The HIV-1 splicing inhibitor, SPL-464, compromises viral replication in vitro and induces a long lasting anti-viral effect in humanized mice infected with HIV-1

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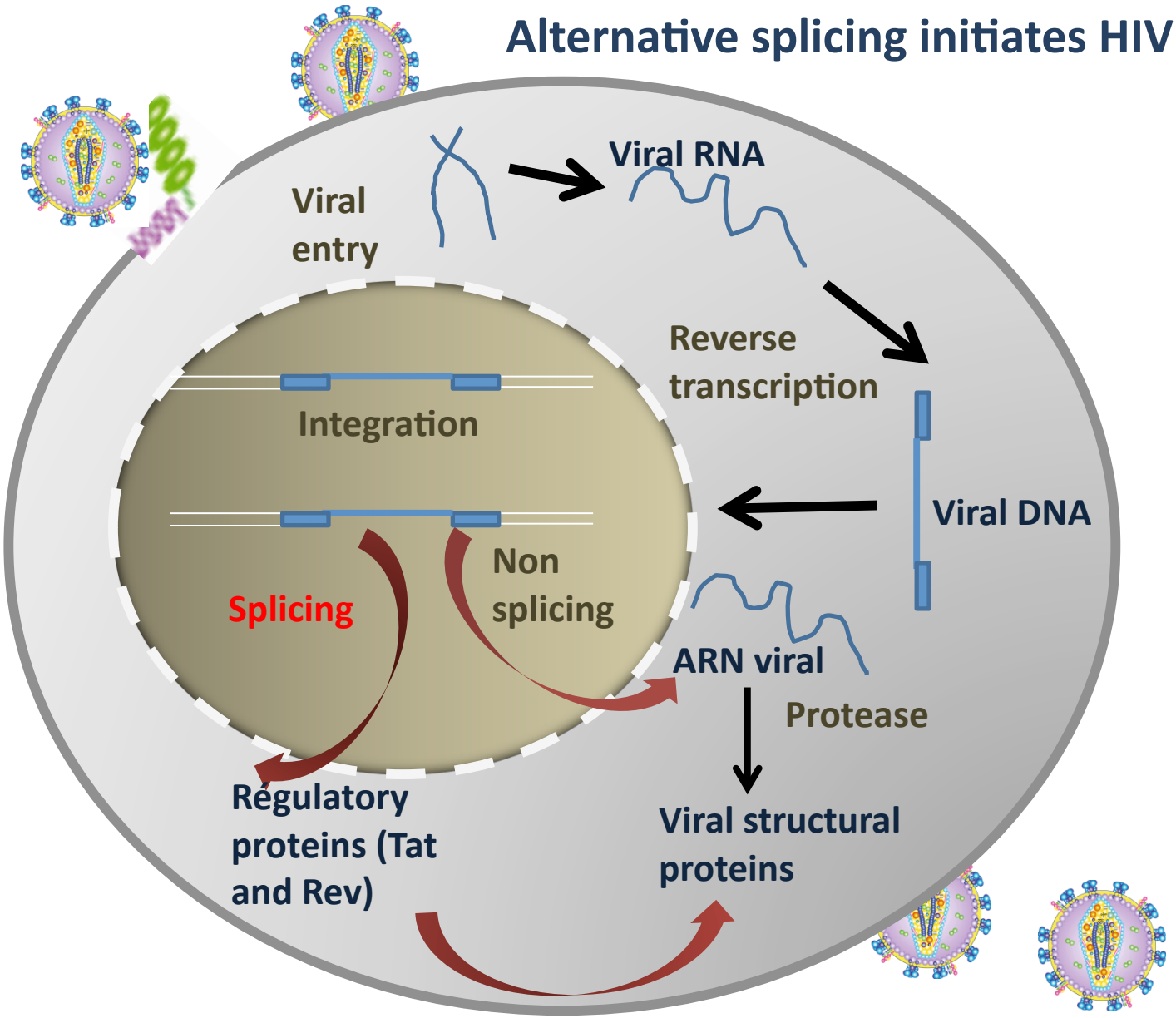
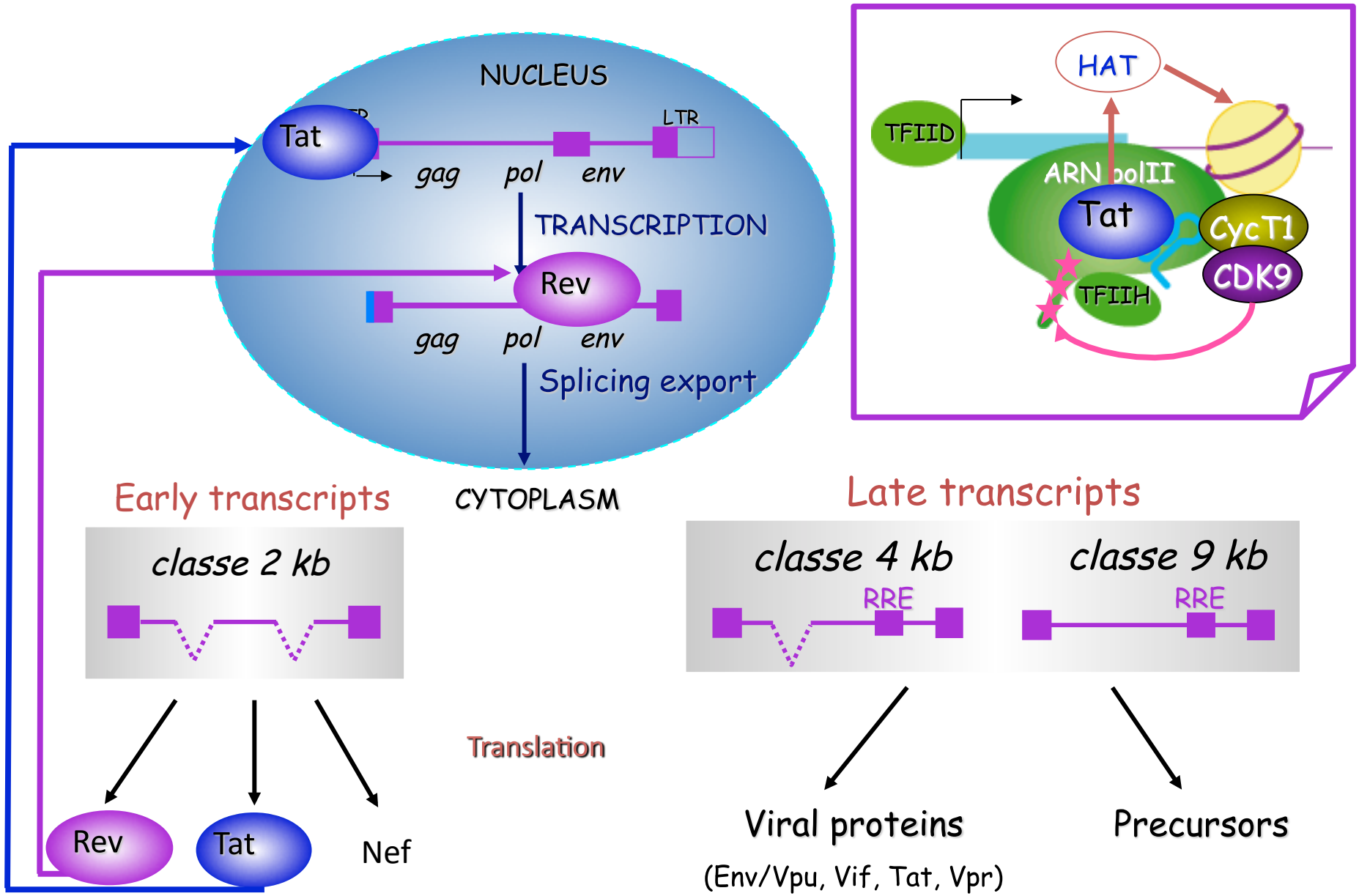
Alternative splicing and HIV life cycle

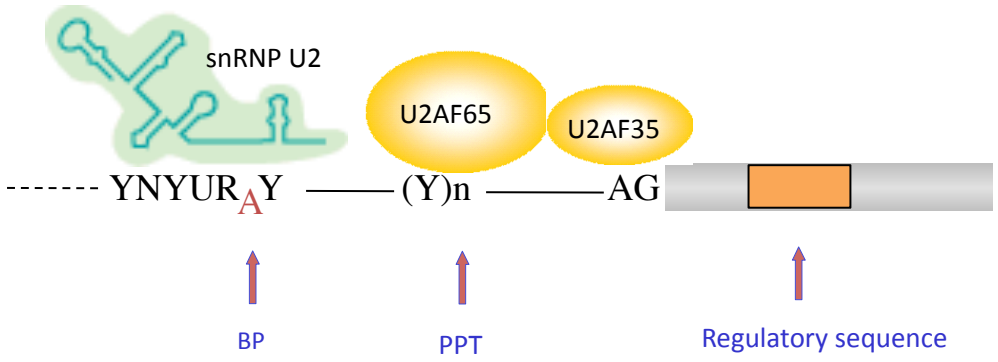
Alternative splicing initiates HIV replication



Alternative splicing is a key process for HIV replication

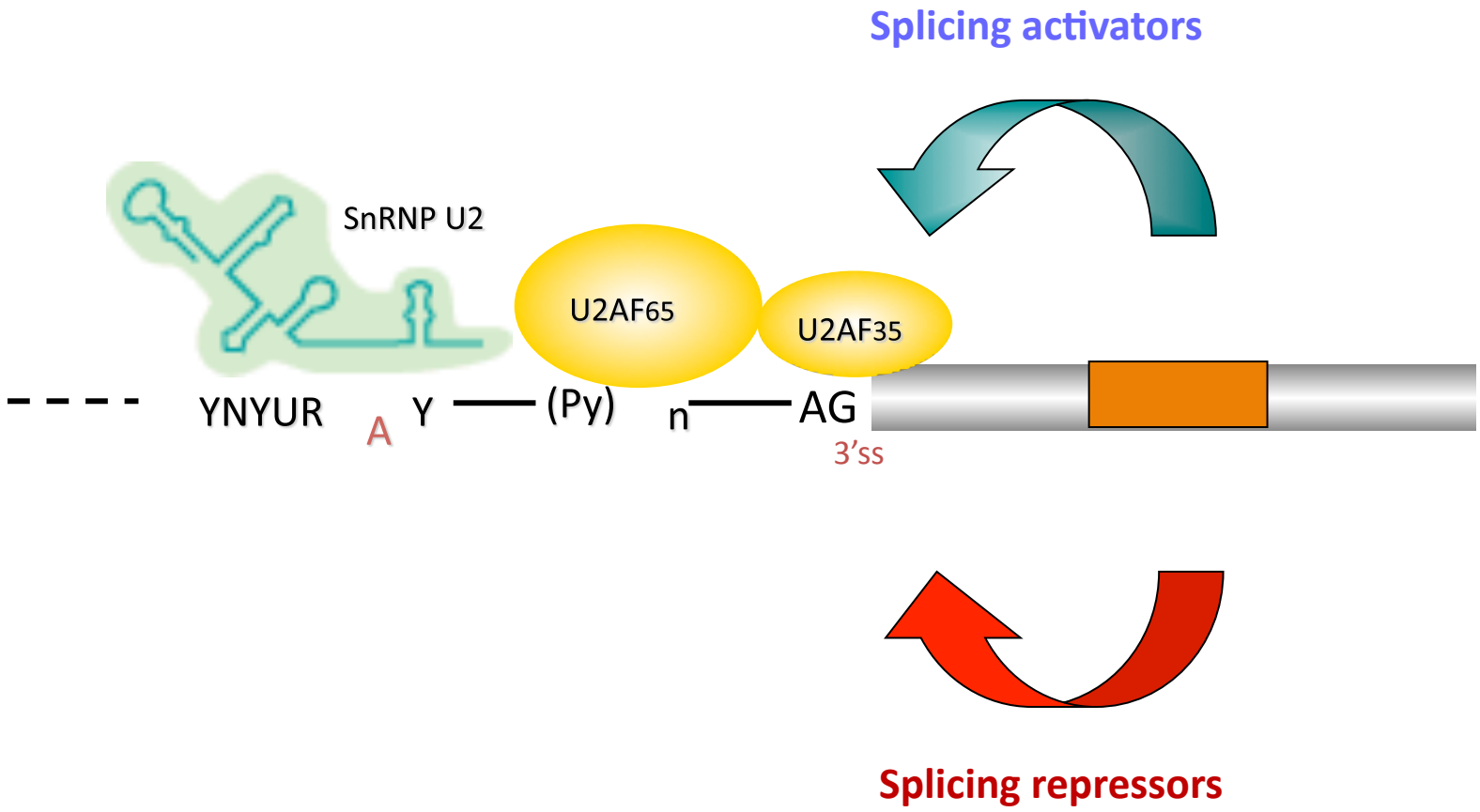

NUCLEUS
Tat
LTR
gag
pol
env
TRANSCRIPTION
Rev
gag
pol
env
Splicing export
CYTOPLASM
HAT
TFIID
ARN polII
Tat
CycT1
CDK9
TFIIH
Early transcripts
classe 2 kb
Late transcripts
classe 4 kb
RRE
classe 9 kb
RRE
Translation
Rev
Tat
Nef
Viral proteins
(Env/Vpu, Vif, Tat, Vpr)
Precursors

HIV-1 RNA has weak 3’ splice sites

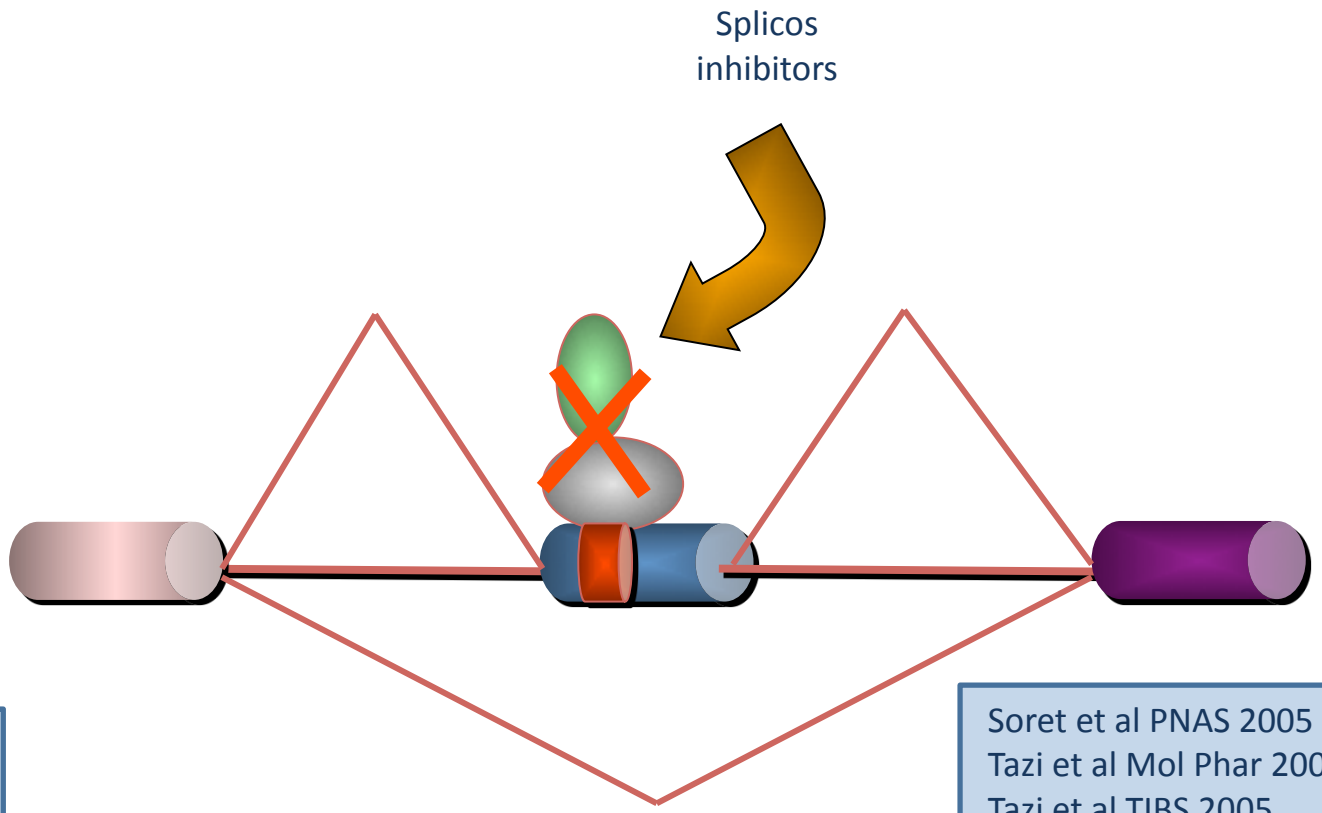


Consensus	Y(n)C/UAG G
A1	AAUUUUCGGGUUUAUUACAG G
A2	UAUUACUUUGACUGUUUUUCAG A
A3	ACAACUGCUGUUUAUCCAUUUCAG A
A4a	AGUUUGUUUCACAACAAAAG C
A4b	AGUUUGUUUCACAACAAAAGCCUUAG G
A4c	AAGUGUUGCUUUCAUUGCCAAG U
A5	AGUUUGUUUCACAACAAAAGCCUUAG G
A7	GGAUAUUCACCAUUAUCGUUUCAG A

HIV-1 RNA splicing depends on splicing regulators that target HIV-1 RNA sequences



Splicos has a propriatory library of small chemical compounds targeting the splicing machinery

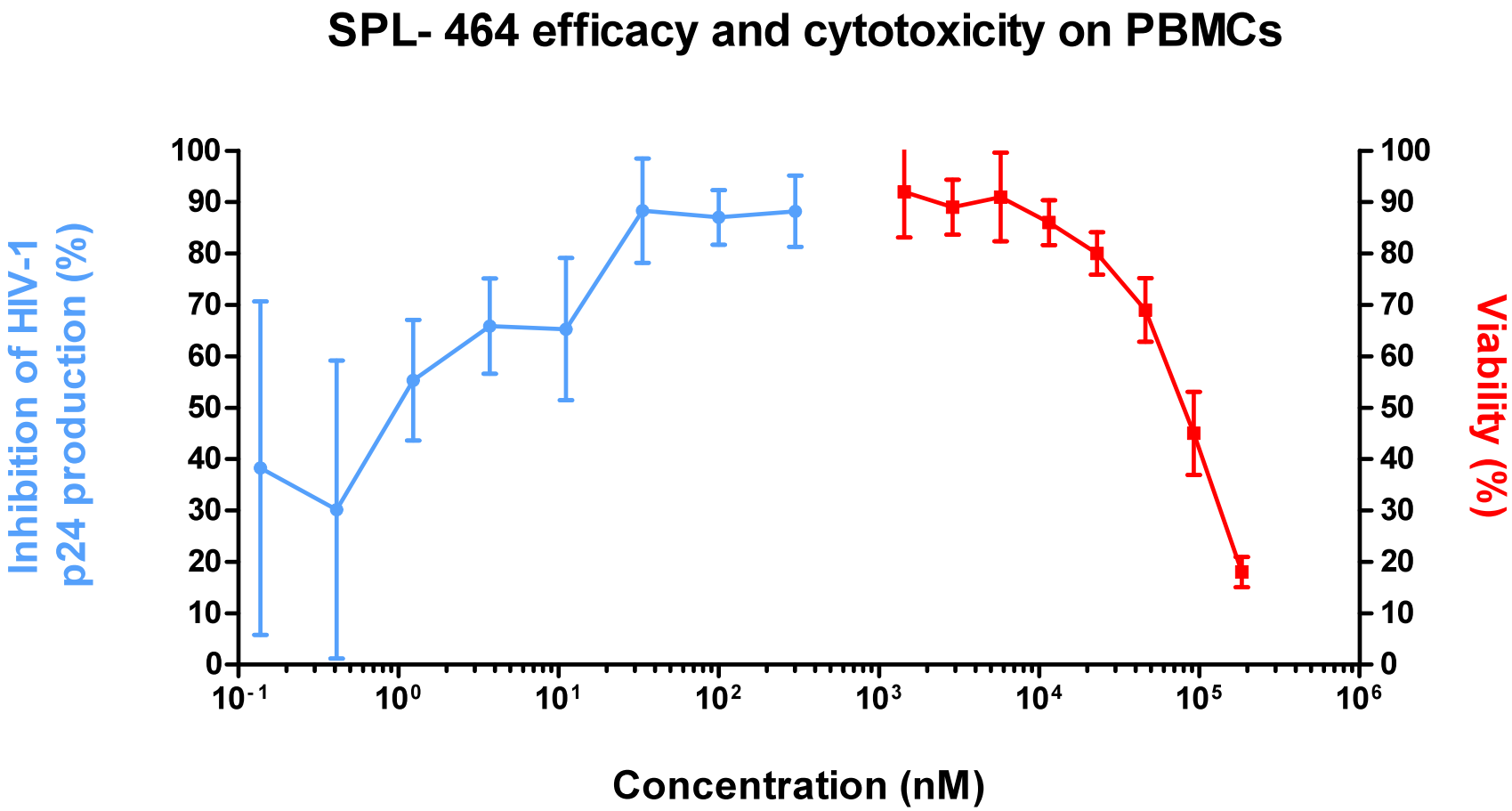


Patents

Lejeune et al 2007
Tazi et al 2005
Tazi et al 2008
Tazi et al 2009
Tazi et al 2009
Tazi et al 2010

Soret et al PNAS 2005
Tazi et al Mol Phar 2005
Tazi et al TIBS 2005
Soret al Prog Mol Subcell Biol 2006
Bakkour et al PloS Path 2007
Keriel et al PloS One, 2009
Tazi et al BBA Mol Bio 2009
Tazi et al FEBS J 2010
2 manuscripts

Lead SPL-464 characterization


SPL- 464 efficacy and cytotoxicity on PBMCs
Inhibition of HIV-1 p24 production (%)
Viability (%)
Concentration (nM)
100
90
80
70
60
50
40
30
20
10
0
10-1
100
101
102
103
104
105
106

- **SPL-464 induces a dose-dependent inhibition of HIV-1 replication in primary macrophages from different donnors**

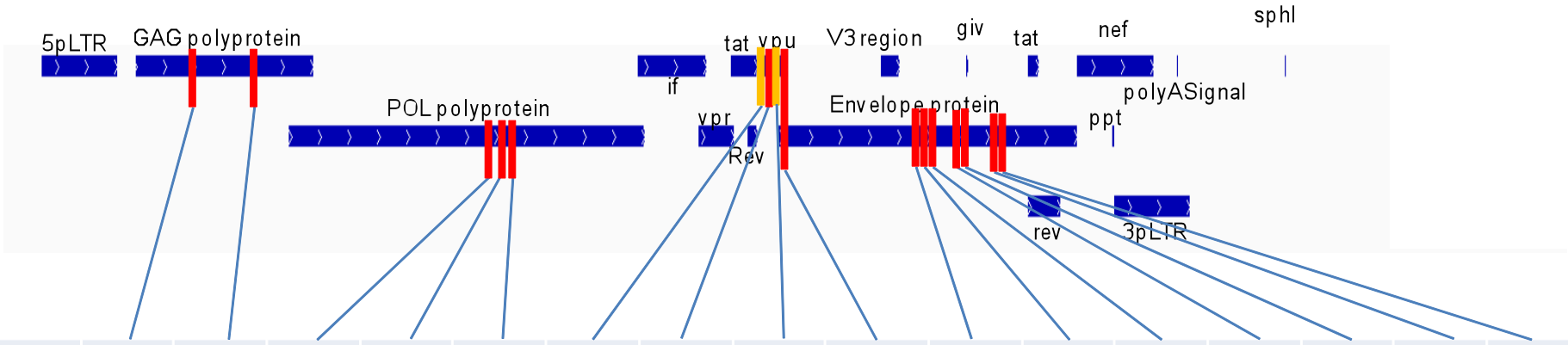
- **SPL-464 inhibits viral replication of different HIV-clades including B and C types**

- **SPL-464 inhibits viral replication of ART escape mutants**

- **SPL-464 inhibits viral replication of HIV-2**

- **After six months of *in vitro* treatment with SPL-464 no resistant viruses have emmerged, whereas drug-resistant viruses are selected following three weeks of treatment with either 3TC or EFV**

Deep sequencing of YU-2 virus after SPL-464 treatment did not reveal any selected mutation

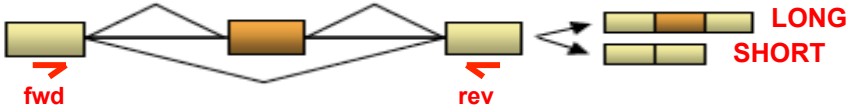


POS	1222	1852	3874	3876	3877	6066	6214	6215	6231	7553	7555	7556	7758	7759	8008	8009
CODON REF/ALT	CAT/CCT	GGG/GTG	TAT/TAC	GTA/GGT		ATG(start)/CTG	GCA/GAG-GCG		Vpu: GAG/AAG Env: ATG/ATA	ACA/ATA	AGA/CCA		TTG/TTA	GGA/AGA	ACT/TGT	
AARef/Alt	H/P	G/V	Y/Y	V/G		M/L	A/E-A		vpu: E/K Env: M(start)/I	T/I	R/P		L/L	G/R	T/C	
REF	A	G	T	T	A	A	C	A	G	C	A	G	G	G	A	C
ALT	C	T	C	G	T	C	A	G	A	T	C	C	A	A	T	G
1_R_10_220						99									84	85
3_R10_221						99				84	84	84	80	61		
9_R10_SPL_235						99		71								
10_R10_R10_235	84	60	84	65	99	99	87	99	74							
13_R10_SPL_236						99										
14_R10_R10_236						99										

Profiling cellular alternative splicing events shifted by Splicos drugs

Robotic liquid handling

High throughput capillary electrophoresis

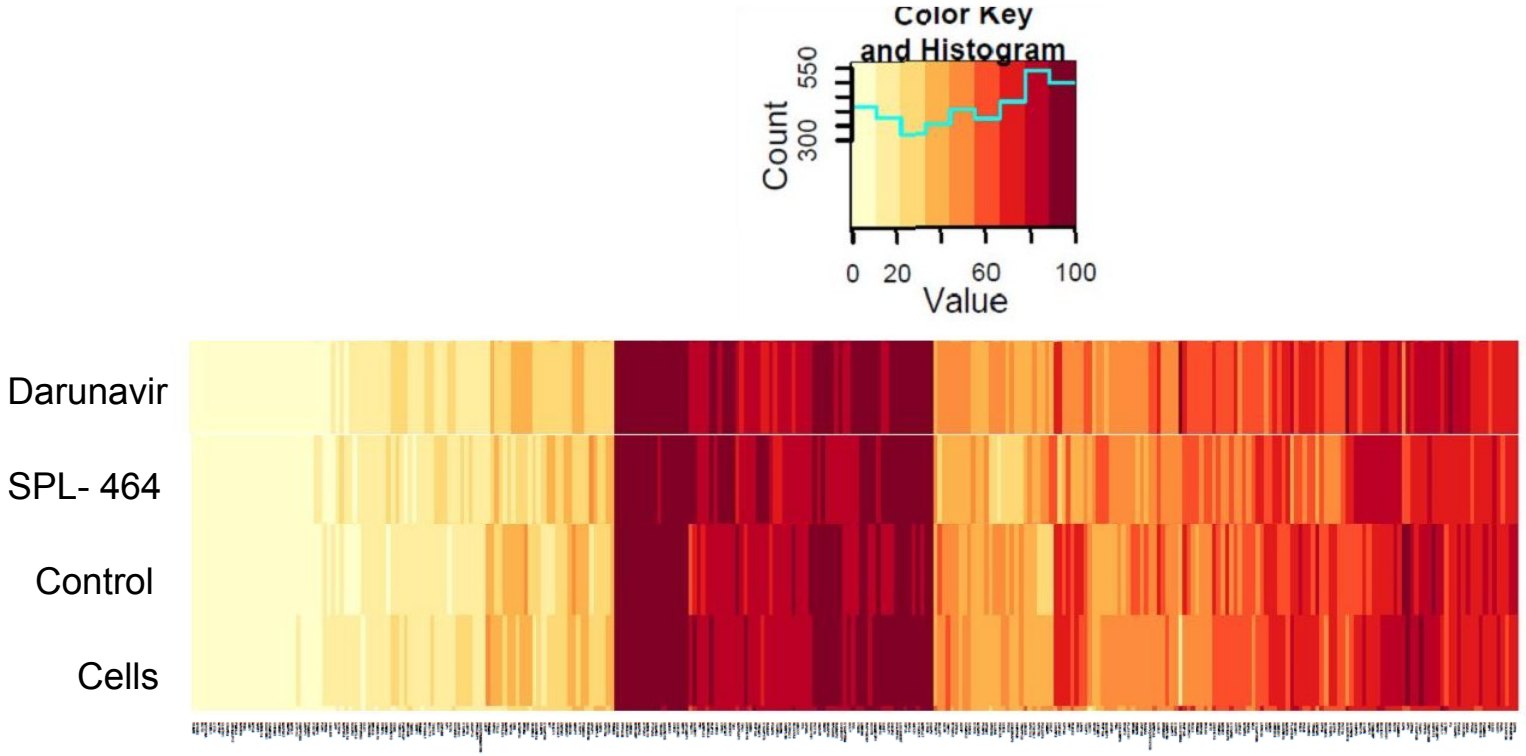


'Percent Spliced In', psi or Ψ

$$\Psi = \frac{[\text{LONG}] \times 100\%}{[\text{LONG} + \text{SHORT}]}$$

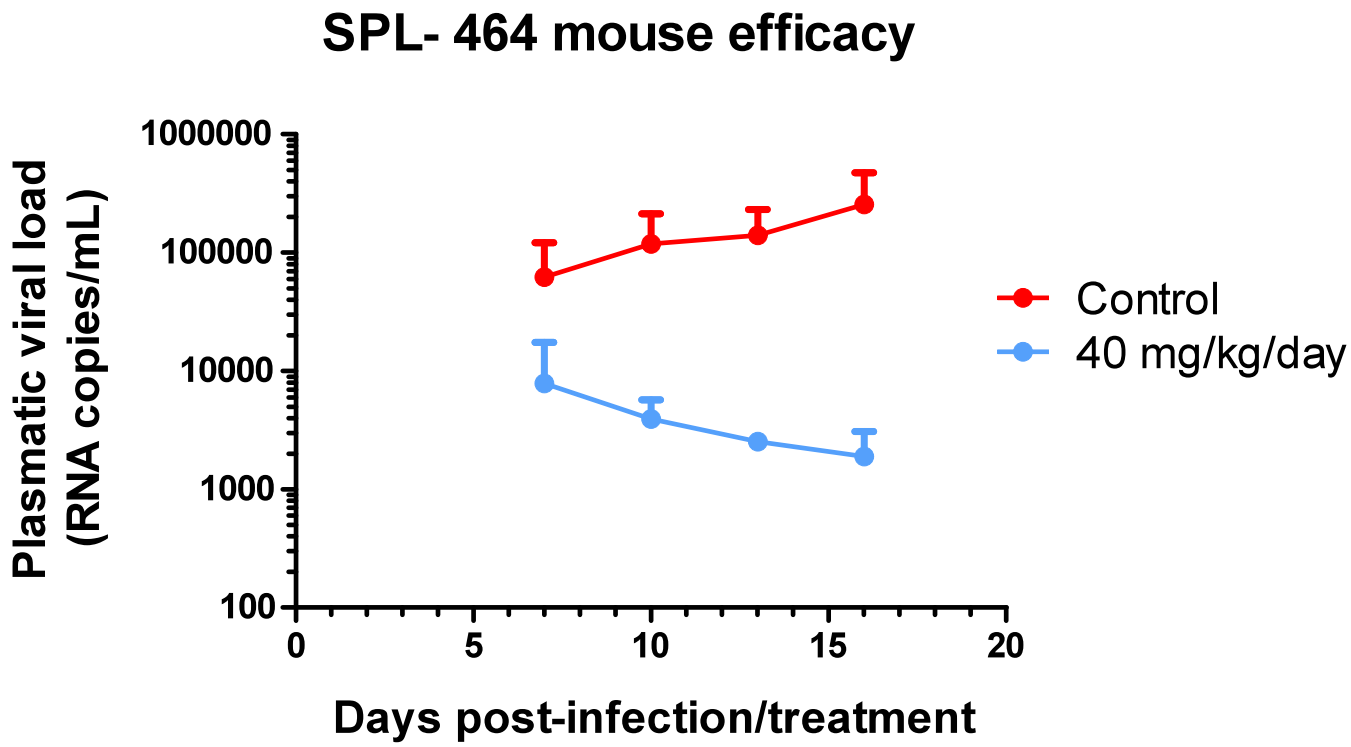
LGFUS
Laboratoire de Genomique Fonctionnelle de L'Universite de Sherbrooke

SPL-464 did not induce global changes of alternative splicing in PBMCs

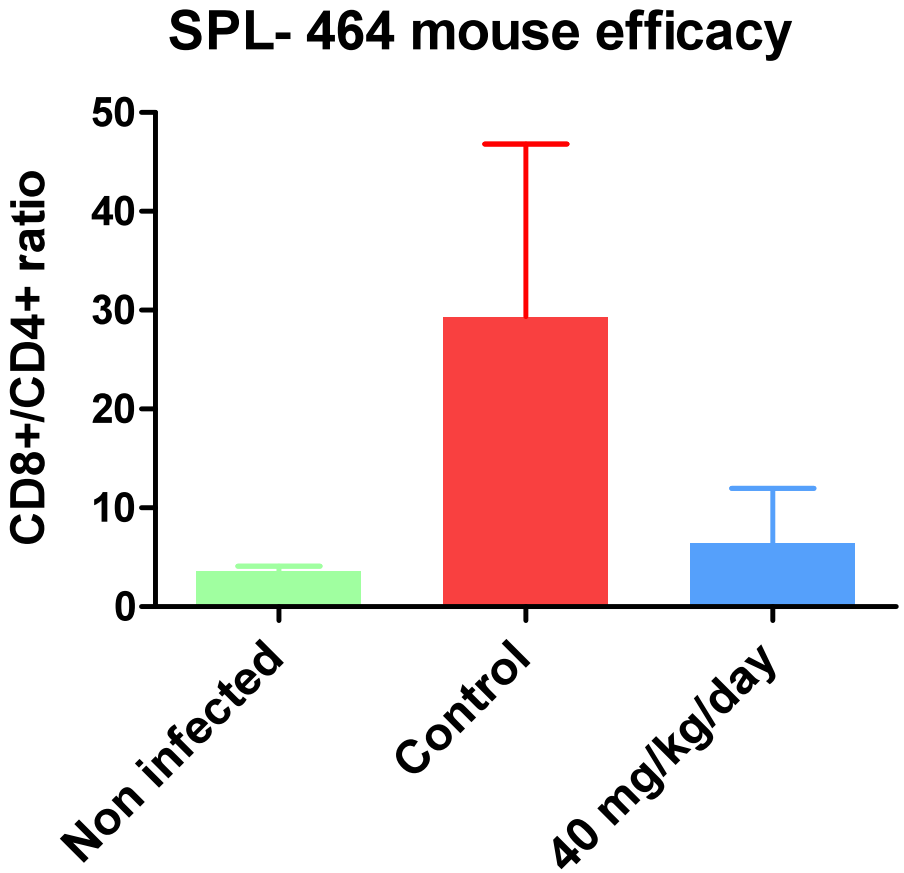


Efficacy of SPL-464 in mouse models

SPL- 464 HIV-1 inhibition on hu-PBL-SCID mouse after 40 mg/kg/day treatment by twice-daily per os administration started simultaneously to HIV-1 infection.

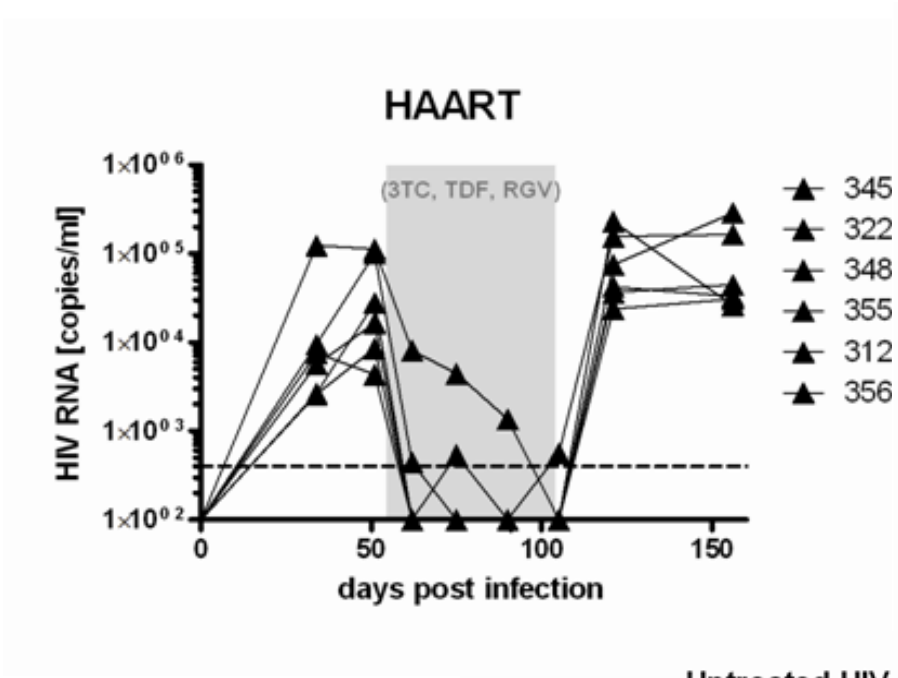


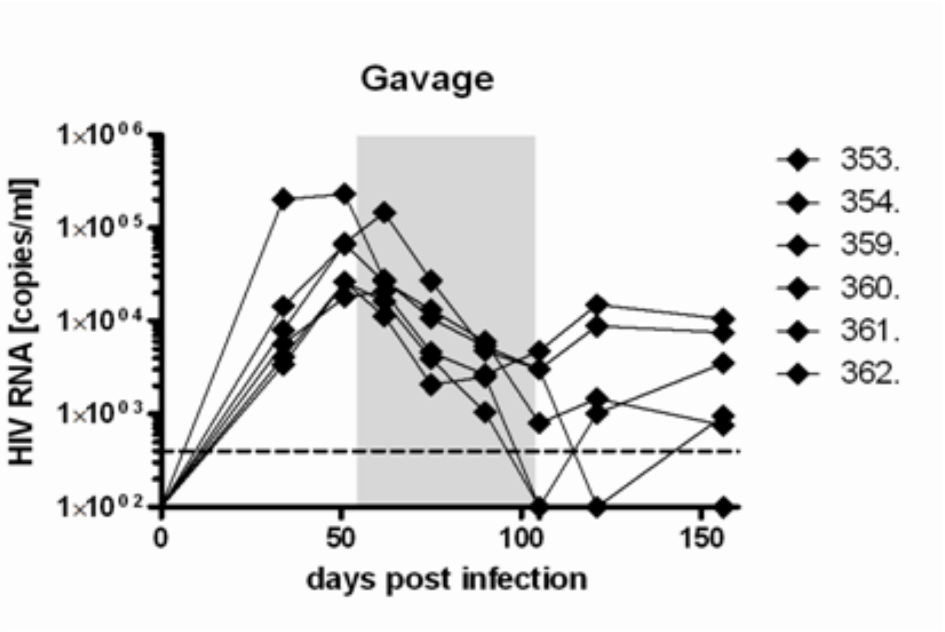
Treatment with SPL- 464 rescues CD8/CD4 ratio in infected mice

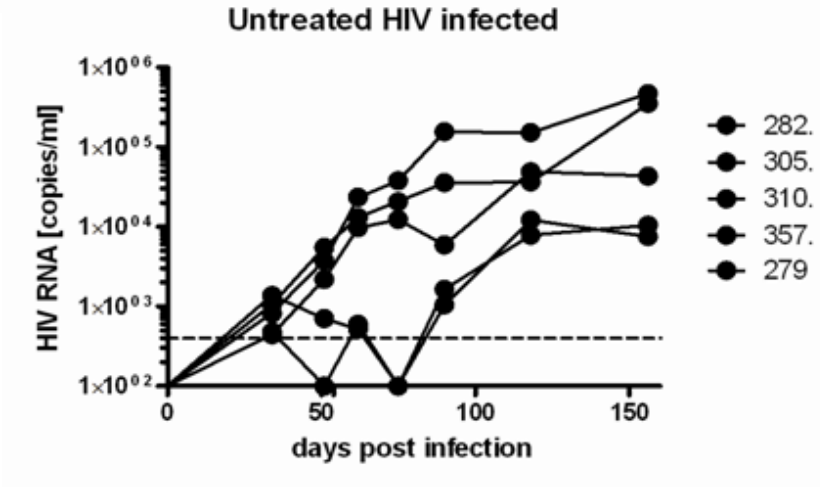


SPL-464 (40mg/kg daily by gavage) reduces viral loads in engrafted humanized NSG mice infected by YU2 HIV-1 strain. Comparison with ART

Long lasting HIV-1 inhibitory effect of SPL-464 in infected humanized mice.







Summary

- SPL-464 is a novel anti-HIV agent active against different HIV-1 clades and mutants as well as HIV-2

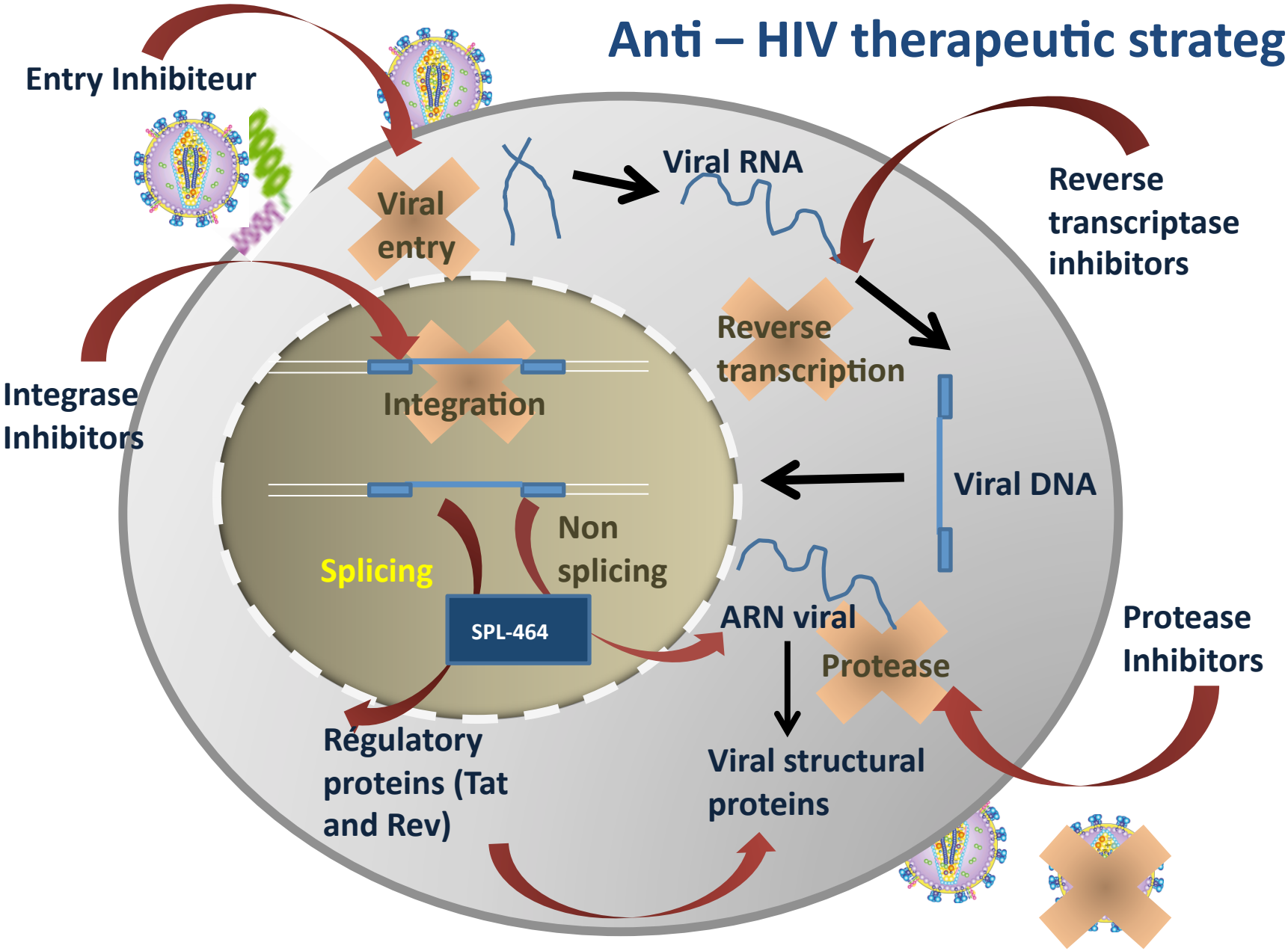
- SPL-464 did not induce emergence of HIV-1 mutants

- SPL-464 has a new mode of action inhibiting HIV-1 splicing but not splicing of cellular genes

- SPL-464 induces a long lasting effect in humanized mice

- SPL-464 rescues CD8/CD4 ratio in infected humanized mice

Anti – HIV therapeutic strategies



Acknowledgements

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