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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 Authors & Referees 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	Flowcytometry data was collected using the BD LSRFortessa™ SORP, BD FACSCelesta, BD FACSCanto™ II or BD FACSAria™ devices with the FACSDiva software version 6.0.3 or 8.0.1.1 or using the BD FACSMelody™ with the BD FACSChorus 1.1.18.0 software. Cell index (CI) impedance measurements were analyzed using the xCELLigence MP system (OMNI Life Science) and the RTCA or RTCA Pro software. ELISPOT spot count data was aquired using the CTL ImmunoSpot S6 Core Analyzer or S5Versa-02-9038 with the CTL Switchboard Software v2.6.1 as well as the ImmunoCapture Image Aquision Software V6.3 or V7.0, the ImmunoSpot 7.0.17.0 Professional or the AID ELISPOT 7.0. TCR sequencing: 300bp paired-end Illumina MiSeq. Demultiplexing: bcl2fastq (Illumina), followed by in-house Python script for single-cell samples. For collection and analysis of qRT-PCR data the 7300 System Sequence Detection Software Version 1.4 (Applied Biosystems) was used. Bioluminescence signals (NFAT T cell activation assay) were aquired using the Tecan Infinite F200Pro reader with the i-control 1.10 software.
Data analysis	Flow cytometry data was analyzed using the FlowJo software version 7.6.5, 10.5.3 or 10.6 (Tree Star). All statistical analysis was performed using GraphPad Prism software version 6 or 8. TCR alignment: In-house bash pipeline using MiXCR 2.1.5 with default repseqio. TCR analysis: single cell samples: In-house Java tool for TCR-pair identification, bulk TCR profiling: In-house bash pipeline using VDJTools and in-house Pythons scripts (tracking of TCRs between samples).

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

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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 sample size for small cohorts is driven by the 3+3 study design and will range from 3 to 6 DLT evaluable patients depending on the number of DLTs which may occur. The actual total sample size at the time of analysis is 89 patients. With 89 patients the probability to observe at least one adverse event of incidence 2% would be 83.4%.
Data exclusions	No data available until data extraction date of 29JUL2019 were excluded. Data shown are preliminary and not fully source-data verified.
Replication	Replication was not attempted
Randomization	No randomization was performed. Allocation of patients to open cohorts was perfomed by sponsor in consultation with treating investigators to provide the best treatment option for each patient
Blinding	This is an open-label phase I safety trial. Investigators were not blinded.

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

Used antibodies (specificity/ fluorochrome/ clone/ manufacturer/ catalogue number/dilution/ lots):

anti-human CD3/ FITC/ UCHT1/ BD/ 561806/ 1:5/ 8086745/ 7188737
anti-human CD3/ FITC/ SK7/ BD/ 345763/ 1:25/ 8016855/ 27948
anti-human CD3/ APC-R700/ SK7/ BD / 659110/ 1:100/ 9113554/ 8234916
anti-human CD3/ APC-Cy7/ UCHT1/ BD/ 561800/ 1:50/ 3199137
anti-human CD3/ BV421/ UCHT1/ BD/ 562426/ 1:50/
anti-human CD4/ BV421/ OKT4/ BioLegend/ 317434/1:20/ B247270/ B227581
anti-human CD4/ BV510/ OKT4/ Biolegend/ 317443/ 1:25/ B252920/ B195295
anti-human CD4/ APC-Cy7/ OKT4/ Biolegend/ 317418/ 1:100/ B258921/ B238460/ B203215/B223630
anti-human CD4/ APC/ REA623/ Miltenyi Biotec/ 557834/ 1:50/ 5181106254

anti-human CD8/ BV510/ RPA-T8/ BD/ 563256/ 1:20/ 3161529/ 8117809
anti-human CD8/ BV421/ RPA-T8/ BD/ 562429/ 1:100/ 7082750/ 5267719
anti-human CD8/ BB515/ RPA-T8/ BD/ 564527/1:33/ 7242958/ 8143569
anti-human CD8/ BUV395/ RPA-T8/ BD/ 563795/ 1:50/ 8220831/ 8137897
anti-human CD8/ APC/ SK1/ BD/ 345775/ 1:100/ 5195609/ 6308559
anti-human CD8/ BV711/ RPA-T8/ BD/ 563676/ 1:20/ 5082865
anti-human CD14/ APC-Cy7/ MϕP9/ BD/ 561709/1:50/ 9009999/ 8285873/ 7165785/ 6047808
anti-human CD14/ BV510/ MϕP9/ BD/ 563079/ 1:25/ 4220870
anti-human CD16/ APC-Cy7/ 3G8/ BD/ 561726/ 1:100/ 8333901/ 7130902/ 8215754/ 5295824
anti-human CD16/ BV510/ 3G8/ BD/ 563829/ 1:100/ 5065775
anti-human CD19/ APC-Cy7/ HIB19/ Biolegend/ 302217/ 1:50/ B252246/ B208039
anti-human CD19/ BV510/ SJ25C1/ BD/ 562953/ 1:33/ 5118538
anti-human CD27/ BV605/ L128/ BD/ 562656/ 1:50/ 7263551/ 7058985/ 4318538/ 5085687
anti-human CD28/ PerCP-Cy5.5/ CD28.2/ BD / 560685/ 1:25/ 8183515/ 7278835/ 7348626/ 5093561/ 6051578
anti-human CD45RA/ Alexa700/ HI100/ BD/ 560673/ 1:167/ 7180940/ 5061696
anti-human CD45RA/ BUV737/ HI100/ BD/ 560673/ 1:100/ 8173965
anti-human CD134/ BV711/ ACT35/ BD/ 563664/ 1:20/ 7209599/ 8017755/ 4253839
anti-human CD134/ PE/ ACT35/BD/ 555838/ 1:50/ 4128542/ 4128542
anti-human CD137/ PE/ 4B4-1/ BD/ 555956/1:50/ 3161529/ 3161529
anti-human CD197/ PE-CF594/ 150503/ BD/ 562381/ 1:50/ 8179961/ 8036858/ 5135914/ 5295853
anti-human CD279/ BV650/ EH12/ BD/ 564104/ 1:25/ 8316604/ 8179961/ 7258845/ 8036858/ 435776/ 5247639
anti-human HLA-DR,DP,DQ/ FITC/ 9-49/ Beckman Coulter/ 6603424/ 1:5/ 71370108
anti-human HLA-A,B,C/ PE/ DX-17/ BD/ 560168/ 1:5/ 7137018
anti-human TNF/ PacificBlue/ Mab11/ Biolegend/ 502920/ 1:167/ B209342
anti-human TNF/ BV421/ Mab11/ BD/ 562783/ 1:167/ 5309795
anti-human IFNγ/ PE-Cy7/ B27/ BD/ 557643/ 1:100/ 6295905/ 7202642/ 5072641
DAPI/ DAPI/ N/A /BD/ 564907/ 1:2500/ 5079994
Fixable Viability Dye eFluor780/ eFluor780/ N/A/ eBioscience/ 65-0865/1:1666/ 1968231/ 4302689/ 1965980
Fixable Viability Dye eFluor506/ eFluor506/ N/A/ eBioscience/ 65-0866/ 1:200/ E15162-120
IFN-γ Secretion Assay, Detection Kit (APC) human/ APC/ N/A/ Miltenyi/ 130-090-762/ 1:10/ 5180212093/ 5180212093

Used multimers (peptide/HLA/fluorochrome/sequence/manufacturer/catalogue number/dilution/lots)

MAGE-A3-168-176/ HLA-A*0101/ PE/ EVDPIGHLY/ Immudex/ WA3249-PE/ 1:10/ 20190902-PL29/ 20181015-EA31
NY-ESO-1-96-104/ HLA-Cw*0304/ PE/ FATPMEAEL/ Immudex/ WS5342-PE/ 1:10/ 20180925-EA3
NY-ESO-1 94-104/ HLA-B*3501 / APC/ MPFATPMEAEL/ Immudex/ WK5341/ 1:10/ 20180820-FL14
NY-ESO-1 92-100/ HLA-Cw*0304 / APC/ LAMPFATPM/ Immudex/ WS3964/ 1:10/ 20190902-PL32/ 20180925-EA1/ 20160118-LL10

Validation

Commercial available antibodies were selected based on their antigen specificity and suggested application as described on the manufacturer`s website and data sheets. The antibody concentrations for staining were optimized by titrating down each reagent starting at the manufacturer`s recommendation. The optimal amounts of the reagents were defined by (i) minimal unspecific shift of the negative population and (ii) a maximal separation of the negative and positive population.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

The K562 and the SK-Mel-28 cell lines were obtained from ATCC. The SK-Mel-29 was obtained from the Memorial Sloan Kettering Cancer Center, NY-York. The SK-Mel-37 cell line is described in Carey TE et al., Proc Natl Acad Sci, 1976. The Jurkat T cell line that expresses a luciferase reporter driven by an NFAT-response element is manufactured by Promega.

Authentication

Reauthentication of cell lines was performed by short tandem repeat (STR) profiling at ATCC and Eurofins.

Mycoplasma contamination

All used cell lines tested negative for mycoplasma contamination

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used

Human research participants

Policy information about studies involving human research participants

Population characteristics

Patients ≥ 18 years old, no gender discrimination, malignant melanoma disease status stage IIIB,C-IV at enrolment, expression of at least one target antigen as assessed by RT-PCR on historic FFPE tumor sample

Recruitment

Recruitment was performed by treating investigators according to inclusion and exclusion criteria without any bias.

Ethics oversight

National competent authority Paul-Ehrlich-Institue and local Ethics Committee of Mainz (Landesärztekammer Rheinland-Pfalz)

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	ClinicalTrials.gov Identifier: NCT02410733, see also manuscript
Study protocol	The full clinical study protocol is not published online, but a comprehensive description of the clinical trial design, eligibility criteria and endpoints is available at https://clinicaltrials.gov/ct2/show/NCT02410733
Data collection	Data are concurrently captured in eCRF until database closure.
Outcomes	Safety and tolerability as primary outcome is assessed by evaluation of dose-limiting toxicities (DLTs) and adverse events (AEs) reported by relationship, grade, and seriousness according to National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events, version 4.03 (CTCAE v4.03). Anti-tumor activity as secondary outcome is assessed by CT-based tumor assessments according irRECIST1.1 and overall survival

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	For flow cytometry analysis PBMCs, skin infiltrating lymphocytes (SILs) or in vitro stimulated (IVS) cultures were used. PBMCs were isolated by ficoll density gradient from whole blood. For IVS either PBMCs or magnetic microbead purified CD8+ or CD4+ T cells were cultured with tumor antigen encoding peptides or tumor antigen presenting APCs for 6-11 days. SILs were recovered from punch biopsies after 2-3 week culture in high dose IL-2 (50.000 U/mL) containing medium. For intracellular stains, cells were fixed and permeabilized using the eBioscience™ Foxp3/ Transcription Factor Staining Buffer Set.
Instrument	For flowcytometry analysis (phenotype, multimer staining, ICS) the BD LSRFortessa™ SORP, the FACSCelesta or the BD FACSCanto™ II devices were used. For flowcytometry sorting of single antigen-specific T cells either the BD FACSAria™ or the BD FACSMelody™ device was used.
Software	The BD FACSDiva™ software version 6.0.3 (FACSAria) or 8.0.1 (LSRFortessa, FACSCanto II, FACSCelesta) or the BD FACSChorus 1.1.18.0 software (FACSMelody) was used for data collection. For data analysis the Treestar FlowJo version 10.5.3 was used.
Cell population abundance	N/A
Gating strategy	The gating strategies are detailed in the materials and methods section, in the supplementary information or in figure legends.

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