Venetoclax decreases intact proviral DNA frequency in SIV-infected, ART-suppressed Rhesus Macaques

529

Sydney N Bergstresser[1], Tomas R Salinas-Wiche[1], **,** Diane Carnathan[1], Hong Wang[1], Yohannes Abraham[1], George Pavlakas[3], Barbara Felber[4], Mario Roeder[5], Christine Fennessey[6], Brandon Keele[6], Mirko Paiardini[1,2], Deanna A Kulpa[1,2], and Guido Silvestri[1,2]

1 Emory University, National Primate Research Center, Atlanta, GA, United States of America, 2 Emory University, School of Medicine, Department of Pathology and Laboratory Medicine, Atlanta, GA, United States of America, 3 Human Retrovirus Section, Vaccine Branch, Center for Cancer Research, National Cancer Institute at Frederick, Frederick, MD, United States of America, 4 Human Retrovirus Pathogenesis Section, Vaccine Branch, Center for Cancer Research, National Cancer Institute at Frederick, Frederick, MD, United States of America, 5 Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA
6 AIDS and Cancer Virus Program, Frederick National Laboratory, Frederick, MD, United States of America

BACKGROUND

Long-term persistence of CD4+ T cells harboring proviral DNA in people living with HIV (PWH) is mediated by many factors, including the modulation of pro-survival pathways such as anti-apoptotic protein B cell lymphoma-2 (BCL-2). Upregulation of the BCL-2 has been observed in HIV-Gag+CD4+ T cells from anti-retroviral therapy (ART) suppressed PWH, along with an enrichment for intact proviral DNA. These data suggest high levels of BCL-2 may help HIV-Gag+CD4+ T cells with intact provirus to evade apoptosis and support long-term survival, allowing for reservoir persistence. Here, we aim to evaluate the impact of BCL-2 antagonism on the persistence of the viral reservoir in the HIV pre-clinical animal model SIV-infected *Rhesus macaques* (RMs).

METHODS

29 adult *Mamu B*08, B*17* negative RMs were infected with SIVmac239M for 8 weeks followed by daily ART (DTG, TDF, and FTC) for >90 weeks. To induce SIV expression, we employed the previously established model of on-ART SIV viremia- CD8α+ T cell depletion combined with IL-15 treatment. 23 RM were given the CD8α depleting antibody MT807R1(50 mg/kg) followed by 7 doses of heterodimeric IL-15(10-40 ug/kg) across 21 days. 17 CD8α depleted RM were administrated 10 doses of Venetoclax (300 mg, oral gavage) in the same period. 9 Venetoclax treated RM were given 4 SIV env protein monoclonal antibodies (RhmAbs) one day prior to CD8 α depletion to encourage all avenues of clearance of reactivated cells. All RM were followed until elective necropsy.

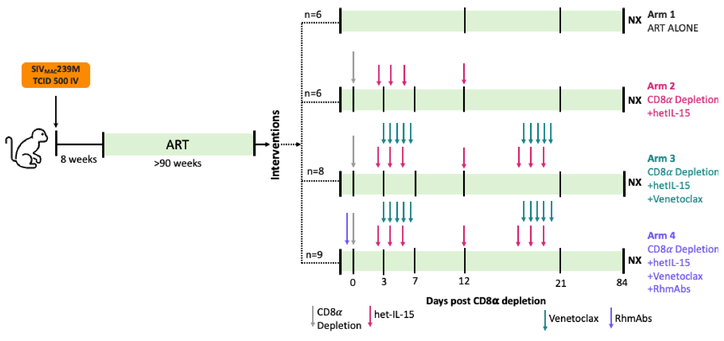


Figure 1: Study design to induce on ART viral reactivation and simultaneously antagonize BCL-2 in reactivated cells to sensitize cells to apoptosis.

Reactivated CD4+ T **cells harboring intact SIV proviral** DNA can be **cleared** under long-term ART **using BCL-2 antagonism**.

RESULTS

Figure 2: CD8α depletion and heterodimeric IL-15 drive on-ART viral reactivation in animals suppressed for greater than 90 weeks . Ultrasensitive viral loads (LOD=15 copies/mL) are displayed throughout intervention period. All animals were undetectable (<60 copies/mL) for a minimum of 6 months prior to interventions.

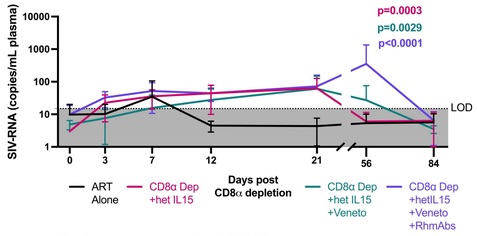
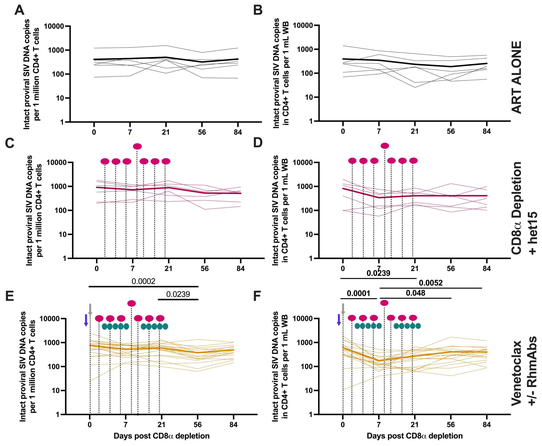


Figure 3: Intact proviral DNA copies decrease following Venetoclax treatment.

The reservoir size was measured by intact proviral DNA assay (IPDA) from total CD4+ T cells enriched from PBMCs. IPDA results are shown for each major treatment arm throughout the course of interventions, with Venetoclax arms (Arms 3 and 4) combined. Panels B,D,F show intact proviral DNA copy changes adjusted to absolute CD4+ T cell counts in whole blood. Panels A,C,E show copy changes reported strictly as copies per million CD4+ T cells.



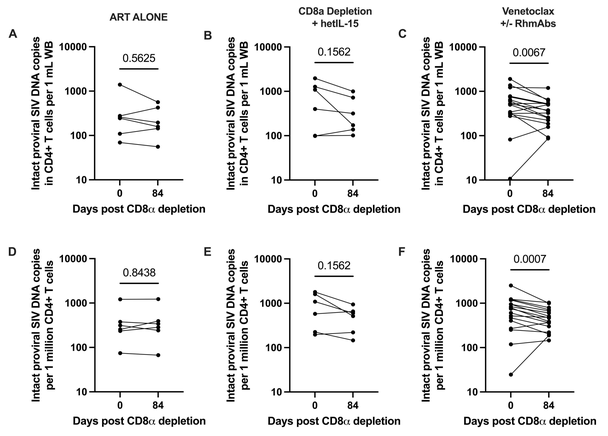


Figure 4: Intact proviral DNA copies decrease only in Venetoclax treated animals from baseline to necropsy.

IPDA results are shown for each major treatment groups, with Venetoclax groups combined. Panels A-C show intact proviral DNA copy changes at baseline (day 0) and necropsy (day 84) when adjusted to absolute CD4+ T cell counts in whole blood. Panels D-F show copy changes reported strictly as copies per million CD4+ T cells.

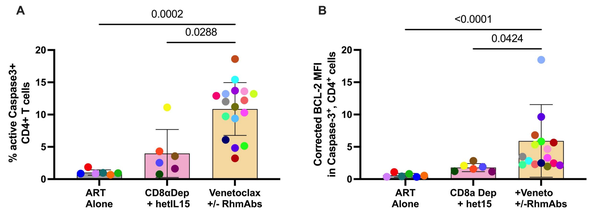


Figure 5: Venetoclax treated animals show increased levels of active Caspase-3 and BCL-2 MFI. To assess for active apoptosis, blood was collected 4 hours after Venetoclax administration on day 7 post depleted (dose #5). Flow cytometry analyses revealed Venetoclax treated animals have a significantly higher frequency of cells with active Caspase-3 in the CD4+ T cells compartment. Additionally, the Caspase-3+ cell population had a higher BCL-2 MFI in Venetoclax treated animals. BCL-2 MFIs were corrected to an internal control before being compared across sampling groups. Venetoclax treatment has been reported to result in increased BCL-2 expression in impacted T cells.

CONCLUSIONS

Here, we use a NHP model of long-term ART suppression and subsequent on ART reactivation to show a proof of concept that BCL-2 antagonism can be used in conjunction with LRAs to achieve reservoir clearance in an *in vivo* model. Latency reversal was achieved using a combination of CD8α depletion and IL-15 signaling via heterodimeric IL-15. BCL-2 antagonism was achieved by administering Venetoclax via oral gavage. Additional clearance of virally reactivated cells was mediated by the addition of monoclonal antibodies specific to SIV env. Firstly, we show on ART reactivation is achievable after more than 90 weeks on suppressive ART with CD8α depletion and heterodimeric IL-15 regardless of Venetoclax administration. These combination of therapies drive reduction of the frequency of intact proviral SIV DNA copies in CD4+ T cells, whether assessing these intact copies strictly on a per million CD4+ T cell basis or on an absolute CD4+ T cell count basis in whole blood. We also show these therapies drive apoptosis within the CD4+ T cell compartment as a significant increase in active caspase-3+ cell frequency were observed four hours post Venetoclax administration. Venetoclax treated T cells have been reported to show increased BCL-2 MFIs (Ludwig et al, *Cell Death and Disease,* 2021, Nagasaki et al, *Blood Cancer Journal*, 2024). Reservoir analyses in tissues is in progress, as along with flow cytometry characterization of the impact of Venetoclax on immunological parameters.

Acknowledgements

We want to recognize the Emory National Primate Center veterinary staff and animal research services (**NIH P51 OD011132**), the Center for AIDS Research (CFAR) Translational Virology Unit (**NIH P30 AI050409**), and Accelevir for providing services. Tomas R. Wiche-Salinas performed Venetoclax pK/dosing pilot studies. ART drugs were supplied by Gilead and ViiV. The Quantitative Molecular Diagnostics Core at the Frederick National Laboratory performed the ultrasensitive viral plasma load assays. Funding for this research was provided by **NIH NIAID R01 AI5064** and **NIAID/NHLBI/NIDDK/NINDS/NIDA/NIMH UM1 AI164562**