The Contributions of Clinical Pharmacology to HIV Cure Research

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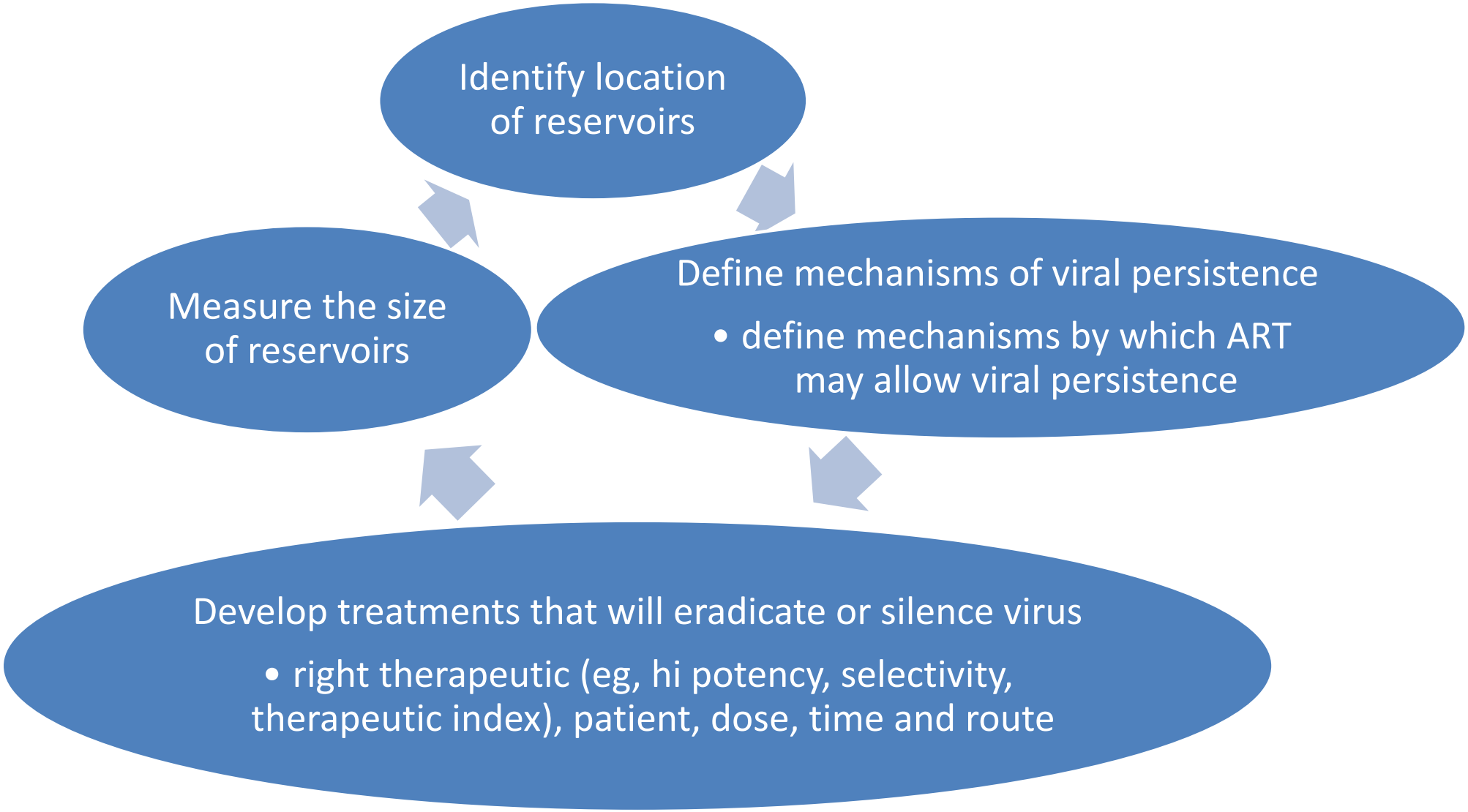
HIV Cure Research – Why Pursue It?

- To improve the long-term health of persons with HIV infection.
- To reduce transmission of HIV.

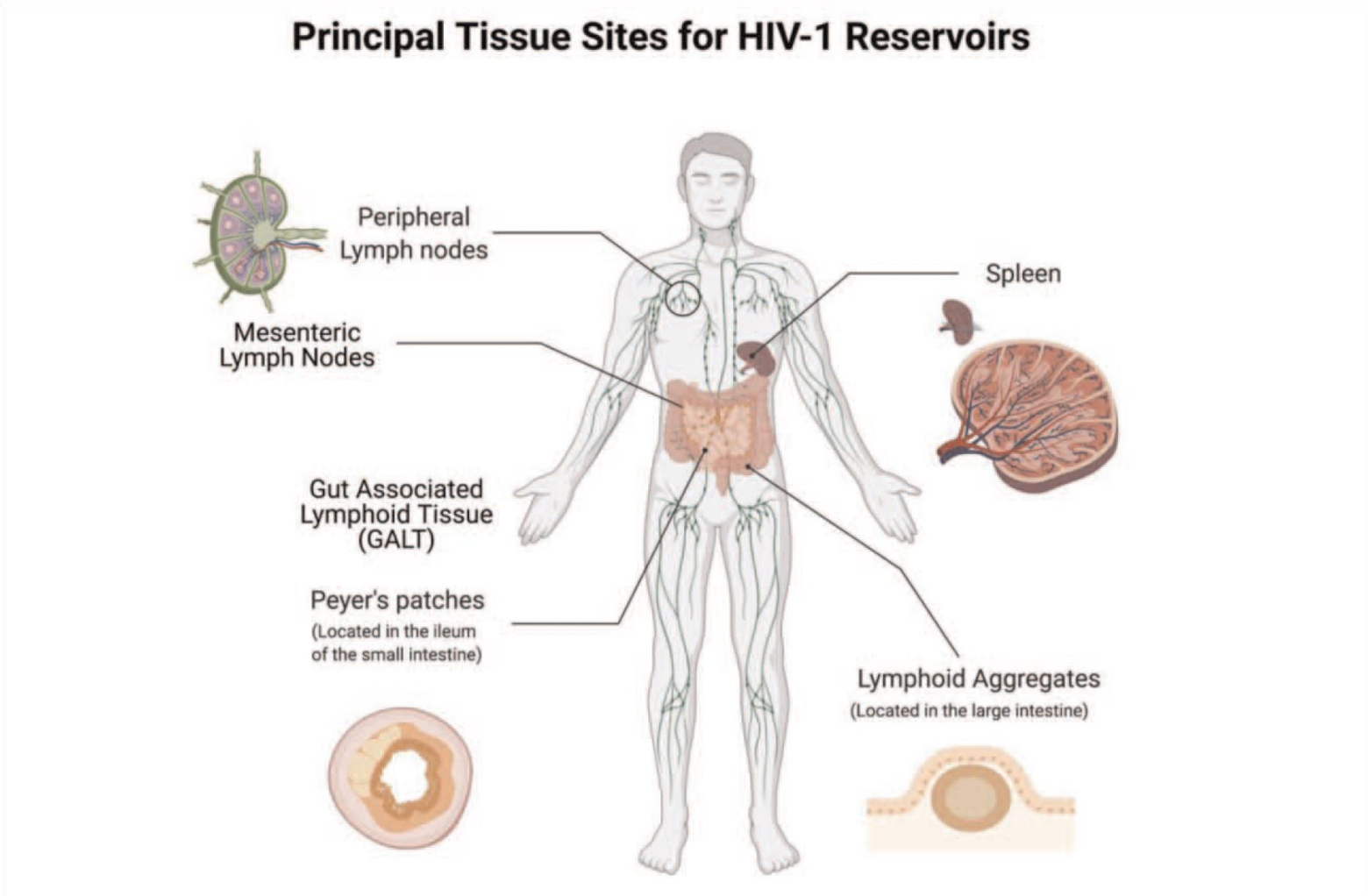
Preeminent Challenges for HIV Cure Research



Adapted from Rose R, *AIDS Res Hum Retro* 2018; 34:3-8 and Fletcher CV, et al. *Clin Pharmacol Ther*; 2021; 110:334-45.

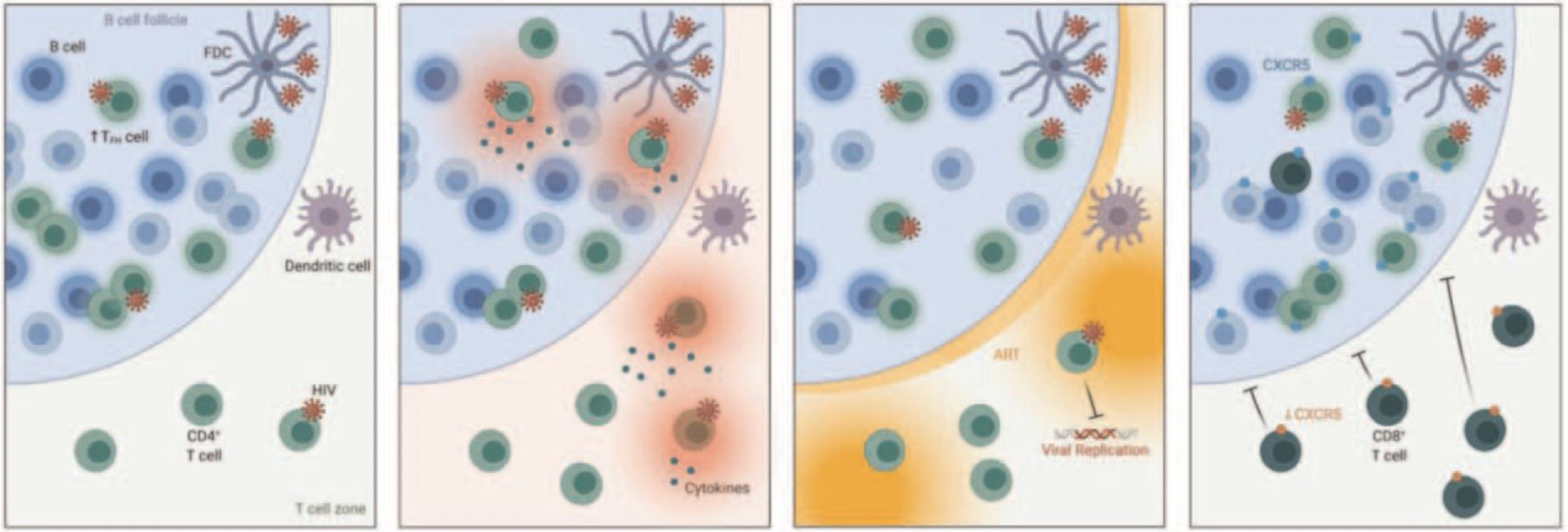
Why Focus on Lymphoid Tissues as Reservoirs?

- The secondary LN and GALT are the primary sites of HIV replication and where >98% of the latent pool of virus resides.



Busman-Sahay K, Starke CE, Nekorchuk, Estes JD. CurrOpinHIVAIDS 2021;16:200-8.

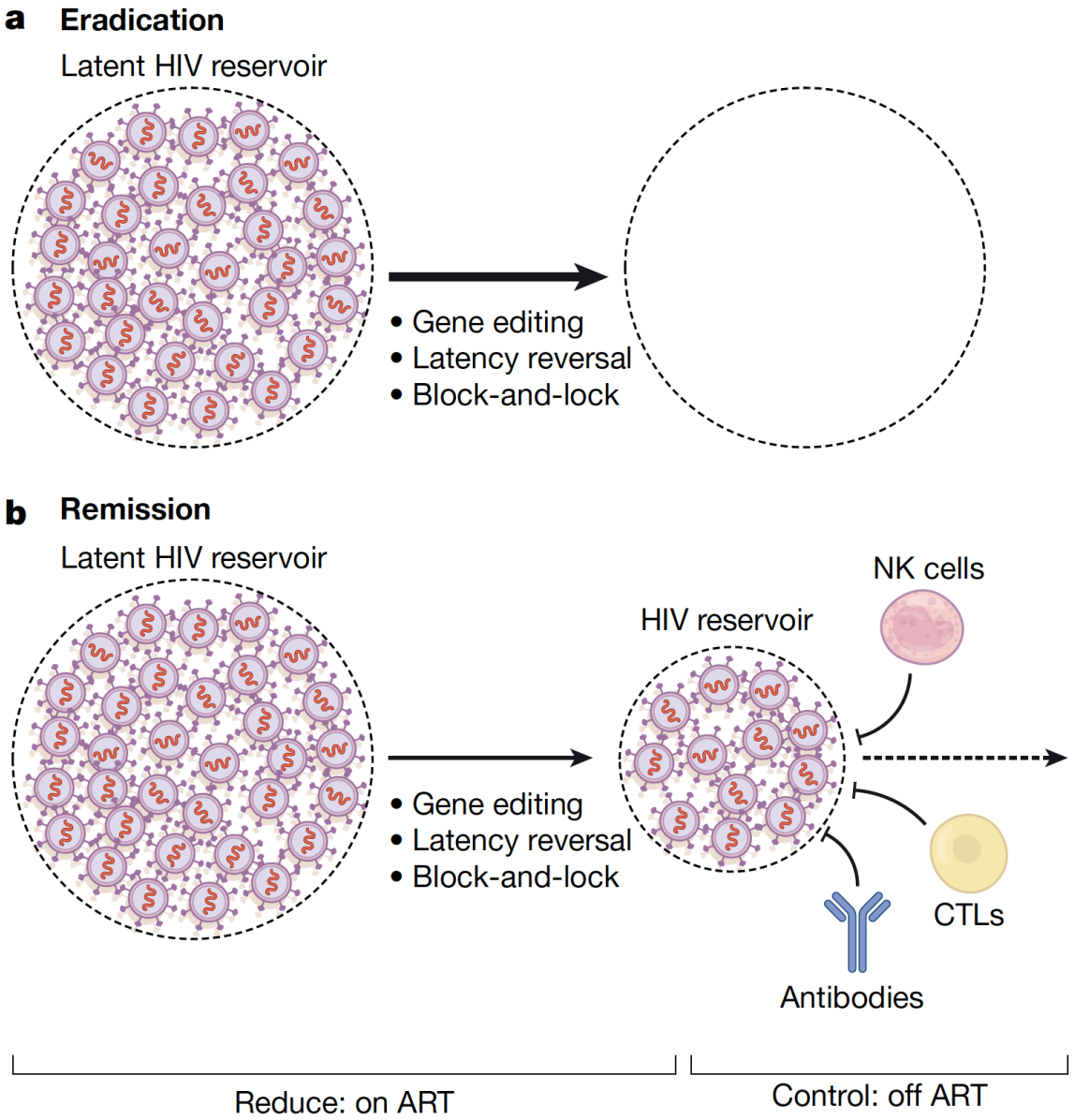
Mechanisms of Persistence in Lymphoid Tissues



1. LT B cell follicles throughout the body contain two unique tissue resident viral reservoirs, T_{FH} cells (infected) and FDCs (non-infected).
2. Heighted inflammation and immune activation persist in LTs (particularly the GALT) during ART, which may contribute to viral reservoir clonal expansion.
3. Antiretroviral (ARV) drug penetration into tissue sites is heterogeneous with incomplete combination ARV exposure to anatomic sites and infected cells.
4. HIV-specific cytotoxic CD8+ T cells have limited accessibility to B cell follicles within LTs.

Busman-Sahay K, Starke CE, Nekorchuk, Estes JD. CurrOpinHIVAIDS 2021;16:200-8.

Pathways to a Cure



Ndung'u T, McCune JM, Deeks SG. Nature, 2019; 576, 397-405.

Pharmacologic Features of HDAC Inhibitors

Characteristic	Vorinostat	Romidepsin	Panobinostat
EC_{50}, ng/mL (nM)	1044 (3950)	2.4 (4.5)	3.5 (10.1)
C_{max} (ng/mL)	317	377	21.6
Inhibitory Quotient ($C_{max\text{-}unbound}$ / EC_{50})	0.09	3.21	0.62
CSF penetration	19.8 ng/mL (75 nM) (2 children with brain tumors)	2% of plasma (in NHPs)	negligible (BLQ in 11 PLWH and 1 child with brain tumor)
Lymphoid tissue penetration	?	?	?

Fletcher CV, et al. *Clin Pharmacol Ther*; 2021; 110:334-45. PMID: 33763860. DOI: 10.1002/cpt.2237

Romidepsin PKPD as a HIV Latency Reversing Agent

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Journal of Antimicrobial Chemotherapy

Pharmacokinetic/pharmacodynamic analysis of romidepsin used as an HIV latency reversing agent

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Objectives: To develop a population pharmacokinetic model for romidepsin given as an HIV latency reversing agent (LRA) and to explore the relationship between romidepsin exposure and its *in vivo* effects on viral gene expression and antiviral immunity.

Methods: A population pharmacokinetic analysis was performed in 15 HIV-1-infected patients who received three weekly infusions of romidepsin (5 mg/m^2) within the BCN02 clinical trial. A full pharmacokinetic profile was obtained for each participant at the first dose, and additional samples thereafter. A population pharmacokinetic model was developed. Bayesian estimates of the individual pharmacokinetic parameters of romidepsin were used to simulate individual time–concentration curves on each occasion. The relationship between romidepsin $AUC_{0-\infty}$ and its *in vivo* effects was assessed.

Results: Romidepsin pharmacokinetics were best described by a three-compartment model with linear kinetics. Body weight influenced romidepsin disposition. A significant relationship was observed between romidepsin $AUC_{0-\infty}$ and increases in expression of exhaustion markers by CD4+ and CD8+ T cells and apoptosis markers in CD4+, but not with histone acetylation levels or HIV-1 cell-associated RNA in CD4+ T cells. For each increase of 100 ng·h/mL in romidepsin $AUC_{0-\infty}$, CD4+ counts decreased by a mean (95% CI) of 74 (42–94) cells/mm^3 after dosing.

Conclusions: A population model describing the pharmacokinetics of romidepsin as an HIV LRA was developed. Higher exposure to romidepsin resulted in higher expression of apoptosis markers and declines in CD4+ count but did not increase viral reactivation levels. These observations have important implications for the optimization of effective kick-and-kill strategies for an HIV-1 cure.

Status of HDAC Inhibitors for HIV Cure Research

- HDAC inhibitors (e.g. panobinostat, romidepsin, vorinostat) have demonstrated in-vivo activity as measured by detection of increased histone H3 acetylation.
- Clinical evaluations have shown evidence of activation of latent HIV provirus in resting CD4+ T cells; however:
  - this finding in not uniform across all PLWH who received these drugs
  - the magnitude of reactivation as measured by levels of plasma HIV-RNA is modest
- No HDACi, when administered alone, has resulted in a decrease in the size of the latent reservoir.
  - A modest reduction in reservoir size, measured by total CD4+ T cell HIV-DNA, was seen with RMD plus a therapeutic vaccine.
- No study to date that employed an ATI, was the time to viral rebound meaningfully prolonged.

Factors Affecting Clinical Pharmacologic Responses of HIV Cure Therapeutics

Factor	Findings	Ref
Intrinsic		
Biological sex	• Women have lower plasma HIV-RNA than men in the absence of ART. • Cell-associated HIV-RNA, residual plasma HIV-RNA, T-cell activation and PD-1 expression were lower in women. • Women have smaller inducible RNA reservoirs.	1, 2
Age	• Peripheral blood HIV-DNA (proviral reservoir) was lower in children who achieved virologic control before 1 year of age vs. 1-5 and >5 years. • In adults 31-66 years, HIV-DNA was lower for those who achieved plasma HIV-RNA <40 copies/mL at a younger age.	3, 4
HIV subtype	• Persons infected with HIV-1-subtype B had larger viral reservoirs than non-subtype B-infected individuals.	5

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Factors Affecting Clinical Pharmacologic Responses of HIV Cure Therapeutics

Factor	Findings	Ref
Intrinsic		
Reservoir size	• Total cell associated HIV-DNA (measuring reservoir size) positively correlated with level of reactivation in ex vivo cultures of resting CD4+ T cells from aviremic PLWH.	6
Complications	• Chronic immune activation persists during suppressive ART and has been associated with, for example microbial translocation and concomitant herpes virus and other infections. • Greater immune activation is associated with an increased reservoir size.	7

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Factors Affecting Clinical Pharmacologic Responses of HIV Cure Therapeutics

Factor	Findings	Ref
Extrinsic		
Potency Physio-chemical PKPD	• Higher lipophilicity (LogP) and acid dissociation constant (pKa) and lower water solubility (higher hydrophobicity) were associated with better penetration in lymphoid cells. • A concordance is observed between high LN penetration and high CNS penetration. Higher protein binding of HDACi is associated with poorer CSF penetration. • A large reservoir of viral DNA persisted in lymphoid tissues during ART in SIV-infected NHPs; ART levels in lymphoid tissues were lower than in peripheral blood. • Daily dosing of VOR resulted in blunted RNA expression; exposure-response analysis found clockwise hysteresis relationship indicating the timing of drug administration can affect response.	8, 9, 10, 11

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Factors Affecting Clinical Pharmacologic Responses of HIV Cure Therapeutics

Factor	Findings	Ref
Extrinsic		
ART initiation	• Early ART was associated with lower total HIV-1 DNA in perinatally-infected children. • ART initiation within the first year of HIV infection was associated with a lower reservoir size in 1057 PLWH on suppressive ART for a median of 5.4 years.	12, 13
Concomitant medications	• Alcohol negatively affects HIV care and worse outcomes for women at higher levels of alcohol use.	14
Adherence	• Quantitation of tenofovir-diphosphate in dried blood spots, predicted future viremia in PLWH. • Viral blips and low-level viremia are associated with a larger reservoir size.	13, 15

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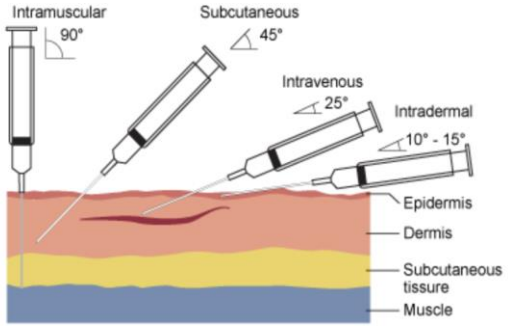
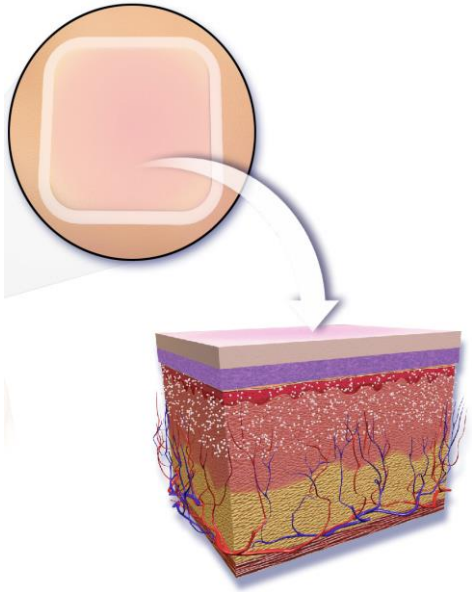
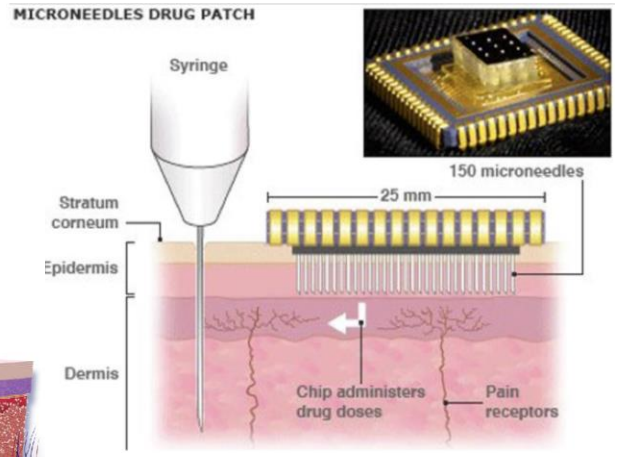
Drug Delivery Technology

Long-acting depot injections

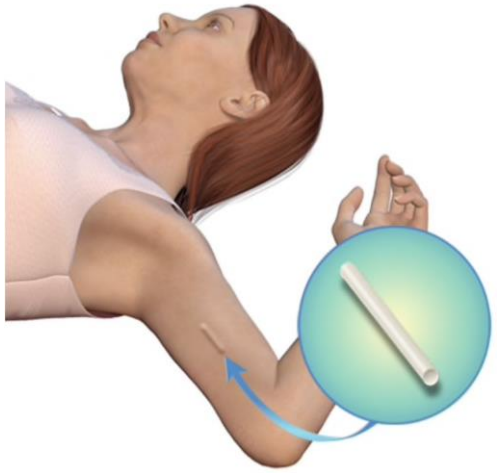


Microneedle drug patch

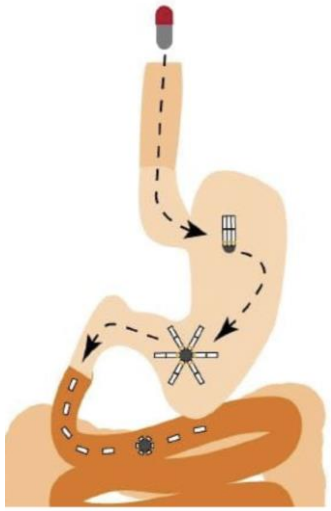




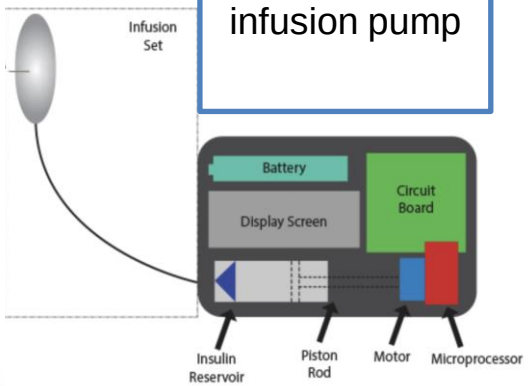
Subdermal implant



Novel oral formulations



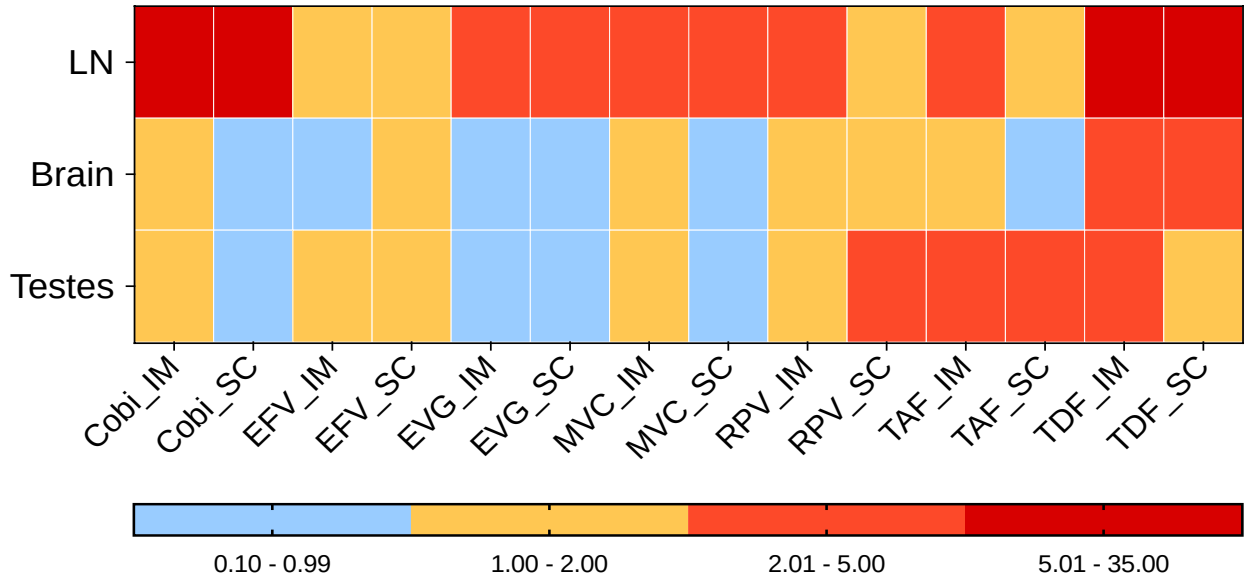
Wearable infusion pump



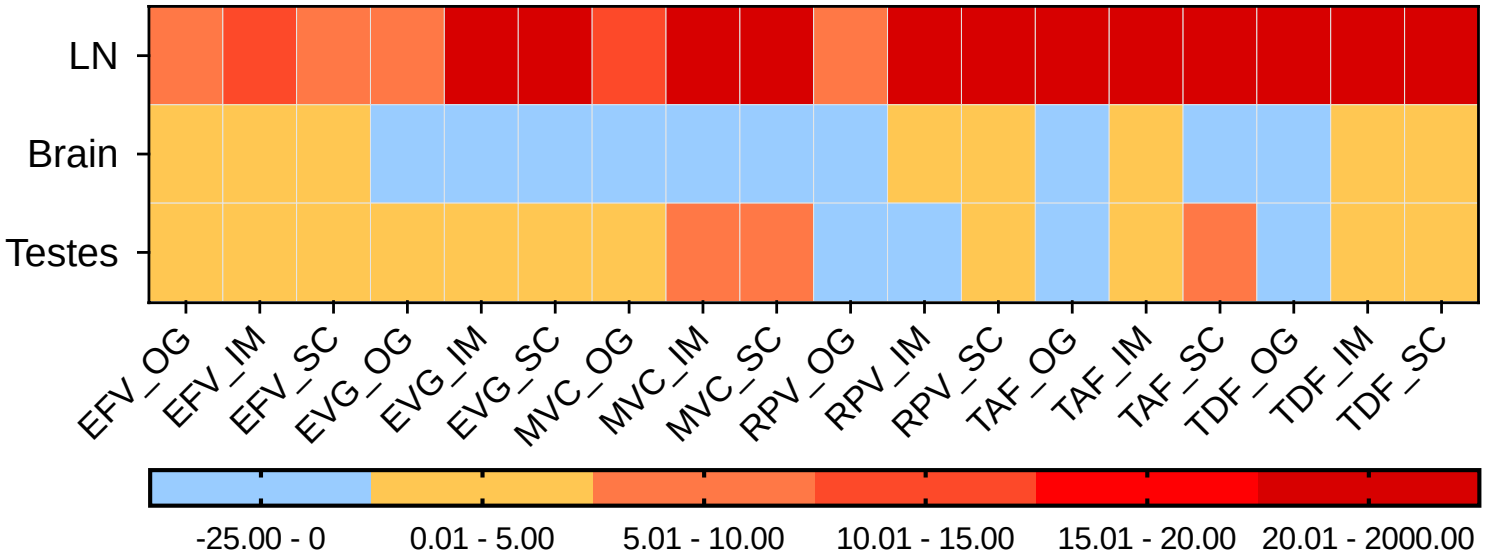
Vaginal rings

ARV Tissue Delivery: IM and SC Compared with Oral

A. Tissue Penetration Ratio



B. Tissue Inhibitory Quotients



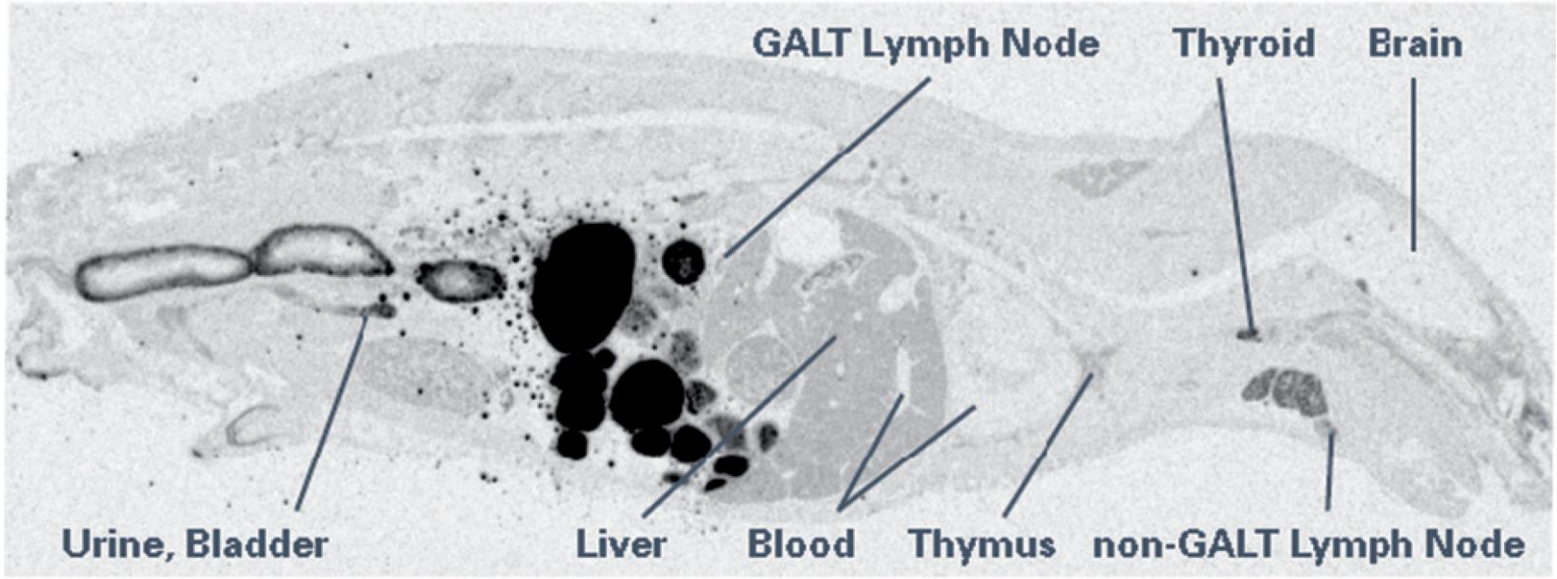
Dyavar SR … **Fletcher CV**. J Antimicrob Chemother 2021; 76 (10): 2651-58

ARV Tissue Delivery – IM and SC vs. Oral: Observations

- A change in the route of drug administration from oral to IM or SC can change tissue uptake, as measured by the tissue penetration ratio (TPR).
  - The TPR controls for any change in tissue concentration from only a change in plasma concentration for that route of administration. Thus, a change in TPR arises from factors affecting tissue distribution.
- Generally, IM and SC administration improved lymphatic tissue uptake.
- IM and SC administration, however, can result in a decrease in concentrations in some tissues, such as the brain and testes.
- Future studies may investigate a change in route of administration as a therapeutic maneuver to enhance lymphatic tissue concentrations and limit viral persistence.

Dyavar SR … **Fletcher CV**. J Antimicrob Chemother 2021; 76 (10): 2651-58

Tissue Distribution of Maraviroc



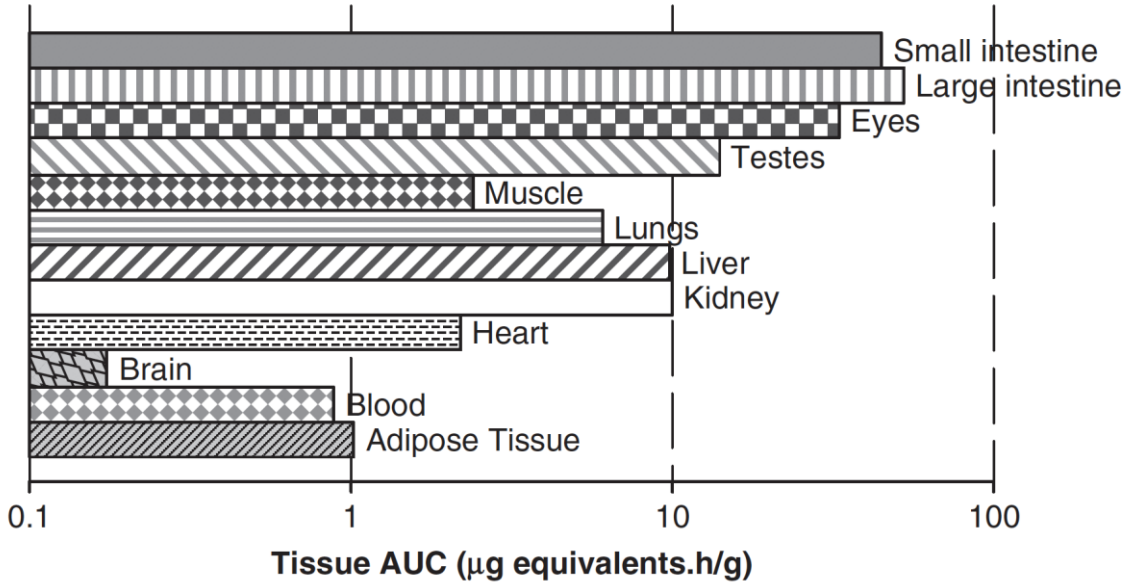


Table III. Mean concentrations of radioactivity in selected tissues following intravenous doses (3 mg kg^{-1}) of [^{14}C]-labelled maraviroc to male Long Evans rats.

	Radioactivity concentration (µg equivalents g^{-1})	
Tissue type	1 h	4 h
Lymph node: GALT	0.87	0.43
Lymph node: axial and sublingual	0.78	0.40
Lymph (and chyle): GALT	n.d.	n.d.
Thoracic duct contents: lymph (and chyle)	0.19	BLQ
Blood: vena cava	0.13	0.06
Liver	1.3	0.33
Kidney cortex	0.85	0.18
Kidney medulla	0.91	0.20
Urine	22	2.0
Bile	6.1	1.8

BLQ, below the limit of quantitation (0.05 µg equivalents g^{-1}); GALT, gut-associated lymphoid tissue; n.d., not determined due to a high concentration in the surrounding tissue.

Walker DK et al. Xenobiotica 2008;38:1330-9.

Persistent HIV Transcription and ARV Levels in Lymph Nodes During Plasma Viral Suppression

- Participants of the RV254 acute infection cohort in Bangkok.
  - Objective: to describe clinical, immunological and virological characteristics of persons with acute HIV infection.
  - Intervention: ART was immediately initiated under a separate protocol
- Group 1 (n=6): initiated and continued ART with 2 NRTI and DTG and MVC.
- Group 2 (n=12): initiated ART with 2 NRTI and EFV and were switched from EFV to DTG.
- Cell-associated HIV-RNA+ and total HIV-DNA+ cells were measured in PBMCs by PCR and viral DNA and RNA in the lymph node by RNAscope.
- ARV levels in peripheral blood, PBMCs, and lymph node MNCs were measured by LC/MS/MS.

Participant Characteristics (at lymph node biopsy)

Characteristics	Groups 1+2 (N=18)	Group 1 (n=6)	Group 2 (n=12)	P-value
ARV duration, weeks	109 (24 - 252)	44 (24 - 54)	135 (86 - 252)	<0.001
DTG duration, weeks	59 (24 - 82)	44 (24 - 54)	63 (42 - 82)	0.002
ARV regimen				
ABC/3TC/DTG/MVC	3 (16.7)	3 (50)	-	
TDF/3TC/DTG/MVC	3 (16.7)	3 (50)	-	
ABC/3TC/DTG	11 (61.1)	-	11 (91.7)	
TDF/3TC/DTG	1 (5.6)	-	1 (8.3)	
Plasma HIV-RNA (<20)	18/18	6/6	12/12	

Fletcher CV et al. Submitted.

HIV Viral Load in PBMCs and in Lymph Nodes

HIV Measure	Groups 1+2 (N=18)	Group 1 (n=6)	Group 2 (n=12)	P-value
HIV in PBMCs				
Cell-associated RNA (LTRgag copies/10^6 cells)	0 (0 - 57)	0 (0 – 4.7)	0 (0 – 133.1)	0.394
HIV in LN				
vRNA (+ cells/g tissue)	9.14 (5.3-27.6 E+04)	7.14 (5.3-10.9 E+04)	9.98 (5.5-27.6 E+04)	0.111
vDNA (+ cells/g tissue)	26.23 (10.5-76.9 E+04)	16.91 (13.5-54.3 E+04)	30.34 (10.5-76.9 E+04)	0.512

Values are presented as median (min-max)

Fletcher CV et al. Submitted.

Persistent HIV Transcription and ARV Levels in Lymph Nodes During Plasma Viral Suppression: Observations

- Ongoing viral expression as measured by RNAscope in LN was seen in all participants despite suppression of plasma HIV-RNA in 100% to < 20 copies/mL.
- A trend was observed for lower levels of RNA+ cells in LN in the DTG + MVC group vs. DTG group.
- Plasma and PBMC concentrations of ARVs were consistent with prior data.
- CBV-TP levels in the LN were commonly not quantifiable and $<IC_{50}$.
  MVC LN levels, however, were uniformly quantifiable and 39-fold higher than IC_{90}.
- The trend for lower LN RNA+ expression in the DTG+MVC group may be consistent with enhanced anti-HIV potency in the LN from MVC.

Fletcher CV, et at. Submitted.

Desired Clinical Pharmacologic Attributes of Agents for HIV Cure Research

Characteristic	Attributes
P'dynamic	• high IQ conferring high potency and barrier to resistance; • high therapeutic index; not antagonistic and additive or synergistic with other agents; • rapid onset of effect; and • predictable dose-concentration-effect relationship.
P'kinetic	• High organ/tissue/cell/site-of-action distribution (LN ≈ plasma), and understanding effect of admin route; • high bioavailability; long half-life; low intra- and inter-patient PK variability; low probability as victim or perpetrator of drug-food or drug-drug interactions; and • convenient dosing yielding high adherence and forgiveness of missed doses.
Formulation	• fixed dose combinations; • suitable pediatric formulations; and • amenable to delivery as a long-acting formulation.

Fletcher CV, et al. *Clin Pharmacol Ther*; 2021; 110:334-45. PMID: 33763860.

My Crystal Ball: ARV Clinical Pharmacology Contributions to HIV Cure-Remission

- Formulation:
  - *Prodrugs, nanoformulations*
- Mechanism of action:
  - *Potent, selective agents with novel mechanisms of action and additive-to-synergistic with existing agents*
- Pharmacokinetics
  - *Improved tissue/organ distribution (C_t or $C_c = C_p$)*
- Pharmacodynamics
  - *Full suppression of viral replication in all tissues, compartments, reservoirs*
  - *Novel, highly synergistic combinations and new, precision medicine approach to combinatorial therapeutics*

Thank You

References: Factor Affecting Clinical Pharmacologic Responses

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