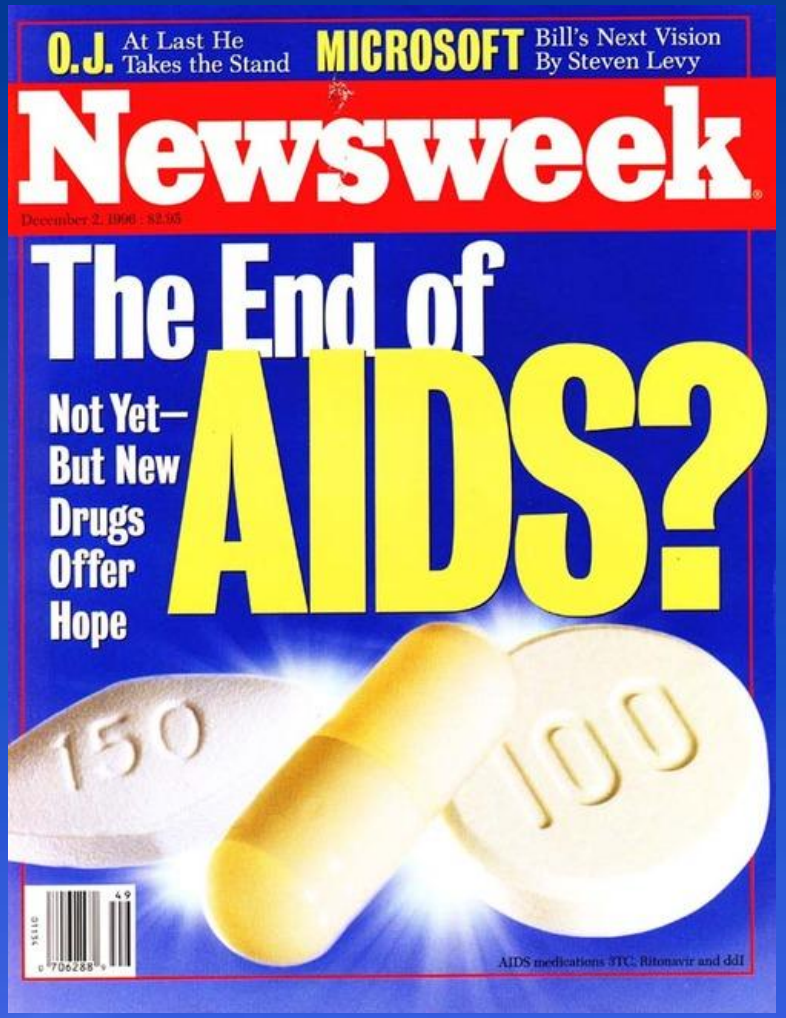
Translational Approaches to Curing HIV infection

Andrew D Badley MD
Director HIV immunology Lab
Chair Research Core Oversight
Professor on Medicine
Mayo Clinic and Foundation

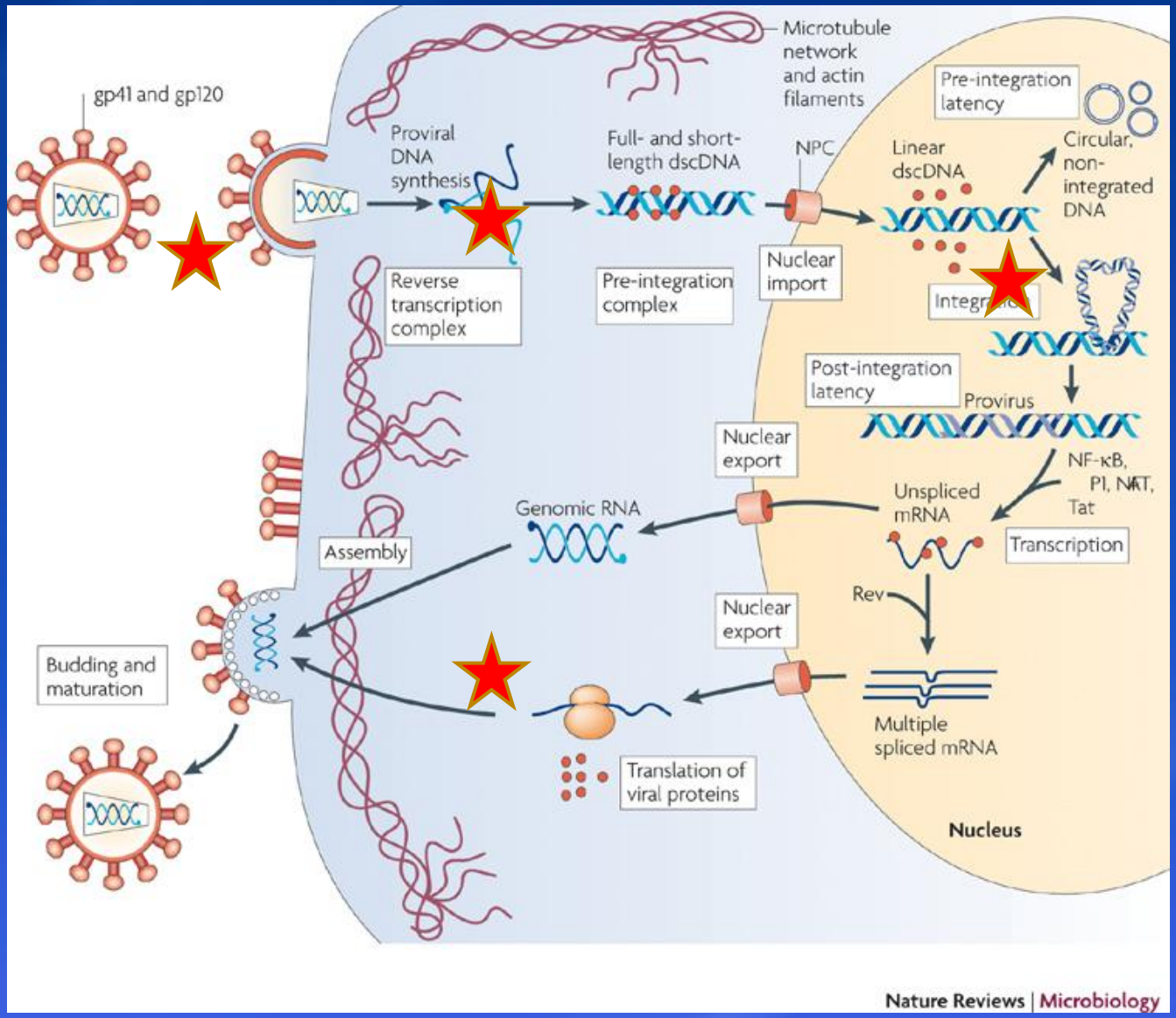
©2013 MFMER

1996


TIME
"CLONE ON THE RANGE" BY DOUGLAS COUPLAND
FICTION BONUS:
Will There Ever Be Another You?
A SPECIAL REPORT ON CLONING

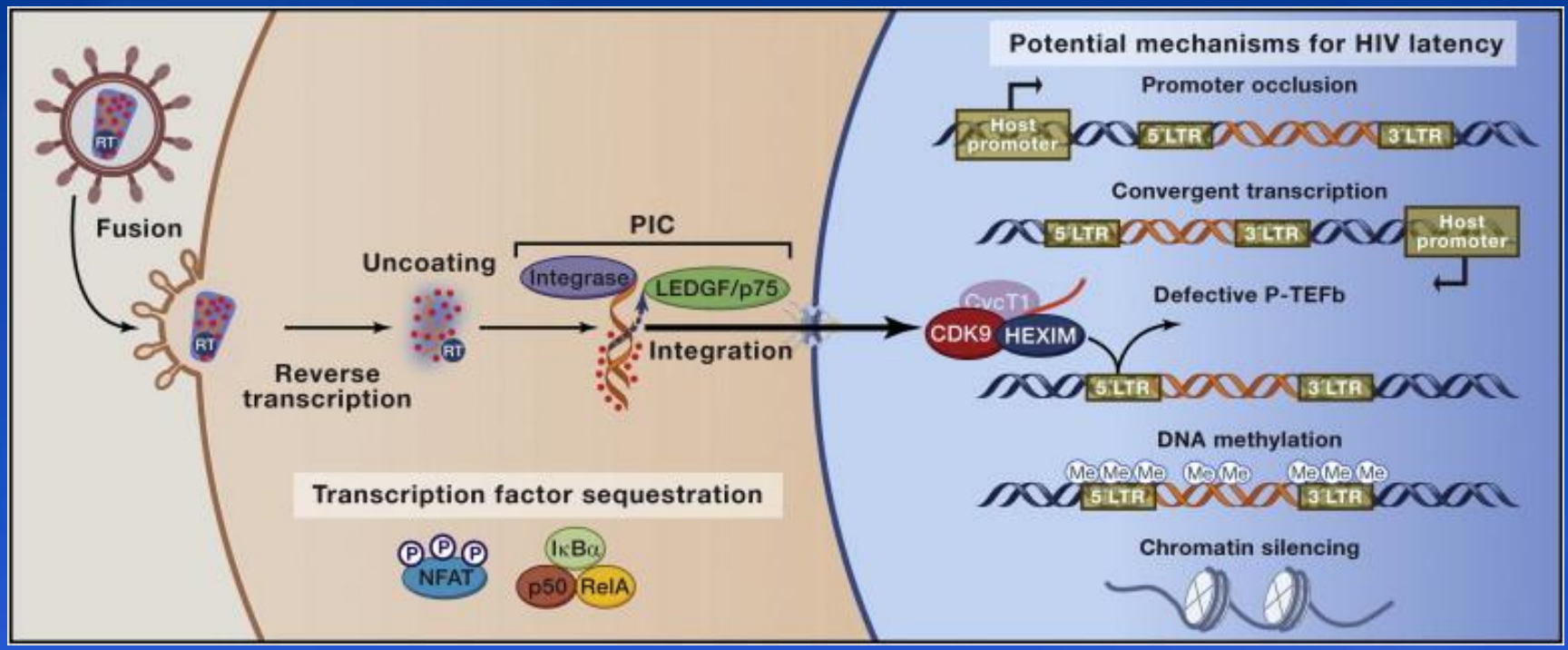

O.J. At Last He Takes the Stand
MICROSOFT Bill's Next Vision By Steven Levy
Newsweek
December 2, 1996 : $2.95
The End of AIDS?
Not Yet— But New Drugs Offer Hope
150
100
AIDS medications 3TC, Ritonavir and ddI

Why don't drugs cure HIV : The HIV Life cycle



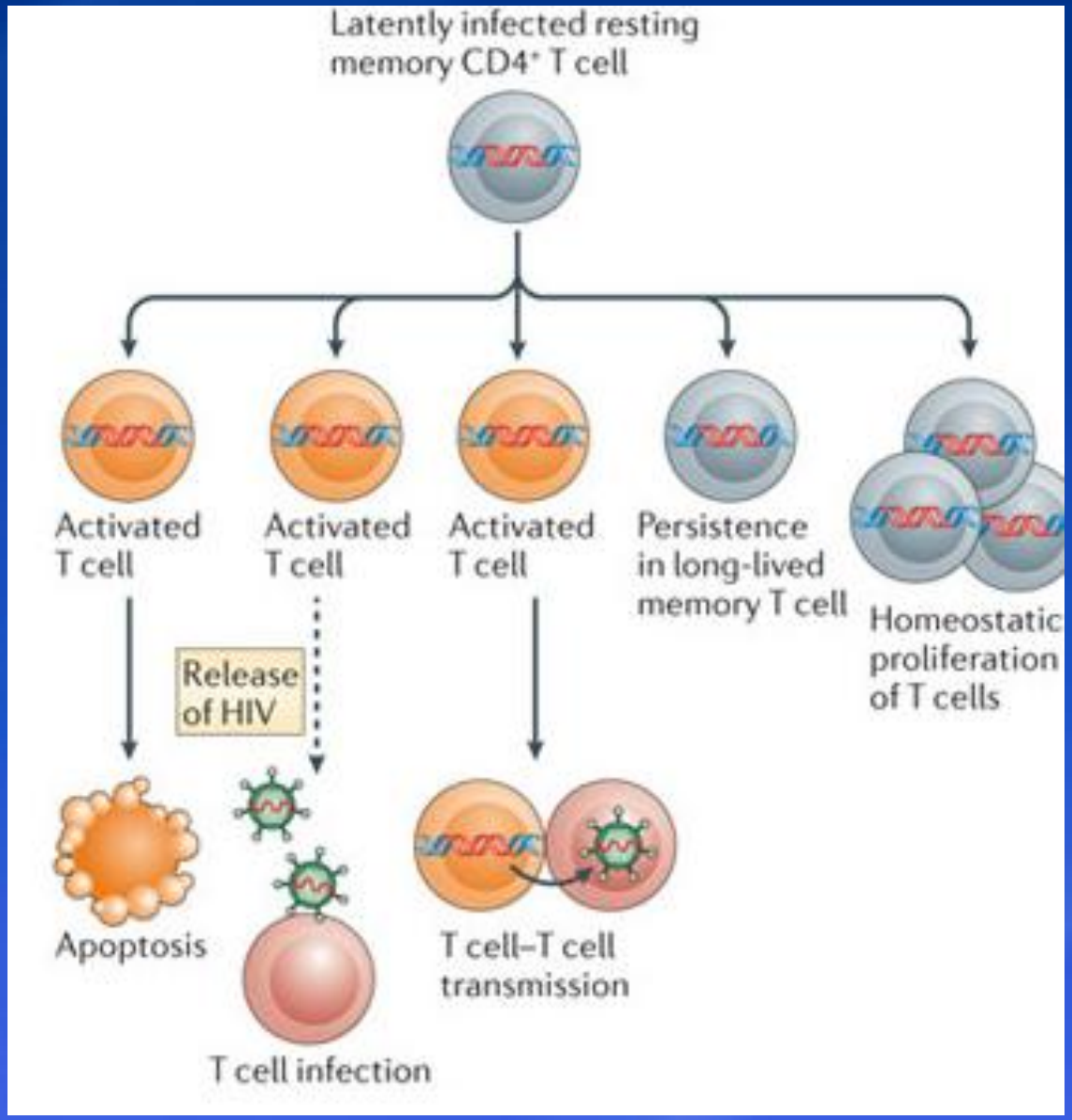
©2013 MFMER

Mechanisms of Latency



©2013 MFMER

Sequelae of latency



Cure IS possible

- Timothy Ray Brown, the Berlin patient
  - Allo Tx times 2, ART through chemo (3 years).
  - Now off ART, >6 years post Tx, no intact HIV sequences

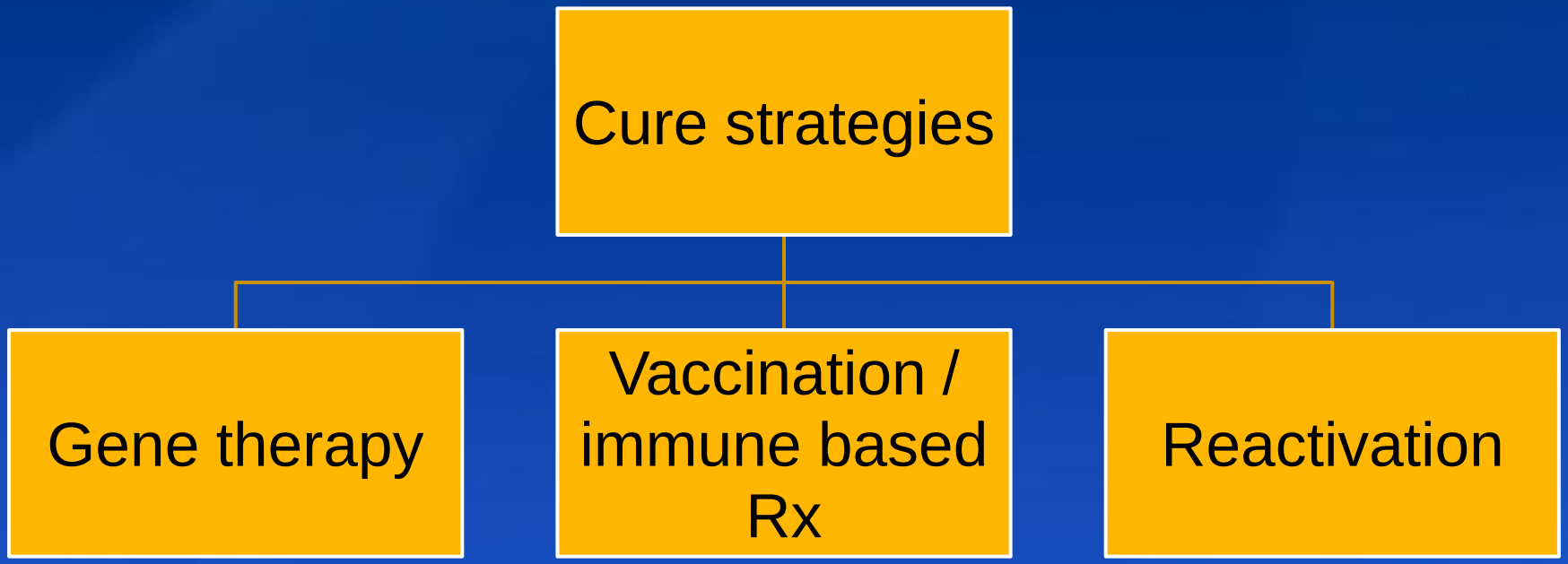


How does BMT cure HIV infection??

- Cytotoxic chemo Rx – kills lymphoid and myeloid cells containing HIV; ART blocks new infection
- Anti HIV effect of ChemoRx
- GVHD = Graft VS HIV Effect ?
- CCR5 delta 32

- What is minimum??

MAYO CLINIC



- Low accessibility
- Oncogenic ?

- Toxicity concerns
- Curative vs preventative

- Low cost, high accessibility

MAYO CLINIC

Gene therapy Approaches

- Knockdown
  - Eg CXCR4 / CCR5
- Overexpression
  - chimeric TRIM5α
- Engineered receptors
  - Eg CAR



Medscape Medical News from the:
7th International AIDS Society (IAS) Conference on HIV Pathogenesis, Treatment and Prevention

This coverage is not sanctioned by, nor a part of, the International AIDS Society.

Medscape Medical News > Conference News

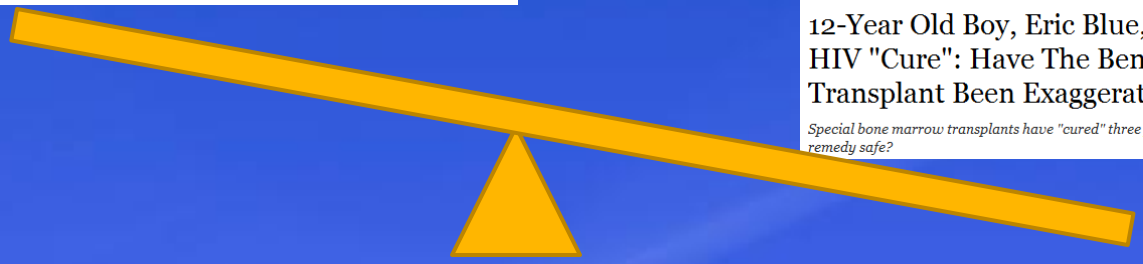
Stem Cell Transplant Patients Renew Excitement for HIV Cure

Neil Canavan
Jul 09, 2013

HOME > CONDITIONS

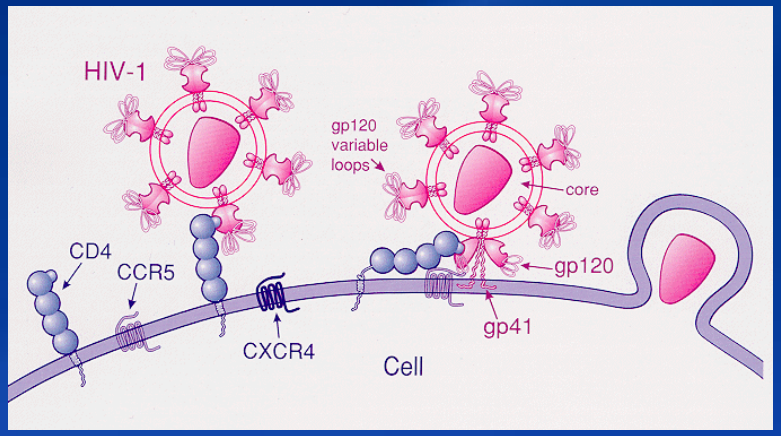
12-Year Old Boy, Eric Blue, Dies After Receiving HIV "Cure": Have The Benefits of Bone Marrow Transplant Been Exaggerated?

Special bone marrow transplants have "cured" three people of HIV and cancer, but is the celebrated remedy safe?

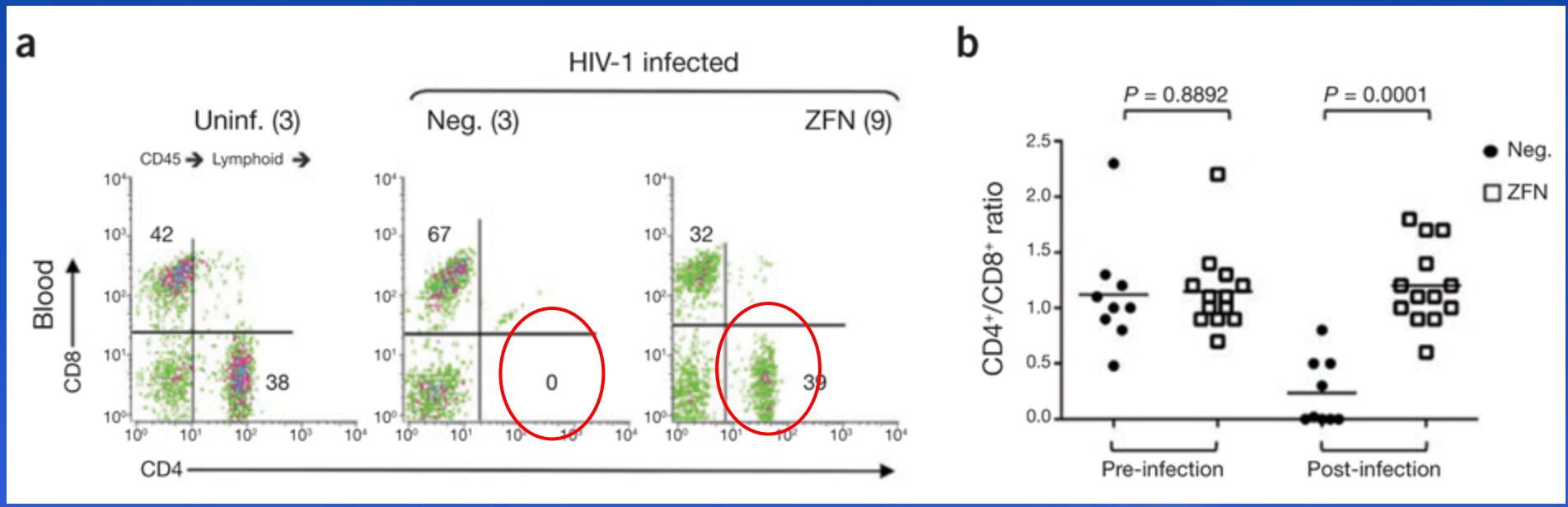


MAYO CLINIC

CCR5 knockdown in BLT mice.







Holt N et al.. Nature Biotech. 28, 839–847 (2010).

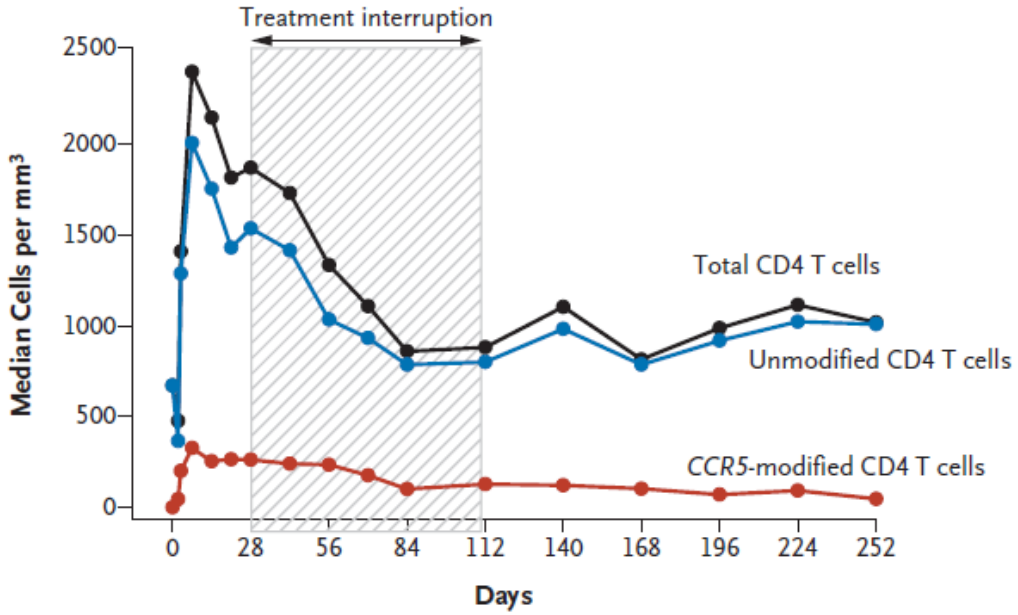
©2013 MFMER | slide-10

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MARCH 6, 2014 VOL. 370 NO. 10

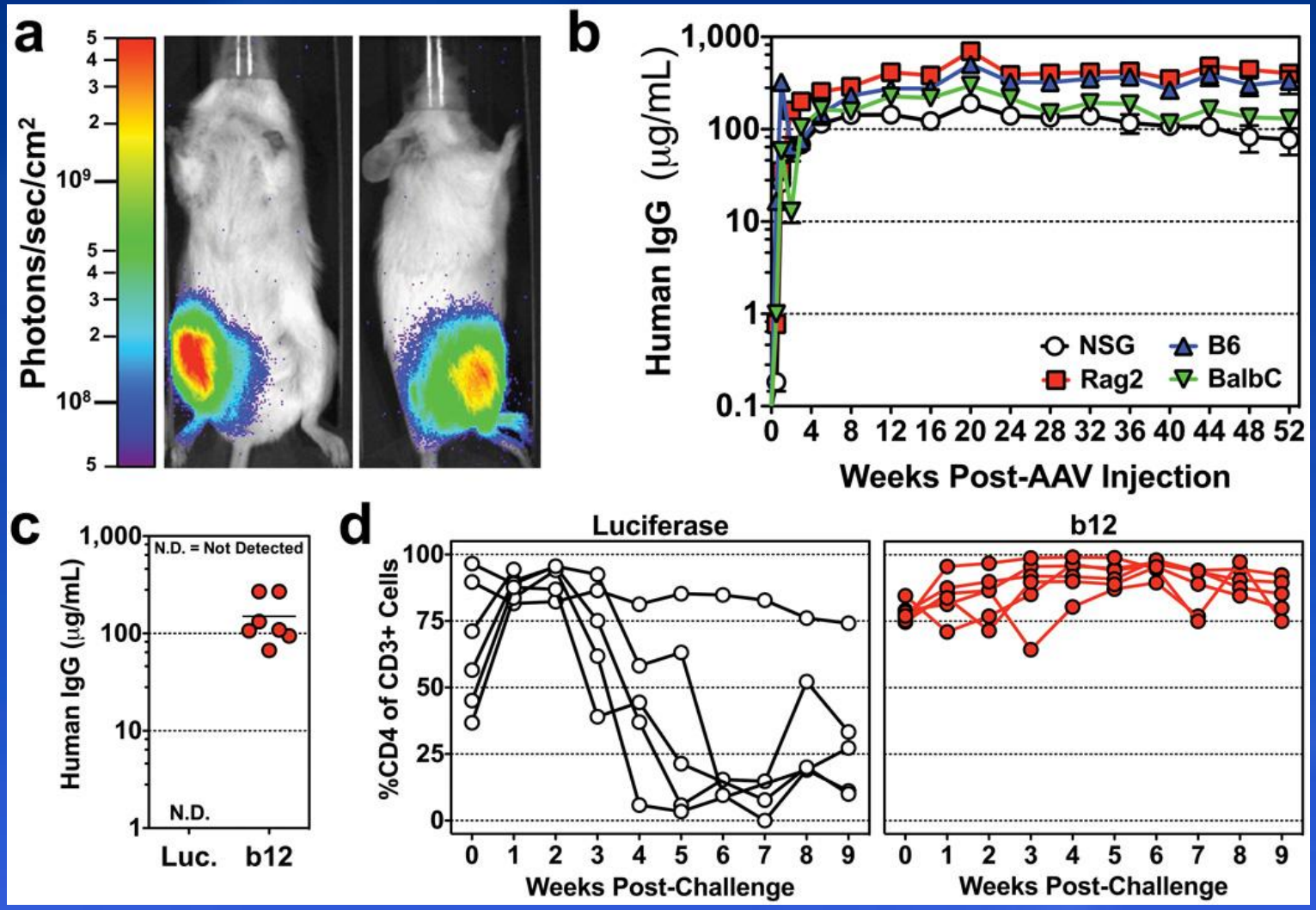
Gene Editing of *CCR5* in Autologous CD4 T Cells of Persons Infected with HIV

Pablo Tebas, M.D., David Stein, M.D., Winson W. Tang, M.D., Ian Frank, M.D., Shelley Q. Wang, M.D., Gary Lee, Ph.D., S. Kaye Spratt, Ph.D., Richard T. Surosky, Ph.D., Martin A. Giedlin, Ph.D., Geoff Nichol, M.D., Michael C. Holmes, Ph.D., Philip D. Gregory, Ph.D., Dale G. Ando, M.D., Michael Kalos, Ph.D., Ronald G. Collman, M.D., Gwendolyn Binder-Scholl, Ph.D., Gabriela Plesa, M.D., Ph.D., Wei-Ting Hwang, Ph.D., Bruce L. Levine, Ph.D., and Carl H. June, M.D.



MAYO CLINIC

Over expression of b12 anti HIV antibodies, in BLT mice



Nature. 2011 November 30; 481(7379): 81–84.

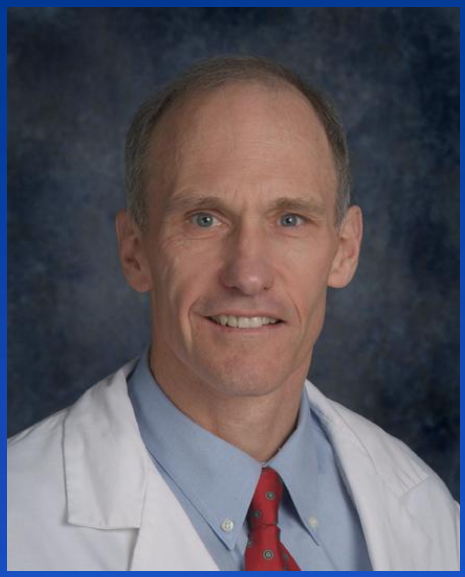
iAntibody-based Protection Against HIV Infection by Vectored ImmunoProphylaxis

MAYO CLINIC

My Bet for a Nobel Prize

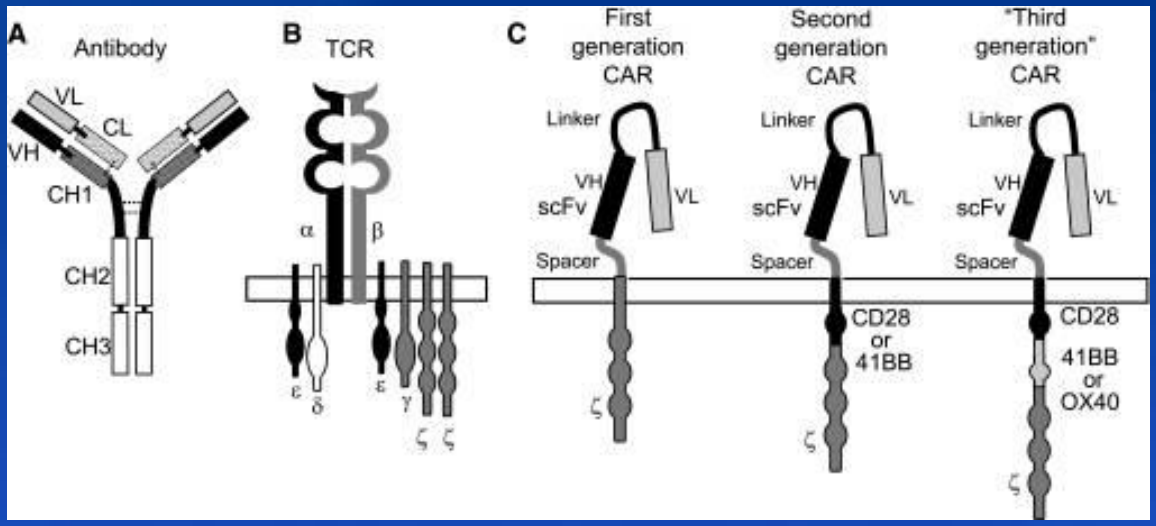
- CAR
- ALL
- CLL
- Melanoma
- Glioma
- Numerous others…..
- Novartis and Penn.

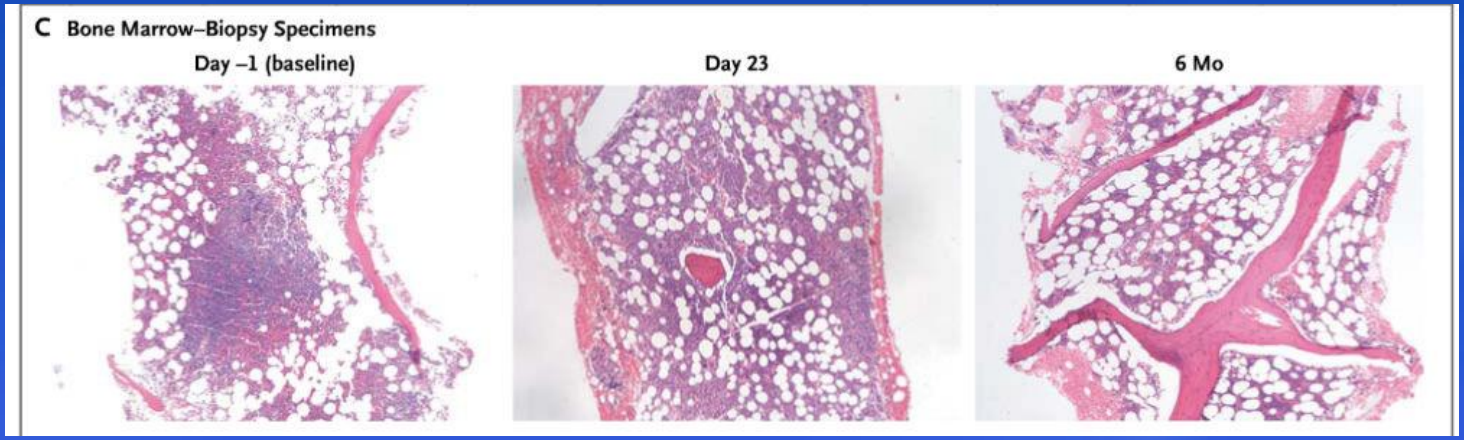




MAYO CLINIC

CAR generations





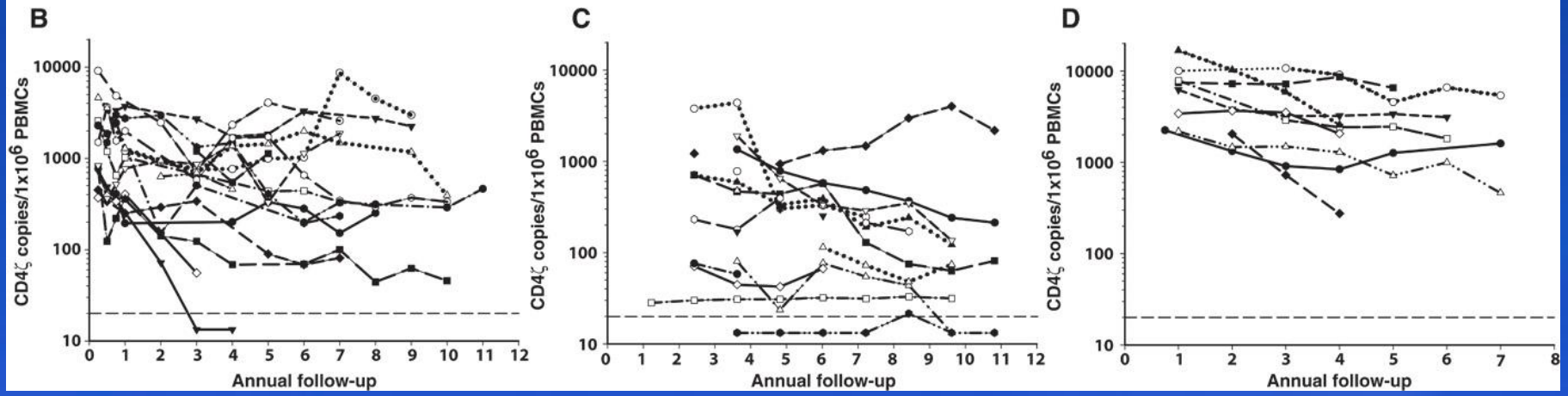
CAR in CLL; NEJM 2011

MAYO CLINIC

Persistence of CD4ζ-modified CAR T cells over 11 years after infusion.

A

Annual	1	2	3	4	5	6	7	8	9	10	11	Total
Detected	*20*	*25*	*33*	*31*	*28*	*25*	*24*	*13*	*8*	*4*	*1*	*212*
Tested	*20*	*25*	*35*	*33*	*29*	*26*	*24*	*15*	*9*	*4*	*1*	221

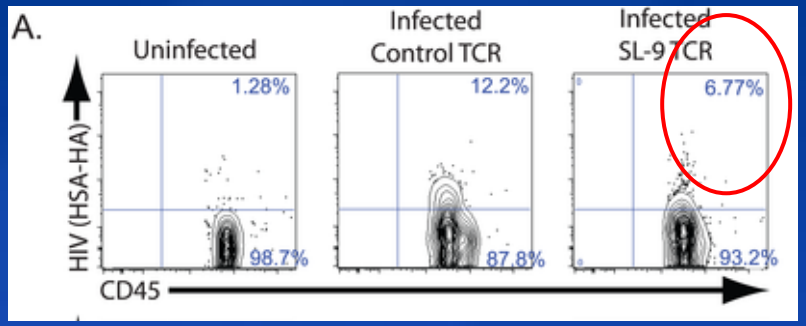


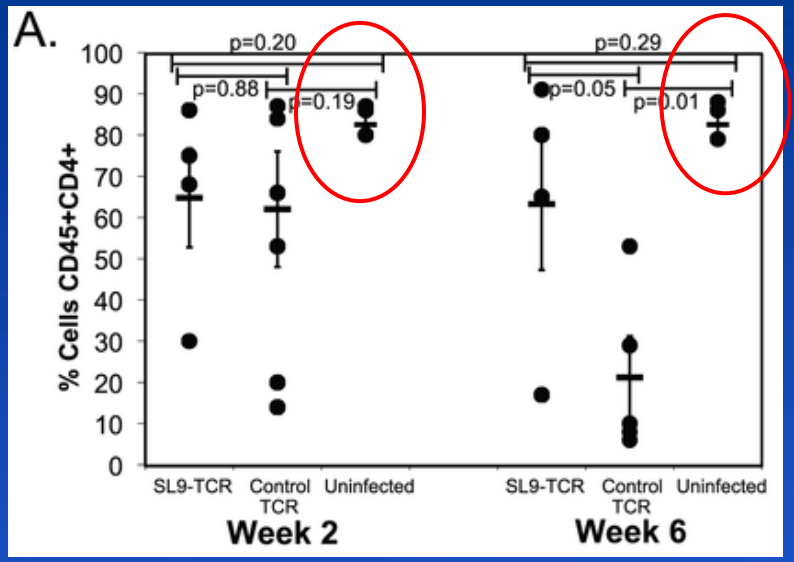
Scholler J et al. Sci Transl Med 2012;4:132ra53-132ra53

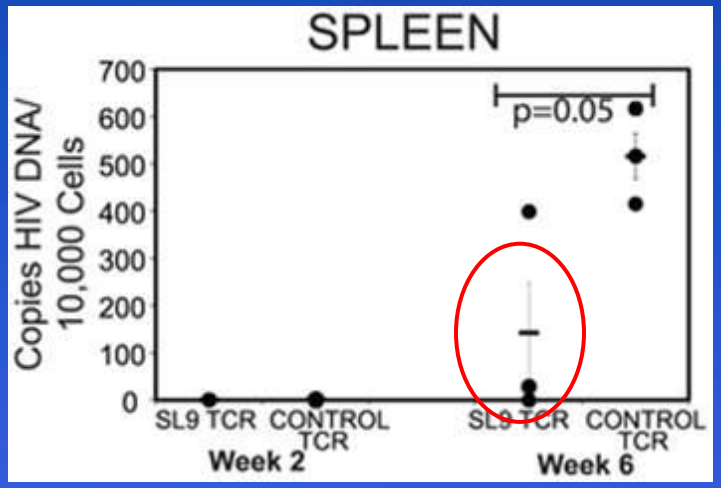
MAYO CLINIC

Science Translational Medicine AAAS

Human CD34+ repopulated NSG mice; 3rd generation CAR







MAYO CLINIC

Kitchen SG, Levin BR, Bristol G, Rezek V, et al. (2012) In Vivo Suppression of HIV by Antigen Specific T Cells Derived from Engineered Hematopoietic Stem Cells. PLoS Pathog 8(4): e1002649. doi:10.1371/journal.ppat.1002649
http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1002649

PLOS | PATHOGENS

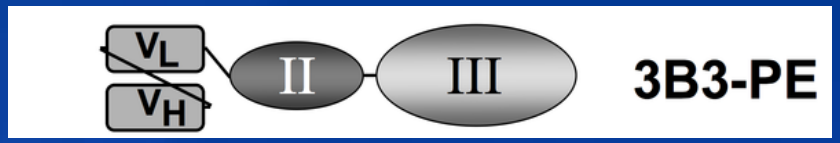
Vaccination / Immune based therapy

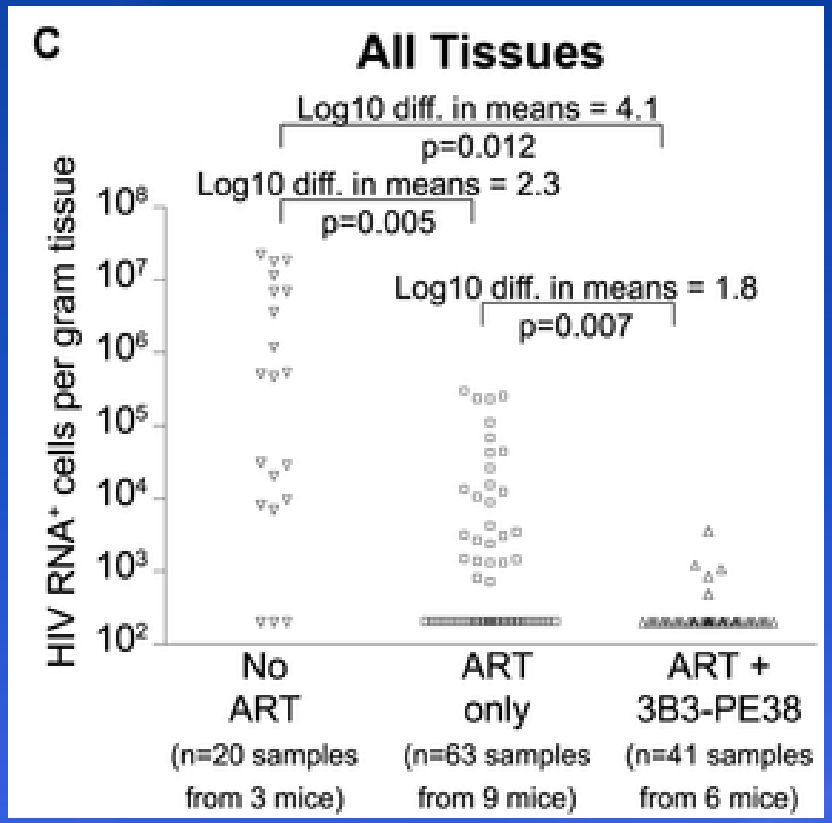
- Interleukin therapy - disappointing
- Other biologics
- Therapeutic vaccination - disappointing
- Preventative vaccination

MAYO CLINIC

©2013 MFMER

αgp120 immunotoxin; BLT mice

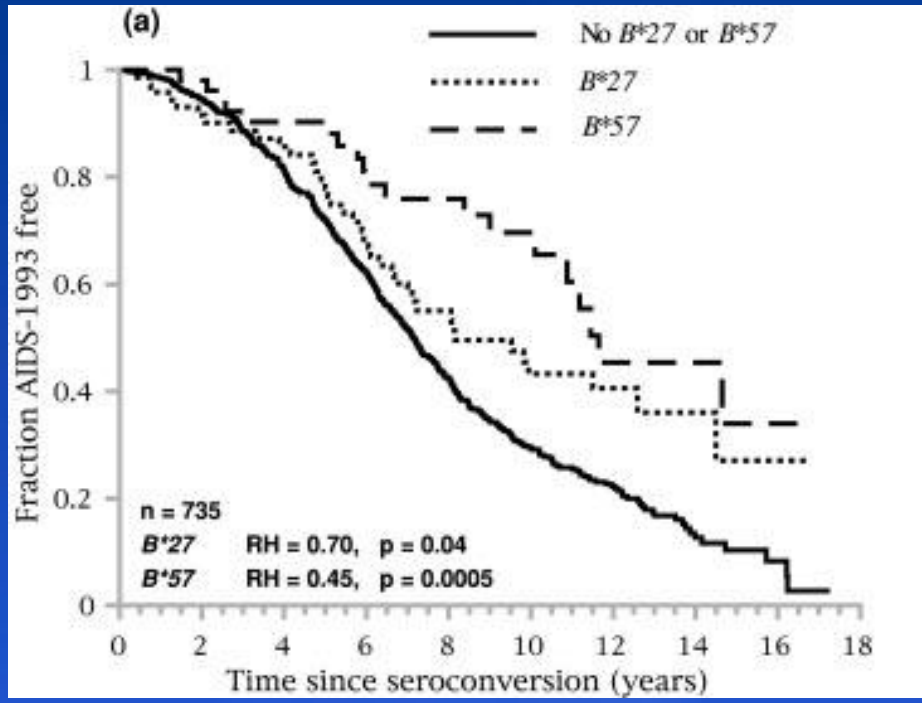




©2013 MFMER

Host Factors in HIV Control

- Known factors
  - Chemokine family: CCR5 delta 32 homozygosity prevents HIV, heterozygosity delays progression
  - MHC locus: HLA-B5701, HLA-B27 slower HIV disease
  - Not yet established - Host restriction factors: Apobec3G, Trim5a, tetherin, others-role in LTNP/EC not yet defined



MAYO CLINIC

RV-144: Evidence that an AIDS Vaccine Can Prevent HIV-1 Infection in Humans

The NEW ENGLAND JOURNAL of MEDICINE

Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand

Supachai Rerks-Ngarm, M.D., Punnee Pitisuttithum, M.D., D.T.M.H., Sorachai Nitayaphan, M.D., Ph.D., Jaranit Kaewkungwal, Ph.D., Joseph Chiu, M.D., Robert Paris, M.D., Nakorn Premsri, M.D., Chawetsan Namwat, M.D., Mark de Souza, Ph.D., Elizabeth Adams, M.D., Michael Benenson, M.D., Sanjay Gurunathan, M.D., Jim Tartaglia, Ph.D., John G. McNeil, M.D., Donald P. Francis, M.D., D.Sc., Donald Stablein, Ph.D., Deborah L. Birx, M.D., Supamit Chunsuttiwat, M.D., Chirasak Khamboonruang, M.D., Prasert Thongcharoen, M.D., Ph.D., Merlin L. Robb, M.D., Nelson L. Michael, M.D., Ph.D., Prayura Kunasol, M.D., and Jerome H. Kim, M.D., for the MOPH–TAVEG Investigators*

ABSTRACT

BACKGROUND

The development of a safe and effective vaccine against the human immunodeficiency virus type 1 (HIV-1) is critical to pandemic control.

METHODS

In a community-based, randomized, multicenter, double-blind, placebo-controlled efficacy trial, we evaluated four priming injections of a recombinant canarypox vector vaccine (ALVAC-HIV [vCP1521]) plus two booster injections of a recombinant glycoprotein 120 subunit vaccine (AIDSVAX B/E). The vaccine and placebo injections were administered to 16,402 healthy men and women between the ages of 18 and 30 years in Rayong and Chon Buri provinces in Thailand. The volunteers, primarily at heterosexual risk for HIV infection, were monitored for the coprimary end points: HIV-1 infection and early HIV-1 viremia, at the end of the 6-month vaccination series and every 6 months thereafter for 3 years.

RESULTS

In the intention-to-treat analysis involving 16,402 subjects, there was a trend toward the prevention of HIV-1 infection among the vaccine recipients, with a vaccine efficacy of 26.4% (95% confidence interval [CI], −4.0 to 47.9; P=0.08). In the per-protocol analysis involving 12,452 subjects, the vaccine efficacy was 26.2% (95% CI, −13.3 to 51.9; P=0.16). In the modified intention-to-treat analysis involving 16,395 subjects (with the exclusion of 7 subjects who were found to have had HIV-1 infection at baseline), the vaccine efficacy was 31.2% (95% CI, 1.1 to 51.2; P=0.04). Vaccination did not affect the degree of viremia or the CD4+ T-cell count in subjects in whom HIV-1 infection was subsequently diagnosed.

CONCLUSIONS

This ALVAC-HIV and AIDSVAX B/E vaccine regimen may reduce the risk of HIV infection in a community-based population with largely heterosexual risk. Vaccination did not affect the viral load or CD4+ count in subjects with HIV infection. Although the results show only a modest benefit, they offer insight for future research. (ClinicalTrials.gov number, NCT00223080.)

From the Department of Disease Control, Ministry of Public Health, Nonthaburi (S.R.-N., R.P., C.N., S.C., C.K., P.T., P.K.); Vaccine Trials Center (P.P.) and Data Management Unit (J.K.), Faculty of Tropical Medicine, Mahidol University, Bangkok; Thai Component (S.N.) and U.S. Army Medical Component (J.C., R.P., M.S., M.B.), Armed Forces Research Institute of Medical Sciences, Bangkok — all in Thailand; the Division of AIDS, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD (E.A.); Sanofi Pasteur, Swiftwater, PA (S.G., J.T., J.G.M.); Global Solutions for Infectious Diseases, South San Francisco, CA (D.P.F.); the Emmes Corporation, Rockville, MD (D.S.); the Global AIDS Program, Centers for Disease Control and Prevention, Atlanta (D.L.B); U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Rockville, MD (M.L.R., N.L.M., J.H.K); and U.S. Army Medical Materiel Development Activity, Ft. Detrick, MD (J.H.K.). Address reprint requests to Dr. Kim at the U.S. Military HIV Research Program, Walter Reed Army Institute of Research, 1600 E. Gude Dr., Rockville, MD 20850, or at jkim@hivresearch.org.

*The names and affiliations of the Ministry of Public Health–Thai AIDS Vaccine Evaluation Group (MOPH–TAVEG) investigators are listed in the Appendix.

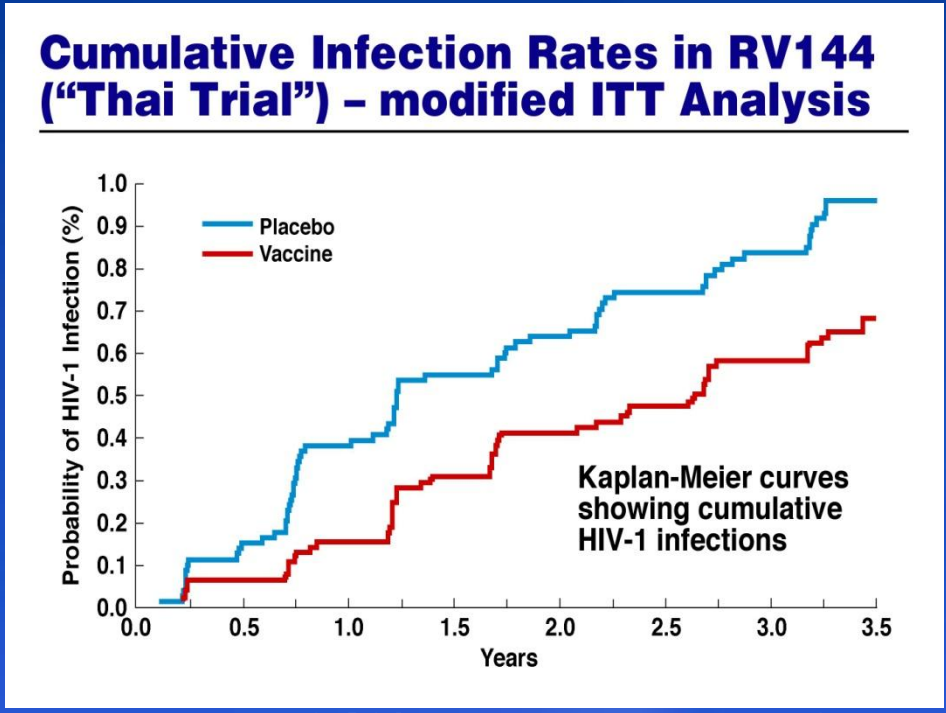
This article (10.1056/NEJMoa0908492) was published on October 20, 2009, at NEJM.org.

N Engl J Med 2009;361.

Copyright © 2009 Massachusetts Medical Society.

Downloaded from www.nejm.org at HHS LIBRARIES CONSORTIUM on October 24, 2009 .
Copyright © 2009 Massachusetts Medical Society. All rights reserved.

Prime boost : Recombinant gp120

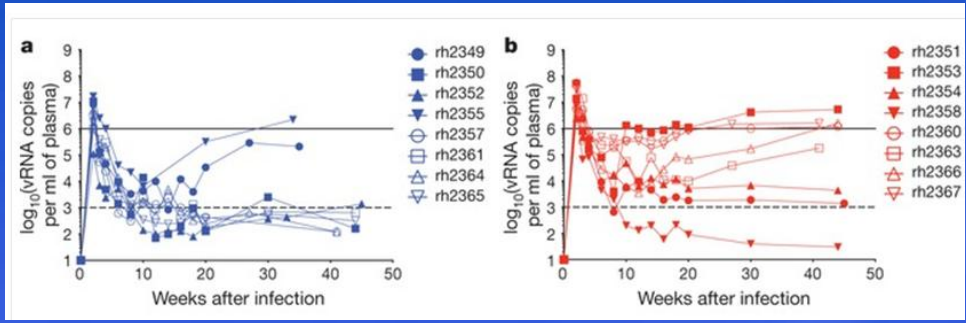


- **Modest 31% reduction in infection**
- **limited duration**

Proof of concept for a protective vaccine

Most promising approach to HIV Vaccination: Genotype specific

- HLA-B57 = Mamu B
- Vaccinogen Gag epitope in Mamu B vs non-MamuB
- Non-MamuB – rapid progression after SIV challenge
- MamuB - protected.



Nature. 2012 November 1; 491(7422):

Shock and Kill hypothesis

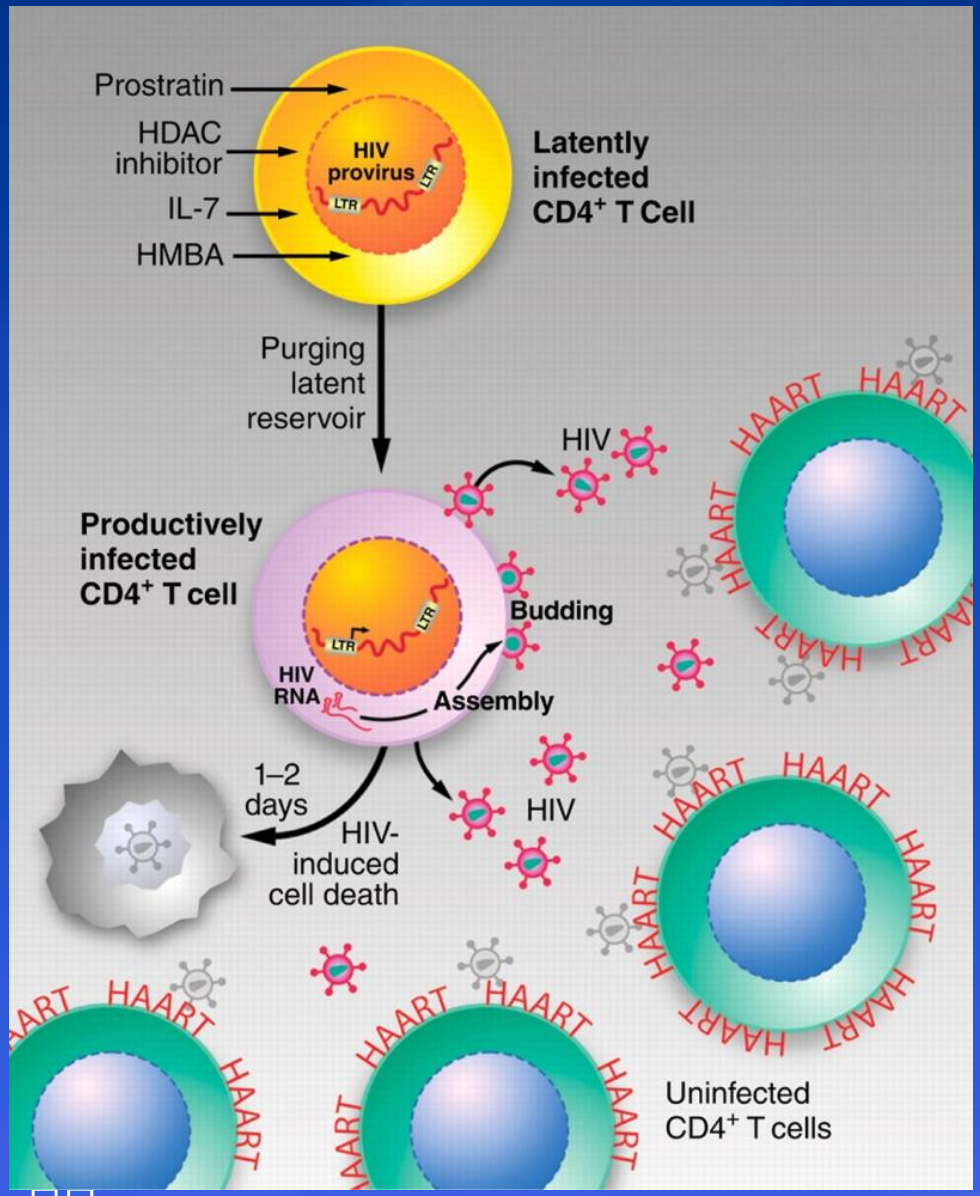


Table 2 Compounds that activate latent infection

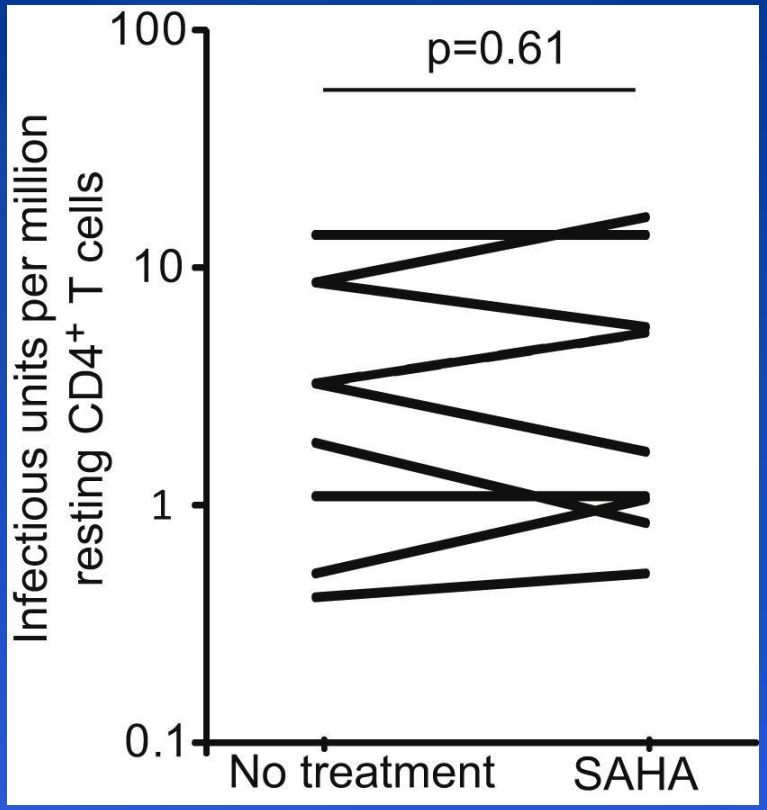
Mechanism of action	Name	Clinical trials[a]	Reference
Histone deacetylase inhibition (HDACi)	Valproic acid		43,48,66,116,117
	Trichostatin A		43,116,117
	Vorinostat	2	43,48,63,116–119
	Sodium butyrate		116,117
	Oxamflatin		66,116
	MCT-1 and 3		66
	MRK1, 10, 11, 12, 13, 14		120
	MC compounds		118
	Givinostat		121
	Givinostat analogs		121
	Scriptaid		116,117
	NCF-51		122
	Belinostat		121,123
	Panabinostat	1	123,124
	Entinostat		117,118,124
	Apicidin		116,117
	CG05, CG06		119
	Droxinostat		116
	M344 Romedepsin	In devpt	125
Methylation inhibitors	5-aza-2'deoxycytidine (Aza-CdR)		126
	BIX-01294		127
	Chaetocin		127,128
			129
NFκB activators	Prostratin		117,125,127,130
	TNFα		130
Protein kinase C modulators	Bryostatin		131
Akt/HEXIM-1 modulators	Hexamethylbisacetamide (HMBA)		132,133
	Disulfiram	1	134
BET bromodomain inhibitors	JQ1		135
Immune modulation	IL-7	6	130,136
	IL-15		137
	Anti-PD1	In devpt	138
	Anti-PDL1	In devpt	
Combinations	AV6 + Valproic acid		139
	Bryostatin + Valproic acid		131
	HDACi + Prostratin		117,125,130
	Prostratin + IL-7		

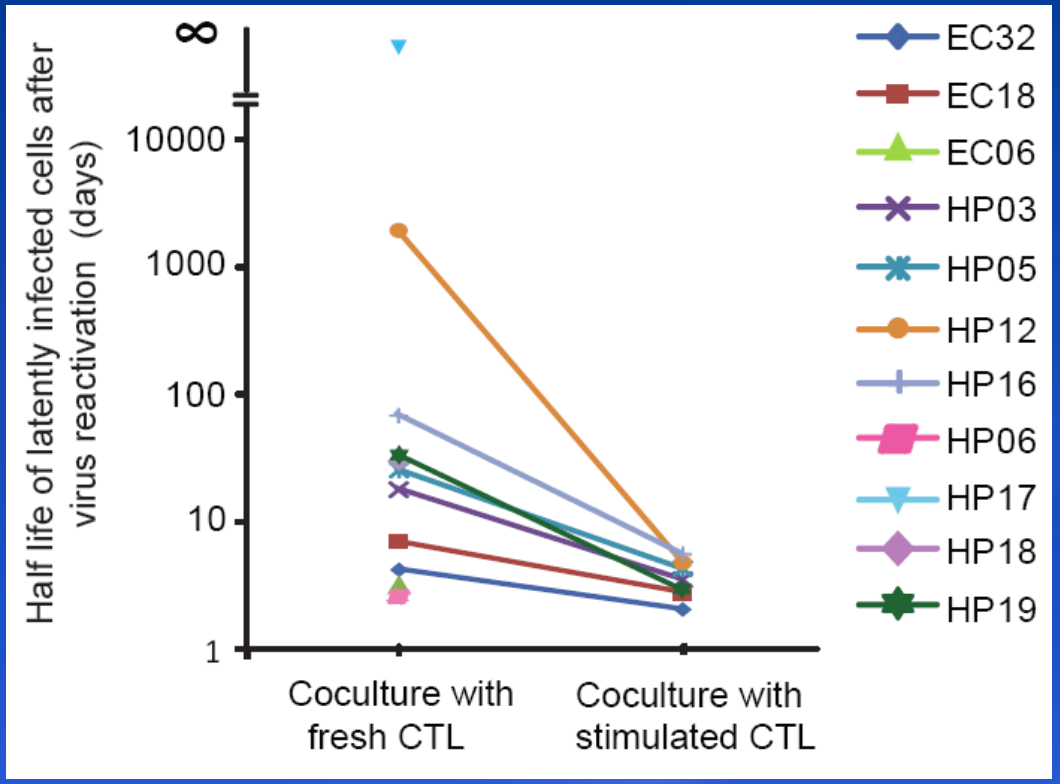
All compounds have demonstrated activity *in vitro* in either latently infected cells lines, latently infected primary T cells, and/or resting CD4+ T cells from HIV-infected patients on cART.
[a]Completed or currently active trials in HIV-infected patients on cART (source clinicaltrials.gov).

D. D. Richman et al., Science 323, 1304 -1307 (2009)

Badley Cell Death and Disease 2013

Does HIV reactivation with decrease viral burden ?





Immunity. 2012 Mar 23;36(3):491-501. Epub 2012 Mar 8.
Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation.

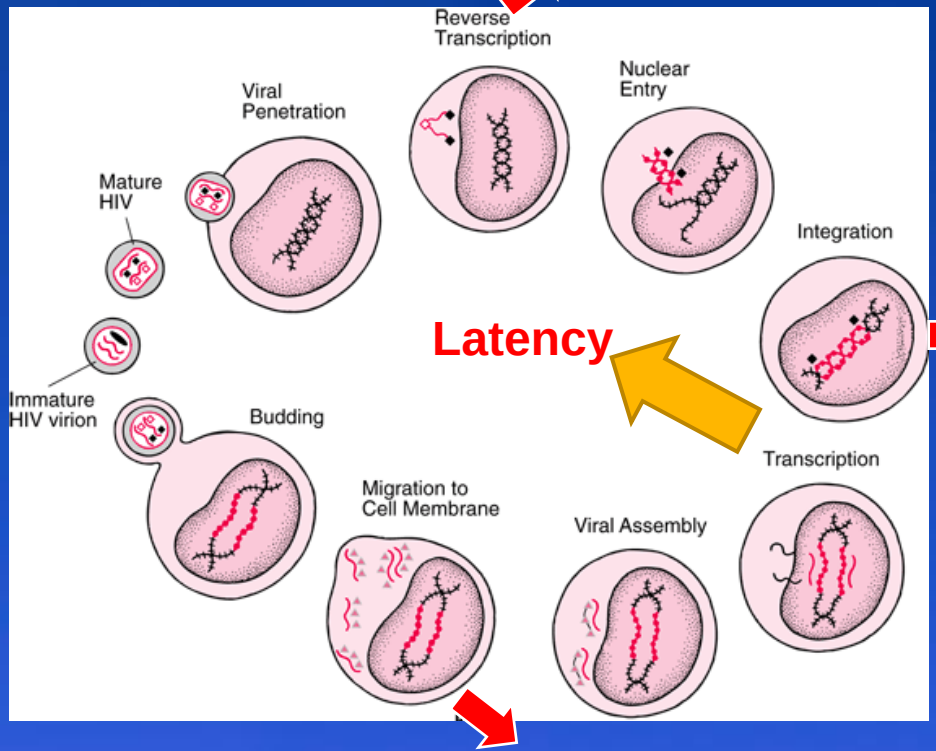
MAYO CLINIC

How do HIV infected CD4 T cells die?

Cell

Abortive HIV Infection Mediates CD4 T Cell Depletion and Inflammation in Human Lymphoid Tissue

Gilad Doitsh,[1] Marielle Cavrois,[1,5] Kara G. Lassen,[1,5] Orlando Zepeda,[1] Zhiyuan Yang,[1] Mario L. Santiago,[1,4] Andrew M. Hebbeler,[1] and Warner C. Greene[1,2,3,*]



Before integration

LETTER

doi:10.1038/nature12274

HIV-1 causes CD4 cell death through DNA-dependent protein kinase during viral integration

Arik Cooper[1], Mayra García[1], Constantinos Petrovas[2], Takuya Yamamoto[2], Richard A. Koup[2] & Gary J. Nabel[1]†

Coincident with Viral replication

JOURNAL OF VIROLOGY, July 2007, p. 6947–6956
0022-538X/07/$08.00+0 doi:10.1128/JVI.02798-06
Copyright © 2007, American Society for Microbiology. All Rights Reserved.
Vol. 81, No. 13

Human Immunodeficiency Virus Type 1 Protease Cleaves Procaspase 8 In Vivo[▽]

Zilin Nie,[1] Gary D. Bren,[1] Stacey R. Vlahakis,[1] Alicia Algeciras Schimnich,[1] Jason M. Brenchley,[3] Sergey A. Trushin,[1] Sarah Warren,[1] David J. Schnepple,[1] Colin M. Kovacs,[4] Mona R. Loutfy,[4] Daniel C. Douek,[3] and Andrew D. Badley[1,2]*

MAYO CLINIC

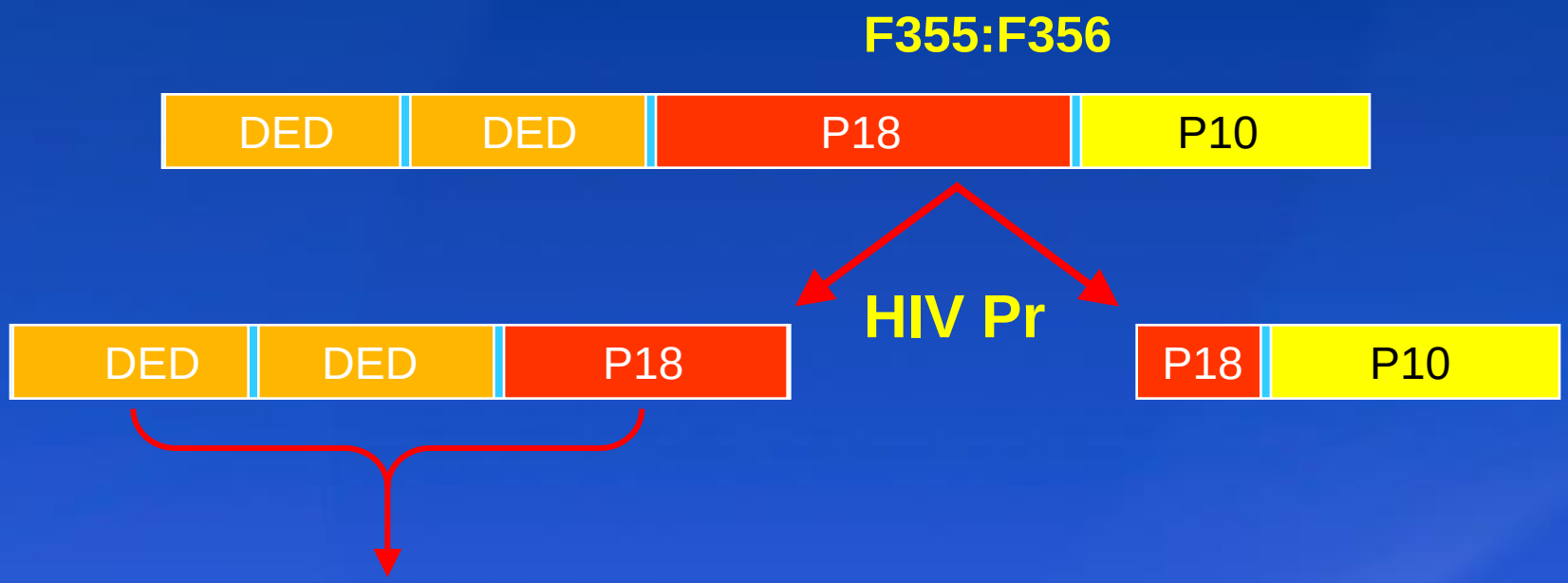
HIV Protease

- Discovered in early 80's
- Expression in cells is cytotoxic
- Target for therapy / cytotoxicity exploited as screening tool.
- Degenerate substrate specificity
- Cleaves Actin, Desmin, eIF4G, Bcl2

MAYO CLINIC

©2013 MFMER

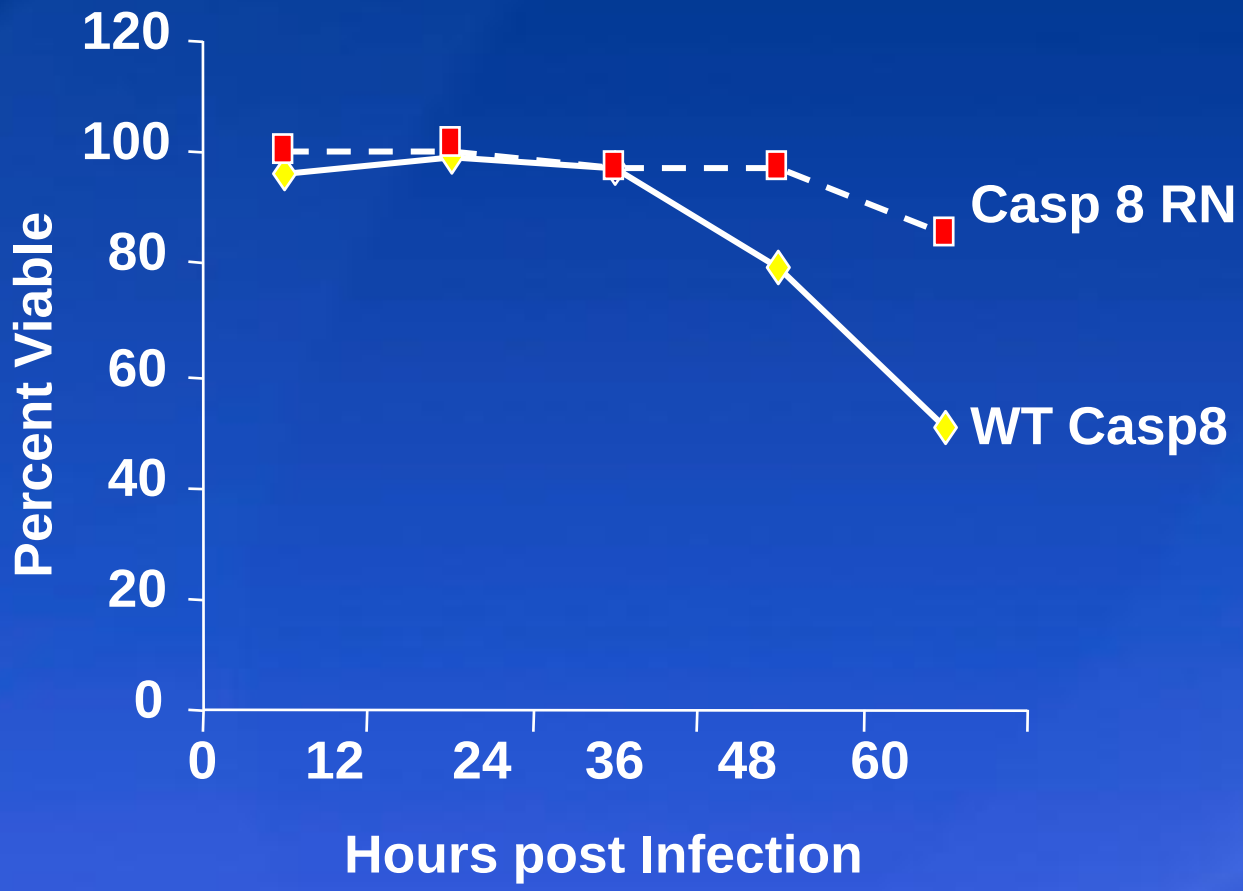
Pr cleaves Procaspase 8 between 355:356

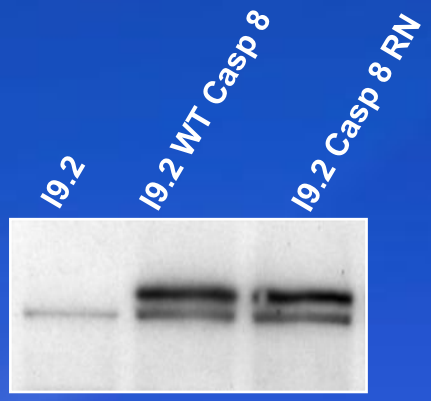


Casp8p41

MAYO CLINIC

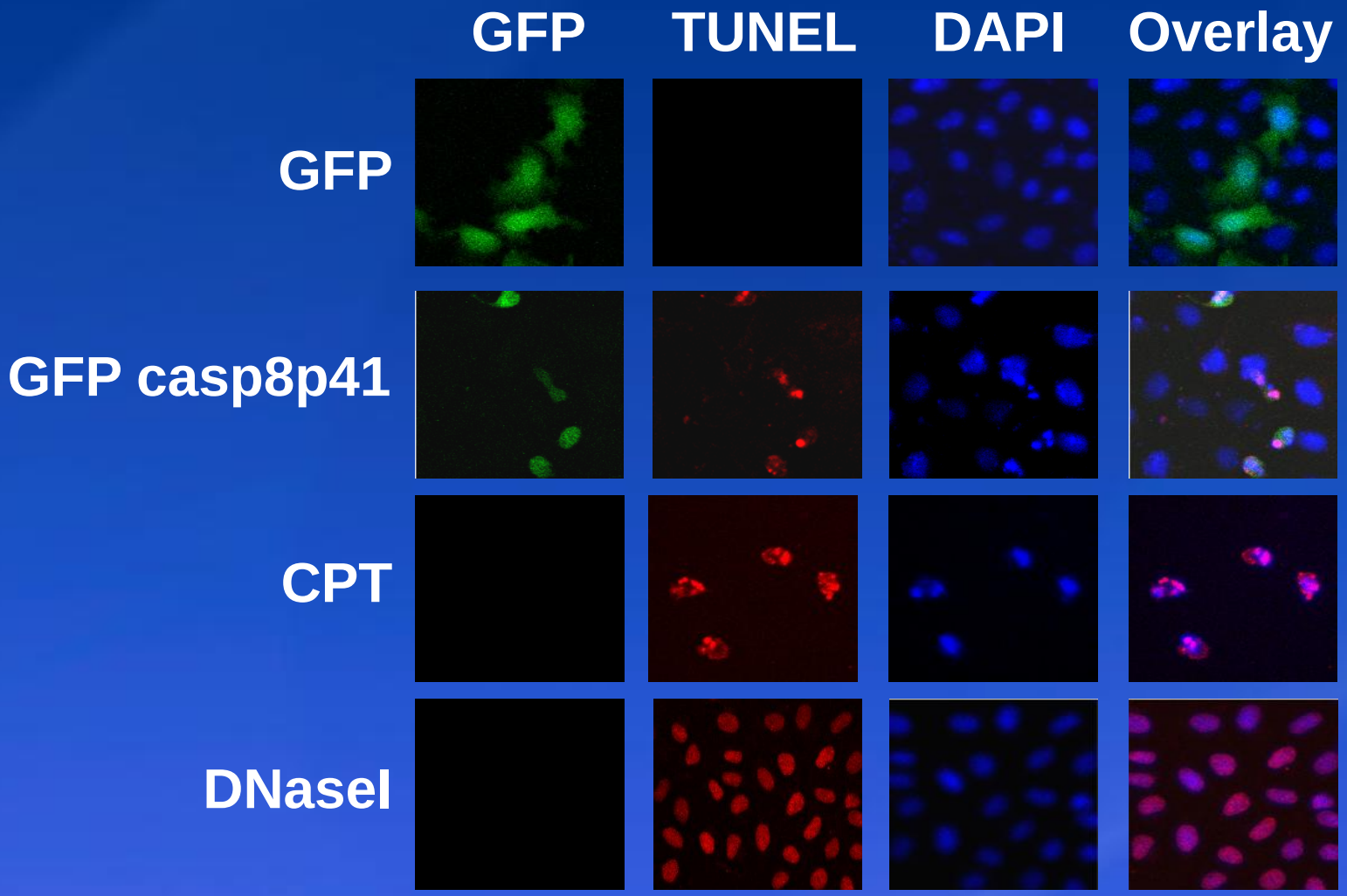
Mutating the Pr cleavage site in procaspase 8 abrogates HIV induced death





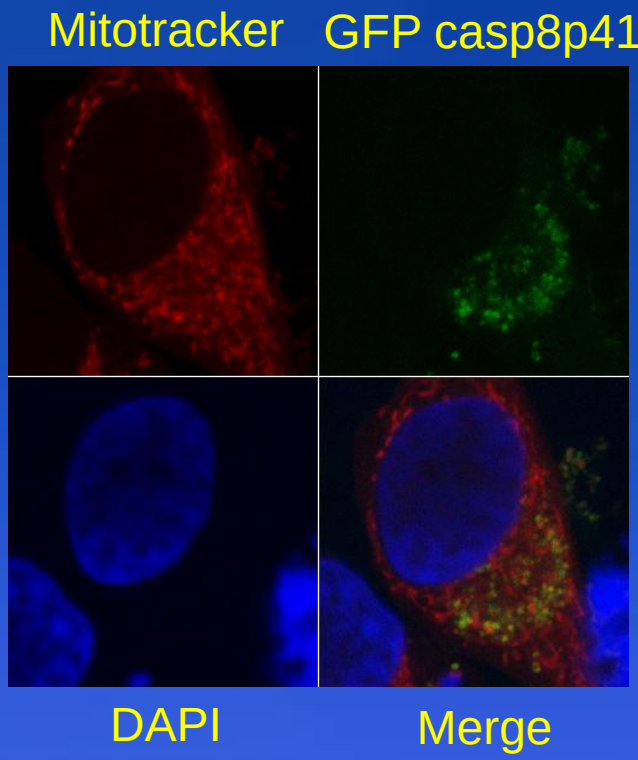
MAYO CLINIC

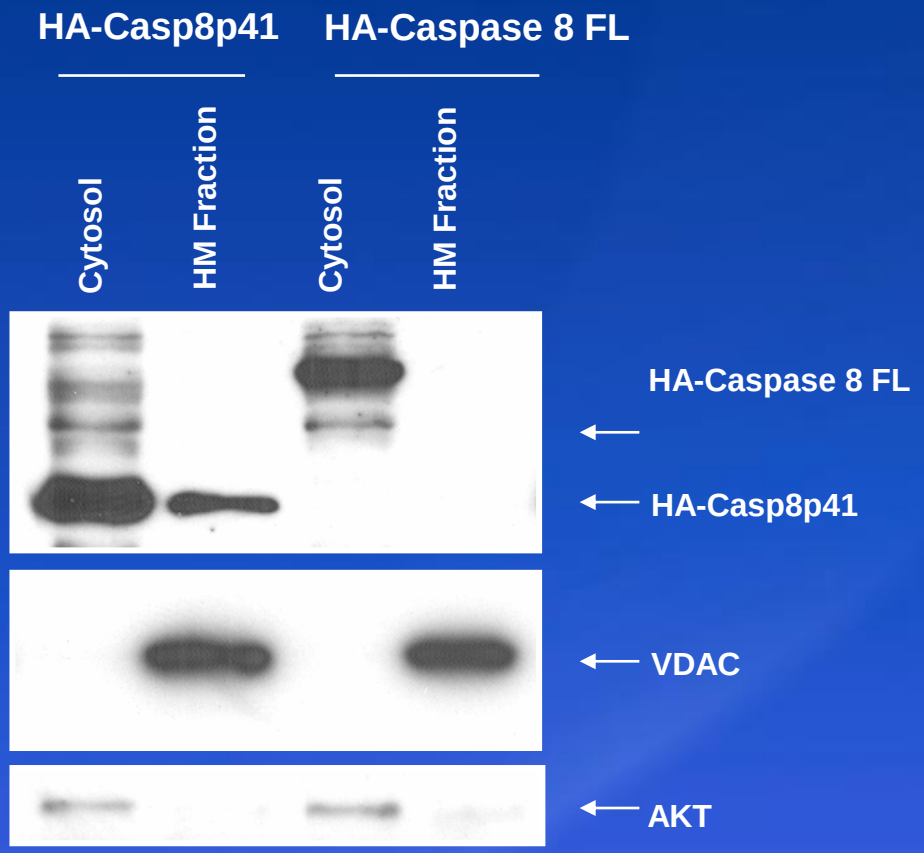
Casp8p41 Induces Apoptosis



HeLa Cells

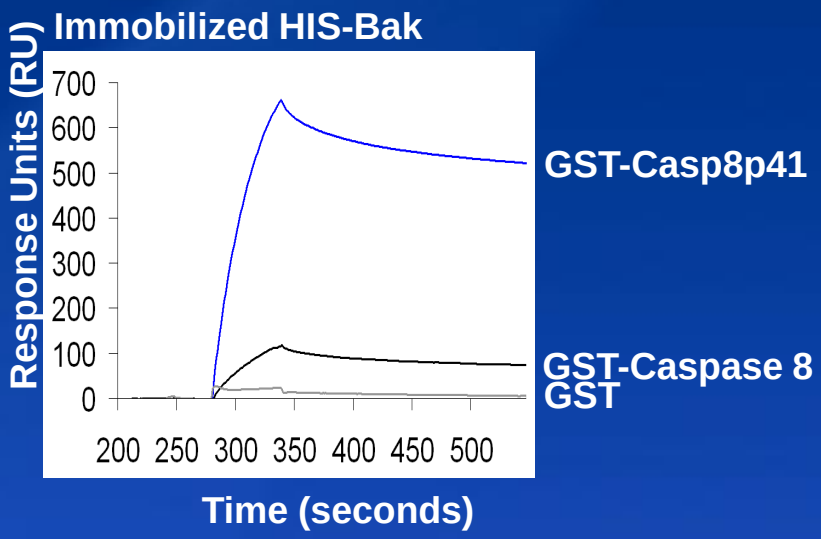
Caspase8p41 colocalizes with mitochondria

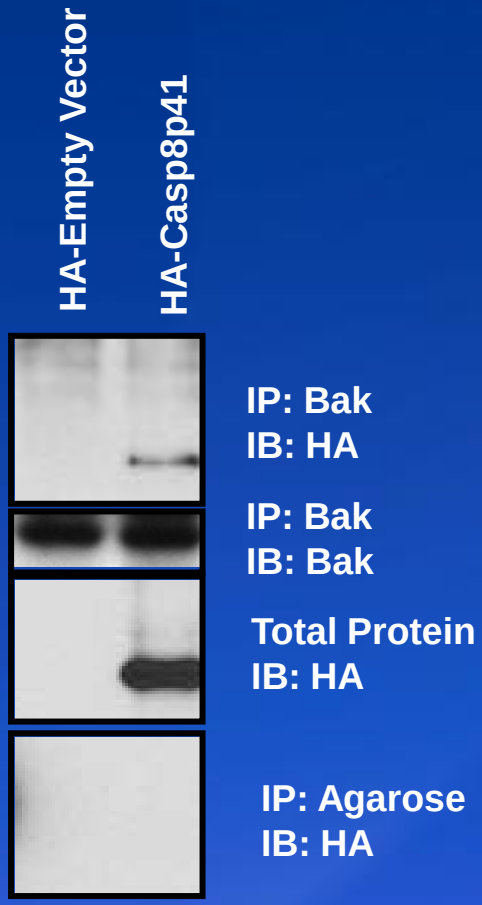


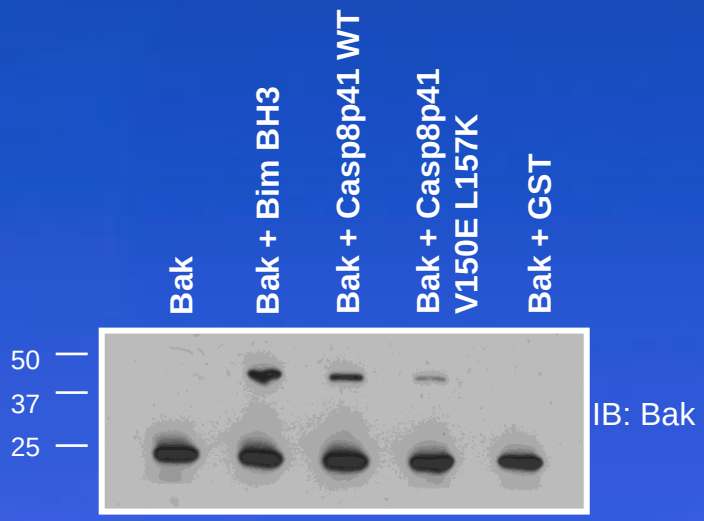


MAYO CLINIC

Casp8p41, Not Caspase 8 Binds Bak

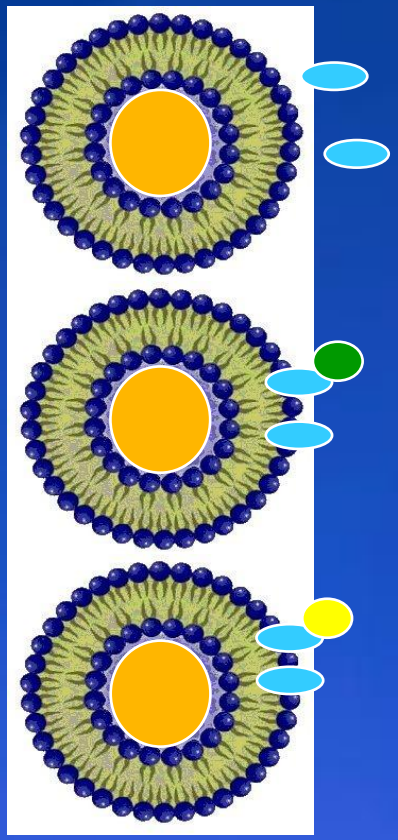






MAYO CLINIC

Casp8p41 stimulates Bak pore like function



Bak alone

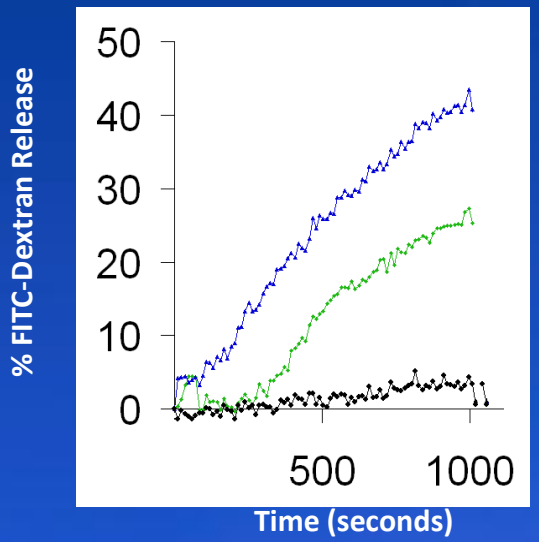
→ No Bak oligomerization

Bak & Bim

→ Dextran FITC release

Bak & Casp8p41

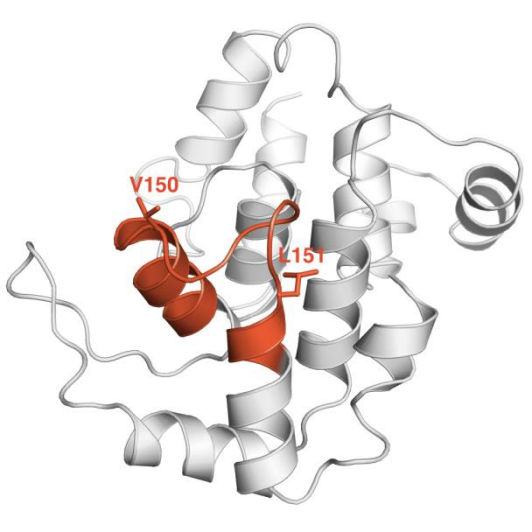
→ ????



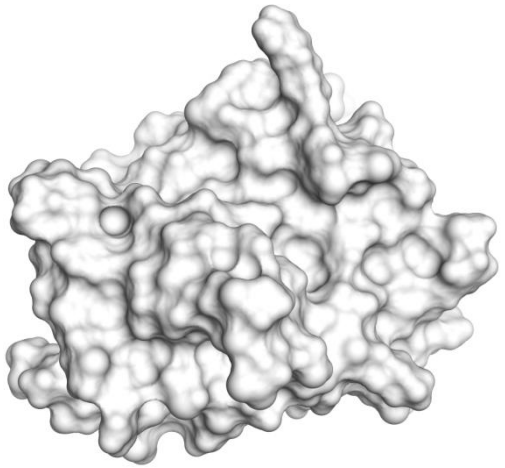
MAYO CLINIC

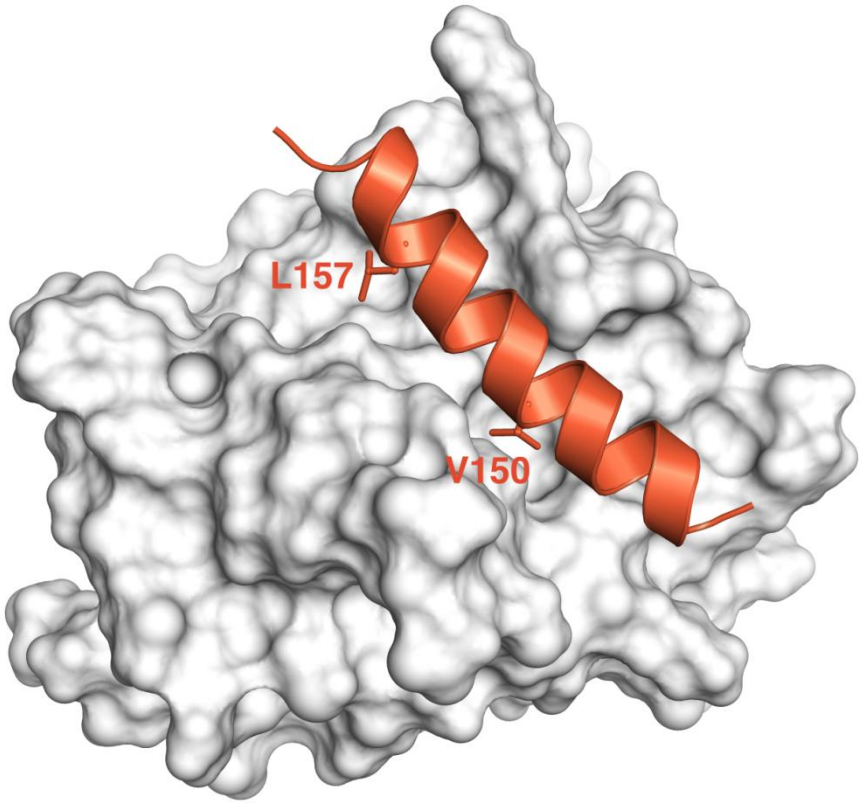
Molecular Dynamic Simulation

Casp8p41



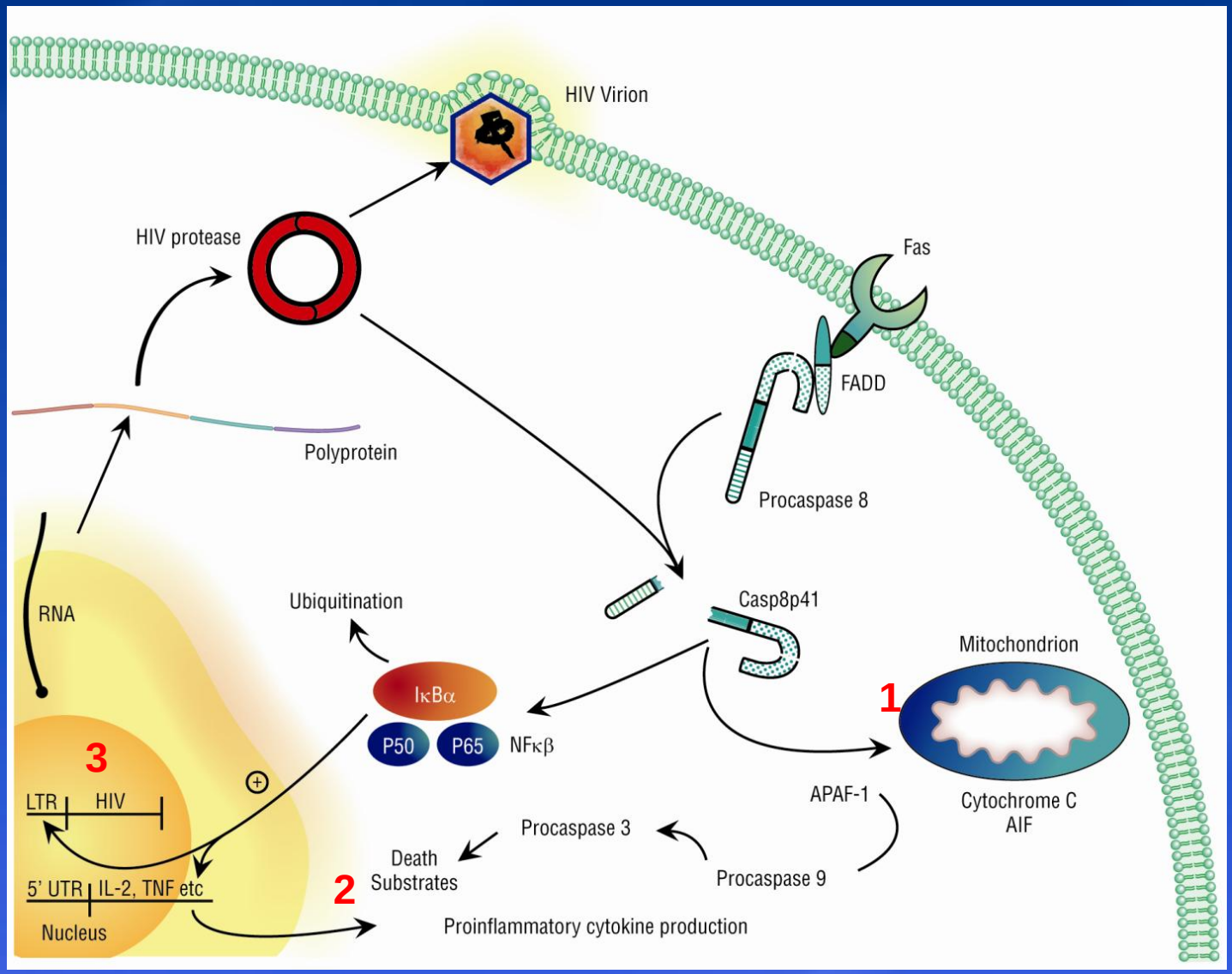
Bak





MAYO CLINIC

Casp8p41

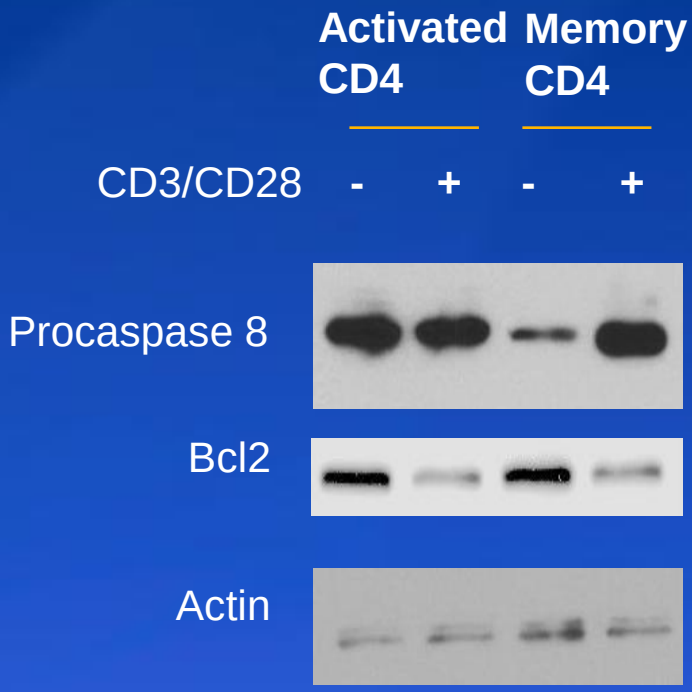

HIV Virion
HIV protease
Fas
FADD
Polyprotein
Procaspase 8
RNA
Ubiquitination
Casp8p41
Mitochondrion
IκBα
P50
P65
NFκβ
1
3
LTR
HIV
APAF-1
Cytochrome C
AIF
Procaspase 3
Death
Substrates
Procaspase 9
5' UTR
IL-2, TNF etc
2
Nucleus
Proinflammatory cytokine production

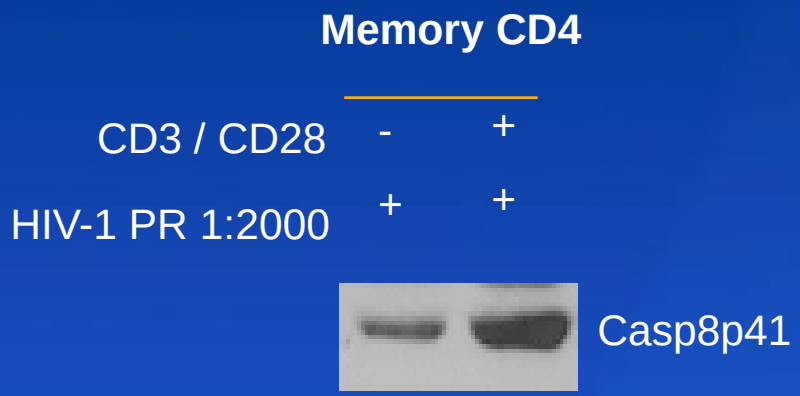
Casp8p41

- Casp8p41 is a novel cleavage fragment of procaspase 8
- Molecular signature of cells in which Protease has cleaved procaspase 8 (αCasp8p41)
- Predictive of ongoing CD4 losses
- Mechanistically linked to discordance
- Novel paradigm in Caspase biology
- May provide insight into how to kill cells reactivating HIV

MAYO CLINIC

Why don't latently HIV infected memory CD4 T cells die following HIV reactivation

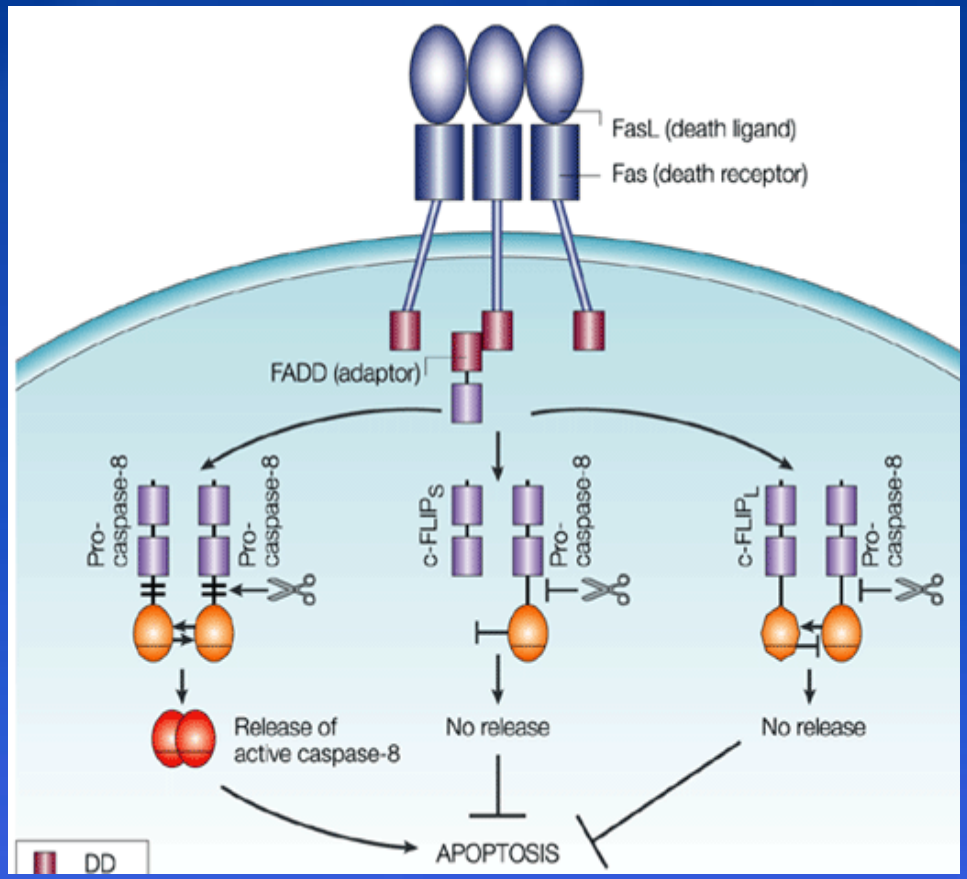


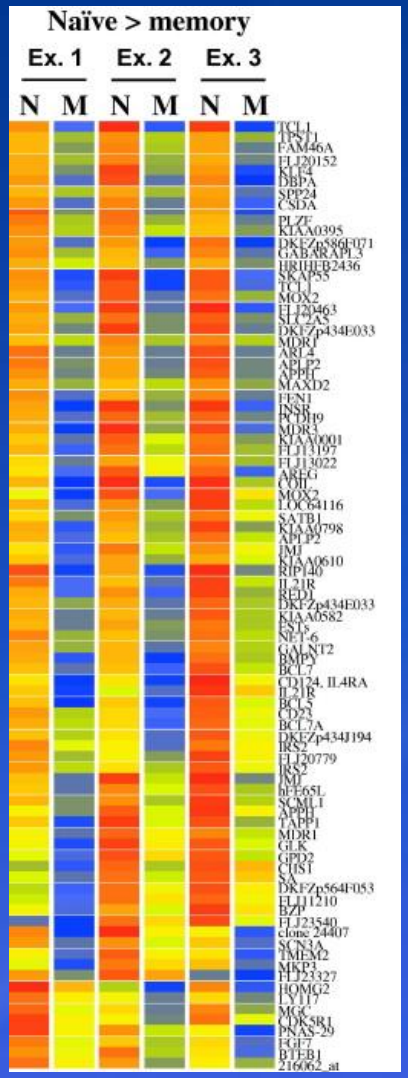


Also Upregulated Bcl2, Decreased Fas / FasL

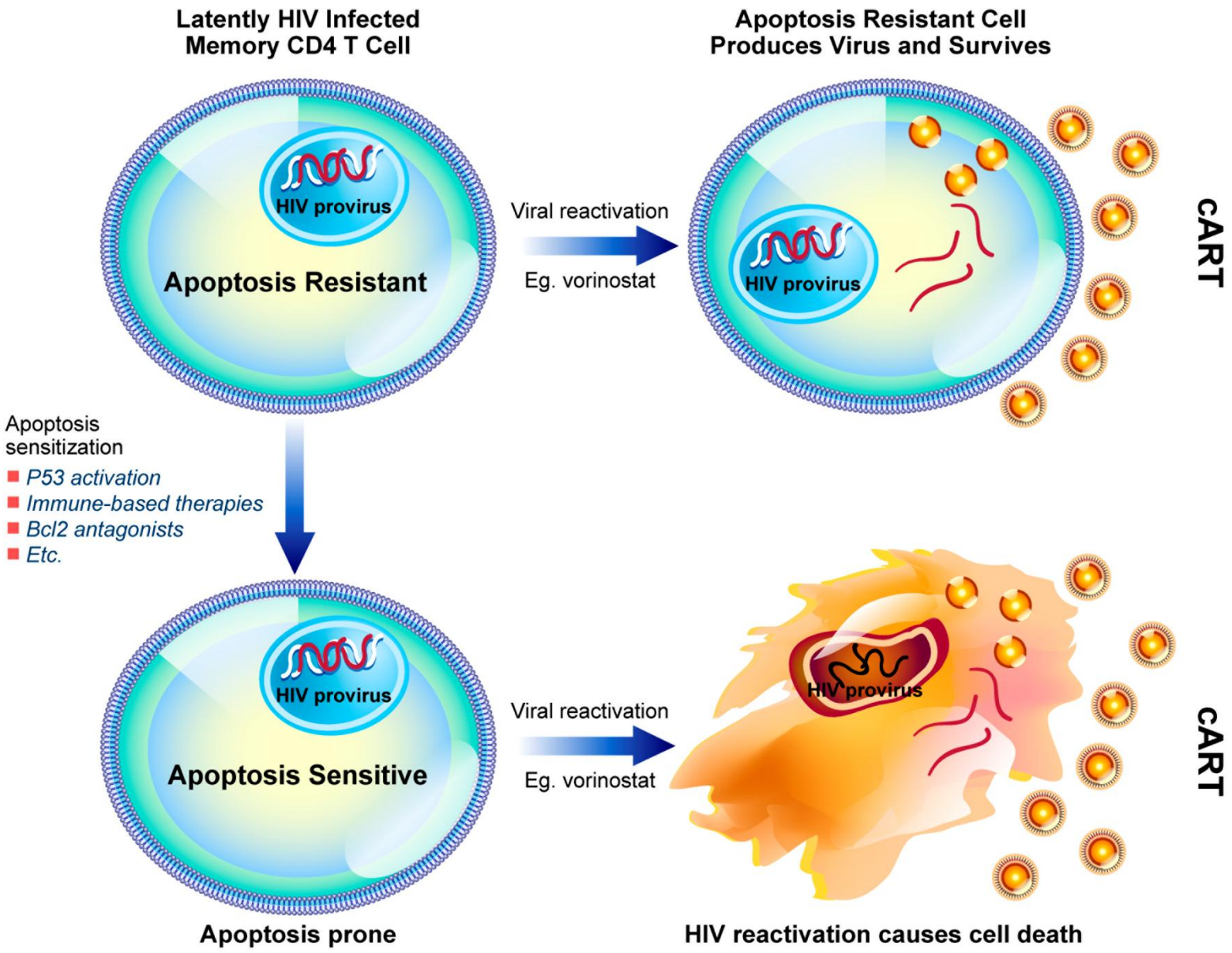
MAYO CLINIC

Memory cells upregulate antiapoptotic and down regulate proapoptotic proteins.





MAYO CLINIC


Latently HIV Infected
Memory CD4 T Cell
Apoptosis Resistant Cell
Produces Virus and Survives
HIV provirus
Apoptosis Resistant
Viral reactivation
Eg. vorinostat
HIV provirus
cART
Apoptosis
sensitization
P53 activation
Immune-based therapies
Bcl2 antagonists
Etc.
HIV provirus
Apoptosis Sensitive
Viral reactivation
Eg. vorinostat
HIV provirus
cART
Apoptosis prone
HIV reactivation causes cell death

Approaches to cure	Status
• Gene editing – eg CCR5	★★
• Overexpression of restriction factors	★
• CAR's	★★★
• Vaccination	★★
• Immune based therapy	★
• Shock and Kill	★
• Prime shock and kill	★★



MAYO CLINIC

Acknowledgements

Badley Lab Members (past and present)
Gary Bren
Stacey Rizza
Alicia Algeciras-Schimnich
Zilin Nie
Sergey Trushin
Nathan Cummins
Sekar Natesampillai
Amy Sainski
Dave Schnepple
Brett Shepard
Sergei Trushin

Mayo Collaborators
Scott Kaufmann
Greg Gores
Tom Griffith
Joseph Yao
Paul Leibson*
Yuan-Ping Pang
Zami Temesgen
David Katzmann
Marina Ramirez-Alvarado
Haiming Dai
Peter Wettstein

Other Collaborators
Guido Kroemer
Danny Douek
Jason Brenchley
Steve Deeks
Nawal Boukli
Guido Silvestri
Tom Griffith
David Lynch
Catherine Brenner
Alan Landay
Mike Lederman

MAYO CLINIC

©2013 MFMER