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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

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	OnCore CTMS Enterprise Research system (Windows) was used to collect adverse events and for data outcome reporting. FlowJo software package v_10.0 and 10.6.1 (Windows, English) was used to collect and analyze cell populations.
Data analysis	Data were analyzed in R version 3.6.3 (2020-02-29), using Windows 10 x64 operating system, ming w32, user interface "RTerm" language English, United States, and Prism 9.0 (121) October 22, 2020. FEST analysis http://www.stat-apps.onc.jhmi.edu/FEST/ version 2018-05-22. Adaptive Biotechnologies immunoSEQ analyzer version 3.0 was used to track clones and assess clonality of T cells. ELISA absorbance data was analyzed using SkanIt Software 4.1. STAR: ultrafast universal RNA-seq aligner was accessed as free open source software from http://code.google.com/p/rna-star/. Fusioncatcher Versions 1.0, and Version 1.20 -- November 8, 2019 were used for genomic analysis.

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 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

Source data on expression, allelic frequency, and predicted MHC affinity for all identified neoantigens are provided in the supplemental excel spreadsheet uploaded as tab S4. TRVB information for 20 patients are available as the project "TIL trial for NSCLC" in the immuneACCESS public database: https://clients.adaptivebiotech.com/immuneaccess . Tumor whole exome sequencing and RNA-Seq raw data files (accession code: phs002486) is available in the public NIH

dbGaP data browser https://www.ncbi.nlm.nih.gov/projects/gapprev/gap/cgi-bin/preview1.cgi?GAP_phs_code=UgsrhvZ3dGC4uQSh. The study protocol is uploaded as a supplementary file. In such circumstances that source data is not inferred from specified Tables/Figures, it is available upon request as a comma-delimited format from the study authors.

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 trial sample size justification is described in the study protocol, page 59. We expected a total of 14 evaluable patients for the study. Sample size justification was based on continuous monitoring for toxicity using a Pocock-type stopping boundary. We consider the maximum-tolerated toxicity would be 17%. We considered the probability of early stopping to be at most 10%. With 14 patients, these settings resulted in a Pocock-type stopping boundary (Table A1 of Protocol). This boundary is equivalent to testing the null hypothesis, after each patient, that the maximum-tolerated toxicity rate is equal to 17%, using a one-sided level 0.053 test.
Data exclusions	No data is specifically excluded from each analysis. Data is omitted in sections where no data was available. Specifically, Pt 13 and 15 did not receive a measurable post-TIL CT scan and Pt 03 did not have a RECIST evaluable tumor lesion post-TIL. A data cutoff of September 2020 was applied for adverse events, survival time, sample collection, and response analysis. Timepoints with evaluable biosamples are indicated in Extended Data Table 2. Because of variations in sample collections for some patients, "Week 10" and "Week 20" permitted timepoints from +/-3 weeks.
Replication	Genomics was performed once on one biological sample each. Genomics could not be repeated due to expense of sequencing and availability of tumor sample. ELISpot or ELISA experiments were performed once with triplicate wells, on one biological sample each, and performed twice if sufficient sample was available. The replication was consistent with both experiments and successful.
Randomization	Not relevant, as no treatment types were compared. No randomization was used in this single-arm study.
Blinding	Not relevant in regards to treatment assignment, since all patients received the same treatment on an open-label trial. In regards to comparison of biomarkers, the pathologist Dr James Sallman reviewed tumor markers such as CD3+ staining without prior knowledge of patient outcomes. Because biomarkers were neither a primary or secondary endpoint of the trial, no formal blinding program was implemented.

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
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Flow cytometry antibody / Catalogue/ Lot Number were: Anti- Human Tim-3 (CD366) BV421 565562 8165981 Anti- Human TIGIT PE-Cy™7 25-9500-42 1995496 Anti- Human LAG3 (CD223) PE 565616 8281624 Anti- Human CD95 BV711 563132 8288847 Anti- Human CD8 BUV395 563795 8220831 Anti- Human CD56 BUV563 565704(Discontinued)/612928 9022780 Anti- Human CD45RA FITC 555488 7055682 Anti- Human CD4 BUV737 564305(Discontinued)/612748 8310934

Anti- Human CD314 (NKG2D) APC 558071 8159562
Anti- Human CD3 BUV496 564809(Discontinued)/612940 8296578
Anti- Human CD28 BV480 566110 7054585
Anti- Human CD279 (PD-1) BV480 566112 7171905
Anti- Human CD272 (BTLA) APC 564800 7150655
Anti- Human CD27 PerCP-Cy™5.5 560612 8138674
Anti- Human CD25 PerCP-Cy™5.5 560503 8339603
Anti- Human CD244 FITC 550815 6307734
Anti- Human CD226 BV711 564796 7205804
Anti- Human CD197 (CCR7) BV421 562555 8291773
Anti- Human CD195 BV786 565001 8353941
Anti- Human CD194 PE-Cy™7 557864 7250981
Anti- Human CD19 BV605 562653 8281951
Anti- Human CD152 BV786 563931 8129674
Anti- Human CD14 BV605 564054 8194956
Anti- Human CD127 PE 557938 7242866;
All from BD Biosciences.
The optimized dilutions for the flow cytometry antibodies included:
NKG2D 1:5; CD197 1:10; CD56 1:20; CD195 1:20; CD45RA 1:20; CD194 1:20; CD3 1:50; CD4 1:50' CD14 1:50; CD19 1:50; CD25 1:50; CD95 1:50; CD8 1:100; CD28 1:100; CD127 1:150; CD244 1:05; CD152 1:20; CD223 1:20; CD366 1:20; CD3 1:50; CD4 1:50; CD14 1:50; CD19 1:50; CD279 1:50; CD226 1:50; TIGIT 1:50; CD8 1:100; CD272 1:100; CD27 1:100.
Rainbow Calibration Particles (3.0 - 3.4 µm) from BD Biosciences was used for calibration of flow cytometry between runs.

Human IFN-γ Immunoassay (R&D Systems) Catalog Number DIF50 was used for autologous reactivity testing of TIL cultures, this kit includes a a polyclonal antibody specific for human IFN-γ. Dynabeads Human T-Activator CD3/CD28/CB137 antibody from Gibco cat # 11163D was used to activate T cells for ELISA. OKT3 ((14-0037-82) from Thermo Fisher Scientific was used as positive control for T cell stimulation for ELIspot.
For immunohistochemical detection of PD-L1, the PD-L1 22C3 mouse anti-human monoclonal antibody (SK 006, Agilent) was performed according to manufacturer guidelines utilizing the DAKO EnVision FLEX visualization system on the DAKO Autostainer Link 48 on formalin paraffin embedded tissue. The interpretation of stained tumor cells are analyzed by manual morphometric analysis and reported using the Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining.

Validation

A Certificate of Analysis showing compliance with all BDB release criteria for flow cytometry, using Human FC Optimal Conc uG/Test (0.500 - 1.000) was confirmed for each of the BD antibody catalogue and lot number combinations listed above. These certificates are publically available at https://regdocs.bd.com/regdocs/qcSearchResults. For the flow cytometry antibodies, the CD-Chex Plus (Streck) control (Lots #73030071, 90210071) for internal validation was run for each patient during TIL analysis for CD3, CD4, and CD8.
For immunohistochemical detection of PD-L1 using 22C3 antibody, the Moffitt pathology laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA 1988) pursuant to the requirements of CLIA, and verified the test's accuracy, precision, reportable range of patient test results, reference range, analytical specificity and sensitivity, and other relevant performance specifications.

Human research participants

Policy information about studies involving human research participants

Population characteristics	This information is provided in Table 1.
Recruitment	All participants were recruited by medical oncology or surgeons within a Thoracic multi-disciplinary clinic setting at Moffitt Cancer Center, which has a catchment area of the surrounding 10 counties of Florida. The clinic demographic consists primarily of suburban and urban Caucasian patients with health insurance access. Only two of 20 enrolled patients were African American. Spanish language consent was available for translation within the study budget, however no enrolled patients were primary Spanish language speakers. No sources of recruitment bias were identified.
Ethics oversight	This study was approved by Adverra Institutional Review Board (FWA 0023875) and underwent FDA safety review (IND 17489).

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	NCT03215810
Study protocol	Full study protocol is uploaded as a supplemental file.
Data collection	Patients were accrued from October 2017 to January 2020. Data analysis was performed with data cutoff 10 Sept 2020. OnCore CTMS Enterprise Research system (Windows) was used to collect adverse events and for data outcome reporting. Data was collected in tumor measurement worksheets by assigned MCC 19122 trial data managers at Moffitt Cancer Center. Data managers collected and graded adverse events based on review of the clinical charts from enrolled patients. Adverse event attribution was performed based on source documentation and the treating physicians. CT scans were conducted every 6 weeks, followed by every 12 weeks

after completion of therapy, according to the study calendar, at the cancer center. Target lesion assignment and measurements per RECIST v1.1 were performed and included within the primary radiology report by the interpreting radiologists. Tumor measurement worksheets were completed by the clinical trial coordinators and signed by the treating physicians. Blood samples were collected according to the study calendar.

Outcomes

The primary objective of the study was to demonstrate that treatment with nivolumab in patients undergoing lymphodepletion/TIL/IL2 therapy was safe, with a continuous Pocock-type stopping boundary for serious toxicity of < 17%, with safety reported based upon CTCAE version 4.0 criteria. Our hypothesis the combination of TIL therapy and nivolumab for NSCLC patients will be feasible and achieve an acceptable toxicity profile.
The secondary objectives were to 1) evaluate the efficacy of TIL administered in combination with nivolumab in subjects with NSCLC by assessing the objective response rate (ORR) per RECIST v1.1; 2) to evaluate the efficacy of TIL administered in combination with nivolumab in subjects with NSCLC by assessing duration of response (DoR); 3) to evaluate the T-cell persistence following TIL and nivolumab when administered in combination, using T cell phenotype such as CDR3 sequencing.

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

Patients had peripheral blood draw in 10 mL sodium heparin tubes, which was then separated on a Ficoll gradient and cryopreserved in albumin and DMSO.

Instrument

BD Symphony

Software

Flowjo v10.6.1

Cell population abundance

After cell sorting, we run flow cytometry on sorted populations to determine cell purity. Only post-sort populations with purity more than 95% will be used for the experiments (e.g. immunoseq in this manuscript).

Gating strategy

From FSC/SSC plots, we gated lymphocytes and only gated single cells using FSC-A/FSC-H and SSC-A/SSC-H. Then CD3+ Zombie NIR- CD14/CD19- cells were gated as live CD3+ cells, where CD4+ and CD8+ T cells were gated respectively. The following T cell differentiation subsets were gated within each of CD4 or CD8 T cells: naive T cells--CCR7+CD45RA+CD95-, Tscm--CCR7+CD45RA+CD95+, Tcm--CCR7+CD45RA-, Tem--CCR7-CD45RA-, Teff--CCR7-CD45RA+.
Positive and negative populations were identified using FMO controls.
A figure showing an example of our gating strategy is provided in the supplementary data.

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