Pharmacologic Insights into Reservoirs and Remission

Courtney V. Fletcher, Pharm.D.

Dean, College of Pharmacy

Professor, Department of Pharmacy Practice and Division of Infectious Diseases

University of Nebraska Medical Center

Viral Reservoirs in HIV Infection

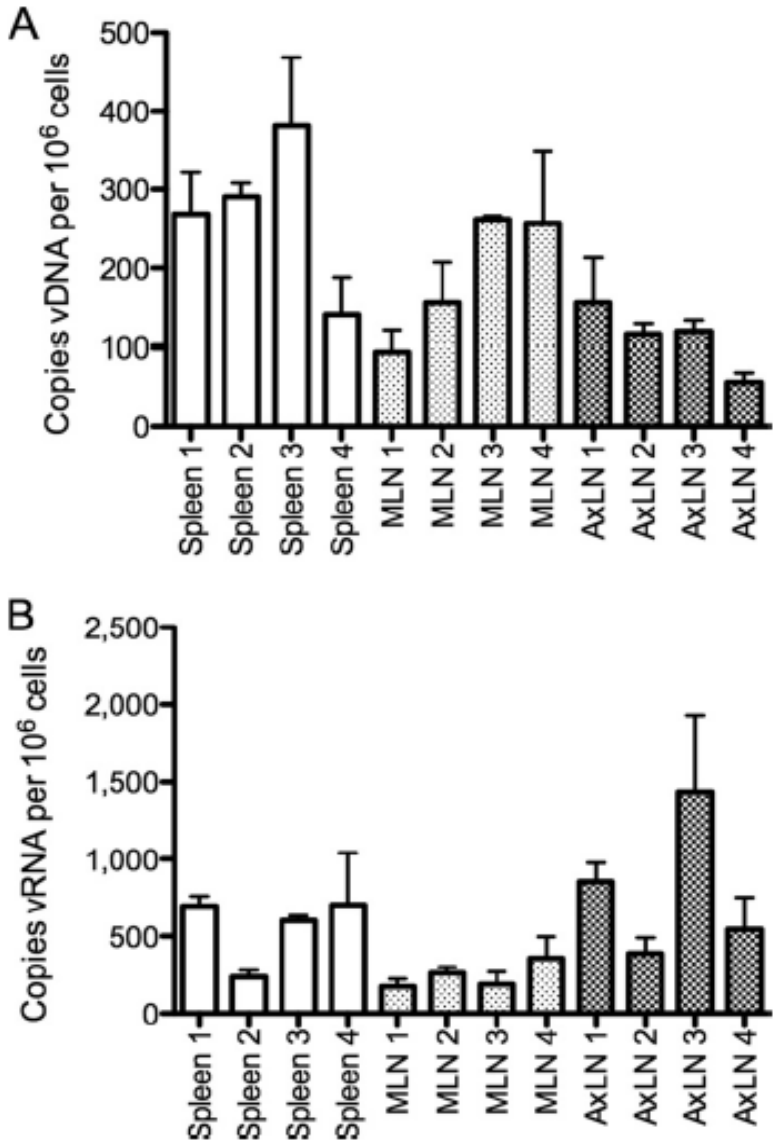
- Evidence supports two distinct, and not mutually exclusive, viral reservoirs that persist during effective, prolonged suppression of HIV.

- Latent Reservoir
  - Comprised of infected lymphocytes in a quiescent state
  - Estimated to decay with a half-life of > 44 months
  - A major obstacle to complete clearance of HIV infection

- Reservoir of Low-Level Ongoing Replication
  - Persistent low level viremia in some patients
  - Secondary LN and GALT are primary sites of HIV replication, and major reservoirs with HIV burden > blood for prolonged time
  - Low level replication in lymphoid tissue may contribute to replenishment of infected resting CD4 T cells

Chun TW and Fauci AS. AIDS 2012;1261-68.

Viral Sanctuaries during HAART in a Non-Human Primate Model for AIDS



- Rhesus macaques treated with TDF, FTC, EFV.
- Plasma viral load at necropsy ranged from 11-28 copies/mL.
- vDNA and vRNA were detected during HAART from numerous anatomical compartments.
- The highest levels of vDNA and vRNA were in lymphoid tissues: spleen, lymph nodes and GI tract tissues.

North TW . . Luciw P. J Virol 2010;84:2913.

The Case for the CNS as a HIV Reservoir

- HIV enters CNS early during systemic infection, initiates local infection and immune activation, and establishes a reservoir of persistent infection; some evidence supports ongoing replication during suppressive therapy.
- Evidence for persistent CNS injury in patients on ARVs despite suppression of plasma HIV-RNA
  - Impaired neuropsychological testing
  - Brain imaging and CSF biomarkers show neuronal injury
- Possible mechanisms
  - Early injury associated with the entry of HIV into the CNS
  - Incomplete suppression of HIV replication
  - HIV persistence in cells of the CNS leading to intermittent release of virus
  - Persistent, local immune activation
  - Drug-related neurotoxicity (?)

Is The Alveolar Macrophage a Reservoir for HIV?

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IMMUNOLOGY

Healthy HIV-1-Infected Individuals on Highly Active Antiretroviral Therapy Harbor HIV-1 in Their Alveolar Macrophages

Sushma K. Cribbs,[1,2] Jeffrey Lennox,[3] Angela M. Caliendo,[4] Lou Ann Brown,[5] and David M. Guidot[1,2]

- 23 HIV-infected persons an ART, non-smokers with no medical comorbidities.
- 16 had (+) and 7 had (-), proviral DNA in their alveolar macrophages
- Those with (+) proviral DNA had lower alveolar macrophage phagocytic index, indicating alveolar macrophage phagocytic immune dysfunction

Lines of Evidence for a Cure or Remission

- Myeloablative allogeneic hematopoietic stem cell transplantation (HSCT)
  - HSCT using cells from a donor homozygous for the delta 32 mutation in gene encoding CCR5; the “Berlin Patient”.
    Hutter G, et al. NEJM 2009;360:692-98.
    Yukl S, et al. PLOS Pathogens May 2013
- Elite Controllers
  - HIV infected persons who can maintain high CD4 cell counts and undetectable HIV RNA in the absence of ART.
    Mendoza D, et al. Blood 2012;119:4645-55.
- Post Treatment Controllers
  - ART initiated in acute/early infection and control of infection sustained after discontinuation of ART (VISCONTI), or sustained for a period of time (27 months, Mississippi Baby).
    Saez-Cirion A, et al. PLOS Pathogens March 2013
    Persaud D, et al. NEJM 2013;369:1828-35.

Potential Therapeutic Strategies

- Pharmacologic
  - Early antiretroviral therapy
  - Latency reversing agents
- Immunologic
  - Immune modulators
  - Antibody therapy (broadly neutralizing antibodies, bispecific antibodies)
  - Therapeutic vaccination
  - Treatment interruption (to boost immunity)
- Cellular
  - Transplantation
  - Gene therapy

Early Initiation of Antiretroviral Therapy

How does the timing of antiretroviral therapy initiation in acute infection affect HIV reservoirs?

Curr Opin HIV AIDS. 2015 Jan;10(1):18-28

Jintanat Ananworanich[a,b], *Karine Dubé*[c], *and Nicolas Chomont*[d]

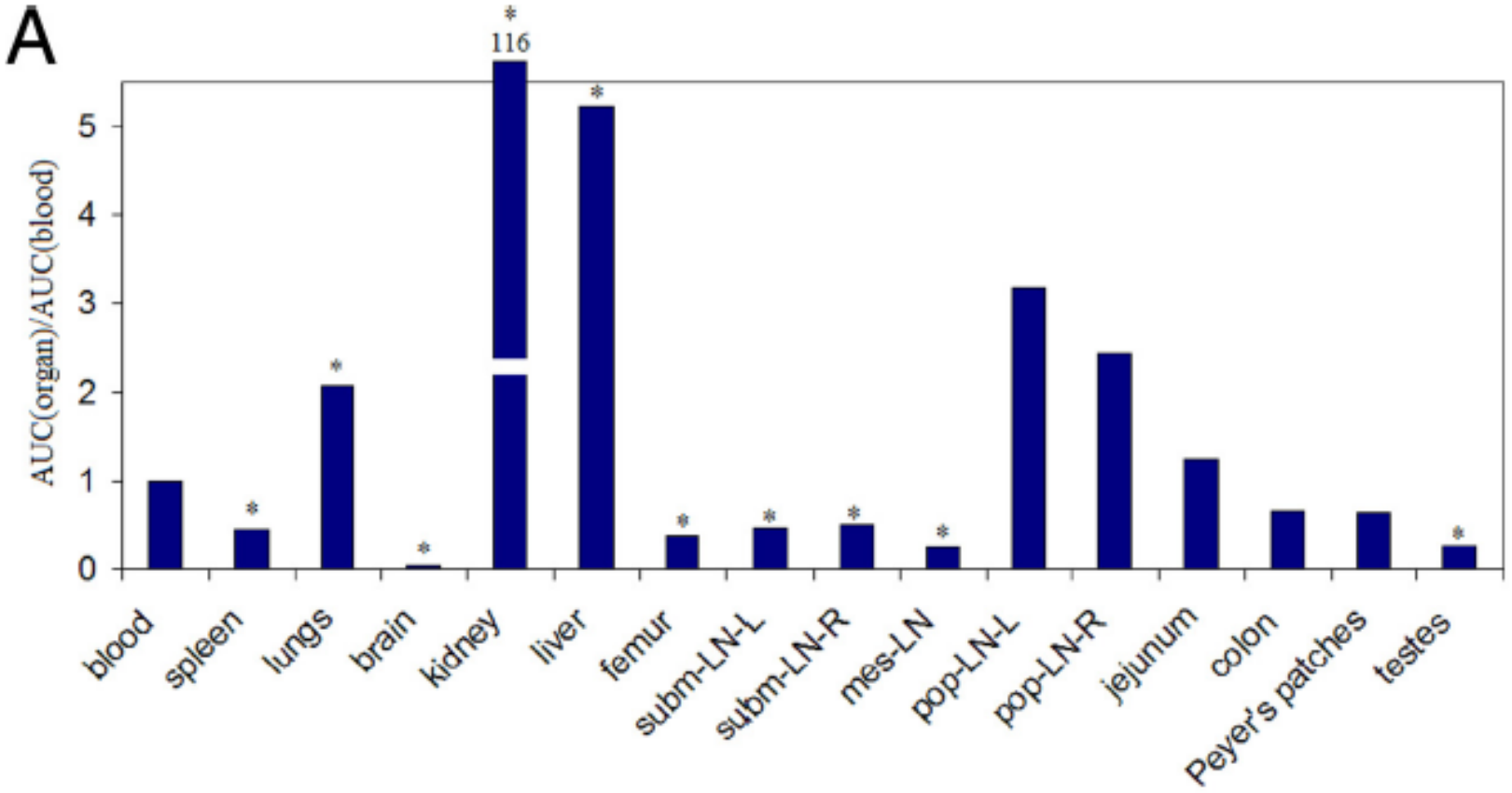
- Latent reservoir established early in infection,
- Early ART in acute infection can reduce the reservoir size,
- No treatment approach started in chronic infection has been shown to limit the reservoir size,
- Early ART may also give a faster decay of the latent reservoir and protect memory CD4 T cell subsets from infection,
- Early ART is associated with a reduction in markers of immune activation vs ART initiation in the chronic phase,
- Long term control (VISCONTI cohort) has been seen with early ART, but no cases seen with with ART started in chronic infection.

Early Antiretroviral Therapy

- When to start:
  - ART treatment (TDF+FTC+DTG) at day 3 in macaques blocked emergence of viral RNA and proviral DNA in peripheral blood; it reduced proviral DNA in LN and GI mucosa, but did not prevent establishment of a reservoir. (Whitney JB et al. Nature 2014 Aug 7;512:74-7.
- What regimen to use:
  - VISCONTI: 2NRTI (1), 3NRTI (7), 2NRTI+PI (5), 2NRTI+NNRTI (1)
  - Mississippi Baby: ZDV+3TC+NVP x 6d, then ZDV+3TC+LPVr
  - Little information on the effects of early ART on establishment of tissue reservoirs; there may be a need to target ART to reservoirs such as the GALT, LN, CNS
- How long:
  - At least 4 years has been suggested (Rouzioux C, et al. Curr Opin HIV/AIDS 2015;Jan 10(1):29-34.)

TFV Tissue Kinetics in Rats by PET

- TFV tissue kinetics evaluated with an fluorine-18 radiolabeled analog
- Lowest distribution: brain, mesenteric LN and testes
- Similar or equivalent distribution: colon and jejunum



Di Mascio M, et al. Antimicrob Agents Chemother 2009;53:4086-95

Highly Intensified ART in Macaques

- 11 macaques treated with: TFV + FTC + RAL for 1.5 months, then DRV + RTV was added, and after 80 days MVC was added. A second group (n=4) was given all 6 simultaneously.
- Plasma viral load was < 40 and < 3 copies/mL.
- Viral DNA was < 2 copies of DNA/$5*10^5$ cells in PBMCs and in rectal biopsies of all animals.
- Viral RNA and DNA was < 2 copies of DNA/$5*10^5$ cells in lymph nodes from 4 of 6 macaques tested.
- These data perhaps support the notion of full suppression of peripheral virus replication and, in the reservoir tissues examined, in the majority of animals.

Shytaj IL. PLoSPathogens 2012;8:e1002774

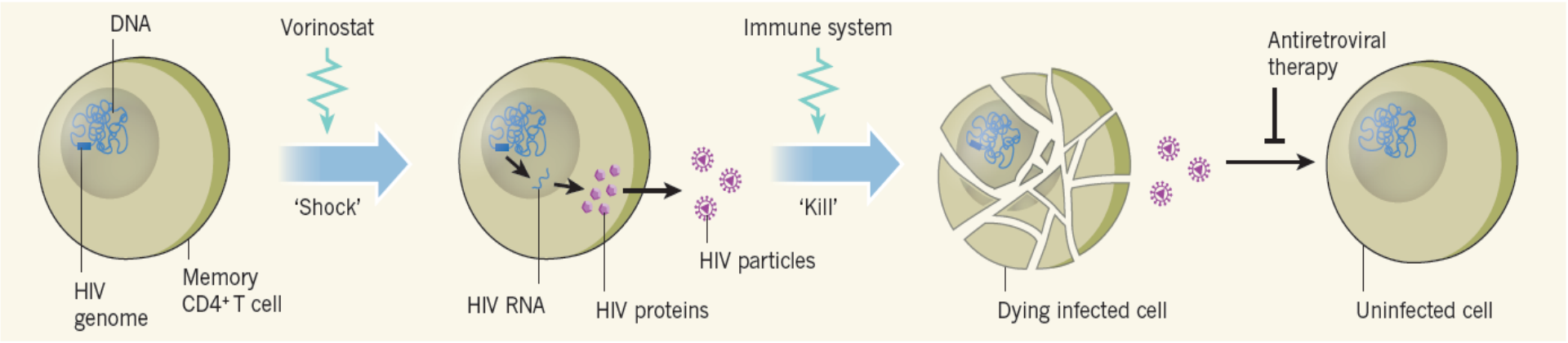
Nanoformulations of ARVs

Time	Lopinavir	Ritonavir	Tenofovir
Plasma drug exposure			
0-168h	18.2	14.0	7.0
Intracellular drug concentrations			
168h	> 8	>132	>25
Inguinal LN intracellular drug concentration			
24h	> 485	50.5	0.7

- Subcutaneous administration of LPV, RTV and TFV packaged together in a lipid nanoparticle (LNP) or as native drug to 4 macaques.
- Concentrations are ratios of the LNP to native formulation.

Freeling JP …Ho R. AIDS 2014;28:2625-2631.

Activation of the Latent Viral Reservoir "Shock and Kill"



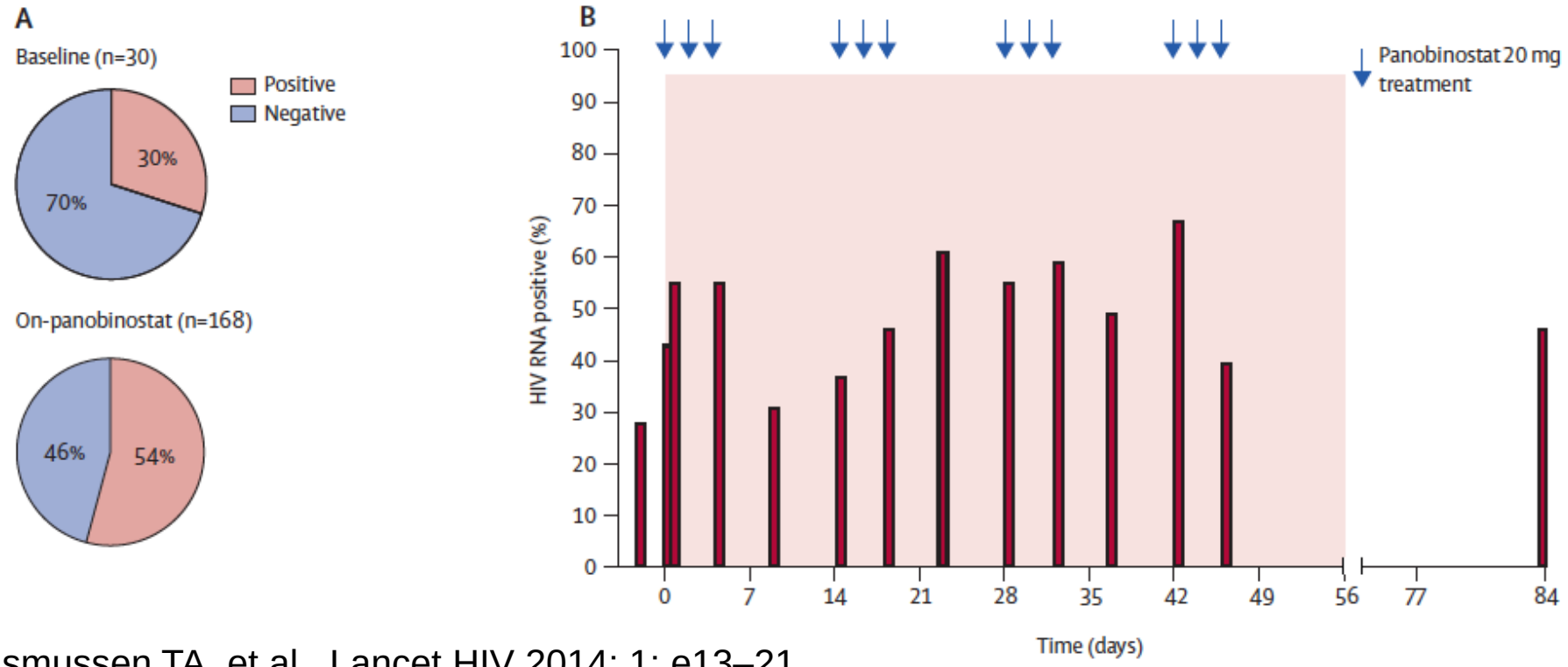
Key steps and assumptions of the induction and clearance approach:

- Activate viral replication by reversing latency,
- HIV RNA synthesis ➔ viral protein production ➔ release of HIV particles,
- Killing of the infected cell by the virus cytopathic effect or the patient's immune system or both,
- Inhibition of released infectious virus by ART.

Deeks SG. Nature 2012;487:439-40.

Panobinostat Phase I/II Clinical Trial in HIV-Infected Subjects

- 15 subjects received panobinostat 20 mg 3x/wk every other wk x 8 wks.
- Cell-associated unspliced HIV RNA increased at all time points; median maximum increase was 3.5x.
- Plasma viremia increased vs baseline; odds ratio 10.5
- There was no cohort wide reduction in total or integrated HIV DNA.

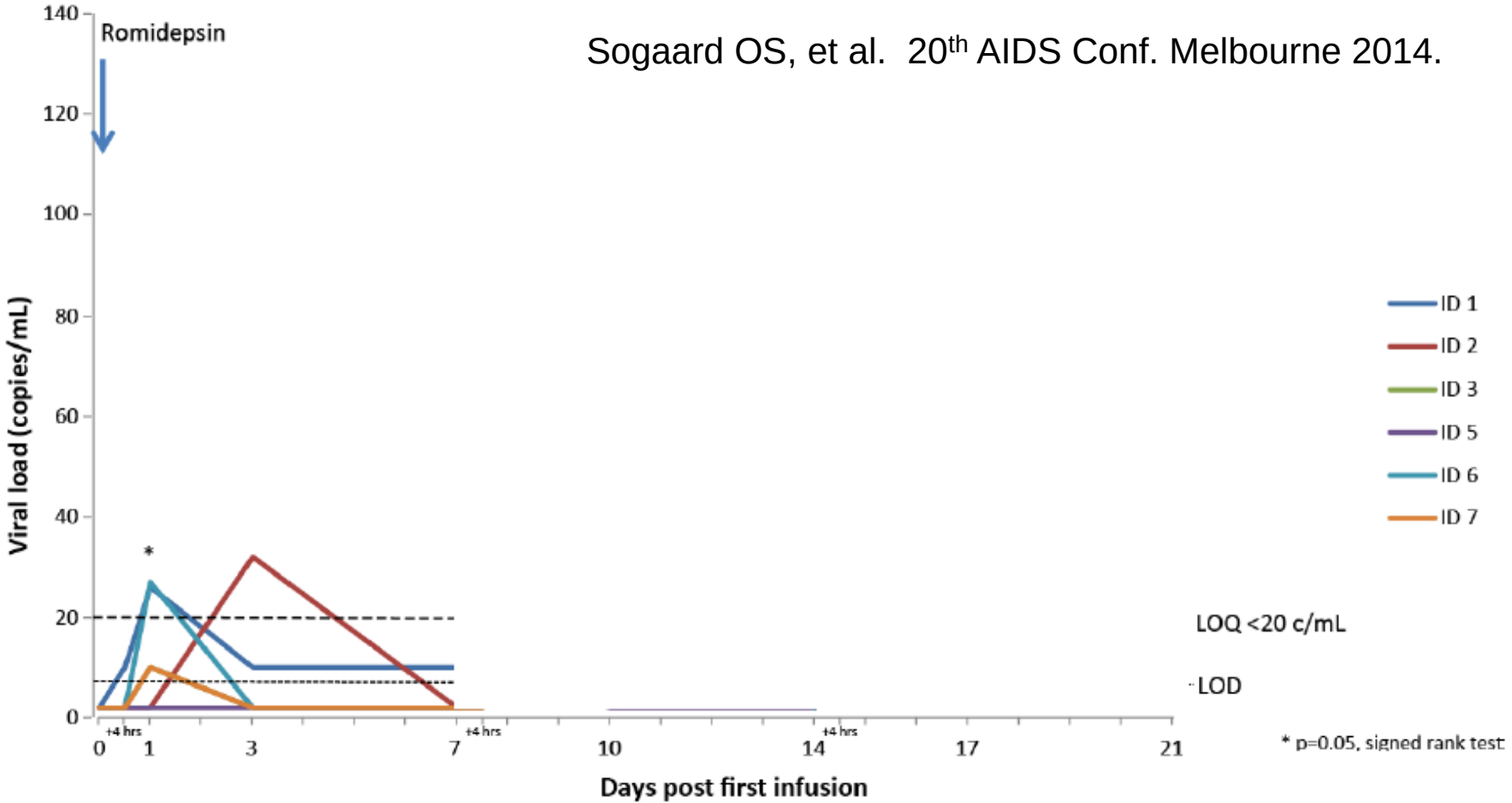


Rasmussen TA, et al. Lancet HIV 2014; 1: e13–21

Romidepsin Phase I Trial in HIV-Infected Persons

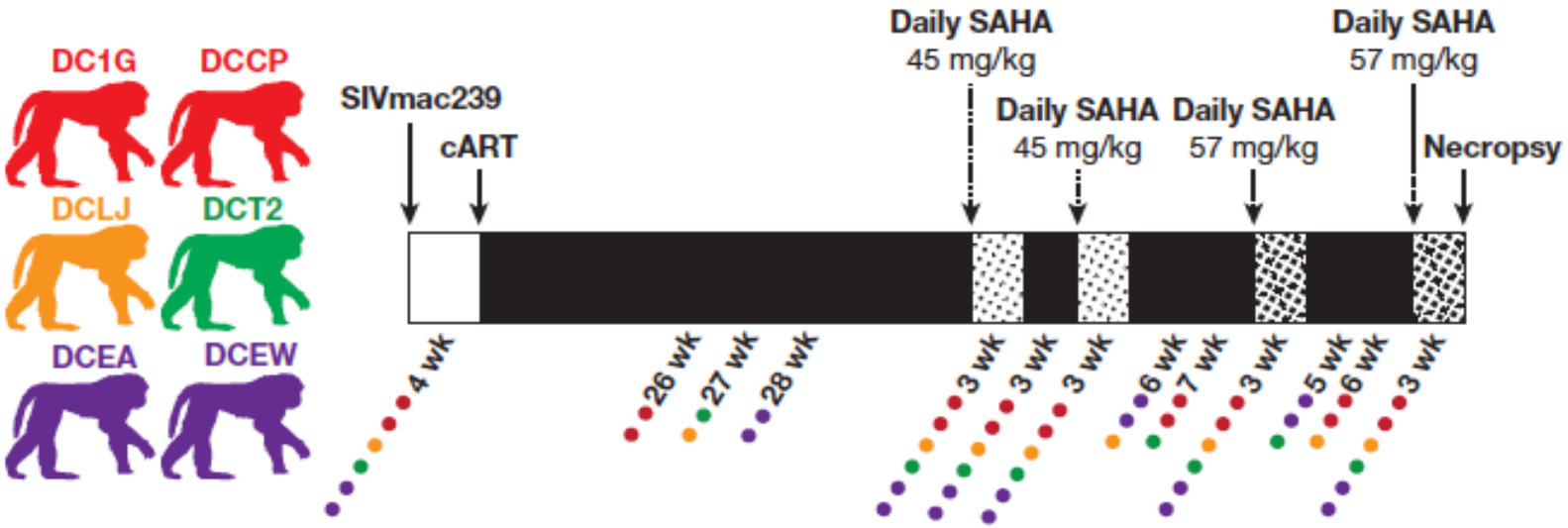
- 6 HIV+ persons received romidepsin (5mg/kg IV, days 0, 7 & 14).
- A transient, quantifiable increase in plasma HIV-RNA was seen.
- As measured by HIV-DNA, the reservoir was not reduced.

Sogaard OS, et al. 20th AIDS Conf. Melbourne 2014.



Effect of Vorinostat (SAHA) on the Residual Virus Pool in Macaques

- ART regimen (TFV+FTC+L870812+L900564+DRV+RTV) to achieve plasma suppression and declines in cell-associated viral RNA and DNA in blood and tissues.
- 2 different doses of SAHA administered; 84 total doses
- In vitro and ex vivo validation, and in vivo assessment of effect on the residual virus pool.



Del Prete G, et al. Antimicrob Agents Chemother 2014;58:6790-6806

Effect of SAHA on the Residual Virus Pool

- Ex vivo SAHA showed evidence of HDAC inhibition and increased vRNA in CD4 cells from ART suppressed animals.
- SAHA in vivo, increased acetylated histone with a dose-dependent response with evidence for sustained modulation and refractoriness after prolonged therapy.
- In vivo virologic effect was demonstrated as an increased ratio of vRNA:vDNA (a measure of viral transcription) in PBMCs.
- Plasma viremia was observed, but not clearly associated with SAHA administration.
- Residual viremia was readily detected at end of treatment indicating SAHA did not eradicate inducible virus.

Del Prete G, et al. Antimicrob Agents Chemother 2014;58:6790-6806

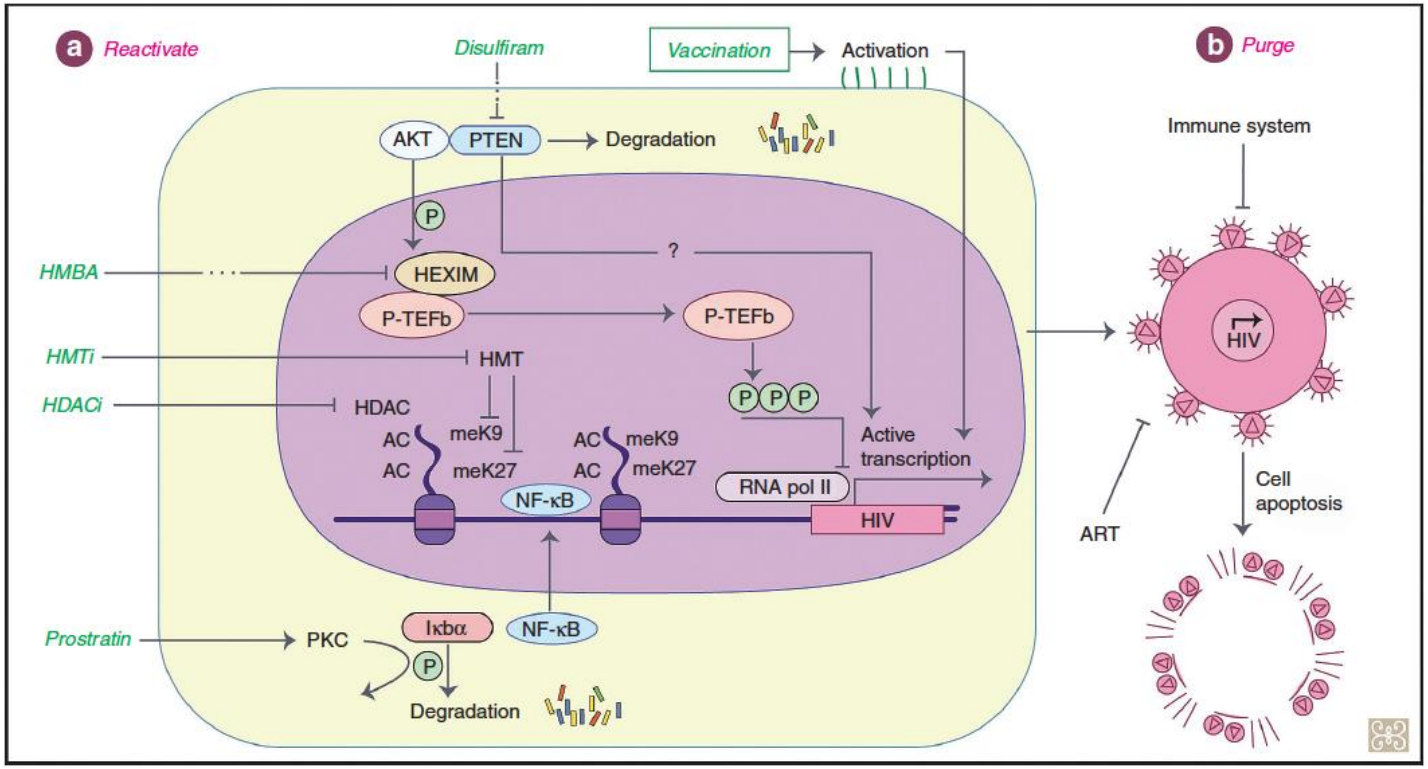
New Developments with HDAC Inhibitors

- HDAC inhibitors impair elimination of HIV-infected cells by cytotoxic T-lymphocytes, thus producing an unintended negative effect for the “shock and kill” strategy. (Jones RB…Walker BD. PLoSPathogens 2014;10:e10904287).
- Reported >98% of latent viruses in chronically-infected patients carry CTL escape mutations that render the cells insensitive to CTLs directed at common epitopes (Deng K…Silicano R. Nature 2015;517:381-85).

- These findings both indicate the need for appropriate boosting of the immune response, and the latter an additional argument for early ART.

Strategies to Disrupt HIV Latency

- HDAC inhibitors (vorinostat, romidepsin, panobinostat)
- Disulfiram (Increases oxidative stress and apoptosis)
- Programmed cell death protein (PD-1) inhibition
- Vaccination
- Activation of protein kinase C (PKC) and/or nuclear factor KB (NF-KB) pathways to induce expression: prostratin, bryostatin



Barton KM et al. Clin Pharmacol Ther 2012; Dec 5, 2012.

Synergistic Latency Reversing Agents

- Synergistic activation of HIV expression with a combination of a P-TEFb releasing compound with NF-KB-inducing agents (Darcis G. Abstract OA2-1. Towards an HIV Cure. Melbourne, 2014).
  - NF-KB-inducing agents and P-TEFb releasing compounds (HMBA, JQ1, I-BET, I-BET151) increased HIV-1 production in a dose dependent manner with minimal cytotoxicity.
  - The combination of both had a strong, synergistic activation of HIV-1, with HIV-1 expression increased in a higher proportion of cells than the compounds alone.
  - Physiologic relevance is supported by combination treatments giving a higher reactivation in ex-vivo cultures of resting CD4 T cells from HIV+ persons with plasma virus suppression.

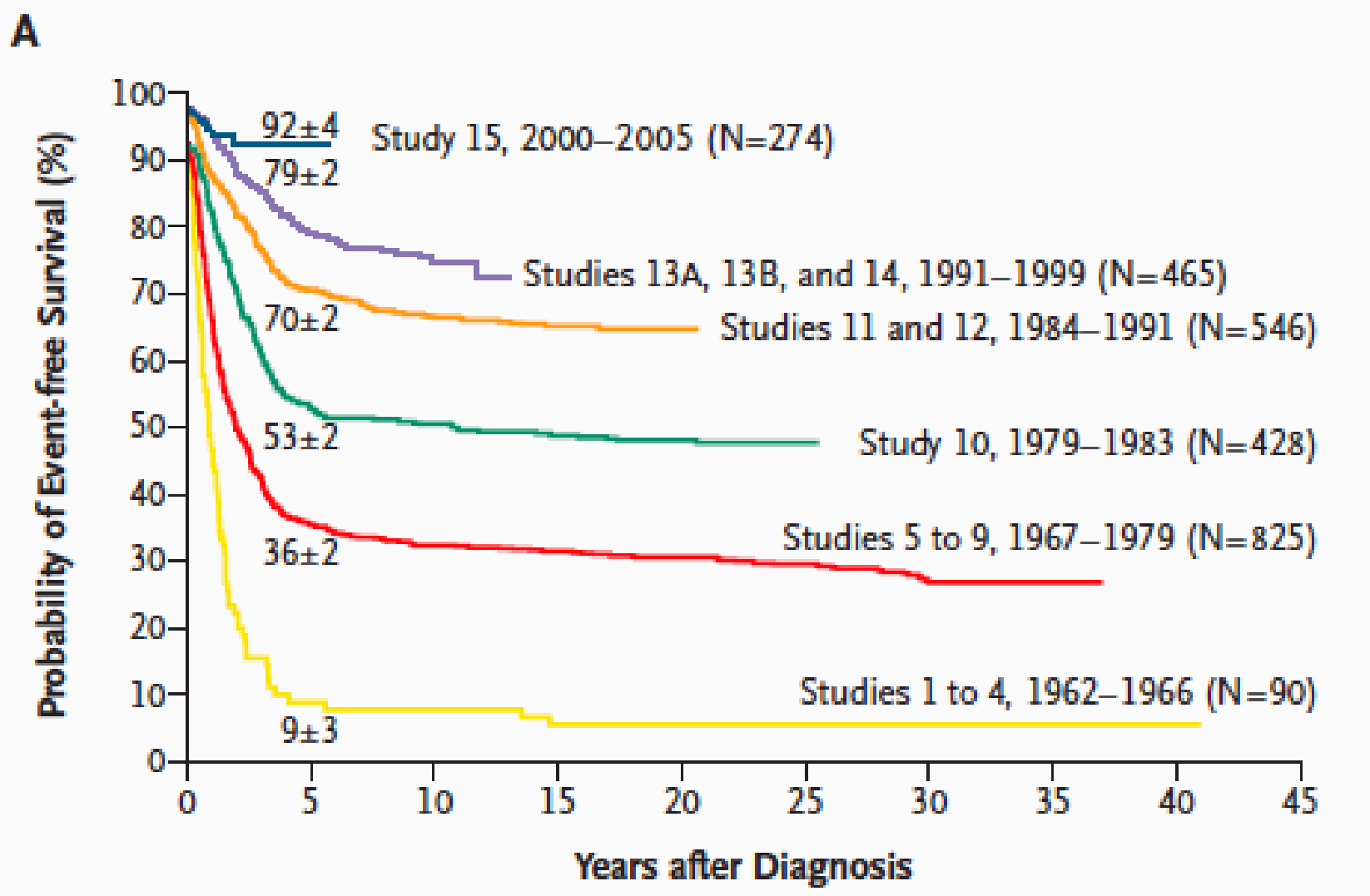
New Latency Reversing Agents

- Benzotriazoles (Planelles V. Abstract OA2-2, Towards an HIV Cure, Melbourne 2014).
  - Reactivate latent HIV in central memory T cells.
  - A candidate 1-hydroxy-7-azabenzotriazole (HOAt) has been shown to reactivate virus particle production in resting CD4 T cells obtained from two patients.
  - Benzotriazole derivatives have been shown to have antiproliferative properties. The HOAt analogs did not induce T cell proliferation, activation or cytokine production, which is a highly desirable property for a latency reversing agent.

Reservoirs and Remission: pharmacologic perspectives

- The pharmacologic characteristics of early and fully suppressive ART need to be established.
- Full suppression of viral replication in tissues or pharmacologic sanctuary sites is a necessary antecedent to the success of any remission-to-cure strategy.
- Strategies to activate viral expression and eliminate infected CD4 cells will be more difficult that originally thought, and will require rigorous study to demonstrate safety and efficacy.
- A similarly rigorous process is necessary to establish the ethical basis of inducing viral replication in persons who meet the current goals of HIV therapy.

Treatment of Childhood Acute Lymphoblastic Leukemia



Pui CH and Evans WE. NEJM 2006;354:166.

Interventions that Improved the Response to Childhood ALL

- Combination systemic chemotherapy
- PK guided, high dose methotrexate
- Early treatment intensification
- Individualized (vs. standard dose) combination chemotherapy
- Application of methotrexate pharmacodynamics in various phenotypes and genotypes of ALL
- Triple agent intrathecal therapy (can eliminate the need for prophylactic cranial irradiation)
- Application of host pharmacogenetics (e.g., mercaptopurine dose based on polymorphisms in thiopurine methyltransferase)

Pui CH et al. Leukemia 2010;24:371.

Thank You