HIV infection of the CNS: Implications for cure

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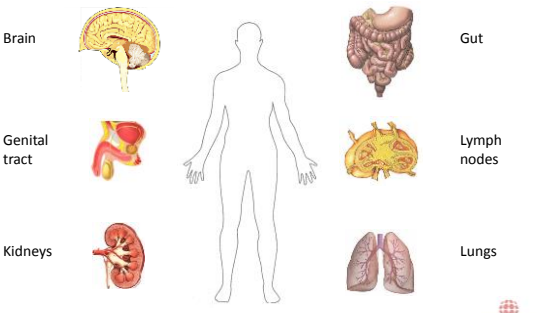
HIV-1 cure

- 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



Is the CNS a viral reservoir?

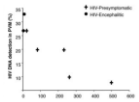
Indirect evidence of a viral reservoir in the CNS

- Ongoing immune activation
  - Levels of Neopterin remain elevated following suppressive therapy *Hagberg et al., AIDS Res and therapy 2010; Eden et al., JID 2007; Yilmezet al., JAIDS 2008*
- Evidence of axonal injury (NFL levels) in patients on suppressive cART *Krut et al., 2014 PLoS One*
- 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 p24-ve perivascular macrophages by LCM, PCR of gag
→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
→1-3 % astrocytes +ve for HIV-1 Env DNA

Does HIV persist in CNS cells from virally suppressed patients?

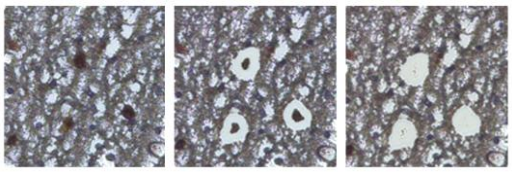
Virally suppressed patient cohort for determining HIV persistence in the CNS

Subject code	Age	Sex	cART	CD4	Neurocog Impair.	Viral Load		Tissues				
						CSF	Plasma	Brain	CSF	Plasma	PBMC	Spleen
N89	53	M	EFV, KTA, TZV	78	Mild	<50	<50	Yes	Yes	Yes	Yes	Yes
N69	60	M	3TC, DDI, IDV	38	Mild	<50	<50	Yes	Yes	Yes	Yes	Yes
T82	66	M	ABC, EFV, KTA	464	None	<50	<50	Yes	Yes	Yes	Yes	Yes
C47	55	M	3TC, ATV, TFV	182	None	<50	<50	Yes	Yes	Yes	Yes	Yes
C06	39	M	ABC, RTV, ZDV,	75	None	<50	<50	Yes	Yes	Yes	Yes	Yes
C58	41	F	Yes/?	5	None	8000	75300	Yes	Yes	Yes	Yes	Yes
T71	40	F	ATR, CBV, KTA	5	Mild	408	157009	Yes	Yes	Yes	Yes	Yes

Laser capture micro-dissection (LCM) approach

- 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

IHC, LCM & PCR workflow



6 µm sections
-GFAP
-CD68

LCM
- 1
10
50
1000,
3000 cells
slabs (#cells unclear, no vessels)
- Lysates prepared

PCR:
- GAPDH/ V3,
- 5 reactions/ lysate
- sample GAPDH +ve subjected to V3 PCR

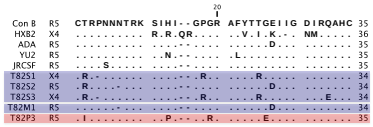
DNA sequencing -batch sequencing

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

Patient ID	Sample type /cell #	# +ve PCR reactions		Sequence determined	# Unique sequences
		GPDH	V3		
C47	1	0/5	0/5	N	-
	10	3/5	0/3	N	-
	50	4/5	0/4	N	-
	1000	4/5	0/4	N	-
	>3000	5/5	0/5	N	-
	Slabs	5/5	0/5	N	-

HIV DNA detected in CNS and PBMC of 1/5 virally suppressed patients

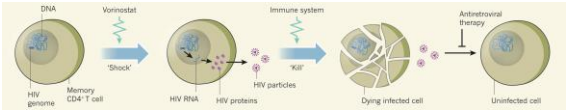
Patient ID	Sample type /cell #	# +ve PCR reactions		Sequence determined	# Unique sequences
		GPDH	V3		
T82	1	0/5	0/5	N	-
	10	4/5	0/4	N	-
	50	4/5	0/4	N	-
	1000	5/5	0/5	N	-
	>3000	5/5	2/5	Y	1
	Slabs	5/5	3/5	Y	3
T82	PBMC	-	-	1	1



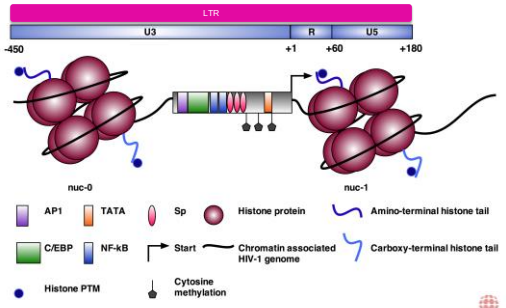
Summary of DNA findings in virally suppressed patients

- 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

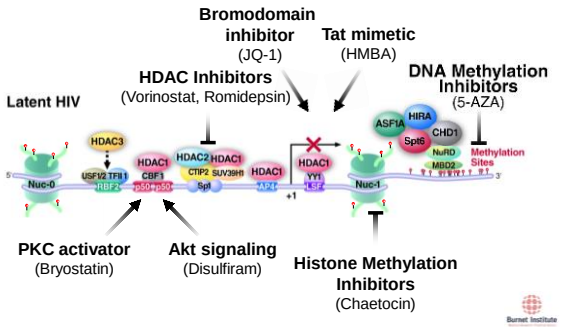
Current cure strategies relevant to CNS "Shock and Kill"



Nucleosome organisation



Mode of action of Latency-reversing agents



LRA class, CNS penetration and potency

Name	Type	CNS penetrance	Potency (ACH2)
Panobinostat	HDACi (Pan)	?	52×
Romidepsin	HDACi (Class I)	-/+	9×
Vorinostat	HDACi (Pan)	+++	7×
Entinostat	HDACi (Class I)	-/+	53×
HMBA	Tat mimetic	++	25×
Disulfiram	Akt signaling	+++	4×
JQ-1	BRDi	++	7×
Chaetocin	HMTi	?	25×
Bryostatin	PKC activator	++	13×

Churchill et al., 2015, J Neurovirol

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

Patient Cohort

Patient	Risk factor	Last CD4 count (cells/μl)	Antiretroviral(s)	HIV-1 encephalitis	Isolates available
CB1	MH	10	ddI (prior AZT)	Severe	CSF, BR, PBMC
CB3	MH	5	ddI (prior AZT and ddC)	Severe	CSF, SC, PBMC
MACS1	MH	2	None	Severe	BR, SPLN
MACS2	MH	52	AZT	Moderate	BR, LN
MACS3	MH	95	None	Moderate	BR, LN

- BR - Brain, SPLN - Spleen, LN - Lymph node, CSF - Cerebral spinal fluid,
- PBMC - Peripheral blood mononuclear cells, SC - Spinal cord

Previously characterized virus isolates from HIV-1 demented patients ***(Gorry et al., 2001)***

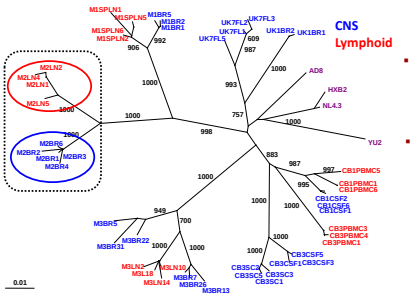
Strategy:

PCR, cloned and sequenced LTR

Analyzed:

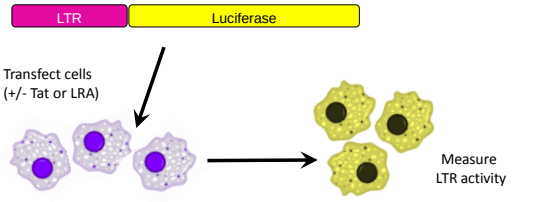
- compartmentalization
- transcriptional activity
- transcriptional factor motif analyses

CNS-derived LTRs are genetically distinct

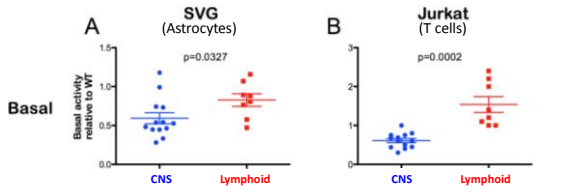


Gray et al., AIDS Res. And Human Retroviruses 2013

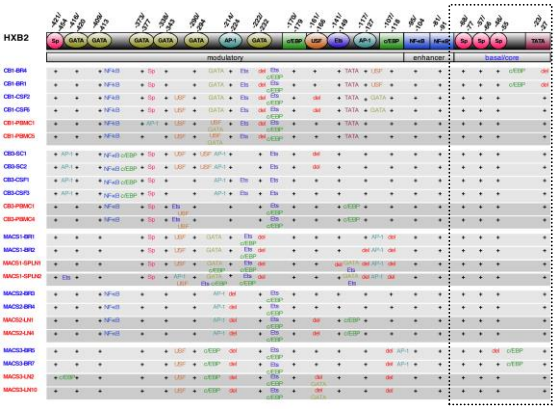
Designing a system to test LTR activity



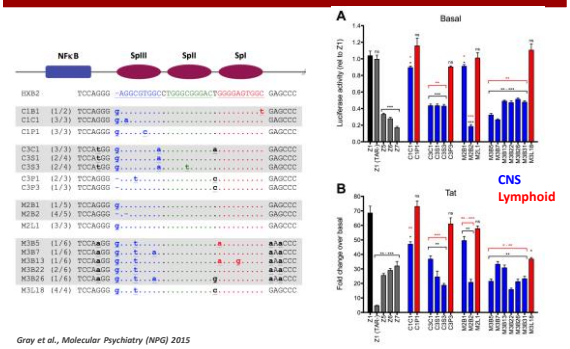
CNS-derived LTRs have lower basal activity



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

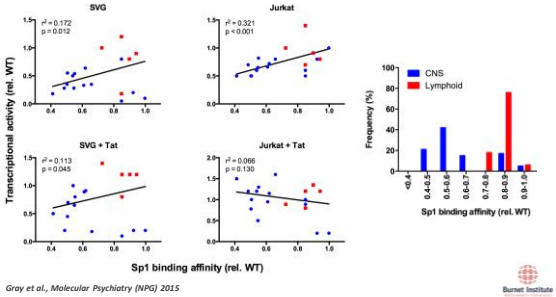


CNS-derived LTRs have mutated Sp motif



Gray et al., Molecular Psychiatry (NPG) 2015

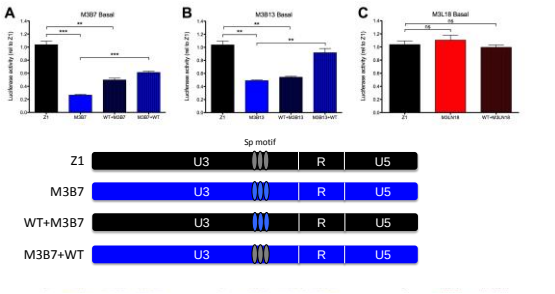
Decreased Sp1 binding to the Sp motif significantly correlated with reduced LTR activity



Gray et al., Molecular Psychiatry (NPG) 2015

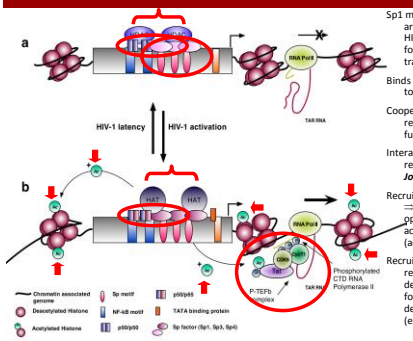
What contribution does the Sp motif have to overall LTR activity?

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



Gray et al., Molecular Psychiatry (NPG) 2015

Sp1 and HIV-1 transcription



Sp1 motif G/C 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 NFkB, required for full core enhancer function ***Perkins 1993, 1994***

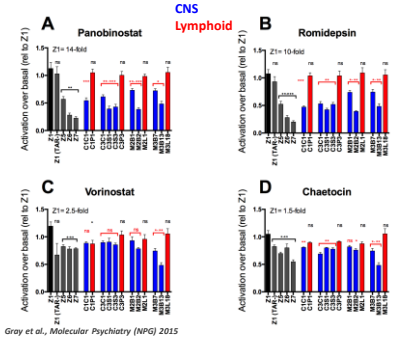
Interacts with Tat-complexes and is required for Tat activation ***Jones 1986, Zeichner 1991***

Recruits HATs to the LTR ⇒acetylation of histones, opening of nucleosomes and activation of transcription (activation from latency)

Recruits c-myc which in turn recruits HDAC 1 to the LTR ⇒ deacetylation of 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)



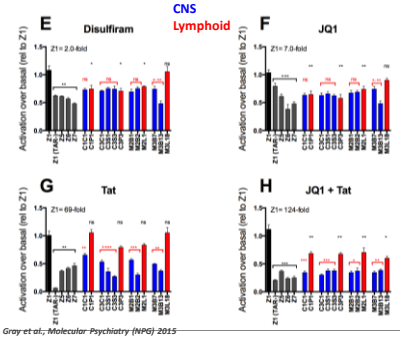
Panobinostat and Romidepsin

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

No significant difference in response was observed for Vorinostat; but varied for Chaetocin

Gray et al., Molecular Psychiatry (NPG) 2015

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



No significant difference in response 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

Gray et al., Molecular Psychiatry (NPG) 2015

Summary

- 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 select 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

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