

**Disruption of latent HIV in vivo during the clearance of actinic keratosis by  
ingenol mebutate**

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Running title: Ingenol disrupts latent HIV in patients

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## **Supplementary Methods**

**Primary CD4<sup>+</sup> T cell isolation and treatments** All patients will receive approximately 30 cc peripheral blood draw on days 0, day 1 or day 2, and day 10 or day 22 after Picato application. Blood was drawn into EDTA tubes by the physicians or personnel who were certified in phlebotomy and processed within two hours. After blood collection, PBMCs were isolated using Ficoll as described before. Some of these PBMCs were run for flow cytometry to measure immune activation. The reminders of the PBMCs were further processed to purify primary CD4<sup>+</sup> T cells with negative isolation using EasySep kit from STEMCELL Technologies Inc. (Vancouver, BC, Canada)<sup>7,8</sup>. The purified CD4<sup>+</sup> T cells were immediately frozen in the liquid nitrogen for RNA/DNA extraction further.

**Immune activation/flow assays** Peripheral blood mononuclear cells (PBMCs) were stained using standard methods in a panel containing anti-CD3-PacificBlue (PacBlue) (antibodies from BD Biosciences), anti-CD25-PE-Cy7, anti-CD38-PE, anti-CCR5-APC, anti-CD69-APC-H7, anti-CD8-PerCP-Cy5.5, anti-CD4-BV650; anti-HLA-DR-ECD (Beckman Coulter). A staining reagent for dead cells (Invitrogen Aqua Live/Dead Fixable Stain) was included. After staining, cells were washed and fixed in PBS containing 1% paraformaldehyde and analyzed with an LSR II cytometer (Becton Dickinson) and FlowJo.

**Luminex assays** Levels of IFN $\gamma$ , TNF $\alpha$ , IL-2 and IL-6 in plasma were measured using Luminex–Bio-rad Multiplex magnetic cytokine bead Kits. The following commercially available antibodies were used from the manufacturers' Kits: Bio-Plex Pro Human Cytokine IL-2 set #171B5003M, IL-6 set #171B5006M, IFN $\gamma$  set #171B5019M, and TNF $\alpha$  set #171B5026M. Appropriate cytokine standards (Bio-Plex Pro Human Cytokine screening panel standards #12007919) and reagent kits (Bio-rad) were used according to the manufacturer's recommendations. Briefly, beads were added to a 96 well plate and washed twice with washing buffer from the kit. Plasma samples were diluted by 4-fold with diluent from the kit and added to the plate containing the multiplex beads coated with capturing antibody for each cytokine. Similarly, serial dilution of standard cytokine mixtures was added to the plate. Plates were incubated at room temperature for 1 hour and rocked on an orbital shaker at 850 rpm. The plate was washed and then biotinylated detection antibody mixture for each cytokine was added and rocked at room temperature for 30 minutes. Then, the plate was washed as above and streptavidin-PE was added. After incubation for 10 minutes at room temperature and washes as above, the assay buffer was added to the wells. Each sample was measured in triplicates. Plates were read using a Luminex 200 instrument (Bio-rad) with a lower bound of 50 beads per sample per cytokine. The cytokine concentration was determined by a Bio-Plex Manager<sup>TM</sup> software (Bio-rad) based on standard curves for each cytokine.

**Suppl. Table 1. Patients participated in this study.**

**Suppl. Table 2. Basal levels of HIV RNA in the primary CD4<sup>+</sup> T cells and in the skin biopsies in the patients without ingenol mebutate application (n=5).**

**Suppl. Table 3. Basal levels of HIV DNA in the primary CD4<sup>+</sup> T cells and in the skin biopsies in the patients without ingenol mebutate application (n=5).**

**Suppl. Figure 1. Application of ingenol mebutate does not induce immune activation in CD4<sup>+</sup> T or CD8<sup>+</sup> T cells in peripheral blood isolated from patients. Expression of**

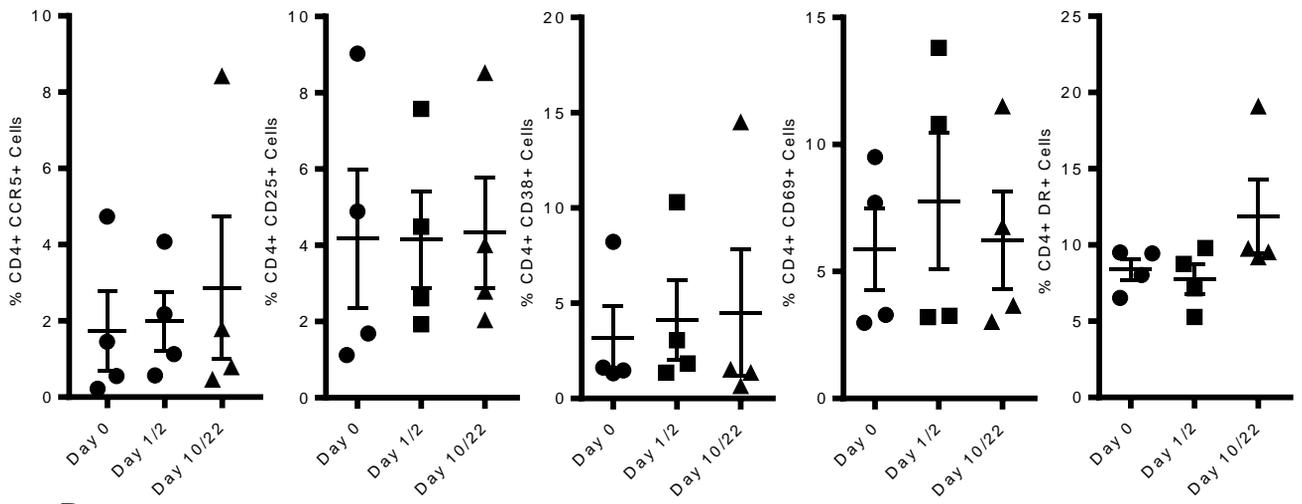
CCR5, CD25, CD38.CD69, or HLA-DR in CD4<sup>+</sup> (A) and CD8<sup>+</sup> (B) T cells was assessed by flow cytometry with PBMCs isolated from patients (n=4).

**Suppl. Figure 2. Application of ingenol mebutate does not induce expression of pro-inflammatory cytokines.** Expression of these pro-inflammatory cytokines in the plasma of the peripheral blood of these patients was determined with Luminex assays (n=5).

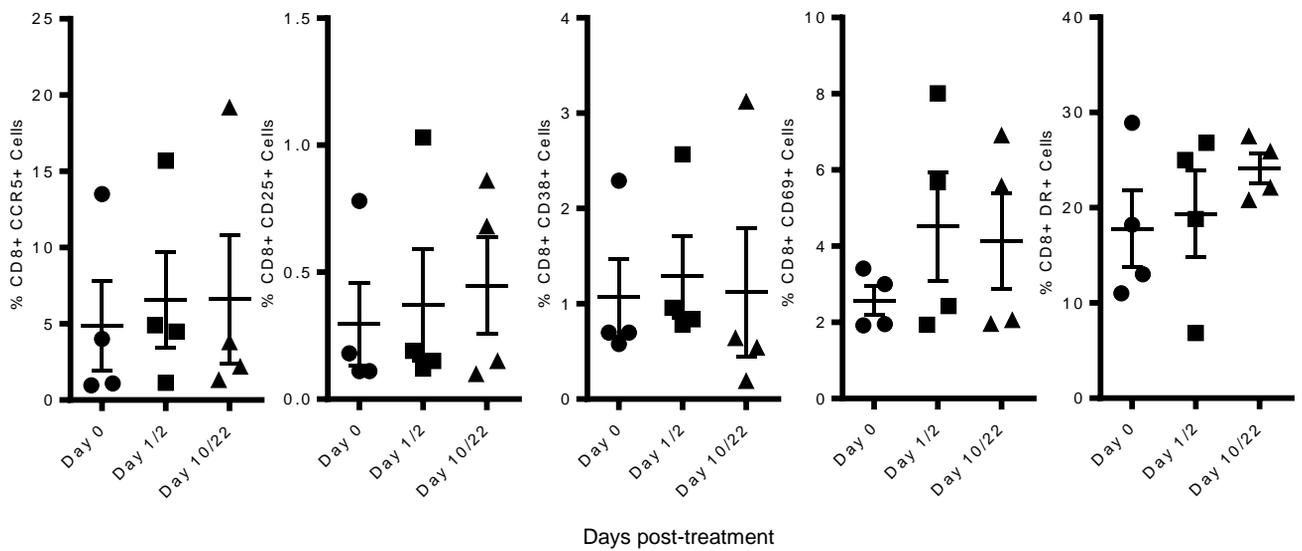
**Suppl. Figure 3. Reactivation of Latent HIV expression correlates with viral reservoirs in both peripheral blood and skin biopsies in patients.** Both linear correlations of HIV RNA copies with DNA levels from CD4<sup>+</sup> T cells and skin biopsies were measured by Pearson coefficient analysis with a two-sided statistical analysis (n=5). A, Initiation of HIV RNA correlates with HIV DNA; B, Elongation of HIV RNA correlates with HIV DNA; C, Full-transcription of HIV RNA correlates with HIV DNA.

**Suppl. Figure 4. Application of ingenol mebutate does not affect CD4<sup>+</sup> and CD8<sup>+</sup>T cells and innate immune response in the skin biopsies from patients.** Expression of CD4<sup>+</sup> T cell, CD8<sup>+</sup> T cell, or macrophage markers in the patient skin biopsies was evaluated by immune staining using anti-CD4, anti-CD8, or anti-CD68/163 antibodies (n=5).

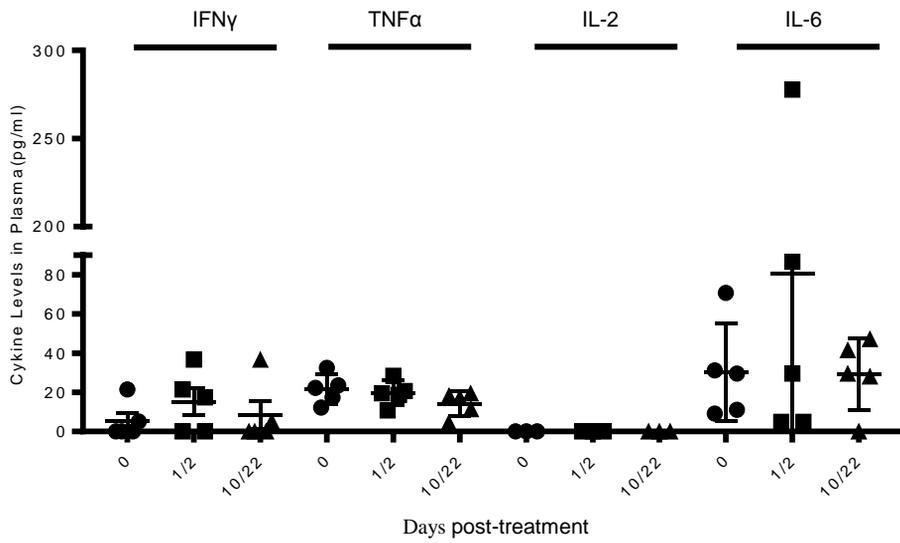
**A**



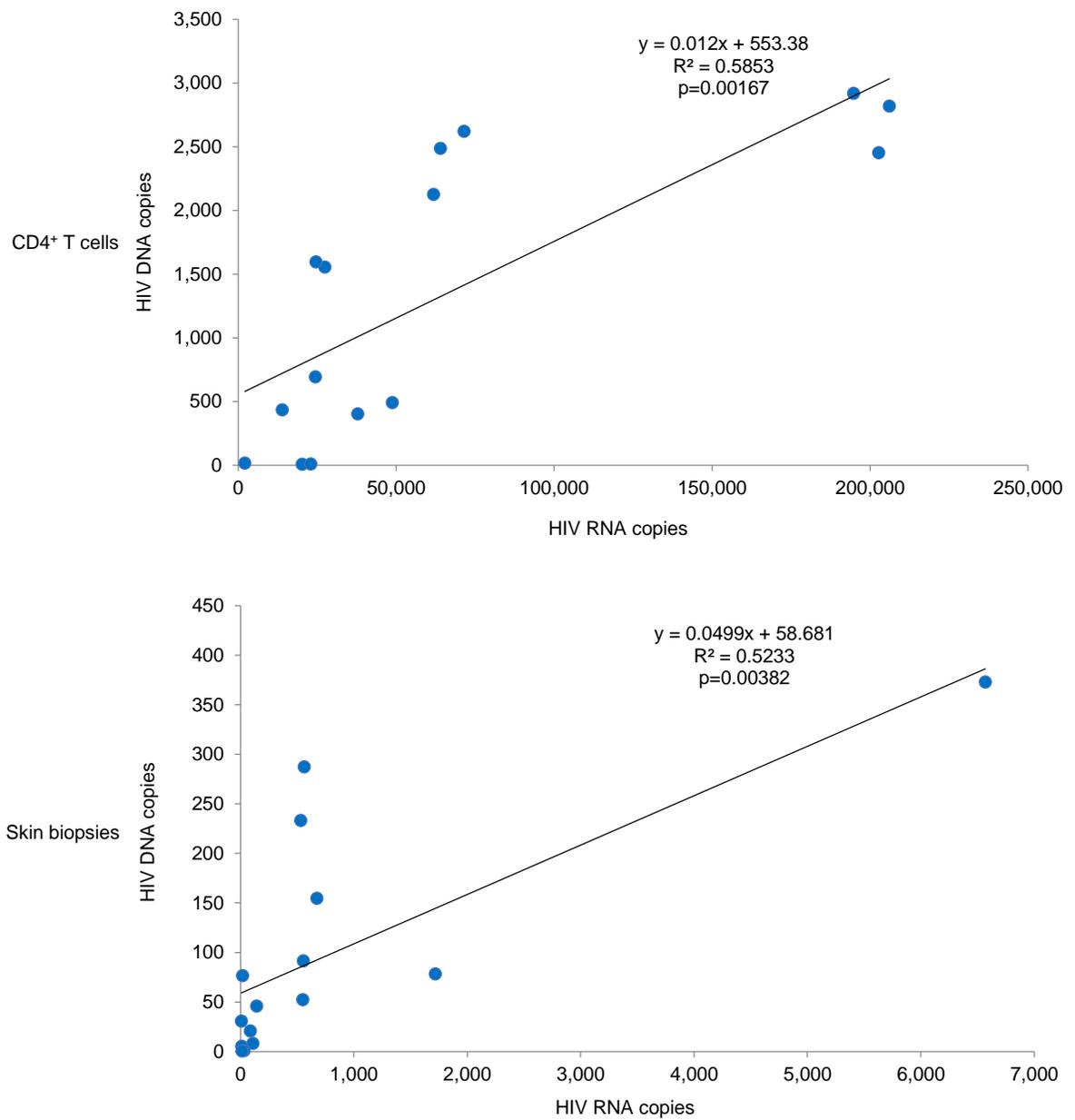
**B**

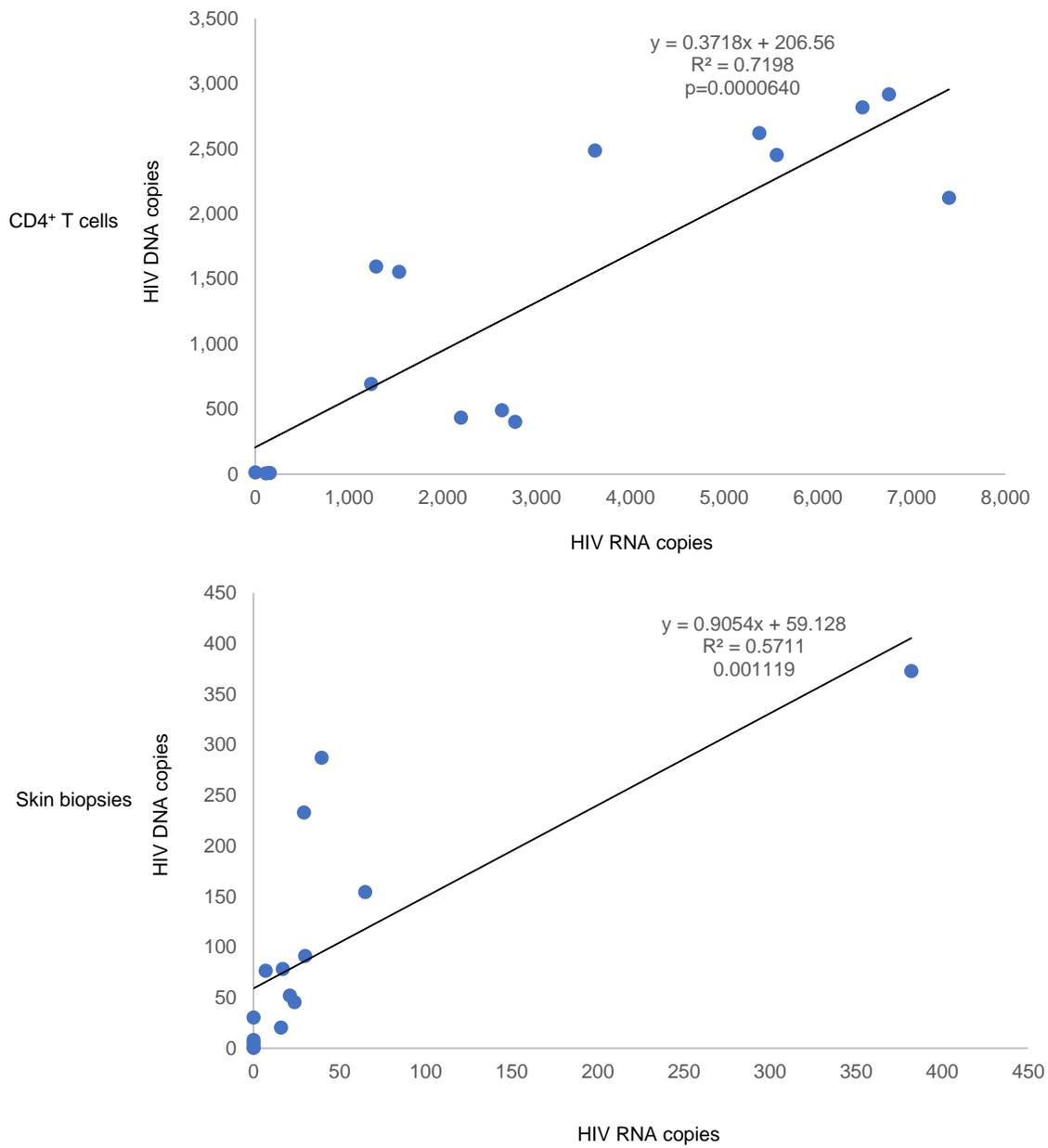


Days post-treatment



A



**B**

C

