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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	For flow cytometry assays, data was acquired with a Fortessa cytometer (BD Biosciences). Cytotoxic assay-based cytolysis was measured with the impedance-based xCELLigence RTCA MP Real Time Cell Analyzer (Acea Biosciences). For fluorescent microscopy studies, slides were digitally imaged using Aperio ScanScope FL Scanner (Leica Biosystems).
Data analysis	For flow cytometry, data was analyzed with FlowJo v10 software. Protein concentrations in the serum and coculture supernatant were determined by electrochemiluminescence immunoassays, where electrochemiluminescence was measured using MESO QuickPlex SQ 120 and protein concentration was calculated using Discovery Workbench 4.0 (MSD). RNA-seq expression data and WES data were processed with the CCR Collaborative Bioinformatics Resource (CCBR) in-house pipeline (https://github.com/CCBR/Pipeliner). Specifically, for WES data processing, the following software were used: Trimmomatic v0.36, BWA-mem v0.7.15, SAMtools v1.317, Picard v2.1.1, and Genome Analysis Toolkit v.3.4. For copy number analysis, Control-FREEC (v11.5) and Sequenza (sequenza-utils v. 2.2 and Sequenza R package v. 3.0) was used. For mutations detected in the WES data, the variants were verified by visualizing WES reads at each locus in the Integrative Genomics Viewer (IGV 2.4.16., http://software.broadinstitute.org/software/igv/). Nonsynonymous mutations were analyzed for functional significance with three in silico protein variant tools: Polymorphism Phenotyping v2, Sorting Intolerant from Tolerant, and Mutation Taster (http://www.mutationtaster.org/). For RNA-seq data processing, the following software were used: Trimmomatic v0.33, STAR v2.5.2b, RSEM v1.3.0, limma-voom v3.34.5, limma (v3.38.3), and ComBat (pacakge sva v3.32.1). For RNA-seq expression data, QIAGEN's Ingenuity® Pathway Analysis (IPA®version Summer Release 2018, QIAGEN Redwood City, www.qiagen.com/ingenuity) was used to identify pathways over-represented among differentially-expressed genes.

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Sequencing data files from WES and RNA have been deposited in the Database of Genotypes and Phenotypes (dbGaP) (accession number: phs002286.v1.p1). Gene sets for pathways analysis were from the Ingenuity Pathway Analysis database (version Summer Release 2018, https://digitalinsights.qiagen.com). WES mutation analysis and copy number variant tables are provided in Supplementary Dataset 1 and Supplementary Dataset 2. Source data for Figures 1 and 2, along with Extended Data 1-10, can be found in Supplementary Dataset 3. External requests for data will be evaluated by the corresponding author and requests may be subject to NIH policy.

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	The primary outcome for this phase 1 study was to determine a safe dose of E7 TCR T cells plus aldesleukin. A 3+3 dose escalation schema was employed to determine a safe dose of E7 TCR T cells. A total of 3 dose levels were tested, therefore the sample size estimation was that as few as 9 and no more than 18 patients would be required to perform the initial safety evaluation. The dose levels were chosen based on previous clinical experience from a phase I clinical trial testing TCR-T cell therapy in patients with metastatic cancer. A total of twelve patients were treated.
Data exclusions	For RNAscope-based E7 TCR imaging studies, some biopsy samples did not pass quality control (including extensive artifacts) and where excluded. For genomic studies, some biopsies did not have adequate tumor content for microdissection or RNA/DNA post extraction and thus had to be excluded. Few samples were collected from Patient 11, and they were not tested. Clinical data for patient 11 was included in the patient characteristics table (Table 1) and the adverse events tables (Table 2 and Supplementary Table 1). Data for patient 11 was excluded from all other studies following cell infusion as it was deemed to be in the best interest of the patient to stop collection of research specimens (including blood). This was not a pre-established exclusion.
Replication	Except for flow cytometry and tissue immunohistochemistry experiments, all attempts at replication were successful. Replication included independent experiments and technical replicates. Flow cytometry and tissue immunohistochemistry experiments could not be replicated due to limited availability of samples.
Randomization	For some analysis, patients were stratified as responding or not responding to treatment or by cell dose level. Randomization is not relevant to our study since this was a first-in-human, phase I study where all correlative research was exploratory.
Blinding	Immunospot assay analysis of IFN-γ, TNF-α, and IL-2 spot counts were analyzed blindly by Immunospot (i.e. the investigators were blinded to group allocation during data collection). Blinding was not relevant since this was a first-in-human, phase I study. All correlative studies were exploratory in nature.

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
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data

Methods

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

Antibodies

Antibodies used

CD3 (UCHT1; BD Biosciences, San Jose, CA, USA), mouse TCR β-chain constant region (H57-595; BD Biosciences), HLA-A*02:01-E711-19 tetramer (peptide sequence YMLDLQPET, MBL), CD4 (SK3, Biolegend, San Diego CA, USA), CD8 (SK1, BD Biosciences), PD-1 (EH12.1 BD Biosciences), LAG-3 (REA351, Miltenyi Biotec, Bergisch Gladbach, Germany), TIM-3 (7D3, BD Biosciences), CD45RA (HI100, Biolegend), CD45R0 (UCHL1, BD Biosciences), Ki-67 (B56, BD Biosciences), CCR4 (L291H4, Biolegend), CCR6 (G034E3, Biolegend), and CXCR3 (G025H7, Biolegend)

Validation

All antibodies are validated and routinely tested as described in the manufacturers product information (BD Bioscience, Biolegend, and Miltenyi Biotec). In addition, we simultaneously stained positive and negative controls for each antibody, which included: CD3 and CD28 activated healthy donor PBMC's, unactivated PBMC's, infusion product, and fluorescence minus one controls.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Except for the 624 cell line, all cell lines (CaSki cells, Human embryonic kidney (HEK) 293 cells, and Jurkat cells (Clone E6-1)) were purchased from ATCC (Manassas, VA, USA). The 624 cell line (parental melanoma) was generated by the National Cancer Institute Surgery Branch and appropriately cited in the methods section.

Authentication

The 293-A2 cell line is an HEK 293-based cell line with stable expression of HLA-A*02:01 that was generated in the National Cancer Institute – Surgery Branch and verified for HLA-A*02:01 expression by flow cytometry.

Mycoplasma contamination

All cell lines used tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified lines were used.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Patients were 18 to 70 years of age with pathologically confirmed diagnosis of metastatic or locally advanced refractory or recurrent HPV-16+ cancer from any primary site. Patients were required to have the germline HLA-A*02:01 allele and prior treatment with at least one chemotherapy or chemoradiation regimen. Patients had a median age of 47 years (range, 31 to 65 years) and sqaumous cell carcinomas (n=11) or adenocarcinomas (n=1). The primary tumor sites were uterine cervix (n=5), head and neck (n=4), anus (n=2), and vulva (n=1). The median number of prior anticancer agents was 4 (range, 3 to 7). There were 7 female and 5 male participants.

Recruitment

Patients were recruited via standard mechanisms used for clinical trials at the Center for Cancer Research, National Institutes of Health, Maryland USA. All advertisements, letters and other recruitment efforts were submitted to the IRB for approval prior to implementation. Selection was based on inclusion and exclusion criteria. No other selection biases were present.

Ethics oversight

The protocol was approved by the National Cancer Institute Institutional Review Board at the National Institutes of Health Clinical Center and was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice Guideline. An independent data and safety monitoring committee regularly reviewed safety data. All the patients provided written informed consent.

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

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

NCT02858310

Study protocol	The full protocol is available on the intramural National Cancer Institute protocol view website and included in the Supplementary Information PDF.
Data collection	Twelve patients were treated from January 27, 2017 to April 20, 2018 at the NIH Clinical Center. All research specimens were collected at the NIH clinical center. Data was analyzed in the laboratory of Dr. Christian Hinrichs, NCI Frederick, and Kite, a Gilead Company.
Outcomes	The primary outcome for this phase 1 study was to determine a safe dose of E7 TCR T cells plus aldesleukin. This was accomplished by conducting a 3+3 cell dose escalation to determine the maximum tolerated dose.

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	Cells were thawed and 2 million PBMC's were washed in iMAG buffer (BD Biosciences). Human BD FC blocker (BD Biosciences) was added to the cells (along with the HLA-A*02:01-E711-19 tetramer were appropriate) in a total volume of 50ul and incubated for 10 minutes at room temperature. The surface antibodies were prepared in 50ul of Brilliant stain buffer (BD Biosciences) and mixed with the 50ul FC block solution on ice in the dark for 30 minutes. The cells were washed with Stain Buffer (BD Biosciences) and PBS and labeled with Fixable Viability Dye (BD Biosciences) in PBS for 30 minutes. The cells were then washed with Stain Buffer. For the Ki-67 intracellular panel, cells were fixed with Fixation/Permeabilization solution (Cytofix/Cytoperm, BD Biosciences) for 20 minutes and washed two times with Perm/Wash Buffer (BD Biosciences) and incubated with Ki-67 antibody on ice for 45 minutes.
Instrument	Flow cytometry data was acquired with a Fortessa cytometer (BD Biosciences).
Software	Flow cytometry data was analyzed with FlowJo v10 software.
Cell population abundance	After negative selection for CD3+ T cells in peripheral blood, cells purity was checked by flow cytometry.
Gating strategy	Analyses were gated on live, singlet, lymphocytes. T cells were identified by CD3 expression. The frequency of E7 TCR-T cells in infusion products and peripheral blood was determined by lymphocytes that expressed mouse TCR β-chain constant region and bound HLA-A*02:01-E711-19 tetramers (both were required). The gating strategy and examples are shown in Supplementary Figure 1. Analyses of E7 TCR-T cell phenotype were performed by gating on lymphocytes that expressed both CD3 and mouse TCR β-chain constant region.

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