HIV infection of the CNS: Implications for cure

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- Impact of combination antiretroviral therapy (cART)
  - Reduced morbidity and mortality, restored life expectancy
  - Treatment remains life-long, Expensive, Side-effects, Access
- Major barrier to cure is persistent viral reservoirs
  - Integrated, replication competent, long-lived, latent
- cART has no/minimal long-term affect on viral reservoirs
- “Shock and kill” cure strategy aims to eliminate latency by reactivating virus using latency-reversing agents (LRA)
- The CNS remains an important, yet understudied, potential viral reservoir

Determining whether the CNS is a viral reservoir will be an important consideration for any HIV cure or eradication strategies

HIV viral reservoirs

- Brain
- Gut
- Genital tract
- Lungs
- Kidneys
- Lymph nodes

Is the CNS a viral reservoir?

Indirect evidence of a viral reservoir in the CNS

- Ongoing immune activation
  - Levels of Neopterin remain elevated following suppressive therapy
    - Hagberg et al., AIDS Res and therapy 2010
    - Eden et al., JID 2007
    - Yılmaz et al., JAMID 2008
- Evidence of axonal injury (NFL levels) in patients on suppressive cART
  - Krute et al., 2014

Symptomatic and asymptomatic CSF ‘escape’

- Dahl et al., JID 2014
- Letendre et al., CROI 2009
- Eden et al., JID 2010
- Peluso et al., AIDS 2012
- Canestri et al., Clin Infect Diseases 2010

Direct evidence of a viral reservoir in the CNS

Pre-symptomatic

- Thompson et al., Am J Path 2011
- Archival brain tissue from pre-symptomatic patients, isolated perivascular macrophages by LCM, PCR of p24
- Detected HIV-1 DNA in PVM, microglia and astrocytes

Asymptomatic

- Churchill et al., Annals of Neurol 2009
- Archival brain tissue from asymptomatic patients, isolated macrophages and astrocytes using LCM
- < 1% astrocytes +ve for HIV-1 Env DNA
Does HIV persist in CNS cells from virally suppressed patients?

**Summary of DNA findings in virally suppressed patients**

- DNA can be detected in CNS macrophages (and possibly astrocytes) isolated from virally suppressed patients
- Does not indicate:
  - Frequency of HIV-1 in CNS cells (size of reservoir)
  - Number of patients with a CNS reservoir
  - Whether a replication competent provirus is present

**DNA sequencing**

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**No HIV DNA detected in the CNS of 4/5 virally suppressed patients**

**Virally suppressed patient cohort for determining HIV persistence in the CNS**

- 5 patients that fulfilled the criteria of suppressed patients (N89, N69, T82, C47, C06) were selected.
- IHC (GFAP (astrocytes)/CD68 (macros))→4 patients were considered 'usable'
- LCM was performed on all viable tissue samples. For each patient macrophages were isolated and triple nested PCR performed for GAPDH and HIV-1 Env V3
**Current cure strategies relevant to CNS “Shock and Kill”**

**Nucleosome organisation**

**Mode of action of Latency-reversing agents**

**LRA class, CNS penetration and potency**

Do unique regulatory mechanisms exist within the CNS which facilitate 'latent' HIV infection and affect responsiveness to LRA

**Patient Cohort**

- **Strategy:**
  - **PCR, cloned and sequenced LTR**
  - Analyzed:
    - compartmentalization
    - transcriptional activity
    - transcriptional factor motif analyses
CNS-derived LTRs are genetically distinct

- LTR sequences form patient clusters
- Compartimentalization between lymphoid and CNS-derived LTRs

CNS-derived LTRs have lower basal activity

CNS-derived LTRs have mutated Sp motif

Decreased Sp1 binding to the Sp motif significantly correlated with reduced LTR activity

Designing a system to test LTR activity

Transfect cells (+/- Tat or LRA)

Measure LTR activity

LTR

Luciferase

Gray et al., AIDS Res. And Human Retroviruses 2013

Gray et al., AIDS Res. And Human Retroviruses 2013

Gray et al., AIDS Res. And Human Retroviruses, 2013

Gray et al., Molecular Psychiatry (NPG) 2015

Gray et al., Molecular Psychiatry (NPG) 2015
What contribution does the Sp motif have to overall LTR activity?

The Sp motif plays a significant role in both basal and Tat-mediated LTR activity.

**Sp1 and HIV-1 transcription**

- **Sp1 motif**
  - GC-rich sequences which are very well conserved across HIV isolates and are essential for both basal and activated transcription.
  - Binds to the basal region of the LTR to activate transcription.
  - Cooperatively interacts with NFκB, required for full core enhancer function.
  - Interacts with Tat complexes and is required for Tat activation.
  - Recruits HATs to the LTR to acetylate histones, opening of nucleosomes and activation of transcription (activation from latency).
  - Recruits c-myc which in turn recruits HDAC 1 to the LTR to deacetylate histones, formation of nucleosomes and decrease in transcription (establishment of latency).

**Do the unique LTRs found in the CNS respond differently to LRA?**

CNS-derived LTRs have reduced responsiveness to Panobinostat/Romidepsin (HDACi).

- **CNS Lymphoid**
  - Reduced response to LRA observed for CNS-derived HIV relative to lymphoid-derived LTRs from the same patient and relative to Z1 wild-type.

CNS-derived LTRs have reduced responsiveness to Tat and JQ1+Tat.

- **CNS Lymphoid**
  - Reduced response relative to lymphoid-derived LTRs from the same patient and relative to Z1 wild-type.

No significant difference was observed for disulfiram and JQ1.

However, for Tat and JQ1+Tat, CNS-derived LTRs had reduced response relative to lymphoid-derived LTRs from the same patient and relative to Z1 wild-type.
- HIV DNA detected in a virally suppressed patient
- CNS-derived HIV had significantly lower responsiveness to select LRA
- These data suggest different treatment outcomes in different compartments/reservoirs
- Implications:
  - Positives - may allow for selective targeting of specific reservoirs
  - Negatives - need to determine LRA activity in all reservoirs
- LTR sequences isolated from the CNS are distinct
- Mutated Sp motif, lower Sp1 binding, lower transcriptional activity
- Unique regulatory mechanisms exist within the CNS that effect the efficiency of LRA to reactivate latent virus
- These data may have implications when selecting LRA for eradication strategies

Summary

Acknowledgements

Burnet Institute
HIV Neuropathogenesis Lab
Melissa Churchill
Wan-Jung Cheng
Emma Roberts
Jacqui Raison
Daniel Cowley
Hung On
Anne Gibbs

SAHMRI
Steve Wesselingh

RMIT University
Paul Garry

Doherty Institute
Sharon Lewin
Han Lu
Michael Moss
Fiona Wrightman
Michael Roche
Damian Purcell
Jonathan Jacobson

Johns Hopkins
Justin McArthur
Carlos Pardo-Villamizar

St Vincents Hospital Sydney
Bruce Brew
Gilles Guillemin

Burnet Institute

APP1051093
APP1009533

R21 MH100954-02 NIMH
NIHU19A1096109
supplement

APP1051093
APP1009533