News from the Reservoirs: Lymphatic Tissues

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Why Focus on Reservoirs and the Lymphoid Tissues?

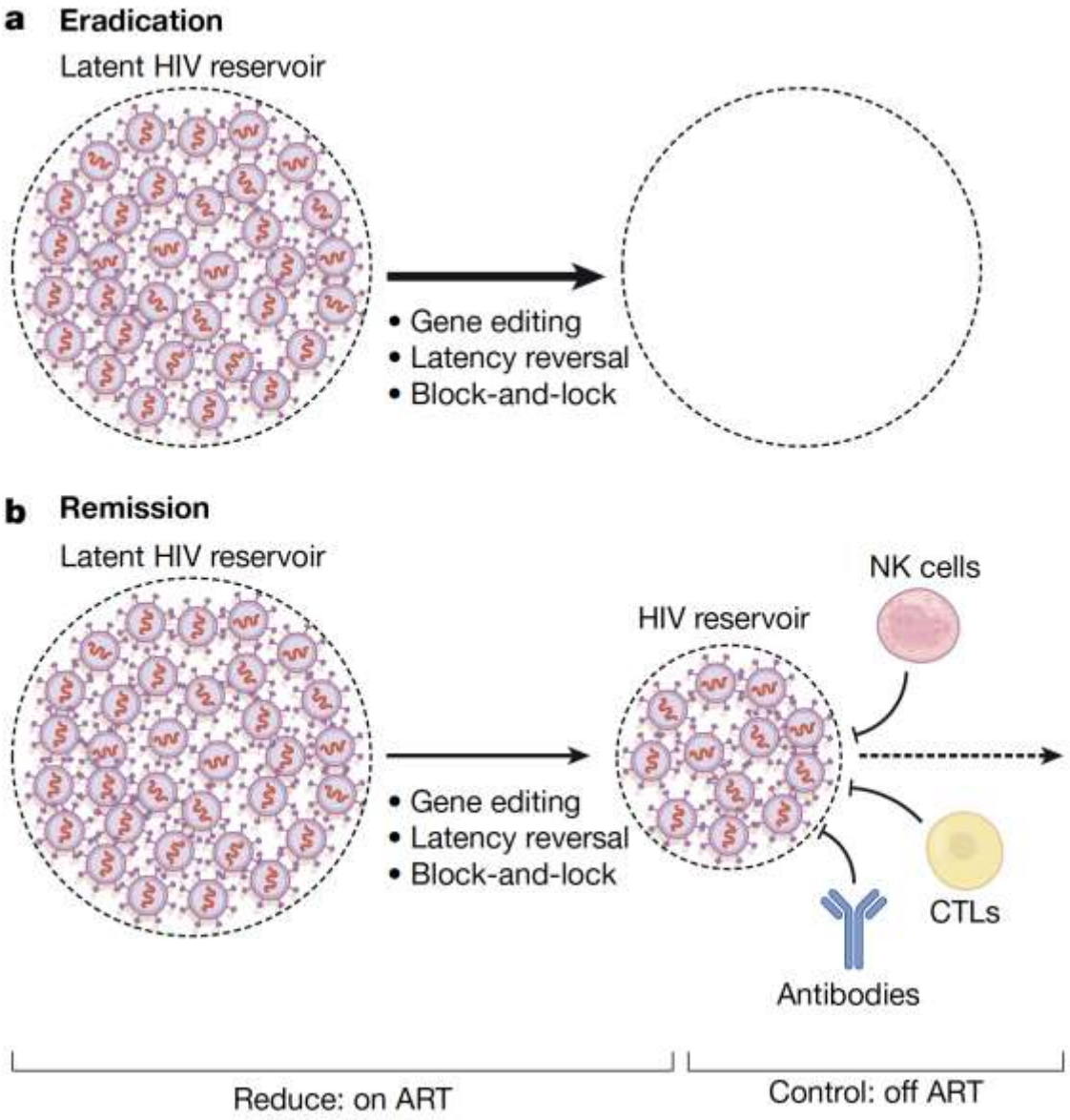
- Antiretroviral therapy (ART) does not:
  - cure HIV infection,
  - fully reconstitute the immune system, or
  - fully restore health.
- 1-2 weeks after HIV acquisition, multiple virus reservoirs are established. The reservoir of latent virus in resting CD4+ T cells is a major obstacle to virus eradication.
- The secondary LN and GALT are the primary sites of HIV replication and where >98% of the latent pool of virus resides.
- These same anatomical sites of viral reservoirs may be pharmacologic sanctuaries.
  - Mechanisms of ARV penetration into LN do not favor high tissue:blood concentrations.

HIV Cure - Remission

- Goal: to find and diminish the size and achieve long-term control of a replication-competent HIV reservoir.
- Three preeminent challenges:
  - Identify the location of the anatomical reservoir/sanctuary from which the virus repopulates blood after ART discontinuation;
  - Define the mechanism(s) by which virus is maintained at low or undetectable levels in such locations;
    - and mechanism by which ART may allow maintenance of ongoing viral replication (CVF).
  - Develop treatment(s) that will eradicate or silence virus without damaging nearby sensitive or irreplaceable tissues (e.g., CNS).

Rose R, Nolan D, Maidji E, Stoddart C, Singer E, Lamers S, McGrath MS. AIDS Res Hum Retro 2018; 34:3-8.

Pathways to a Cure



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

Pharmacologic Features of HDAC Inhibitors

Characteristic	VOR	RMD	PNB
EC_{50}, ng/mL (nM)	1044 (3950)	2.4 (4.5)	3.5 (10.1)
CC_{50} , ng/mL (nM)	> 6608 (>25000)	57.9 (107)	> 875.5 (>2500)
TI (CC_{50}/EC_{50})	6.3	24.1	249.6
Dose in mg, route	400, Oral	14/m^2, IV	20, Oral
C_{max} (ng/mL), T_{max}	317, 4h	377, 4h	21.6, 1h
Protein binding	71%	92-94%	90%
Metabolism	Gluc + hydrolysis	CYP3A4	CYP3A4
T1/2 (hours)	2h	3h	37h

Fletcher CV, et at. *Clin Pharmacol Ther*; in press.

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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 at. *Clin Pharmacol Ther*; in press.

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

Fletcher CV, et at. *Clin Pharmacol Ther*; in press.

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

Fletcher CV, et at. *Clin Pharmacol Ther*; in press.

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

Fletcher CV, et at. *Clin Pharmacol Ther*; in press.

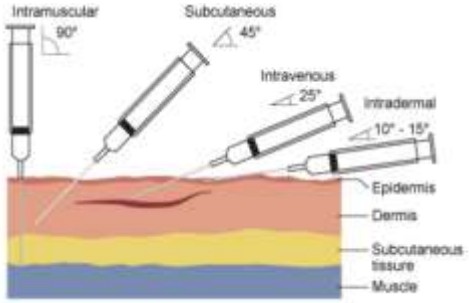
Factors Affecting Clinical Pharmacologic Responses of HIV Cure Therapeutics

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

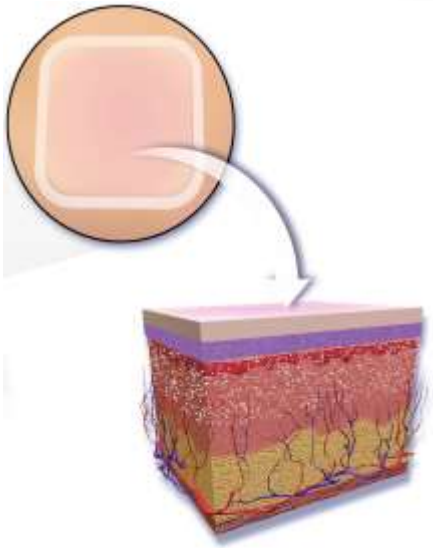
Fletcher CV, et at. *Clin Pharmacol Ther*; in press.

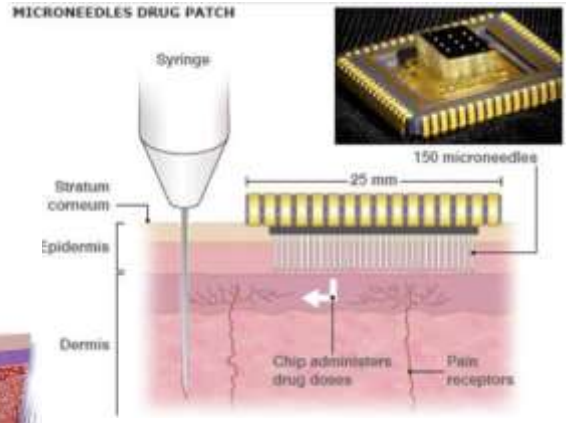
Drug Delivery Technology

Long-acting depot injections



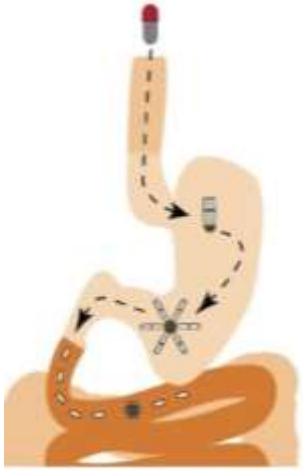
Microneedle drug patch



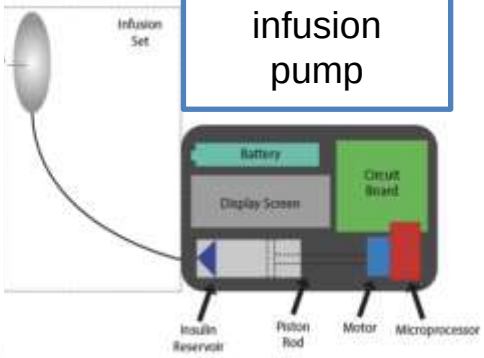


Subdermal implant

Novel oral formulations



Wearable infusion pump



Vaginal rings

DTG, CAB and RPV CSF Concentrations: Oral and IM (long-acting) Administration

Drug	Oral	IM (Long Acting) q 4 week (n=3)	q 8 week (n=15)
Dolutegravir (1)			
Total CSF (ng/mL)	13.2		
CSF to Plasma (%)	0.41		
Cabotegravir (2)			
Total CSF (ng/mL)		12.7	10.6
CSF to Plasma (%)		0.34	0.30
Rilpivirine (2, 3)			
Total CSF (ng/mL)	0.8	1.67	1.84
CSF to Plasma (%)	1.4	1.32	1.07

1. Letendre SL, et al. Clin Infect Dis 2017;59:1032-7.
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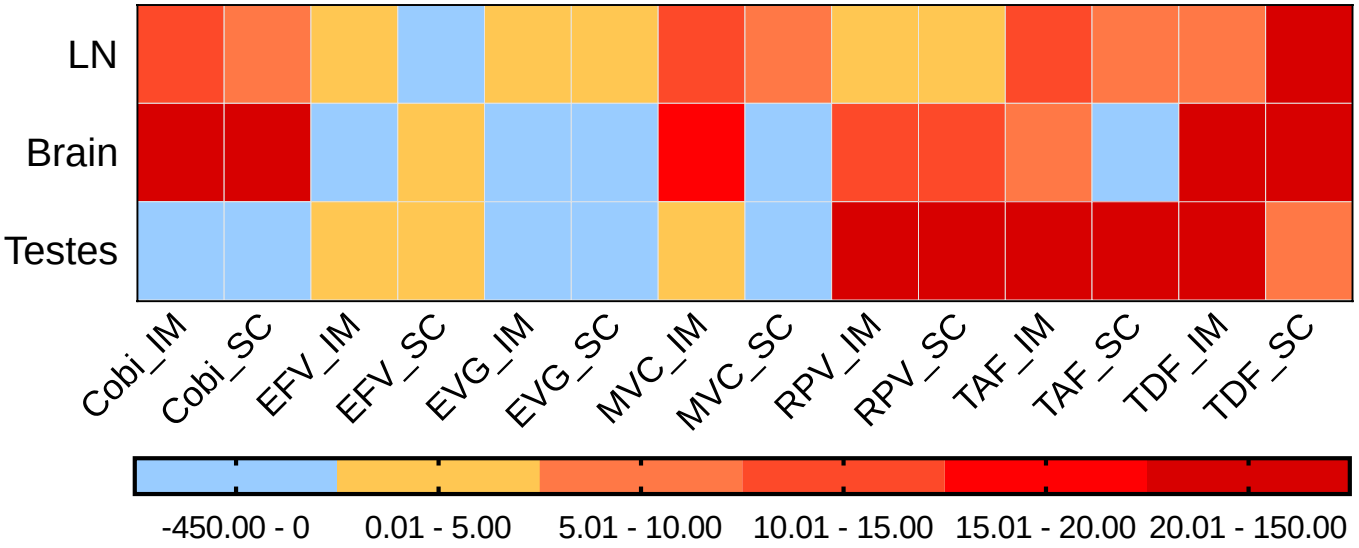
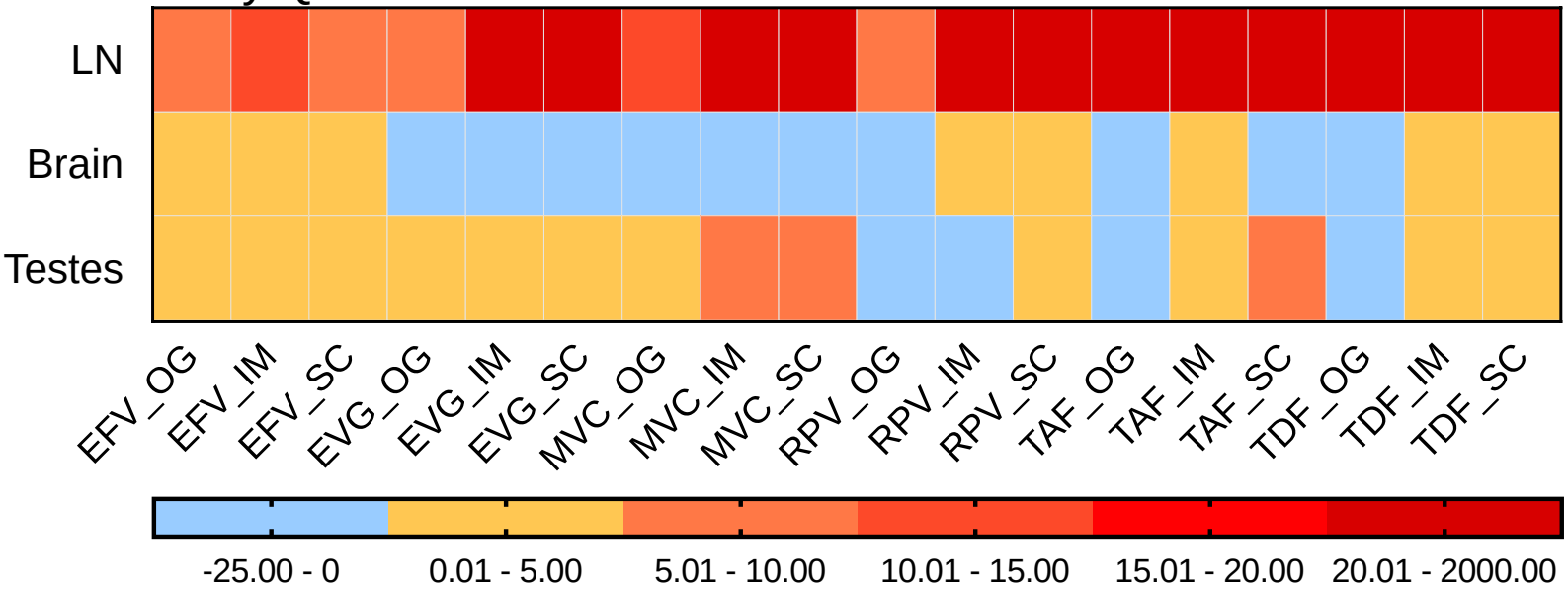
ARV Tissue Delivery: Oral vs SQ vs IM

A. Tissue Penetration Ratio



B. Tissue Inhibitory Quotients



Lymphoid Tissue Pharmacokinetics of TAF vs. TDF in HIV-infected Persons

Matrix	**Tenofovir-diphosphate (fmol/10^6 cells)** Median (and Interquartile Range)	
	TAF (n=13)	**TDF (n=45)**
PBMC	595 (432, 662)	57 (44, 96)
LN	130 (102, 151)	26 (7, 78)
Ileum	151 (26, 385)	641 (448, 1501)
Rectum	49 (33, 107)	456 (194, 909)

Fletcher CV, Podany AT, Thorkelson A, et al. *Clinical Pharmacology and Therapeutics*, 2020;108(5):971-75.

LN Viral Transcription and ARV Levels During Suppressive ART

- Participants of the RV254 acute infection cohort in Bangkok.
  - Objective: to describe clinical, immunological and virological characteristics of persons with acute HIV infection.
  - Intervention: no HIV treatment per protocol, but intent is to facilitate treatment under other AHI protocols that offer immediate treatment.
- 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.
- HIV-RNA+ and HIV-DNA+ cells were measured by RNAscope.
- Cell-associated HIV-RNA and total HIV-DNA were measured by PCR.
- ARV levels were measured by LC/MS/MS.

LN Viral Transcription and ARV Levels During Suppressive ART: 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 higher levels of RNA+ cells in LN in the DTG group vs. DTG + MVC 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 LN anti-HIV potency from MVC.

Fletcher CV, et at. To be presented at *CROI 2021.*

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 at. *Clin Pharmacol Ther*; in press.

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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