Viral Reservoirs and anti-latency interventions in nonhuman primate models of SIV/SHIV infection

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Outline

- Introduction to animal model
- Functional Cure: immune control
- Induction-eradication treatment

SIV/SHIV infection of macaques as animal model of HIV infection

Monkeys ≠ humans

Value of the model:

- **Most similar animal model:**
  - Host: similar physiology (immunology, drug metabolism, PK/PD)
  - Virus: SIV/ SHIV's similar to HIV
  - Pathogenesis

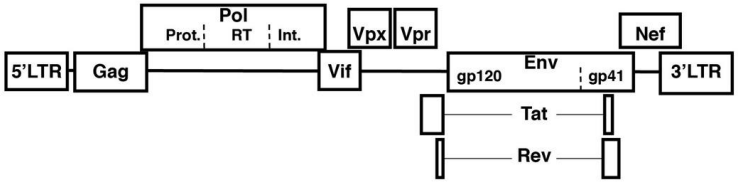
- **Control of variables:**
  - Host
  - Virus
  - Treatments

- **Unique opportunities for tissue analysis**

- **Proof of concept**

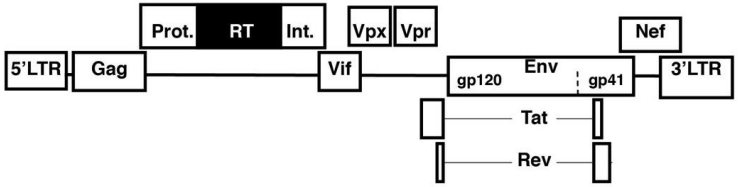
The use of recombinants: “SHIV”

SIV



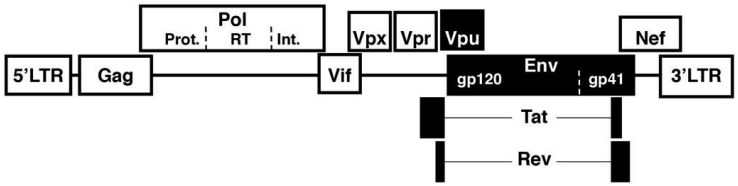
ART
w/o NNRTI

RT-SHIV

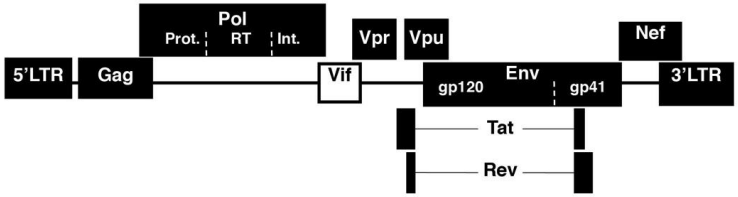


with NNRTI

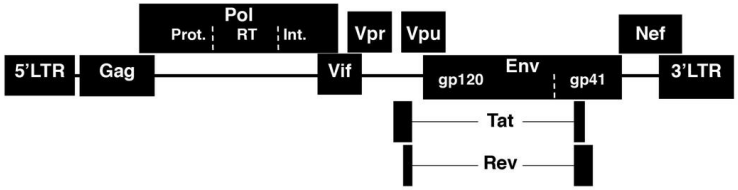
env-SHIV



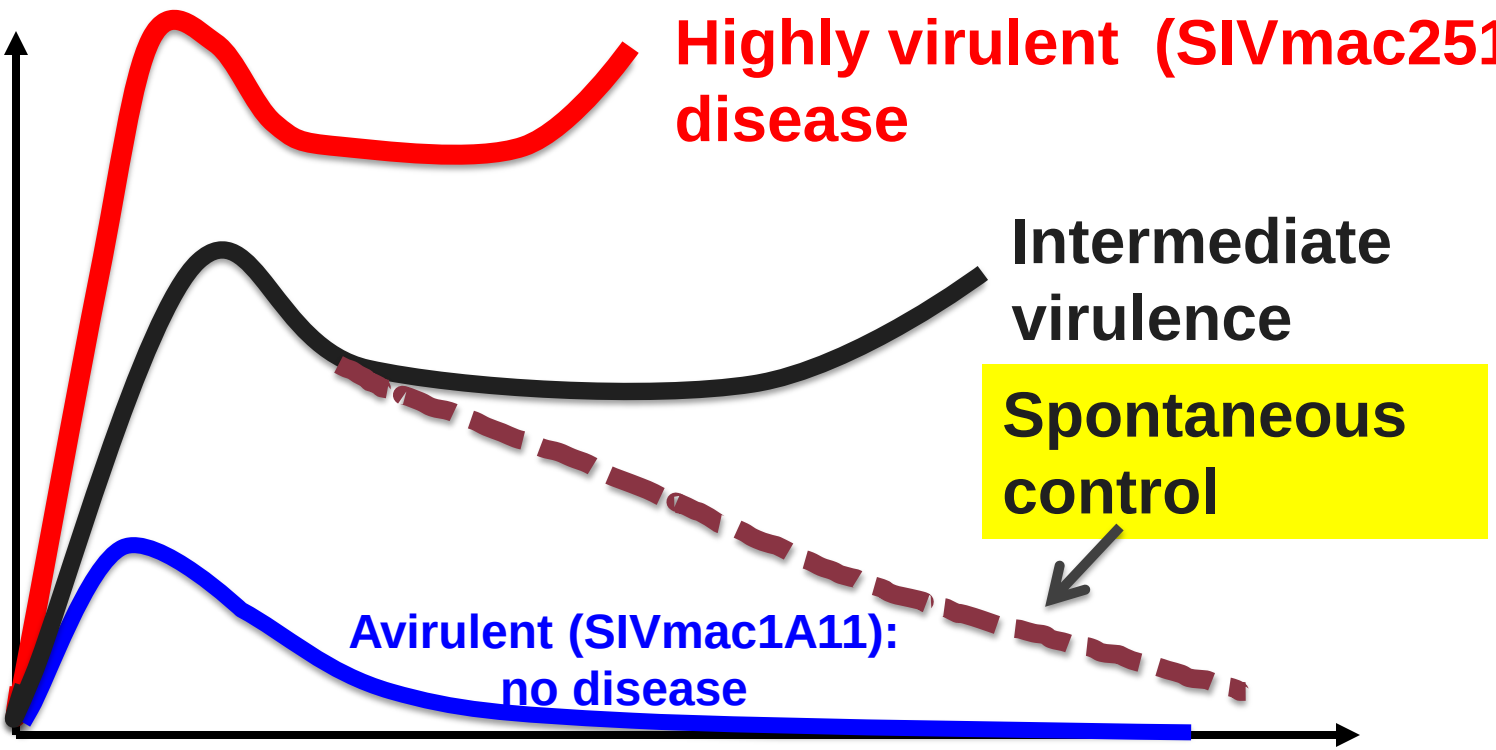
stHIV-1$_{SV}$



HIV-1



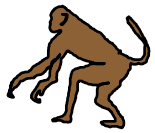
VIRULENCE OF VIRUS



Studies in nonhuman primates

- Functional Cure:
  - Tenofovir monotherapy:
    - Van Rompay et al, Retrovirology, 2012

- Induction-eradication treatment:
  - Ongoing studies with “anti-latency” inducer SAHA



Functional Cure

- **Prolonged** tenofovir (TFV) monotherapy: 8 to 14 years
- All animals: emergence of **K65R mutants**
  - Virulence of K65R mutants ~ wild-type
- Five animals achieved strong **suppression of virus replication**

Early therapy:

- SIVmac251
- TFV ≤ 3 weeks
- Success rate: 4/6

~ 60%

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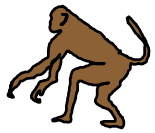
Late therapy:

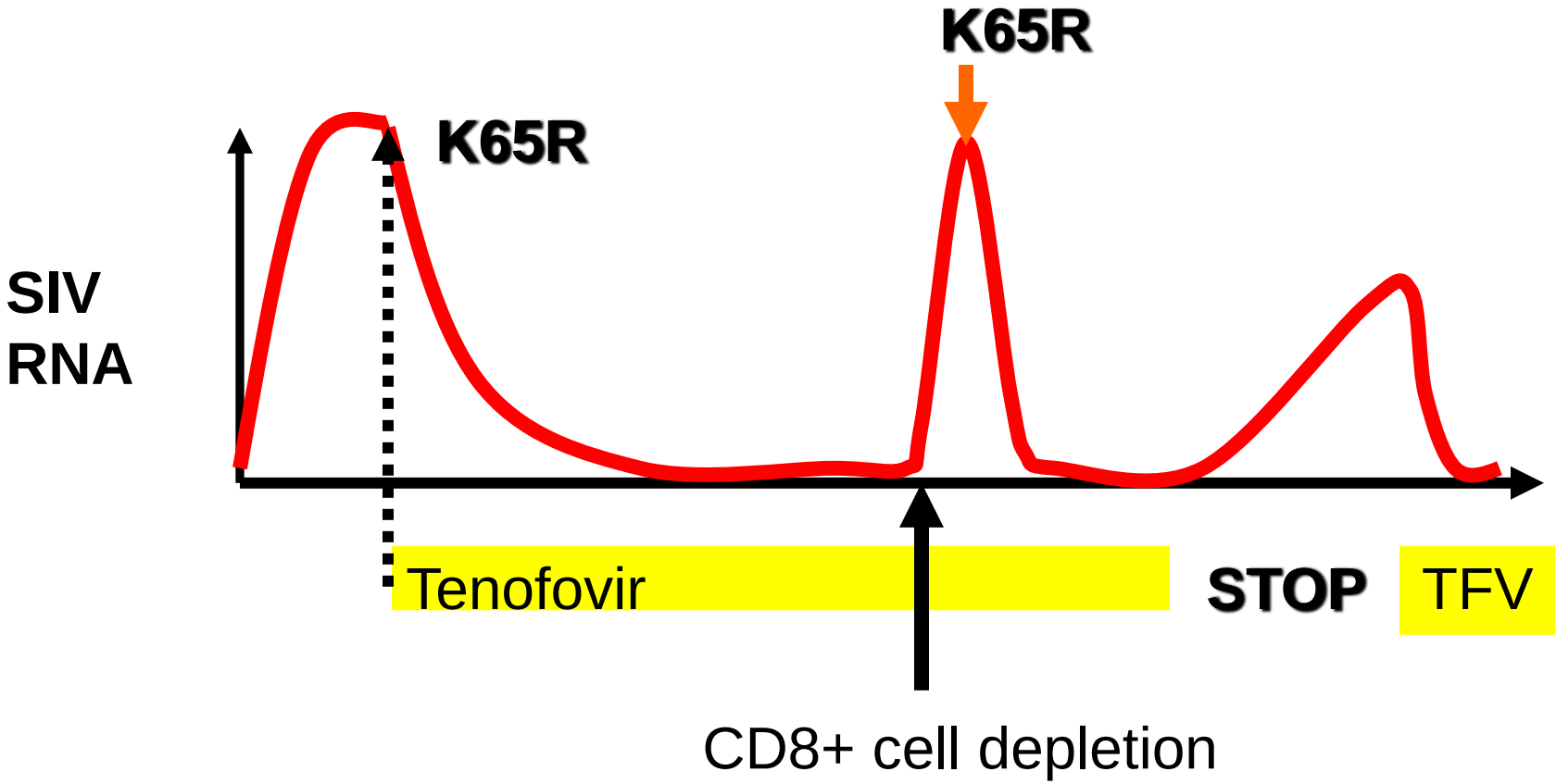
- RT-SHIV
- TFV at 20 weeks
- Success rate: 1/12

~ 10%



Observations on 5 animals
3-6 years of treatment

BOTH antiviral immune responses and continued TFV are necessary to obtain maximum suppression



Van Rompay et al (AAC, 1996, 1998; JVI 2004, Retrovirology 2007)

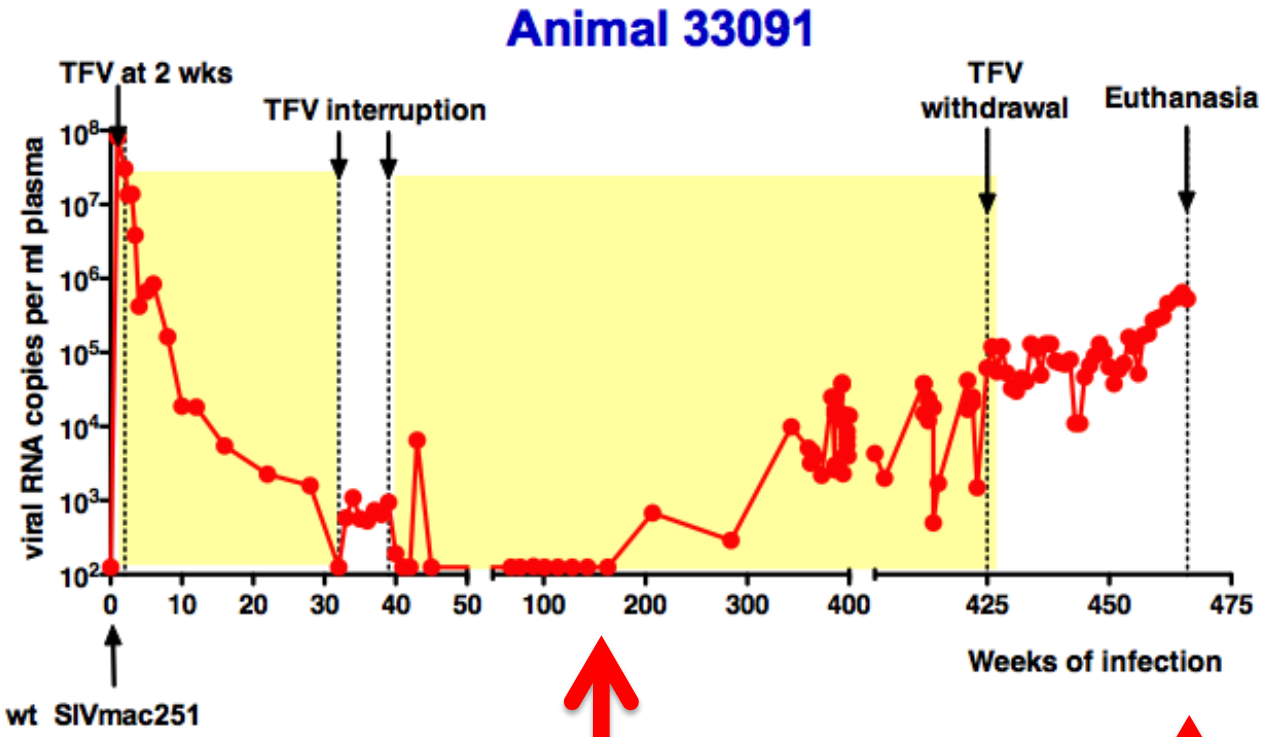
Observations < 6 years of treatment:

BOTH antiviral immune responses
continued TFV are necessa
maximum suppressi

What happens if we continue therapy for longer and then withdraw TFV?

Van Rompay et al (AAC, 1996, 1998; JVI 2004, Retrovirology 2007)

ONE OF FIVE ANIMALS LOST CONTROL: 33091



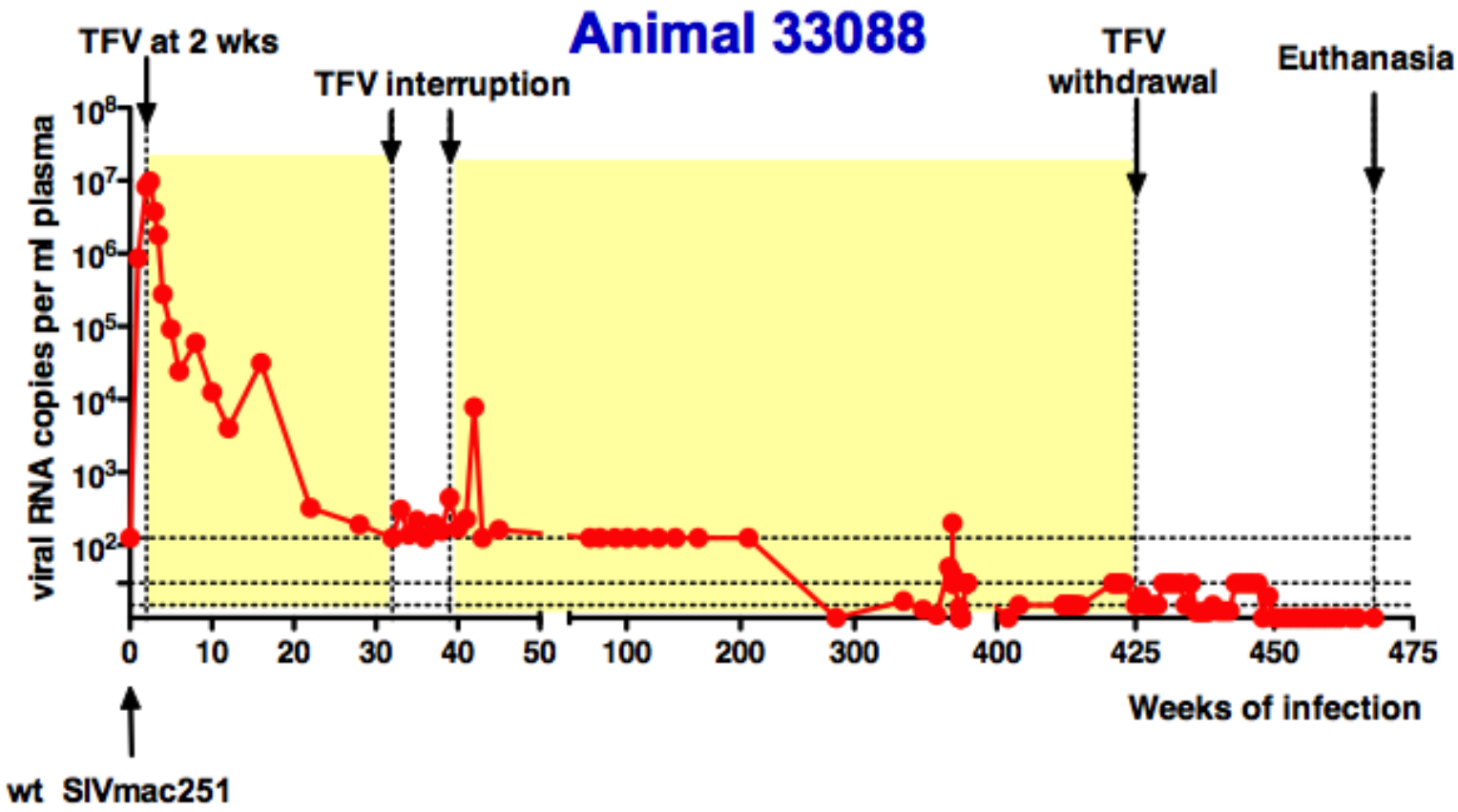
~ 3 years of SIV infection

Euthanasia at ~ 9 years of SIV infection

4 animals: Tenofovir withdrawal

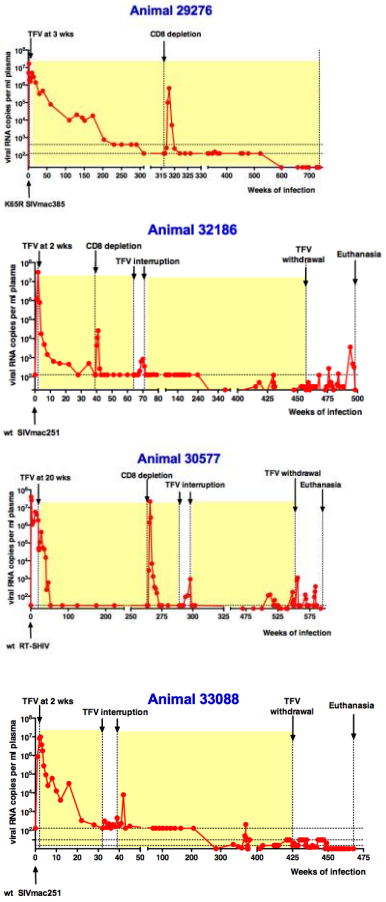
No viral rebound during 1 year

(intermittent viral blips at frequency ~ during treatment)

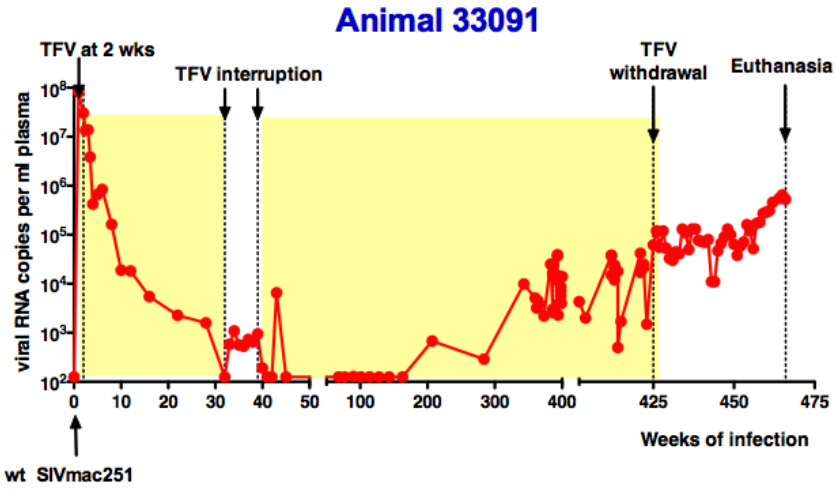


Comparison

Control:
4 animals



Loss of control
Animal 33091



Van Rompay et al, Retrovirology 2012

Virus levels in blood are reflected in tissues

(~ 1 year after TFV withdrawal)

Animal ID	Plasma RNA	PBMC DNA	PBMC RNA	Spleen DNA	Spleen RNA	Ax LN DNA	Ax LN RNA	Jejunum DNA	Jejunum RNA
29276	< 15	4	< 2	2	1	1	2	NA	NA
30577	< 10	7	< 2	< 3	< 3	4	<2	< 2	< 2
32186	20	15	< 1	5	< 1	40	9	60	7
33088	< 10	6	< 1	2	< 1	8	10	< 2	< 2
33091	530,000	280	470	370	5,700	320	13,000	60	640

Cellular vRNA & DNA per 100,000 cells

Sequence analysis revealed K65R only

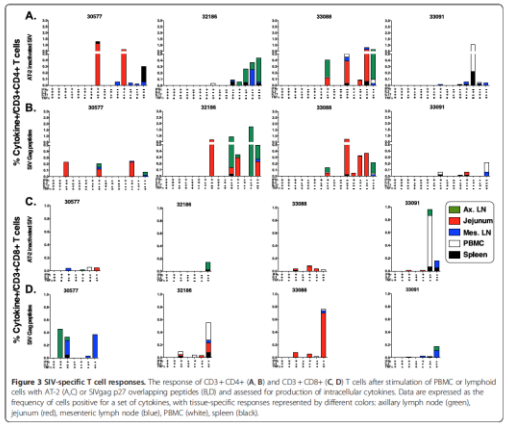
(no evidence of reversion to wild-type after drug withdrawal).

Broad humoral and cellular SIV-specific immunity:

- **Antibody titers**
  - High binding (ELISA)
  - Low neutralization against primary isolates
  - High ADCVI activity
- **Cell-mediated immunity (CMI)**:
  - All animals had CD4+ and CD8+ SIV specific responses (SIVmac239 peptides; AT2-SIVmac239).

Controllers:
- Many cells: multifunctional
- Wide organ distribution incl. gut

Non-controller: 33091:
- Most responding cells: one cytokine
- Mostly blood, none in gut



Van Rompay et al, Retrovirology 2012

Conclusion

Proof of concept: hope for a functional cure

- **Prolonged TFV therapy (> 8 years):**
  - **Controllers resemble long-term nonprogressors (LTNP's)**
  - Gradual ***strengthening*** of immune responses and reduced dependency on tenofovir.

- **Key factors in development of strong immune responses**
  - **Very prolonged treatment**
  - **Low-level replication of K65R** during tenofovir promotes immunity.

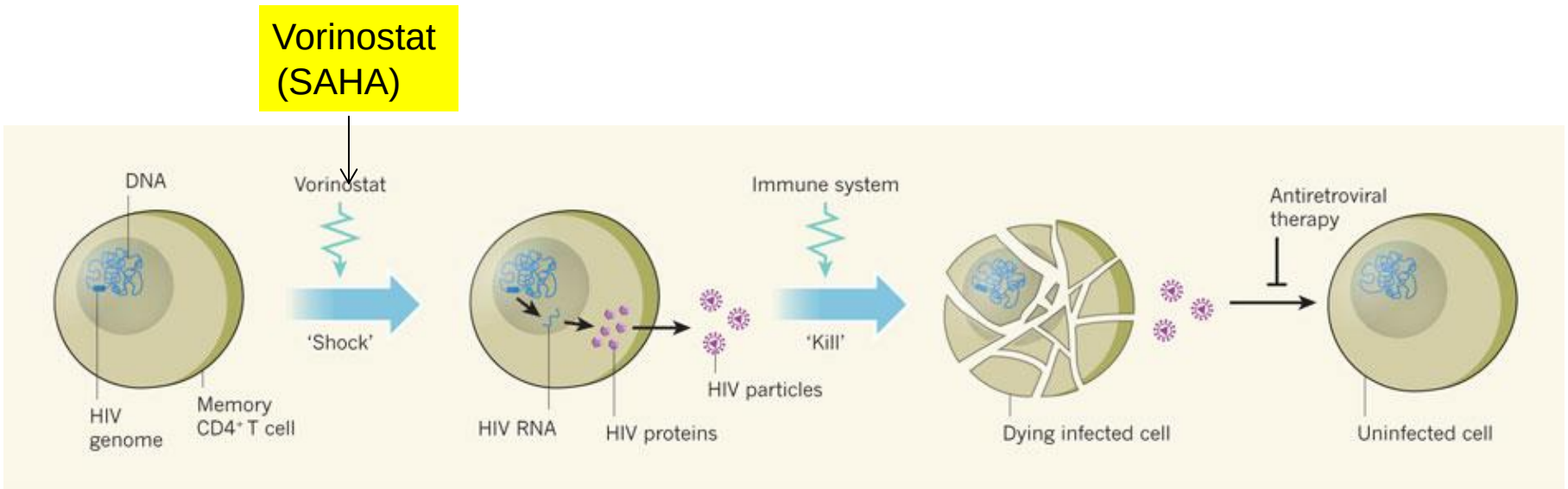
Functional cure:

Challenges/ future studies

- **How do we induce such immune responses:**
  - *faster*
  - *during late-stage infection*
  - *during fully suppressive therapy (wt virus) ~ humans*
- **Durability ?**

Induction & Eradication Treatment
(Shock and kill)

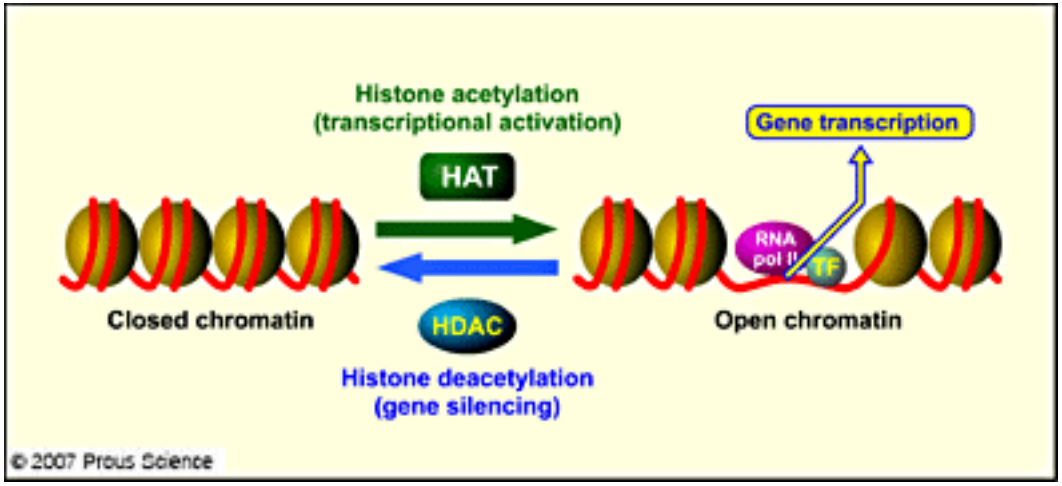
- ART to suppress all virus replication incl. new infections
- Reactivate latent virus
- Kill infected cell (viral cytopathogenicity or immune-mediated)



Deeks, Nature 487, 439-440, 2012

VORINOSTAT (SAHA)

- Histone Deacytelase Inhibitor (HDACi)



(Graul et al., Drug News Perspect. 2007)

- In **HUMANS**: SAHA disrupts HIV latency.
  - *In vitro & ex vivo*
  - *In vivo* single-dose treatment with isolation of resting CD4+ T cells:
    - **Increased histone acetylation**
    - **Increase of HIV RNA in resting CD4+ T cells**

Archin et al, Nature, v.487, 482.

VORINOSTAT (SAHA) in SIV/SHIV macaque model

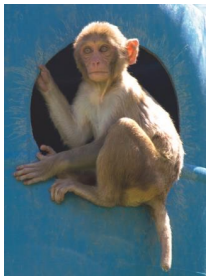
In vitro studies:

- Objective : to identify "surrogate biomarkers" in treated macaque PBMC & lymph node cell cultures that relate directly to pharmacologic properties and mechanism of inducer action.

- Pulse-treatment experiments designed to mimic PK properties of inducers:
  - **SAHA effect on H4 acetylation of CD4+ T cells is significant but short (6 - 10 hr)**

In vivo studies:

- **Pharmacokinetics:** uninfected macaques
- **Pharmacodynamics:** RT-SHIV model

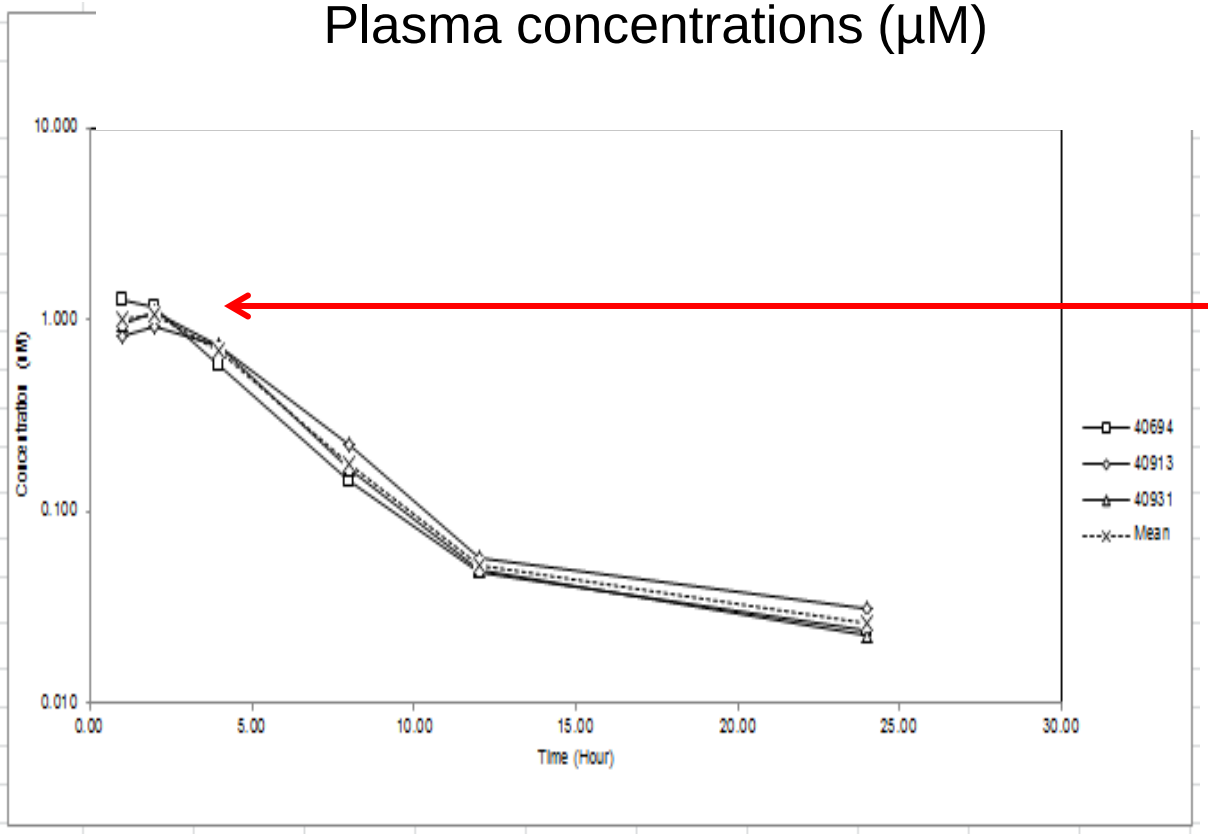


Pharmacokinetics of SAHA

- Oral administration: variable plasma levels
- **Subcutaneous** dosing:

6 mg/kg to 3 juvenile macaques

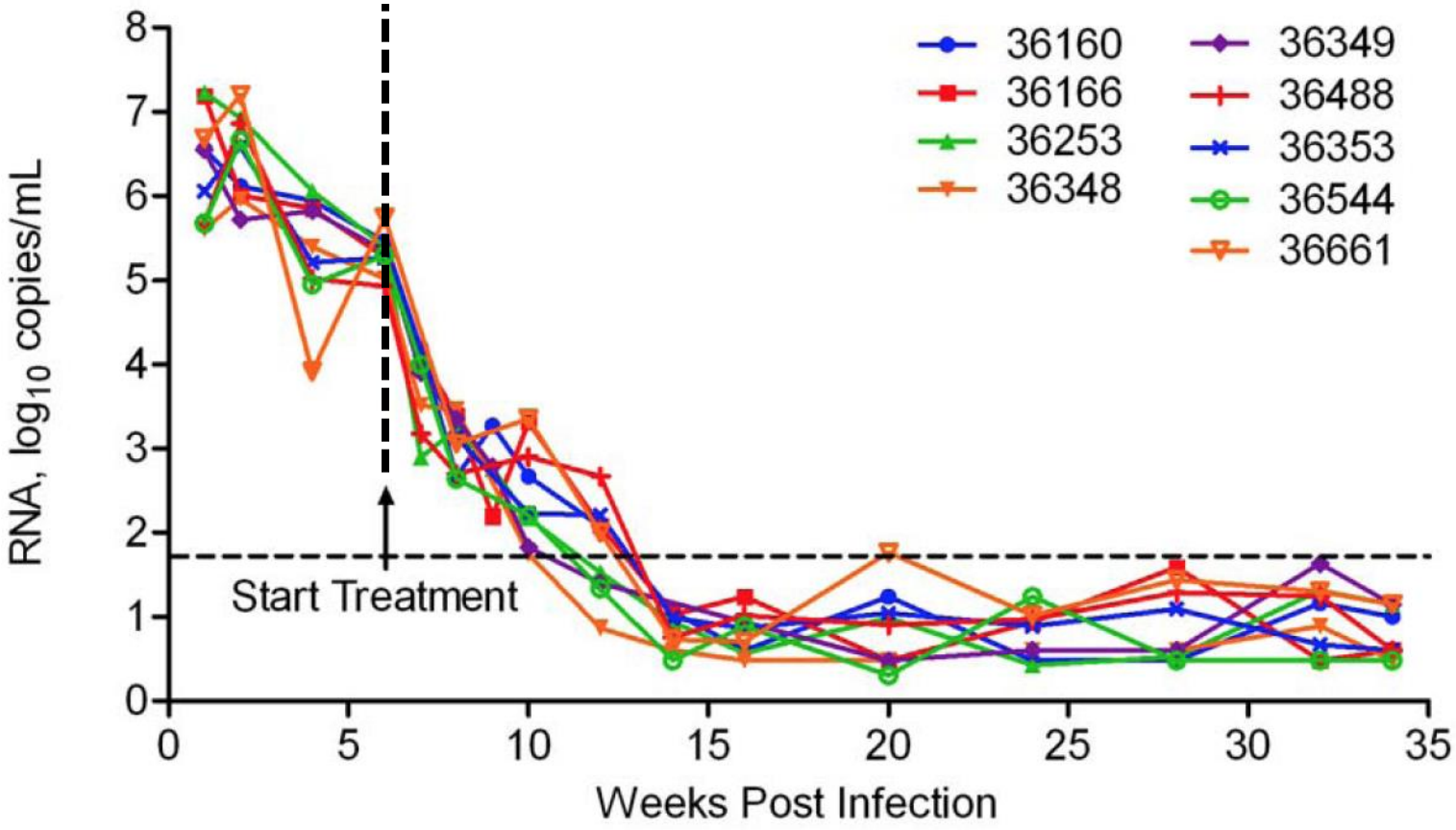
Plasma concentrations (µM)



Cmax ~ 1 µM
similar to human studies

Chris Tan (Merck)

RT-SHIV model: HAART at 6 weeks



Effective HAART regimens (3 or more drugs) can suppress RT-SHIV: TFV, FTC, EFV, ± RAL

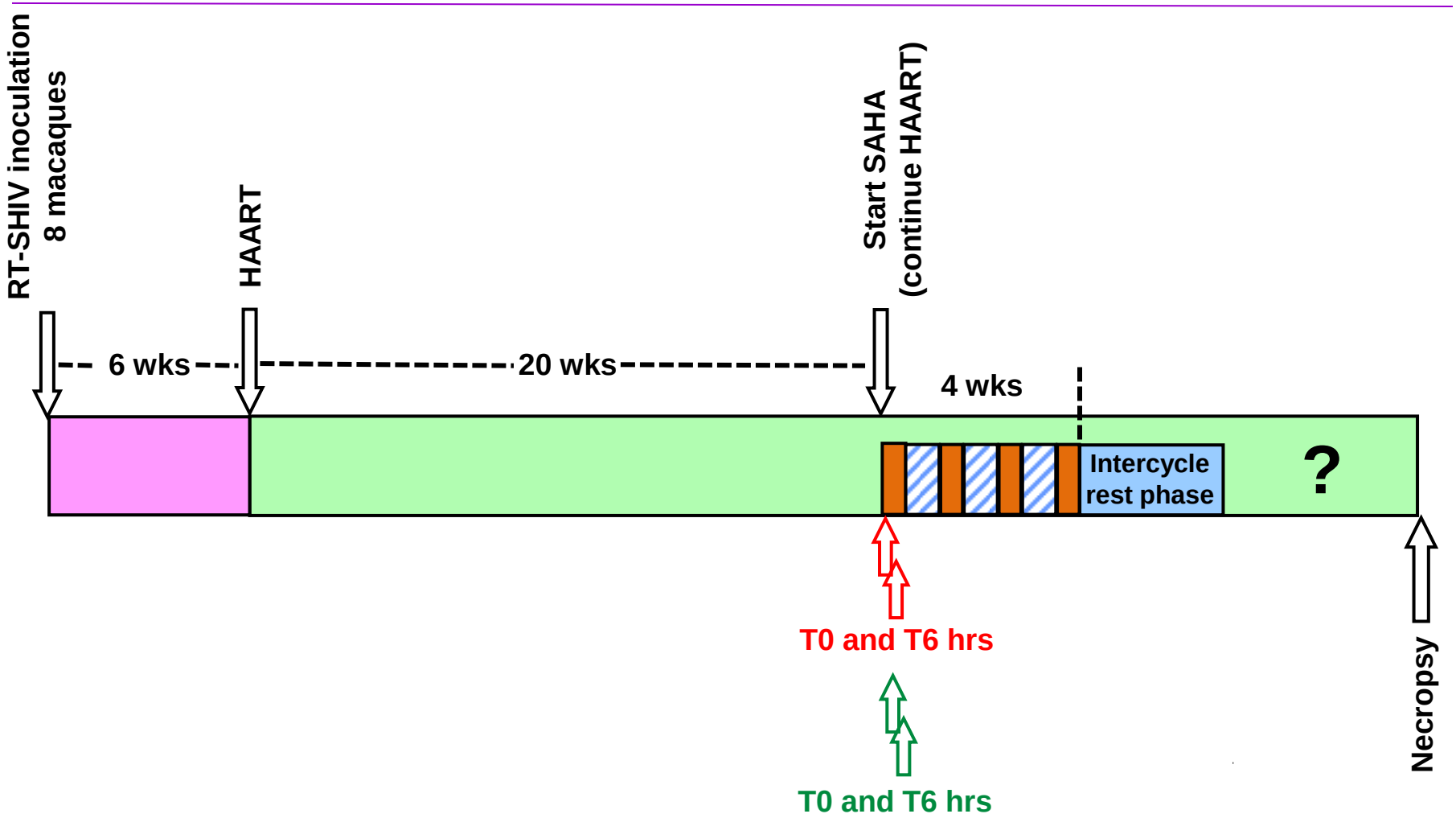
(TW North et al, JVI 2010; J. Deere et al, PLoS One 2010)

Viral load in tissues (per 10^6 copies of cellular DNA)

		HAART										Untreated	
	Monkey	A		B		C		D		E		F	
	Plasma Load	13		25		44		14		29		128,084	
	(copies/ml)	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA
Lymphoid	spleen	414	16	134	53	376	254	1,383	720	3,565	164	7,912	86,239
	mes ln	543	49	1,430	262	3,653	138	1,454	155	2,126	211	77,377	19,553
	ax ln	657	83	8,002	114	185	575	1,971	732	2,680	565	9,298	3,481,052
	ing ln	503	88	236	13	8,319	612	202	5	412	4,452	13,759	443,531
	iliac ln	760	49	136	409	1,061	636	263	nd	975	123	32,108	251,460
	cerv ln	4,208	263	716	321	4	369	617	nd	134	48	4,694	13,898
	thymus	64	28	160	nd	3,610	85	125	10	355	nd	307	952
	bone marrow	18	nd	nd	nd	43,309	nd	129	14	nd	nd	26	249
	tonsil	66	nd	715	116	1,604	2,514	126		669	34	11,206	75,461
GI Tract	duodenum	659	10	203	134	11	17	3	20	230	68	17,914	1,822,459
	jejunum	163	25	112	nd	165	6	12	122	70	31	523	1,126
	ileum	197	16	142	nd	nd	19	762	nd	5,280	64	81	140
	cecum	11	nd	544	63	nd	99	315	10	31	135	2,204	406
	colon	56	nd	395	3	nd	nd	nd	nd	nd	177	298	5,450
Neuro	cerebrum	nd	nd	nd	nd	nd	nd	7	nd	nd	nd	7	nd
	cerebellum	nd	nd	nd	nd	nd	nd	2	nd	nd	nd	0	9
	choroid plexus	21	nd	26	nd	nd	nd	0	nd	nd	nd	15	18
	cerv sp crd	11	nd	nd	nd	nd	nd	0	nd	nd	nd	nd	7
	lumbar sp crd	16	nd	87	nd	797	nd	18	10	nd	nd	nd	8
Repro	testes	44	nd	20	nd	5	nd	12	nd	25	nd	297	2,358
	sem vesicles	25	nd	15	nd	15	nd	16	nd	nd	nd	98	155
	prostrate	22	nd	57	nd	119	nd	58	nd	17	24	394	707
	penis	36	nd	nd	nd	nd	nd	5	nd	8	nd	nd	8
Other	kidney	10	nd	14	nd	nd	nd	nd	10	nd	nd	315	500
	liver	2	nd	nd	nd	nd	nd	nd	nd	nd	nd	12	519
	heart	15	nd	16	nd	9	nd	32	nd	6	nd	29	161
	lung	nd	nd	15	nd	nd	nd	35	nd	7	nd	157	1,219
	bladder	8	nd	10	nd	nd	nd	23	nd	2	nd	217	1,818
		nd=not detected											

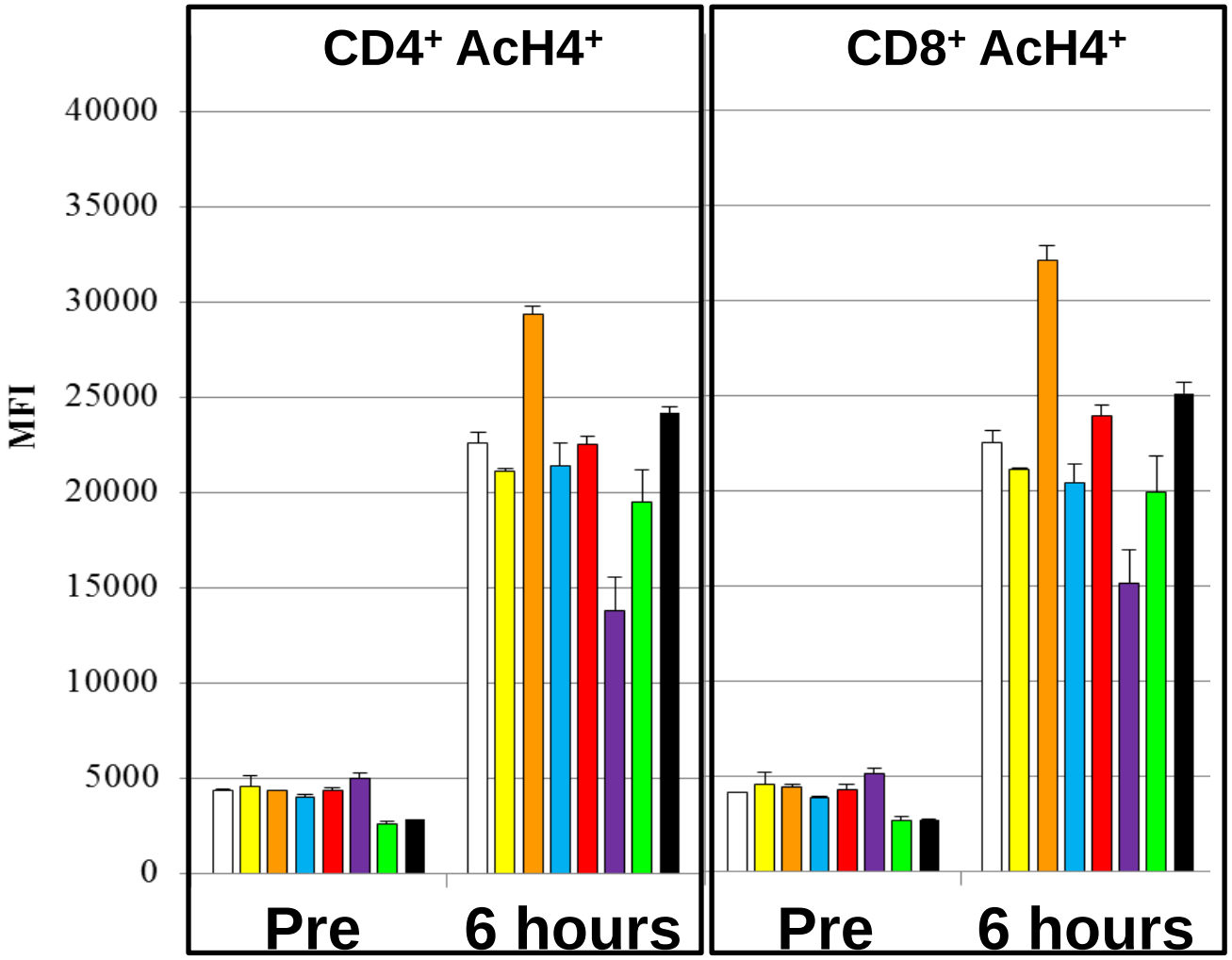
North et al, JVI 2010

Experimental plan (proposed) for anti-latency therapy: SAHA induction of RT-SHIV infected macaques under intensified HAART (TFV, FTC, EFV, RAL)



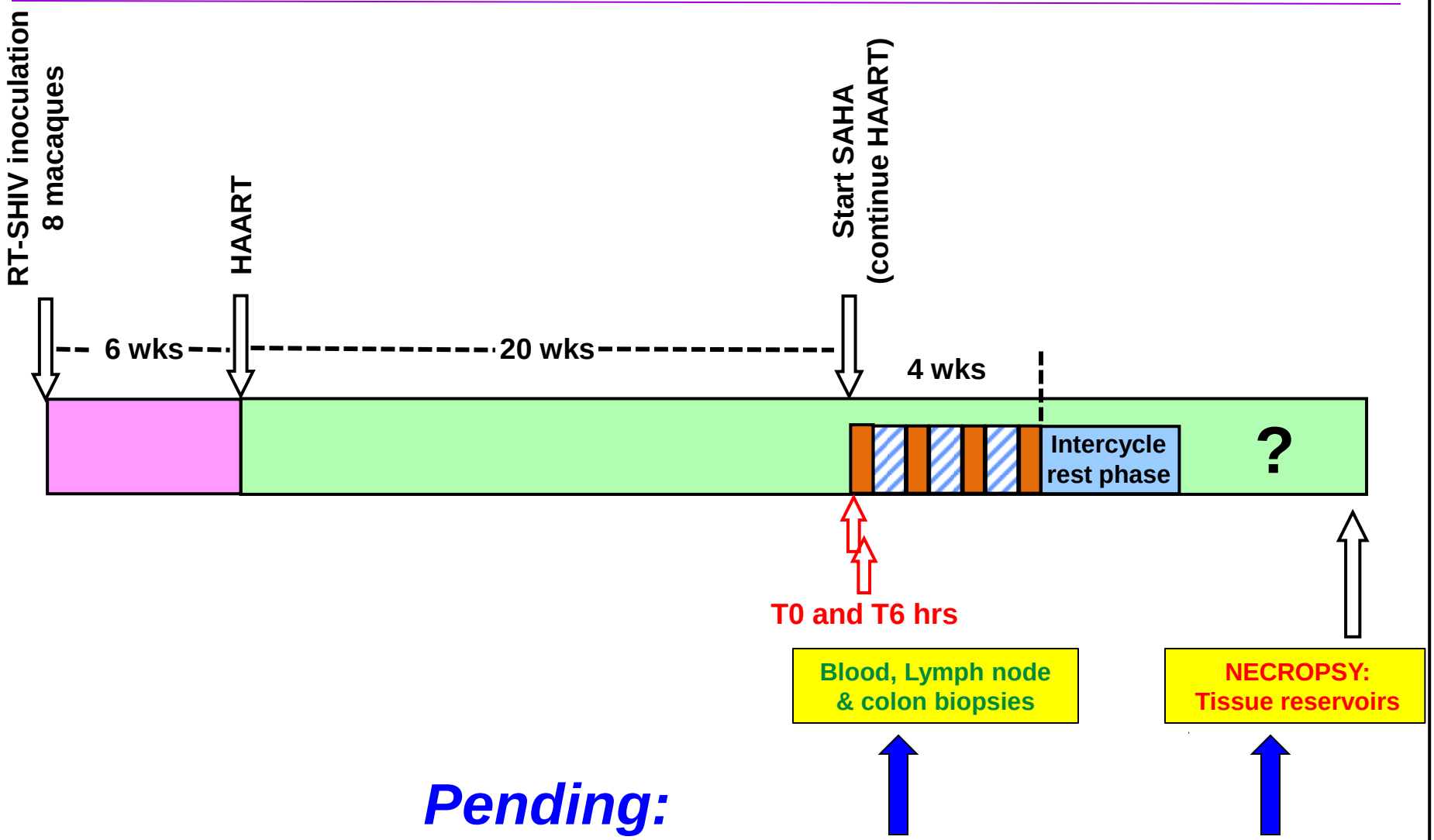
SAHA (Vorinostat) – subcutaneous delivery

H4 histone acetylation of peripheral blood T-cells by flow cytometry



8 RT-SHIV infected rhesus macaques receiving intensified HAART

Experimental plan (proposed) for anti-latency therapy: SAHA induction of RT-SHIV infected macaques under intensified HAART (TFV, FTC, EFV, RAL)



Pending:

vRNA, vDNA during & after induction

Next phase/ future studies:

- Effect on viral reservoirs after treatment
- Effect on viral rebound after removal of all drugs
- Increase efficiency of induction (e.g., combination of inducers).
- Combine with strategies to eliminate infected cells (immunotherapy, immunotoxins, ….)
- Long-term studies

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