Traditional Chinese Medicine TN-01 Reconstitutes T Cells in SIV–infected Rhesus Monkeys

Qiaoli Wang
Jinan University College of Life Science and Technology
Qiongzhen Zeng
Jinan University College of Life Science and Technology
Zhe Ren
Jinan University College of Life Science and Technology
Yuefeng Li
Guangdong Landau Biotechnology Co Ltd.
Rongze Wang
Jinan University College of Life Science and Technology
Yue Feng
Jinan University College of Life Science and Technology
Jianmeng Ye
Guangdong Landau Biotechnology Co Ltd
Xiaowei Song
Jinan University College of Life Science and Technology
Shurong Qin
Jinan University College of Life Science and Technology
Pengjun Zhou
Jinan University College of Life Science and Technology
Yexuan Zhu
Jinan University College of Life Science and Technology
Feng Liang
Jinan University College of Information Science and Technology
Ziyao Li
Jinan University College of Life Science and Technology
Hemei Qi
Chinese Academy of Sciences
Li Qin
Chinese Academy of Sciences
Fujun Jin
Jinan University College of Life Science and Technology
Yifei Wang (twangyf@jnu.edu.cn)

Jinan University https://orcid.org/0000-0002-9918-2865

Research

Keywords: HIV/AIDS, Antiretroviral therapy, Traditional Chinese medicine, Immune reconstruction, Immune functional cure

Posted Date: July 13th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-693617/v1

Abstract

Background: Long-term antiretroviral therapy (ART) cannot recover the T cell counts in a substantial proportion of AIDS patients and viral loads rapidly rebound after ART suspension. Therefore, exploring novel alternative and adjuvant ART has become a priority in HIV treatment. Traditional Chinese medicine (TCM) have beneficial effects in regulating T cells and our previous clinical trial data have shown that TCM TN-01 maintains good health of patients with AIDS with an unclear mechanism. Herein, we preliminary investigated whether TN-01 influences immune reconstruction to enhance the antiviral immune response in simian immunodeficiency virus (SIV)-infected rhesus monkeys.

Methods: The SIV-infected animals were firstly administered with TN-01 alone (5 ml/kg; twice a day, i.g.). More than 2 months later, these 2 SIV-infected rhesus monkeys were intraperitoneally injected with PMPA (30 mg/kg; once a day) and FTC (20 mg/kg; once a day) for about 3 months. Moreover, SIV-infected rhesus monkeys were also administered with TN-01 each 2 weeks from week 23 to week 35 during ART. Flow cytometer were used to assess the phenotype of T cell and plasma was isolated from whole blood for viral load detection.

Results: The flow cytometry analysis revealed that treatment with TN-01 alone or combined with ART significantly upregulated T cells counts and the proportions of T cell subsets. TN-01 treatment also delayed viral rebound after ART suspension.

Conclusions: Our data indicate that TN-01 could enhance the efficacy of ART and promote immune reconstruction, suggesting TN-01 treatment as a potent strategy in immune functional cure of HIV infection.

Backgroud

Acquired immune deficiency syndrome (AIDS) is a type of $CD4^+$ T cells exhaustion mediated immunodeficiency syndrome caused by human immunodeficiency virus (HIV) infection.[1, 2] T cell plays significant roles in suppression of virus replication,[3] and eliminates the virus infected cells.[4, 5] Therefore, functional cure of HIV infection through modulation of T cell reconstitution becomes long-term goal.

Currently, most of HIV-infected people are widely treated by antiretroviral therapy (ART).[6] Despite ART can effectively control virus replication whereas there is a substantial proportion of AIDS patients that do not exhibit any improvements in the normalization of T cell counts and T cell function under long-term ART.[7–10] Besides, viral load rapidly rebounds upon ART interruption in vast majority of HIV-infected individuals.[6, 11] Long-term ART also causes some unacceptable side effects due to the drug toxicity, poor adherence to therapy, and drug resistance.[12, 13] Therefore, it is necessary to develop new drugs or complementary methods to overcome the limitations of ART in treating AIDS and AIDS-related complications.

Traditional Chinese herbal medicine (TCM) plays important roles in treating immune deficiency diseases. [14, 15] For instance, clinical data showed that TCM is effective in treating AIDS via maintaining immune function and reducing the adverse effects of ART.[15] TCM steadily increased $CD4^+$ and $CD8^+$ T cell counts during viral infection, [16, 17] while has no effect on viral load in patients. Interestingly, combination of TCM and ART significantly inhibited HIV infection,[16, 18] suggesting TCM as an adjuvant treatment of ART to achieve the goal of AIDS eradication.[19] Notably, our previous study showed that a unique formula of TCM (TN-01) maintained normal $CD4^+$ T cells counts in AIDS patients. [20]. In this study, we investigated its action on the immune function reconstruction. We revealed that TN-01 upregulated $CD4^+$ and $CD8^+$ T cell counts and enhanced T cells differentiation and activation in SIV-infected rhesus monkeys. Treatment with TN-01 combined with ART therapy also delayed the rapidly rebound of viral load after treatment interruption. Our results highlight the potential function of TN-01 in eradicating HIV infection.

Materials And Methods

Animals

Adult rhesus monkeys in this study were obtained from Guangdong Landau Biotechnology Co. Ltd (Guangzhou, China). All rhesus monkeys were confirmed to be free of Tuberculin, B virus, D-type simian retrovirus, simian T lymphotropic virus type 1 and SIV. All rhesus monkeys were housed at the Non-Human Primate Animal Center of the Guangdong Landau Biotechnology Co. Ltd and acclimatized in a separate cage, with standard primate food and water. All animal experiments were performed in accordance with the animal experiment manual and reviewed and approved by the Institutional Animal Care and Use Committee of Guangdong Landau Biotechnology Co., Ltd (LDACU 20170410-01).

Rhesus monkey model of SIV infection

Rhesus monkey model of simian immunodeficiency virus (SIVmac251, SIV) infection was established as previously described.[21] In brief, rhesus monkeys were infected intravenously (i.v.) with 300 $TCID_{50}$ of SIVmac251. 16 weeks later, the SIV-infected rhesus monkeys were ready to treat with TN-01 alone or TN-01 combined with ART therapy, as described in Fig.1.

Blood collection and preparation

Whole blood was collected from SIV-infected rhesus monkeys at week 0 (before treatment), 2, 4, 6, 8, 10, 12 and 14. Subsequently, T cell counts and the phenotype of T cell were determined using a flow cytometer (FACSCanto; Becton Dickinson). Plasma was isolated from whole blood, and was cryopreserved at -80 °C for viral load detection. In addition, whole blood was collected after combined treatment of TN-01 and ART, and T cell phenotype was then determined by flow cytometry at week 23, 25, 27, 29, 31, 33, 38 and 40. Plasma viral loads were measured every week.

Flow cytometry analysis of T cell phenotype

Flow cytometry analysis of T-cell phenotype was performed according to the protocols as previously described.[21-23] Monoclonal antibodies used in this study including anti-CD3-BV605, anti-CD45-PE, anti-CD4-BV421, anti-CD8-APC-R700 anti-CD95-DX2, anti-CD28-CD28.2, anti-HLA-DR-PE-Cy7, anti-CD69-APC and anti-CCR5-PE, were purchased from BD Biosciences (San Jose, CA, USA). Anti-CD38-FITC was obtained from StemCell Technologies (Vancouver, BC, Canada). Anti-CD25-APC was from Biolegend (San Diego, CA, USA), and anti-CD127-PE was a product of Invitrogen (Carlsbad, CA, USA). $CD4^+$ T cell differentiation was identified in terms of CD28 and CD95 expression, as $CD28^+$ $CD95^+$ $CD4^+$ T cell defined as central memory $CD4^+$ T cell ($CD4^+$ Tcm) and $CD28^+$ $CD95^-$ $CD4^+$ T cell as effector memory CD4+ T cell ($CD4^+$ T_{EM}). $CD28^+$ $CD95^+$ $CD8^+$ T cell was defined as $CD8^+$ T_{CM} cell whereas $CD28^+$ $CD95^-$ $CD8^+$ T cell was $CD8^+$ T_{EM} cell. In addition, expression of CD25 and CD127 were measured to evaluate regulatory T Cells (Tregs). Activation markers HLA-DR, CD38 and CD69 were measured on $CD4^+$ and $CD8^+$ T cells, and $CD4^+$ $CCR5^+$ T cells were defined as SIV-infected cells. All dates were acquired and analyzed on a flow cytometer (FACSCanto; Becton Dickinson).

SIV-1 viral load measurement

Plasma SIV viral load was quantified by SYBR green Real-time reverse transcription-polymerase chain reaction (RT-PCR) based on published study.[24] Primers and probes synthesized by Invitrogen (Carlsbad, CA, USA) were designed as previously study,[25] Alu1217-F, GCA GAG GAG GAA ATT ACC CAG; SIVgagA, CAA TTT TAC CCA GGC ATT TAA TGT T; and Alu1217-PFAM, TCGG GCTTAATGGCAGGTGGACA. Briefly, the viral RNA was isolated using QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA, USA) according to the provided instructions. RNA measurement was performed using One-step qPCR Kit RNA-direct Real time PCR Master Mix (TaKaRa, Tokyo, Japan).

Statistical analysis

Statistical analysis was performed using GraphPad Prism7.0 (GraphPad Software Inc., San Diego, CA, United States). One-way analysis of variance (ANOVA) followed by Turkey post hoc test and unpaired. Student's t-test were used to analyze the statistical significance among multiple groups and between two groups, respectively. Data are reported as the mean ± SD. $P < 0.05$ was considered statistically significant.

Results

Study design

Rhesus monkeys infected SIV before TN-01 treatment (see Methods). We used 2 rhesus monkeys successfully infected with SIV in this study of TN-01 treatment on immune reconstitution, and we named these 2 monkeys as S1 and S3, respectively. The SIV-infected animals were firstly administered with TN-01 alone (5 ml/kg; twice a day, i.g.) from week 0 to week 2, and from week 4 to week 6, respectively (Fig. 1a). Plasma viral loads and immunological efficacy of TN-01 were detected at week 0 (without therapy) and the indicated weeks (up to week 14) (Fig.1a). More than 2 months later, these 2 SIV-

infected rhesus monkeys were intraperitoneally injected with PMPA (30 mg/kg; once a day) and FTC (20 mg/kg; once a day) for about 3 months. Moreover, SIV-infected rhesus monkeys were also administered with TN-01 each 2 weeks from week 23 to week 35 during ART. T cell phenotype and plasma viral loads were detected by flow cytometry at the indicated time points (see materials and methods).

Effects of TN-01 on total T-cell counts

Immune cells are essential for AIDS patients,[16] we therefore assessed the immunomodulatory effects of TN-01 in SIV-infected rhesus monkeys (named S1 or S3). As expected, flow cytometry analysis revealed that $CD4^+$ and $CD8^+$ T cell counts were remained significantly higher than week 0 (Fig. 2a). Similarly, combinatory treatment with ART and TN-01 also increased $CD4^+$ and $CD8^+$ T cell counts after therapy interruption (Fig. 2b). These changes were sustained for 14 weeks, suggesting that TN-01 promoted the efficacy of ART. Together, these results indicated that total $CD4^+$ and $CD8^+$ T cell counts were elevated during TN-01 therapy and these changes were sustained at therapy discontinuation.

Effects of TN-01 on memory T cell

Effector memory T cell plays a key role in immune response against virus infection.[26] We next explored whether TN-01 influenced the different subsets of memory T cell. After 2 weeks, the proportions of $CD4^+$ and $CD8^+$ central memory cells (T_{CM})were significantly decreased ~20% in monkeys treated with TN-01 alone (Fig. 3a). In addition, the proportions of $CD4^+$ and $CD8^+$ effector memory cells (T_{EM})were significantly different from those T_{CM} cells at week 2 and had a steady increment up to week 14 after TN-01discontinuation (Fig. 3b). These changes were sustained at 14 weeks, corroborating that TN-01 influenced proportion of memory T cell. Interestingly, T_{CM} cells showed a steady increase during TN-01 and ART combinatory treatment and remained high level up to 15 weeks after treatment discontinuation (Fig. 3c), which were not observed in T_{EM} cells (Fig. 3d). Together, these results indicated that TN-01 therapy increased the proportions of T_{CM} cells duringSIV-infection, and continuously enhanced the differentiation of T_{CM} cells into T_{EM} cellsto against SIV during persistent infection.

Effects of TN-01 on T cells immune response

To confirm the fore mentioned results obtained from flow cytometry analysis of memory T cell subsets (Fig. 3), we next explored whether TN-01 could influence T cell immune response in SIV-infected rhesus monkeys. The result showed that the percentages of $CD4^+$ and $CD8^+$ Tregs were markedly suppressed after secondary TN-01 discontinuation (Fig. 4a). Moreover, the proportion of $CD4^+$ and $CD8^+$ Tregs were slightly lower than pre 2 weeks by combined with ART therapy (Fig. 4c), suggesting that TN-01 may more effective to recover T cell immune response during SIV-infection. Next, we investigated the effects of TN-01 on the activation of different subsets of $CD4^+$ and $CD8^+$ T cells. At week 6, TN-01 therapy decreased the proportions of $CD38^+$ $CD4^+$ and $CD38^+$ $CD8^+$ T cells (Fig. 4b). The proportions of CD69 expression significantly increased after secondary TN-01 discontinuation and HLA-DR expression slightly

upregulated for 14 weeks (Fig. 4b), suggesting that TN-01 could achieve T cell reconstruction for SIV-infected rhesus monkeys. Interestingly, combination therapy did not change the expression levels of CD69, CD38 and HLA-DR (Fig. 4d). All these results indicated that TN-01 enhanced T cell activation.

Effects of TN-01 on viral load

Next, we assayed the effects of TN-01 on viral load. Flow cytometry analysis showed that the number of SIV-infected T cells ($CD4^+$ $CCR5^+$) was significantly higher after TN-01 therapy (Fig. 5a), whereas the viral load was not obviously reduced (Fig. 5b). Interestingly, TN-01 combined with ART therapy could reduce the proportions of $CD4^+$ $CCR5^+$ cells as compared to TN-01 alone therapy (Fig. 5c). Furthermore, viral load also showed significantly suppression by combination therapy (Fig. 5d), indicating that combination therapy was more benefit to eliminate SIV-infected cells and to delay viral rebound once treatment discontinued. Together, these results indicated that TN-01 combined with ART therapy has the potential to become a functional cure for SIV-infected rhesus monkeys

Discussion

Long-term ART treatment just controls HIV replication and does not restore normal T cell function to eliminate HIV.[27] Therefore, it is necessary to find an effective and long-lasting intervention to enhance efficacy of ART and eliminate HIV completely by immune reconstruction[28, 29]. It has been demonstrated that TCM can safely and effectively treat AIDS,[30, 31] as well as control HIV-infected patients.[16, 18, 19] For instance, clinical data showed that Zhongyan-4 has an immuno-protective effect in HIV-infected patients for 6 months by elevating $CD4^+$ T cells counts and reducing viral load.[17] Our previous clinical trial reports also suggested that TN-01 improves the long-term survival of HIV-infected patients. TN-01 decreased viral loads from 2003 to 2006 and maintained normal $CD4^+$ counts in nine living AIDS patients, suggesting that TN-01 is more conducive for HIV-infected patients to maintain health for long time.[20] In the present works, we examined whether TN-01 influences T cell function. Although only 2 SIV-infected rhesus monkeys were used in this experiment, T cell counts and several T cells subsets were increased after TN-01 treatment. Moreover, combined with ART administration inhibited the rapid rebound of viral load after treatment interruption. Therefore, TN-01 therapy is suggested to be a potential application in treating HIV/SIV infection.

One major concern of this study is the underlying mechanism for TN-01 treatment. Considering that T cell reconstruction does not recover completely during ART treatment, we hypothesized that TN-01 might have been involved in T cell immune function reconstruction during infection. In support of this notion, we found that TN-01 elevated the levels of T cell counts in SIV-infected rhesus monkeys, consistent with our previous study.[20] Besides, TN-01 combined with ART further increased T cell counts. It is known that T_{CM} can differentiate into T_{EM} when encounters the pathogen again,[32] and T_{EM} activation plays crucial role in immune response process against HIV infection. Consistently, we showed that TN-01 treatment alone increased the proportion of $CD4^+$ and $CD8^+$ T_{EM} (see Fig. 1). Interestingly, combined treatment with TN-01 and ART elevated the proportion of T_{CM}, but did not change the proportion of T_{EM}, suggesting that

TN-01 was able to reshape the proportion of memory T cell subsets. This was likely due to that ART effectively suppressed early stage of virus infection and TN-01 did not need to further promote the differentiation of T_{CM} into T_{EM}. In addition, Tregs can balance immune activation in the immune system,[33] and several studies showed that Tregs exerted a negative role in HIV defense by inhibiting the response of $CD4^+$ and $CD8^+$ T cells in HIV-infected patients or SIV-infected rhesus monkeys.[34, 35] We also found that TN-01 reduced the proportions of Tregs, furthering confirming the negative function of Tregs in immune response against HIV infection.

Activation of $CD4^+$ T cell is enhanced in acute HIV infection,[36] and $CD4^+$ and $CD8^+$ T cell activation plays significant role for suppression of HIV replication.[9, 10, 37] We therefore evaluated the effect of TN-01 on T cell activation. CD69 is the earliest activation marker during T cells activation,[38] and CD69 expression becomes a reliable and rapid assessment for the activation and antiviral functions of $CD4^+$ and $CD8^+$T cells.[38] Indeed, we found that TN-01 treatment greatly increased the proportion of CD69 + T cell, implying enhanced T cell immune response by TN-01.

Virus reservoir is the main reason for the virus rebound of HIV patients, and viral load will rapid rebound for 4 weeks or several months after ART interruption.[39] Although virus reservoir was not detected in the present study, our experiments showed that viral load was maintained at a low level after ART treatment interruption in our observations for 8 weeks (from week 36 to week 43). Accordingly, additional investigation is needed to explore virus reservoir and to evaluate an extended observation time after treatment interruption.

Conclusion

Our study showed that TN-01 potentiates immune reconstruction to antagonize SIV infection. Moreover, TN-01 combined with ART therapy was effective in delaying the rapid viral rebound after ART treatment interruption. Therefore, our findings highlight the potential application of TN-01 as an adjuvant treatment of ART therapy in functional cure of HIV/SIV infection.

Abbreviations

ART: antiretroviral therapy; TCM: Traditional Chinese medicine; SIV: simian immunodeficiency virus; AIDS: Acquired immune deficiency syndrome; HIV: human immunodeficiency virus. i.v.: infected intravenously; i.g.: intragastrically

Declarations

Ethics approval and consent to participate

All animal experiments in this study were performed in accordance with the animal experiment manual and reviewed and approved by the Institutional Animal Care and Use Committee of Guangdong Landau

Biotechnology Co., Ltd (LDACU 20170410-01).

Consent for publication

All authors consent to the publication of this manuscript.

Availability of data and materials

Details of data mining, selection, extraction and assessment carried out to support the findings of this study are available from the corresponding author upon request.

Competing interests

No competing financial interests exist.

Funding

This work was supported by the National Natural Science Foundation of China (grants 81872908 and 820722741) and The Key Laboratory of Virology of Guangzhou (grant 201705030003) and Guangzhou Major program of the Industry-University-Research collaborative innovation (grant 201704030087).

Authors' contribution

QZZ drafted the manuscript. QLW, QZZ and ZR searched the literature and conducted the assessment. FJJ and QLW gave suggestions and revised the manuscript. FJJ designed the study. FJJ and YFW gave advice and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank all the researchers and experts for this study.

References

1. Douek DC, Roederer M, Koup RA. Emerging concepts in the immunopathogenesis of AIDS. Annu Rev Med. 2009;60:471-484.
2. Okoye AA, Picker LJ. CD4(+) T-cell depletion in HIV infection: mechanisms of immunological failure. Immunol Rev. 2013;254(1):54-64.
3. Xu H, Wang X, Lackner AA, et al. CD8 down-regulation and functional impairment of SIV-specific cytotoxic T lymphocytes in lymphoid and mucosal tissues during SIV infection. J Leukoc Biol. 2013;93(6):943-950.
4. Cartwright EK, Spicer L, Smith SA, et al. CD8(+) Lymphocytes Are Required for Maintaining Viral Suppression in SIV-Infected Macaques Treated with Short-Term Antiretroviral Therapy. Immunity. 2016;45(3):656-668.

5. Agosto LM, Henderson AJ. CD4(+) T Cell Subsets and Pathways to HIV Latency. AIDS Res Hum Retroviruses. 2018;34(9):780-789.
6. Hatano H, Yukl SA, Ferre AL, et al. Prospective antiretroviral treatment of asymptomatic, HIV-1 infected controllers. PLoS Pathog. 2013;9(10):e1003691.
7. Wilson EM, Sereti I. Immune restoration after antiretroviral therapy: the pitfalls of hasty or incomplete repairs. Immunol Rev. 2013;254(1):343-354.
8. Kelley Colleen F, Kitchen Christina MR, Hunt Peter W, et al. Incomplete Peripheral CD4+Cell Count Restoration in HIV-Infected Patients Receiving Long-Term Antiretroviral Treatment. Clinical Infectious Diseases. 2009;48(6):787-794.
9. Volberding P, Demeter L, Bosch RJ, et al. Antiretroviral therapy in acute and recent HIV infection: a prospective multicenter stratified trial of intentionally interrupted treatment. AIDS. 2009;23(15):1987-1995.
10. Ndhlovu ZM, Kamya P, Mewalal N, et al. Magnitude and Kinetics of CD8+ T Cell Activation during Hyperacute HIV Infection Impact Viral Set Point. Immunity. 2015;43(3):591-604.
11. Davey RT, Bhat N, Yoder C, et al. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. Proc Natl Acad Sci USA. 1999;96(26):15109-15114.
12. Montessori V, Press N, Harris M, et al. Adverse effects of antiretroviral therapy for HIV infection. CMAJ. 2004;170(2):229-238.
13. Wainberg MA, Zaharatos GJ, Brenner BG. Development of antiretroviral drug resistance. N Engl J Med. 2011;365(7):637-646.
14. Wu X-f, Wang J, Li Y, et al. Thoughts on intervention in HIV/AIDS with traditional Chinese medicine. J Tradit Chin Med. 2011;31(4):265-268.
15. Liu Z-B, Yang J-P, Xu L-R. Effectiveness and safety of traditional Chinese medicine in treating acquired immune deficiency syndrome: 2004-2014. Infectious diseases of poverty. 2015;4:59.
16. Zou W, Wang J, Liu Y. Effect of traditional Chinese medicine for treating human immunodeficiency virus infections and acquired immune deficiency syndrome: Boosting immune and alleviating symptoms. Chin J Integr Med. 2016;22(1):3-8.
17. Wang J, Yang FZ, Zhao M, et al. Randomized double-blinded and controlled clinical trial on treatment of HIV/AIDS by Zhongyan-4. Chin J Integr Med. 2006;12(1):6-11.
18. Wang J, Zou W. Practices, challenges, and opportunities: HIV/AIDS treatment with traditional Chinese medicine in China. Front Med. 2011;5(2):123-126.
19. Liu ZB, Yang JP, Xu LR. Effectiveness and safety of traditional Chinese medicine in treating acquired immune deficiency syndrome: 2004-2014. Infect Dis Poverty. 2015;4:59.
20. Wang Y, Jin F, Wang Q, et al. Long-Term Survival of AIDS Patients Treated with Only Traditional Chinese Medicine. AIDS Res Hum Retroviruses. 2017;33(2):90-92.

21. Zhan XY, Wang N, Liu G, et al. Plasmodium infection reduces the volume of the viral reservoir in SIV-infected rhesus macaques receiving antiretroviral therapy. Retrovirology. 2014;11:112.
22. Liu G, Li Y, Qin L, et al. SIV infection aggravates malaria in a Chinese rhesus monkey coinfection model. BMC infectious diseases. 2019;19(1):965.
23. Maino VC, Suni MA, Ruitenberg JJ. Rapid flow cytometric method for measuring lymphocyte subset activation. Cytometry. 1995;20(2):127-133.
24. Zhan X-Y, Wang N, Liu G, et al. Plasmodium infection reduces the volume of the viral reservoir in SIV-infected rhesus macaques receiving antiretroviral therapy. Retrovirology. 2014;11:112.
25. Hofmann-Lehmann R, Swenerton RK, Liska V, et al. Sensitive and robust one-tube real-time reverse transcriptase-polymerase chain reaction to quantify SIV RNA load: comparison of one- versus two-enzyme systems. AIDS research and human retroviruses. 2000;16(13):1247-1257.
26. Kulpa DA, Talla A, Brehm JH, et al. Differentiation into an Effector Memory Phenotype Potentiates HIV-1 Latency Reversal in CD4(+) T Cells. J Virol. 2019;93(24).
27. Hunt PW. HIV and inflammation: mechanisms and consequences. Curr HIV/AIDS Rep. 2012;9(2):139-147.
28. Pace M, Frater J. A cure for HIV: is it in sight? Expert review of anti-infective therapy. 2014;12(7):783-791.
29. Davenport MP, Khoury DS, Cromer D, et al. Functional cure of HIV: the scale of the challenge. Nature reviews. Immunology. 2019;19(1):45-54.
30. Xu LR, Guo HJ, Liu ZB, et al. Unified-planning, graded-administration, and centralized-controlling: a management modality for treating acquired immune deficiency syndrome with Chinese medicine in Henan Province of China. Chin J Integr Med. 2015;21(4):243-248.
31. Jiang F, Zhang R, Gu Z, et al. Fuzhengpaidu granule regulates immune activation molecules CD38 and human leukocyte antigen-D related on CD4+ and CD8+ T cells in patients with acquired immunodeficiency syndrome/human immunodeficiency virus. J Tradit Chin Med. 2013;33(4):439-443.
32. Groot F, van Capel TM, Schuitemaker J, et al. Differential susceptibility of naive, central memory and effector memory T cells to dendritic cell-mediated HIV-1 transmission. Retrovirology. 2006;3:52.
33. Holmes D, Jiang Q, Zhang L, et al. Foxp3 and Treg cells in HIV-1 infection and immuno-pathogenesis. Immunol Res. 2008;41(3):248-266.
34. Pereira LE, Villinger F, Onlamoon N, et al. Simian immunodeficiency virus (SIV) infection influences the level and function of regulatory T cells in SIV-infected rhesus macaques but not SIV-infected sooty mangabeys. J Virol. 2007;81(9):4445-4456.
35. Aandahl EM, Michaelsson J, Moretto WJ, et al. Human CD4+ CD25+ regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens. J Virol. 2004;78(5):2454-2459.

36. Xia H, Jiang W, Zhang X, et al. Elevated Level of CD4+ T Cell Immune Activation in Acutely HIV-1-Infected Stage Associates With Increased IL-2 Production and Cycling Expression, and Subsequent CD4+ T Cell Preservation. Front Immunol. 2018;9.
37. McBrien JB, Kumar NA, Silvestri G. Mechanisms of CD8(+) T cell-mediated suppression of HIV/SIV replication. Eur J Immunol. 2018;48(6):898-914.
38. Pitsios C, Dimitrakopoulou A, Tsalimalma K, et al. Expression of CD69 on T-cell subsets in HIV-1 disease. Scandinavian journal of clinical and laboratory investigation. 2008;68(3):233-241.
39. Vallejo A, Molina-Pinelo S, De Felipe B, et al. Toll-like receptor 9 1635A/G polymorphism is associated with HIV-1 rebound after four weeks of interruption of antiretroviral therapy. J Acquir Immune Defic Syndr. 2020.

Figures

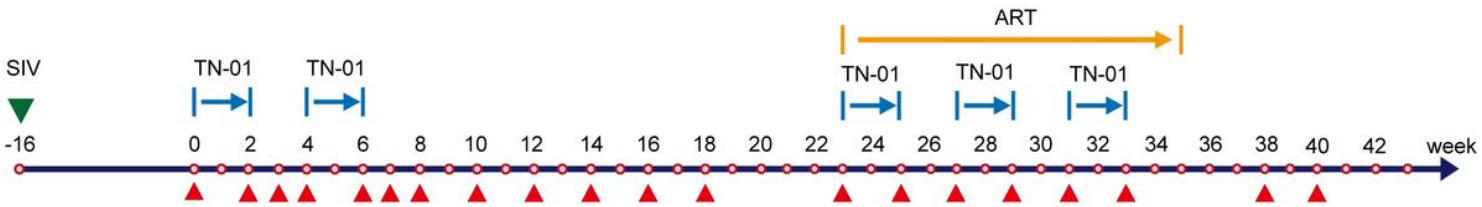

Figure 1

Experimental schedule of SIV-infected rhesus monkey. After rhesus monkeys were infected with SIVmac251 (green arrows) for 16 weeks, then 2 rhesus monkeys were treated with TN-01 (blue arrows) for indicated time periods. Subsequently, SIV-infected rhesus monkeys were treated with ART (yellow arrow) from week 23 to week 35 in the presence or absence of TN-01 (blue arrows) for indicated time period. Flow cytometry detected T cell phenotype (red arrows) and viral loads.

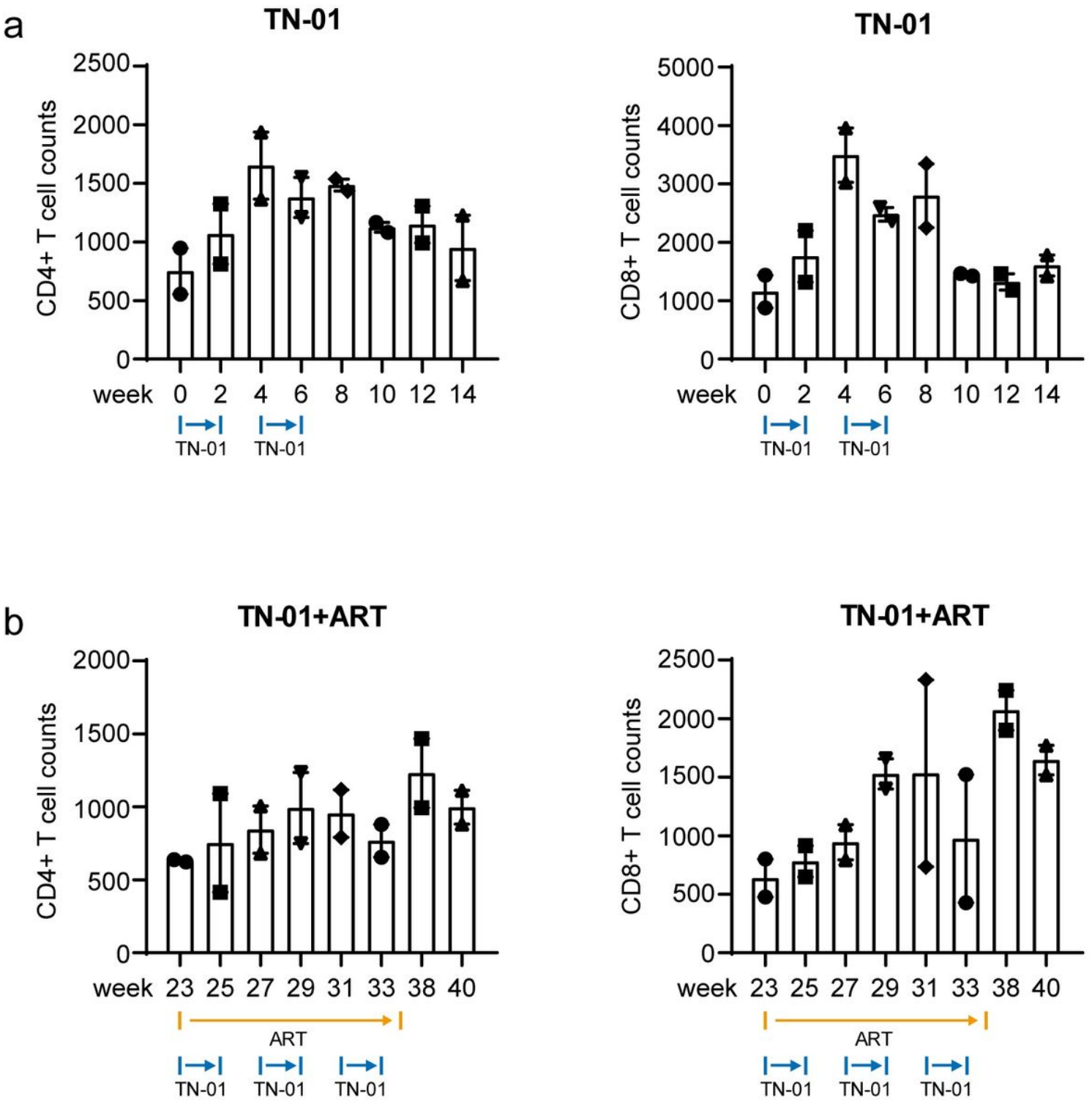

Figure 2

TN-01 increased T cell counts. SIV-infected rhesus monkeys were treated with TN-01 alone (a) or combined with ART therapy (b) for indicated time periods. (a) Flow cytometry was used to assess CD4+ or CD8+ T cell counts on 8 time points (week 0, 2, 4, 6, 8, 10, 12 and 14 in the course). Histograms show the total CD4+ or CD8+ T cell counts. (b) Histograms show total CD4+ or CD8+ at indicated time periods (week 23, 25, 27, 29, 31, 33, 38 and 40 in the course).

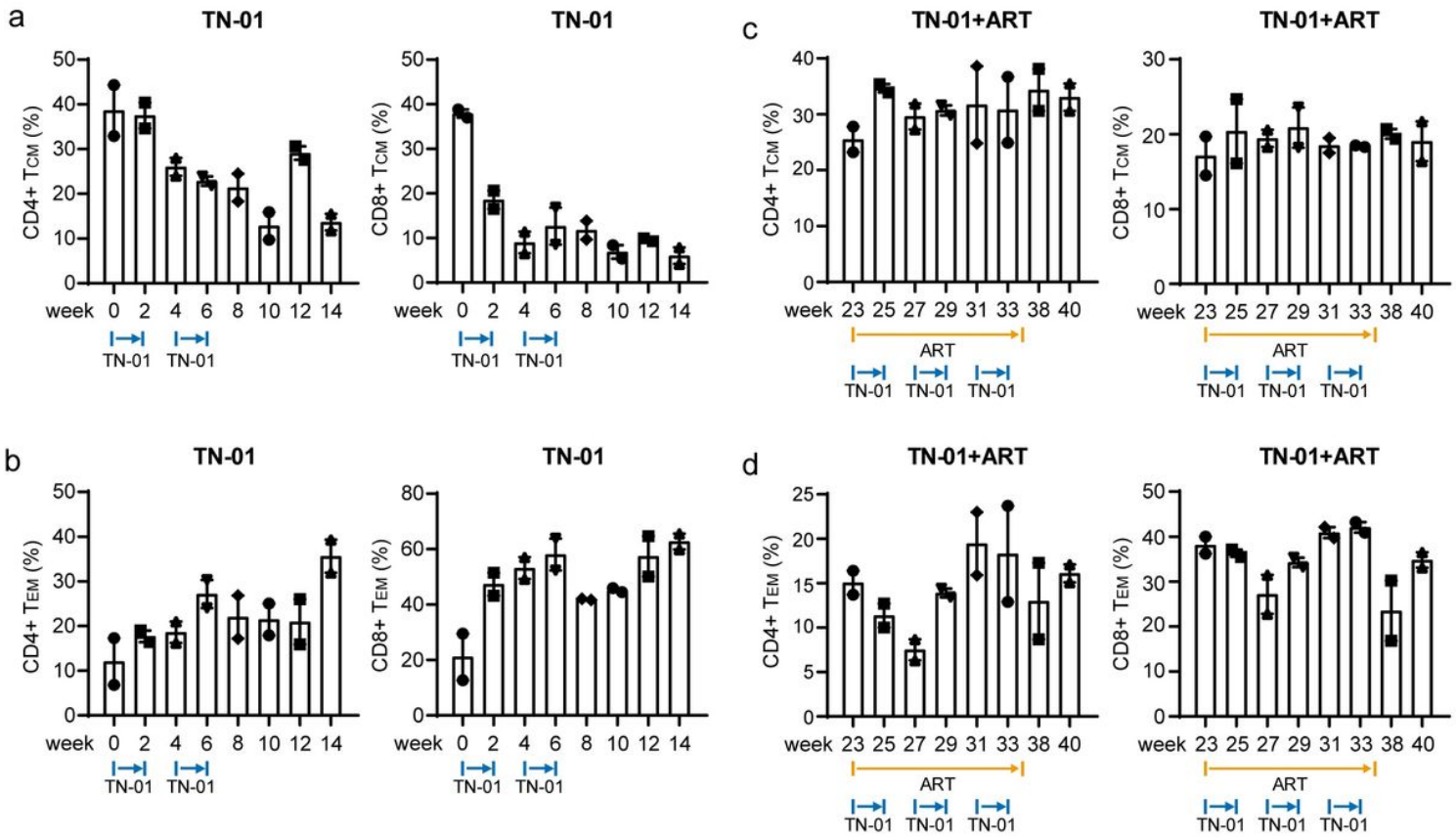

Figure 3

Increasing T cells differentiation after TN-01 treatment. SIV-infected rhesus monkeys were treated as FIG.1. Histograms show the percentages of several CD4+ or CD8+ TCM (a) and CD4+ or CD8+ TEM (b) with TN-01 treatment. (c-d) The percentages of indicated CD4+ or CD8+ T cells subsets by TN-01 combined with ART therapy.

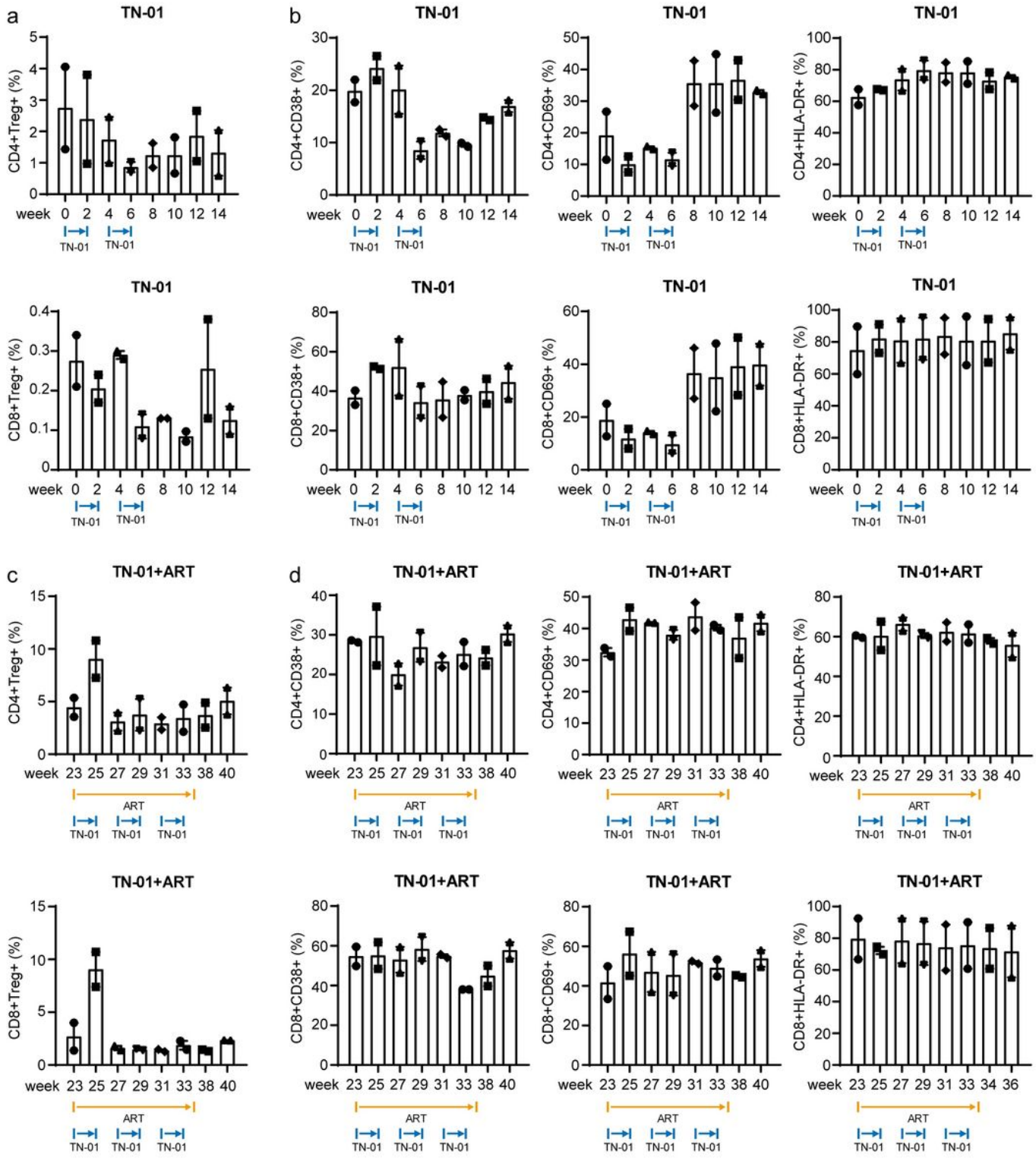

Figure 4

High levels of activation phenotype expression on CD4+ and CD8+ T cells after TN-01 therapy. TN-01 alone (a-b) or TN-01 combined with ART therapy (c-d) treated as Fig. 1. Flow cytometry analysis of indicated phenotype on CD4+ and CD8+ T cells. (a-c) Histograms show the levels of Treg. (b-d) Histograms show the percentages of CD4+ and CD8+ T cells activation marks CD38, HLA-DR and CD69.

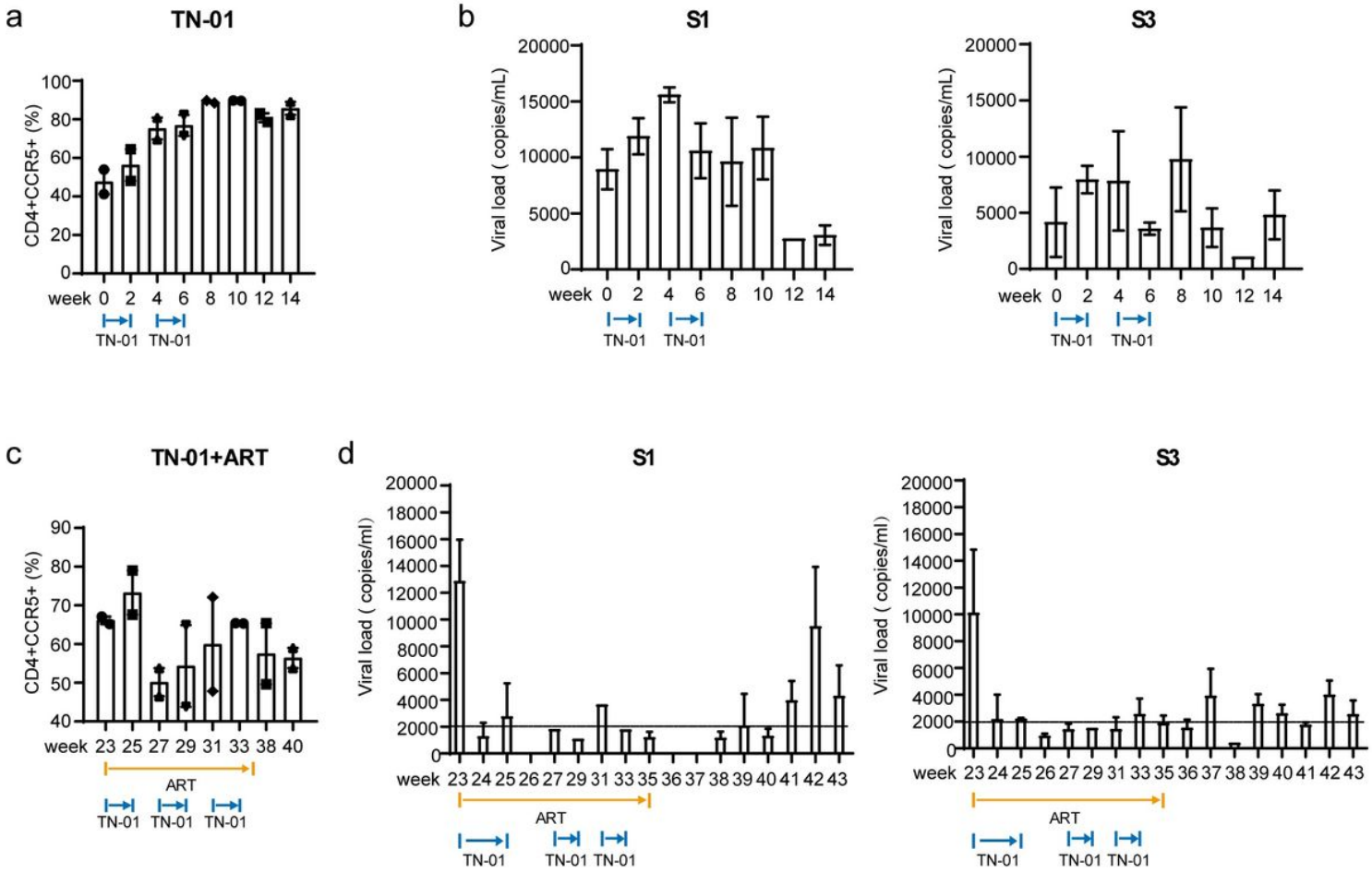

Figure 5

TN-01 combined with ART therapy restrain viral loads rebound. SIV-infected rhesus monkeys were treated with TN-01 alone (a-b) or TN-01 combined with ART therapy (c-d) for indicated time periods. (a-c) CCR5 expression on CD4+ T cells were analyzed by flow cytometry. Histograms show the percentages of CD4+ CCR5+ T cells. (b-d) Histograms show the levels of viral load on indicated time points.