Strong HIV-1 Latency Reversal by *C. megalobotrys* Extract in ART-suppressed PBMCs and Humanized Mice

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BACKGROUND

- Latency reversing agents (LRAs) have had limited success *in vivo*, indicating a need for more potent agents (PMIDs: 22836995; 31129856).
- "Mukungulu," an extract prepared from the bark of *Croton megalobotrys* and traditionally used for HIV/AIDS management in Northern Botswana (Africa), is an LRA in latent HIV cell lines and contains protein kinase C-activating phorbol esters (PMID: 28970153). However, the properties of Mukungulu *ex vivo* in ART suppressed cells from PLWH and/or *in vivo* are not known.
- Here, we seek to investigate in detail the latency reversing properties of Mukungulu and its active components as well as monitor Mukungulu's latency-reversing activity and tolerability in both CD4+ cells and PBMC from ART-suppressed PLWH as well as in humanized mice.

METHODS

- Cell pellets and culture supernatants from 20 million PBMC in triplicate from 12 PLWH suppressed on ART for > 3 years were assessed for p24 protein by Simoa (PMID: 33796087) after 72 hours treatment with Mukungulu (1 µg/mL) or anti-CD3/CD28 positive control. Isolated CD4 T-cells (5 million) were also tested in 3 donors.
- Intact and defective proviral DNA were assessed by IPDA (PMID: 30700913).
- 12 BLT-Humanized male mice were infected with HIV_{SUMA}, suppressed with ART for 7 weeks (PMID: 36460646), and injected i.p. with 5 µg/mL Mukungulu (N=7) or PBS vehicle (N=5) and measured after 24 hours for pVL and vRNA from human cells. Analysis was done with Prism software.

‘Mukungulu’ is a **potent LRA** *ex vivo* and *in vivo*. It induces virus production from the intact but not defective viral reservoir of CD4+ cells with **~2.5 more activity than anti-CD3/28**.

RESULTS

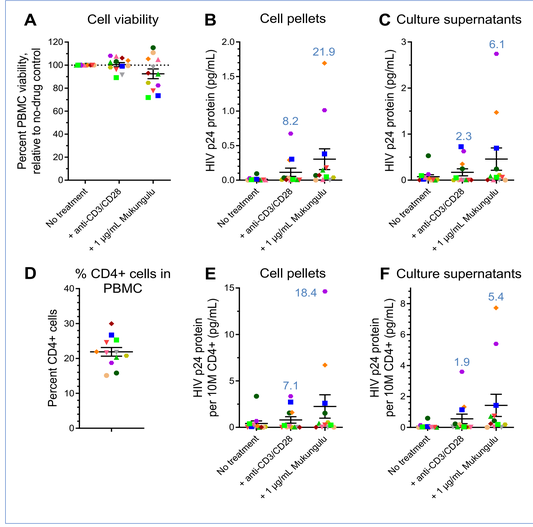

Figure 1. ‘Mukungulu’ reverses HIV-1 latency in PBMC from PLWH stably suppressed on ART. A, Percent cell viability relative to no-drug control. **B,** Detection of viral Gag-p24 protein in cells pellets of PBMCs isolated from twelve (12) aviremic individuals on ART, following 72 hours stimulation as measured by SIMOA. **C,** Detection of HIV-1 p24 protein in supernatants of the donor primary cells. **D,** Percent CD4+ cells within PBMC cultures as measured by flow cytometry. **E-F,** Normalization of Gag-p24 levels per 10 million CD4+ cells from cell pellets (**E**) and culture supernatants (**F**). Colors/shapes denote individual donors. Blue values denote average fold-increases over no-treatment controls.

‘Mukungulu’ induced 0.30 ± 0.15 and 0.46 ± 0.24 pg/mL of p24 protein in pellets and supernatants, respectively, without cytotoxicity, compared to only 0.11 ± 0.06 and 0.17 ± 0.08 pg/mL of p24 induced by anti-CD3/CD28 (p = 0.06 and 0.07).
Moreover, this latency reversal correlates well with percent CD4+ cells in each donor sample, supporting that the extent of latency reversal correlates strongly with the percentage of CD4+ cells in PBMC.

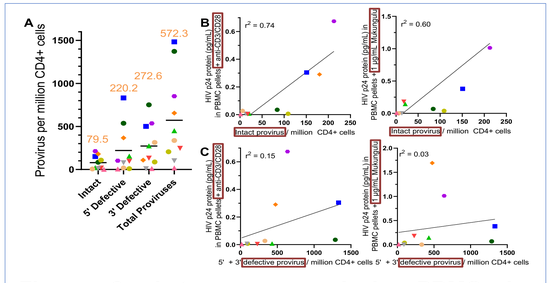

Figure 2. Latency reversal in PBMC by ‘Mukungulu’ correlates with intact but not defective provirus levels of CD4+ subsets. A, Our IPDA results showed an average of 79.5, 220.2, and 272.6 intact, 5’-defective, and 3’-defective provirus copies per million CD4+ cells, respectively, from the 10 PLWH. **B-C,** We observed that HIV-1 Gag-p24 levels from PBMC pellets and supernatants induced by anti-CD3/CD28 and Mukungulu crude extract correlated with intact provirus reservoir size (r^2 = 0.74 and 0.60, respectively.

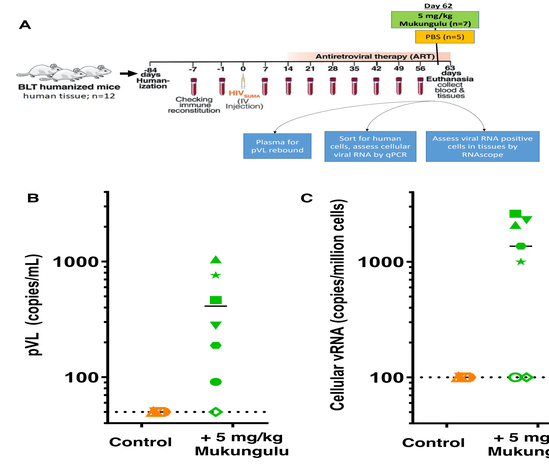

Figure 3. ‘Mukungulu’ extract reverses latency reversal in HIV-infected humanized mice. A, Schematic illustration of the BLT humanized mice model (adapted and modified from PMID: 36460646). **B,** Plasma viral load (pVL) rebound measurements in ART-suppressed BLT humanized mice, treated with Mukungulu. **C,** Cell-associated viral RNA induction and detection in ART-suppressed mice, treated with Mukungulu. Thus, in humanized mice, ‘Mukungulu’ induced 1336 vRNA copies / million human cells plus a pVL of 391 copies / mL, compared to no change by PBS, without obvious toxicities.

CONCLUSIONS

- Mukungulu is an exceptionally potent LRA *ex vivo* and *in vivo*.
- It induces virus production from the intact viral reservoir of CD4+ cells with ~2.5 more activity than anti-CD3/CD28. However, this reactivation is dependent on PBMCs and not isolated CD4+ cells, indicating that additional cell-types contribute to its mechanism of latency reversal.
- This could inform efforts of purging viral reservoir and improve LRA-based HIV-1 cure strategies.

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Study participants

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