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☐ Initial submission ☐ Revised version ☒ Final submission

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.

Experimental design

1. Sample size

Describe how sample size was determined.

The sample size for this study was selected to provide adequate power to test the hypothesis that the true success rate was 5% versus the alternative that the success rate is 30%. A two stage design allowed for an interim assessment of success while controlling the overall type I error. Stage I included 8 participants and stage 2 included an additional 7 participants (totaling 15 participants). The minimax design minimized the maximum number of participants undergoing antiretroviral treatment interruption across both stages. The targeted Type I and Type II error rates for the minimax design were both 0.05 and under the current design, the actual Type I and Type II error rates were 0.034 and 0.147respectively, corresponding to an approximate 3.4% alpha and 85% power under the assumed alternative. The criterion to proceed from stage 1 and 2 was that at least 1 of 8 participants achieved VL < 50 at 12 weeks post treatment interruption. As none did, the study did not progress to stage 2, and a total of 8 participants were enrolled.

2. Data exclusions

Describe any data exclusions.

No data were excluded from the analysis.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Not applicable. Eight human participants were enrolled and underwent treatment interruption.

4. Randomization

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

Not applicable. This is a phase 2, single-arm, open-label study.

5. Blinding

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

Not applicable. This is a phase 2, single-arm, open-label study.

Note: all studies involving animals and/or human research participants must disclose 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
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☒	☐ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☒	☐ Clearly defined error bars

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.	Stata Statistical Software Release 13 (StataCorp, College Station, TX, USA) and GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com.

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 for-profit company.	Not applicable. No unique materials were used. There were no interventions in the study.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Alexa Fluor 700-labeled anti-human CD3 mAb, BD Biosciences, cat#557943, clone UCHT1, lot#4140529. BUV496-labeled anti-human CD4 mAb,BD Biosciences, cat#564651, clone SK3, lot#6130797. Allophycocyanin H7-labeled anti-human CD45RA mAb,BD Biosciences, cat#560674, clone HI100, lot#6099627. BUV395-labeled anti-Ki-67 mAb, BD Biosciences, cat#564071, clone B56, lot#6127768. BV785-labeled anti-human CD8a mAb, BioLegend, cat#301046, clone RPA-T8, lot#B205873. BV650-labeled anti-human CD127 mAb, BioLegend, cat#351326, clone A019D5, lot#B199762. Pacific Blue-labeled anti-human Perforin mAb, BioLegend, cat#308118, clone dG9, lot#B168011. All Abs were tested for their specific staining with PBMCs from healthy individuals. For the Ki-67 Ab, PBMCs were cultured with or without Staphylococcal enterotoxin B to see specific staining of cells in cell cycle. All the Abs were used in our published article (Sci. Transl. Med. 9, eaag1809, 2017).

10. Eukaryotic cell lines

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

Not applicable. Cell lines were not used.

b. Describe the method of cell line authentication used.

Describe the authentication procedures for each cell line used OR declare that none of the cell lines used have been authenticated OR state that no eukaryotic cell lines were used.

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

Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination OR state that no eukaryotic cell lines were used.

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.

Provide a rationale for the use of commonly misidentified cell lines OR state that no commonly misidentified cell lines were used.

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

Not applicable. Research animals were not used.

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.

8 human research participants were enrolled. There were 1 female and 8 male, and their median age was 29 years old. They all initiated antiretroviral therapy in acute HIV infection and had been virally suppressed for a median of 2.8 years prior to enrollment. 6 had CRF01_AE HIV infection and 2 had CRF01_AE/B infection.

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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.	Freshly thawed peripheral-blood mononuclear cell from the participants were subject to flow cytometry staining.
6. Identify the instrument used for data collection.	BD LSRII with 5 lasers (Blue, Green, Red, Violet, and UV)
7. Describe the software used to collect and analyze the flow cytometry data.	BD FACSDiva Software Version 8.0.1 for data collection. FlowJo V10.0.8 for data analysis.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Not applicable. No FACS soring experiment was performed.
9. Describe the gating strategy used.	Lymphocytes were gated on low level of FSC and SSC. Then, doublets were excluded based on high outlier events for FSC-W and SSC-W against FSC-A and SSC-A, respectively. Live lymphocytes were selected based on negative staining for LIVE/DEAD Fixable Aqua Dead Cell Stain and look at HLA-A*1101 NEF tetramer+ cells. CD3+CD8+CD45RA- cells within the live lymphocytes were used to gate on Ki-67+ cells. The Ki-67+ cell gating was based on naive CD8+ T cell population as negative staining reference. All flow cytometry experiments were done with the same control PBMCs from a healthy individuals. The Ki-67+ cell gate was adjusted to 2.2% in the control sample for overall experiments.

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