

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see [Reporting Life Sciences Research](#). For further information on Nature Research policies, including our [data availability policy](#), see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

▶ Experimental design

1. Sample size

Describe how sample size was determined.

This was a single patient case report, therefore the sample size was 1.

2. Data exclusions

Describe any data exclusions.

No data were excluded from the analyses.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

All attempts for replication of the results, where applicable, were successful.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

This is a single patient case report. No randomization was done.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

This is a single patient case report, so there was no blinding in group allocation.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

- | n/a | Confirmed |
|-------------------------------------|---|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The <u>exact sample size</u> (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement indicating how many times each experiment was replicated |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used and whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Test values indicating whether an effect is present
<i>Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.</i> |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A clear description of statistics including <u>central tendency</u> (e.g. median, mean) and <u>variation</u> (e.g. standard deviation, interquartile range) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Clearly defined error bars in <u>all</u> relevant figure captions (with explicit mention of central tendency and variation) |

See the web collection on [statistics for biologists](#) for further resources and guidance.

► Software

Policy information about [availability of computer code](#)

7. Software

Describe the software used to analyze the data in this study.

GraphPad Prism version 6 and 7, FlowJo version 10, Microsoft Excel 365 ProPlus, Allele-Specific Copy Number Analysis of Tumors (ASCAT) version 2.5, Novoalign MPI (from novocraft version 3.02.10), Picard's MarkDuplicates Tool version 1.127, Samtools version 0.1.18, Varscan2 version 2.3.6, Annovar version 2014Nov12, STAR version 2.5.4a, GATK SplitNTrim tool version 3.4-0, GATK toolbox version 3.4-0, samtools mpileup version 0.1.18, R statistical environment version 3.3.0., Sequenza v2.1.2, PyClone v1.3.0, netMHCpan 3.0 algorithm,

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

► Materials and reagents

Policy information about [availability of materials](#)

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

Individualized cellular immunotherapy, limited research material. Materials available for research institutions with MTA or for-profit companies with license.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The following anti-human flow cytometry antibodies were purchased from BD Biosciences: CD3-APC-H7 (clone: SK7, 641406), CD3-APC-Cy7 (clone: SK7, 557832), CD3-APC (clone: SK7, 340661), CD137(4-1BB)-APC (clone: 4B4-1, 550890), CD4-PE (clone: RPA-T4, 555347), CD4-PE-Cy7 (clone: SK3, 557852), CD8-PE-Cy7 (clone: RPA-T8, 557746), CD8-APC-H7 (clone: SK1, 560179), CD62L-APC (clone: DREG-56, 559772), CD45RO-FITC (clone: UCHL1, 555492), CD279(PD-1)-APC (clone MIH4, 558694), mouse IgG1 κ (isotype control)-APC (555751). The following antibodies were also used in this report from Beckman Coulter: TCR V β 3-FITC (clone: CH92, IM2372), TCR V β 13.1-PE (clone IMMU 222, IM2292), TCR V β 17-PE (clone: E17.5F3.15.13, IM2048).

For the assessment of the TCR repertoire, the Beta Mark TCR V β Repertoire KIT (Beckman Coulter, IM3497) was used. Also, the following anti-mouse flow cytometry antibodies were used: TCR β chain-PE (clone: H57-597, BD Biosciences, 553172), TCR β chain-FITC (clone: H57-597, BD Biosciences, 553171).

The following unconjugated antibodies were used for HLA-blocking experiments: pan-HLA-class I (clone: w6/32, eBioscience, 16-9983-85), pan-HLA-class II (clone Tu39, BD Biosciences, 555556), HLA-class II-DR (clone L243, Biolegend, 307612), -DP (clone B7/21, Leinco Technologies, H260), -DQ (clone SPVL3, Beckman Coulter, IM0416), mouse IgG2a, κ (BD Biosciences, 554645).

For ELISPOT and ELISA assays the following antibodies were used: anti-human IFN- γ capture antibody (clone: 1-D1K, Mabtech, 3420-3-1000), anti-human IFN- γ detection antibody (clone: 7-B6-1, Mabtech, 3420-6-1000), anti-human IFN- γ capture antibody (clone: 2G1, ThermoFisher Scientific, M700A), biotinylated anti-human IFN- γ detection antibody (clone: B133.5, ThermoFisher Scientific, M701B)

For immunohistochemical staining the following antibodies were used: CD3 (Clone 2GV6, Prediluted, Ventana Medical Systems, 790-4341), CK7 (Clone SP52, Prediluted, Roche, 790-4462), PD-1 (Clone NAT105, Prediluted, Ventana Medical Systems, 760-4895) and PD-L1 antibody (Clone SP142, 1:3 dilution, Spring Bioscience, MA4421).

Anti-human CD3 antibody (clone OKT3, eBioscience, 16-0037-85) was used in multiple research assays for T cell stimulation (ELISPOT, activation before transduction, rapid expansion protocol) and the anti-CD3 antibody (clone OKT3, Miltenyi Biotec, 170-076-116) for the clinical rapid expansion protocol.

All antibodies were commercially available and validated by the manufacturers.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

293GP: Surgery Branch, NCI
HEK-CIITA: Surgery Branch, NCI
COS-7: ATCC
3T3-CD40L: Surgery Branch, NCI

b. Describe the method of cell line authentication used.

293GP: by DNA fingerprinting for human. For gal/pol expression was authenticated by production of transient vector production.
HEK-CIITA: by FACS for expression of HLA-class II molecules
3T3-CD40L: by FACS for expression of CD40L

c. Report whether the cell lines were tested for mycoplasma contamination.

All tested negative for mycoplasma contamination

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

HEK and 293GP cells lines served the purpose that they were intended for.

► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

No animals were used in the study.

Policy information about [studies involving human research participants](#)

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Single patient report of a 49-year-old woman with metastatic ER+, Her2- breast cancer.

Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

▶ Data presentation

For all flow cytometry data, confirm that:

- 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- 2. 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).
- 3. All plots are contour plots with outliers or pseudocolor plots.
- 4. A numerical value for number of cells or percentage (with statistics) is provided.

▶ Methodological details

- | | |
|--|---|
| 5. Describe the sample preparation. | It is described in Material and Methods |
| 6. Identify the instrument used for data collection. | BD FACS Canto I, FACS Canto II and FACS Aria III sorter |
| 7. Describe the software used to collect and analyze the flow cytometry data. | BD FACS DIVA version 8.0.1
FlowJo version 10 |
| 8. Describe the abundance of the relevant cell populations within post-sort fractions. | Populations were validated for purity by a post-sort analysis by FACS |
| 9. Describe the gating strategy used. | Every flow cytometry analysis was initiated as follows:
FSC-A/SSC-A to gate the lymphocyte population
FSC-W/FSC-H to select single cells
SSC-W/SSC-H to further select single cells
Gating of the Propidium Iodide negative cells to select live cells
followed by the gating as described in the figures. |

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