary endpoint for the Phase 2 portion is ORR per iRECIST; secondary Phase 2 endpoints are ORR per RECIST v1.1, DOR and DCR per iRECIST and RECIST v1.1, progression-free survival (time from initial treatment infusion to documented progression or death from any cause per iRECIST and RECIST v1.1), overall survival, pharmacokinetic parameters, and safety at the RP2D. Tumor assessments were performed by investigators, with responses requiring confirmatory imaging as described above.

Plants

Seed stocks: N/A

Novel plant genotypes: N/A

Authentication: N/A

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ 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).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation: *Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*

Instrument: *Identify the instrument used for data collection, specifying make and model number.*

Software: *Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*

Cell population abundance: *Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*

Gating strategy: *Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*

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

Magnetic resonance imaging

Experimental design

Design type: N/A

Design specifications: N/A

Behavioral performance measures: N/A

Acquisition

Imaging type(s): N/A

Field strength: N/A

Sequence & imaging parameters: N/A

Area of acquisition: N/A

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	N/A
Normalization	N/A
Normalization template	N/A
Noise and artifact removal	N/A
Volume censoring	N/A

Statistical modeling & inference

Model type and settings	N/A
Effect(s) tested	N/A

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)	N/A
Correction	N/A

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis