

# Diagnosis and Pathogenesis of HIV, Antiretroviral drug resistance

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## Topics

- diagnosis of HIV
  - laboratory diagnosis
- pathogenesis of HIV
  - acute infection
  - chronic infection
- antiretroviral drug resistance
  - definitions, mechanism
  - genotypic resistance testing
  - viral and host factors influencing resistance

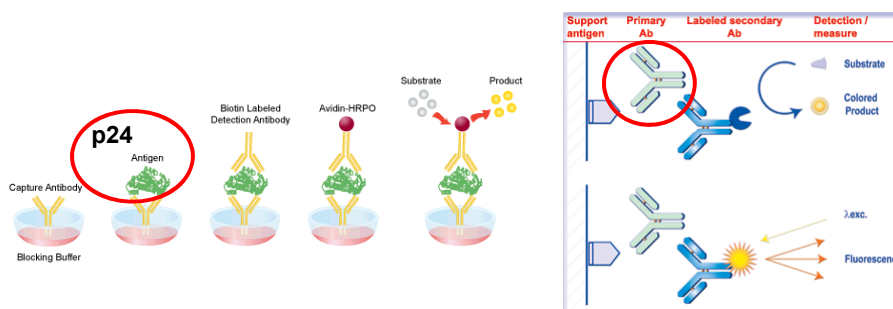
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## Diagnosis of HIV-1

### 1. screening test

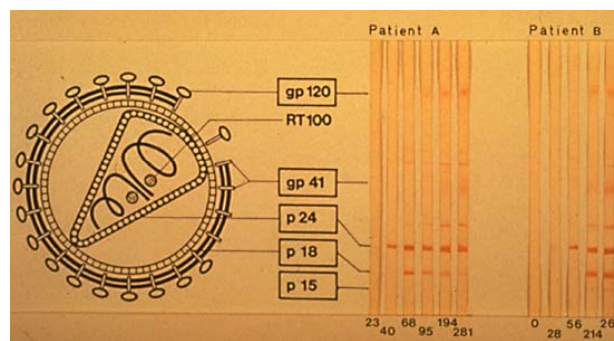
- antibody/antigen (p24) detection assay
- ELISA (enzyme linked immunosorbent assay)



[www.leinco.com](http://www.leinco.com) / [www.interchim.com](http://www.interchim.com)

## Diagnosis of HIV-1 2. confirmatory test

- antibody detection assay
- Western blot



www.aids-info.ch

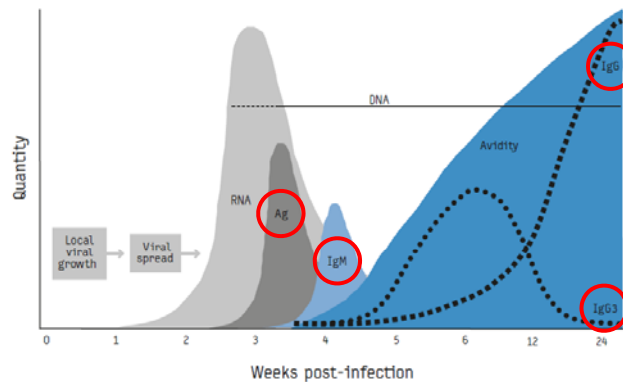
## Sensitivity / Specificity

- sensitivity  $\geq 99.7\%$ 
  - % of positive results when HIV is present
- specificity  $99.8 - 99.99\%$ 
  - % of negative results when HIV is **not** present
- false positive  $1/250,000$  (95% CI,  $1/173,000 - 379,000$ )
  - % of positive results when HIV is **not** present
- false negative  $\leq 0.3\%$ 
  - % of negative results when HIV is present

R Chou *et al.*, *Ann. Intern. Med.* 2005

## Window period

Typical evolution of key viral and serological markers during the first weeks following infection with HIV-1 (schematic diagram)



Viral markers: RNA, Ribonucleic acid; DNA, Desoxyribonucleic acid; Ag, Antigen.  
Immunological markers: IgM/IgG, Immunoglobulin M/G antibodies.

G Murphy & JV Parry, EUROSURVEILLANCE 2008

## Window period

Table 1. HIV testing assays and their "window periods."

HIV test	Assay method	"Window period" estimates, weeks <sup>a</sup>	"Window period" reduction, days <sup>b</sup>
First-generation EIA	Viral particles used to bind patient HIV Ab, detected by marker conjugated to anti-human Ab	~6	...
Second-generation EIA	Same as first-generation EIA except uses purified HIV Ag or recombinant virus	~4-6	10
Third-generation EIA	"Antigen sandwich": synthetic peptide used to bind patient HIV Ab followed by marker conjugated to additional HIV Ag; able to detect IgM	~3-4	6
Fourth-generation EIA	Uses third-generation EIA methodology plus monoclonal Ab to p24 Ag to detect patient p24 Ag	~2	5
Pooled HIV NAT	First combines multiple individual samples into one common pool, then uses PCR or other amplification techniques to detect patient viral nucleic acids	<1-2	3
Individual HIV NAT	As above, except that samples are tested individually rather than diluted by pooling	<1-2	3

NOTE. Data are from [5, 6, 16, 19]. Ab, antibody; Ag, Antigen; HIV NAT, HIV nucleic acid testing.

<sup>a</sup> "Window periods" listed for HIV acquisition, in a study of exposure [20].

<sup>b</sup> Compared with an immunoblot, a fourth-generation EIA.

**60-65% positive 4 weeks after infection**  
**80% positive 6 weeks after infection**  
**90% positive 8 weeks after infection**  
**95-99% positive 12 weeks after infection**

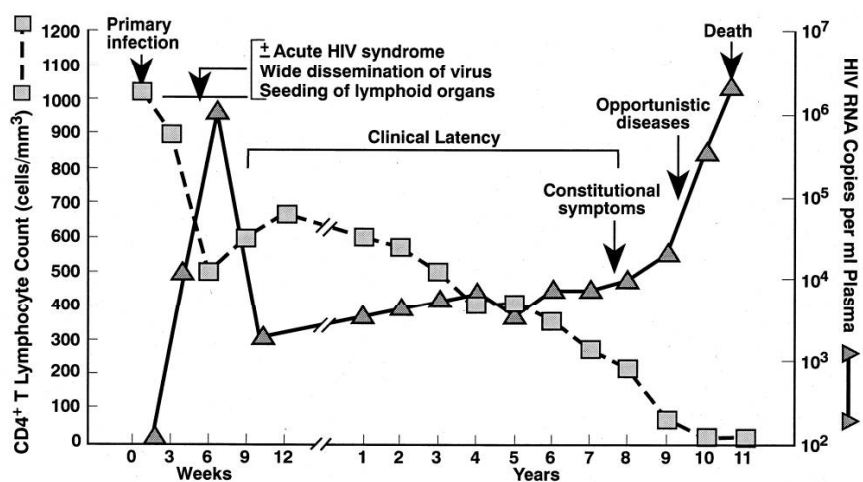
all individuals within 6 weeks of at least 6 months following the seroconversion period rather than the "window period" for

J Stekler et al., CID 2007

## Topics

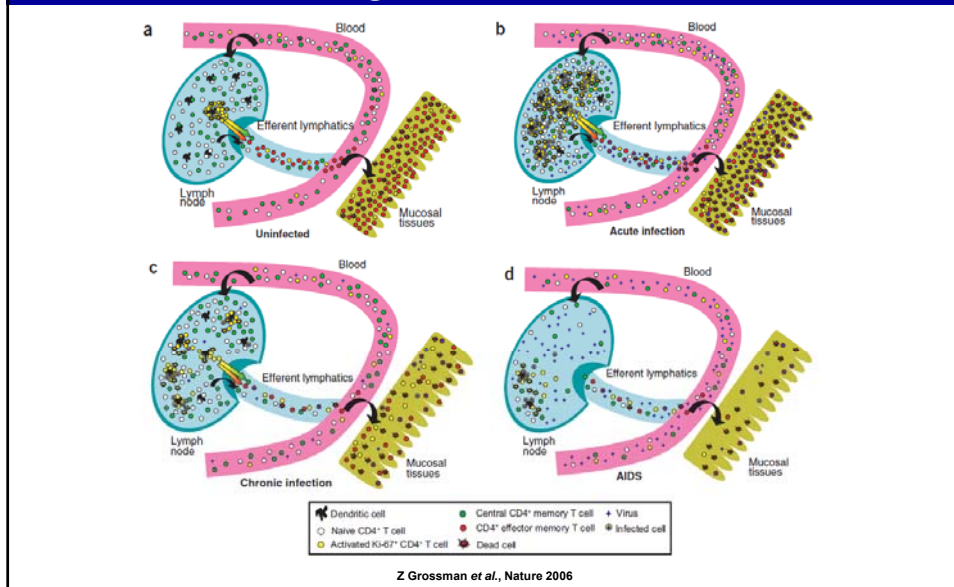
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## Natural course of HIV-1 infection

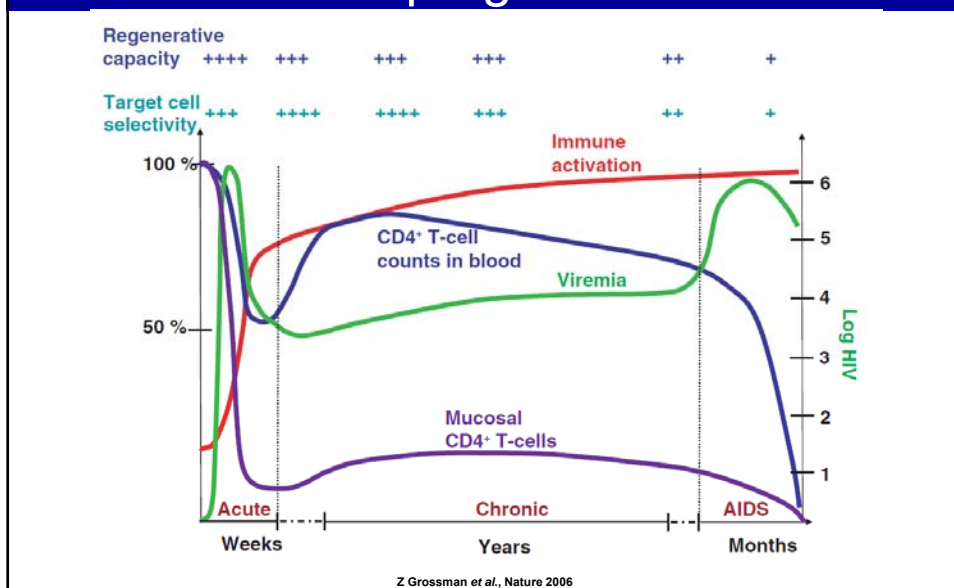


Fields, Virology

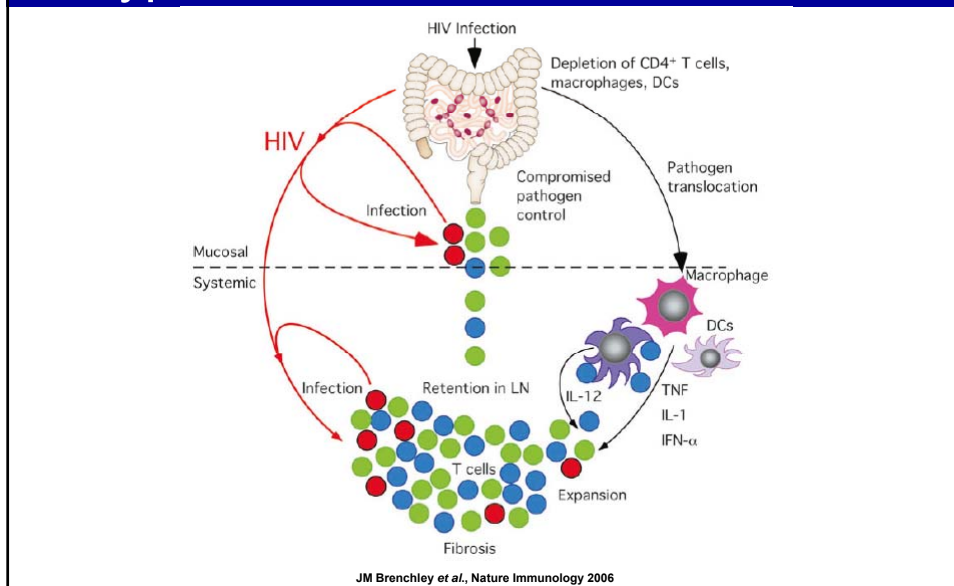
## Turnover and infection of CD4<sup>+</sup> T cells at various stages of HIV infection



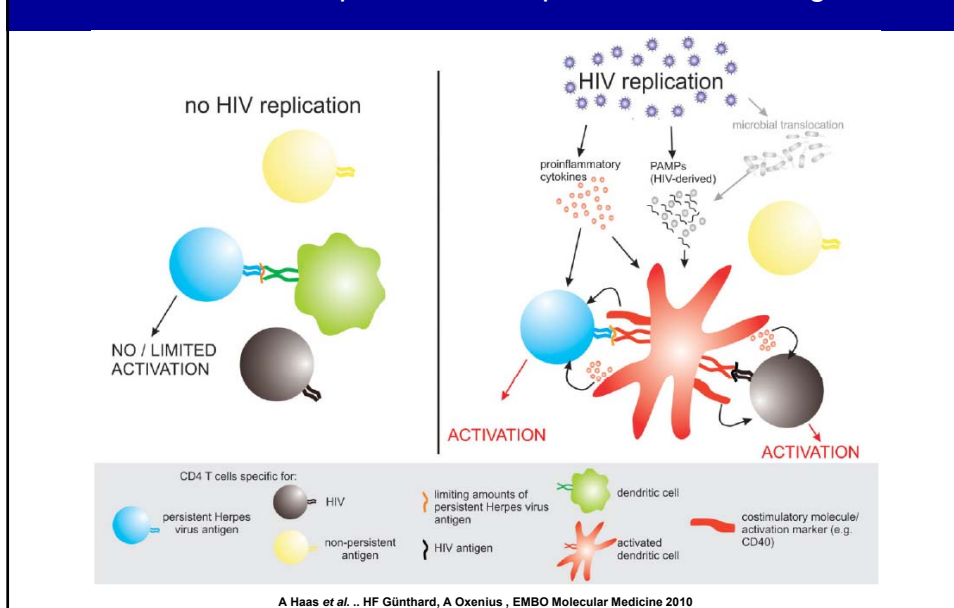
## Quantitative and qualitative measures of HIV disease progression



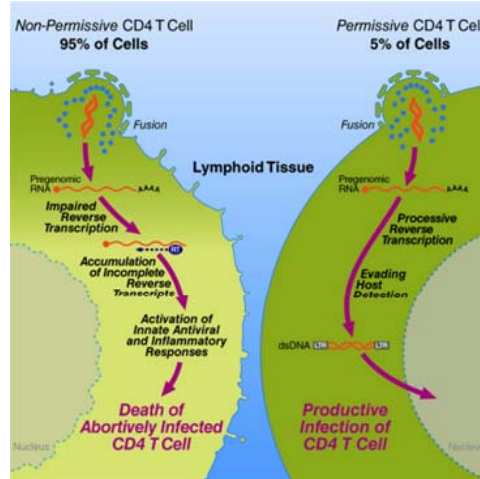
## “Cycle of HIV disease pathogenesis: a hypothesis”



## “Proposed mechanisms of how HIV replication induces activation of CD4<sup>+</sup> T cells with specificities for persistent viral antigens”

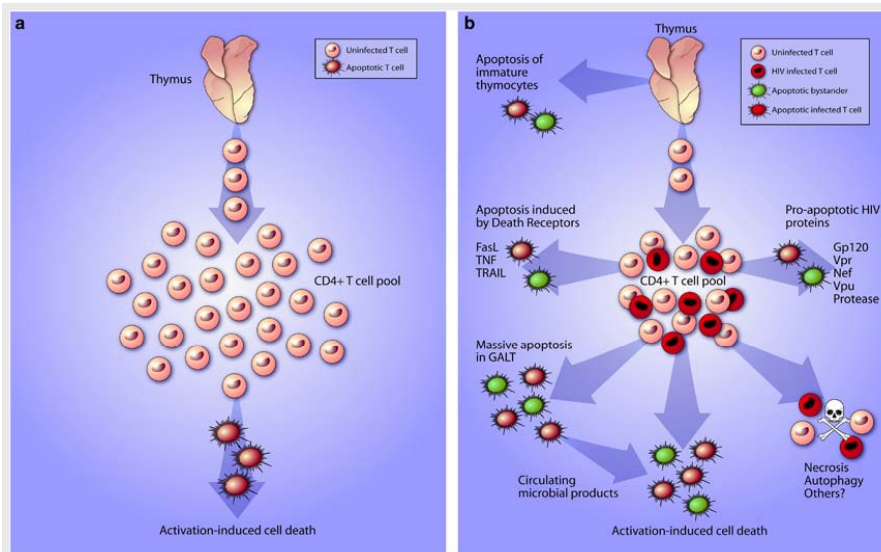


“Abortive HIV infection mediates CD4 T cell depletion and inflammation in human lymphoid tissue”



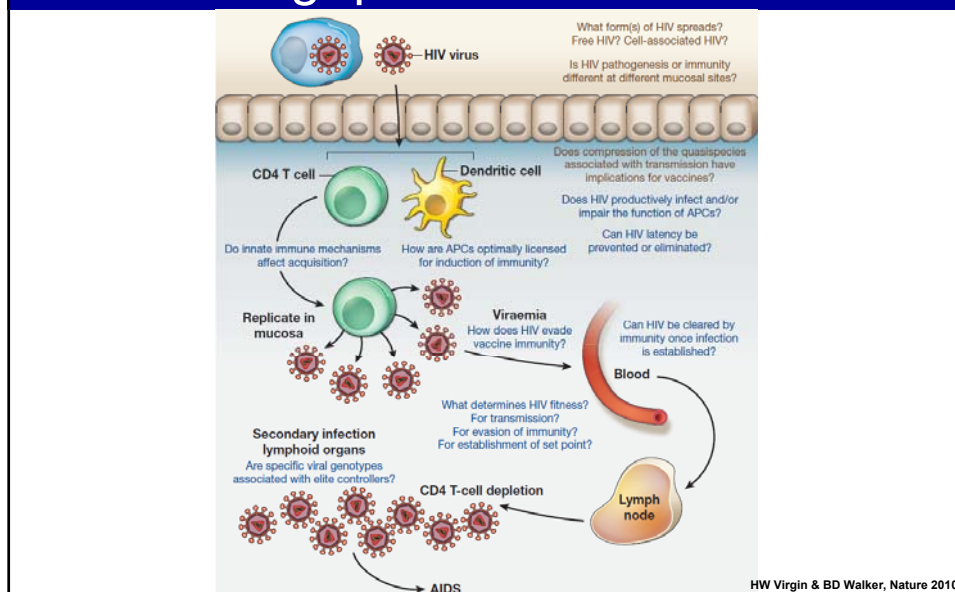
© Doitsh et al., Cell 2010

Mechanisms of CD4<sup>+</sup> T-cell depletion



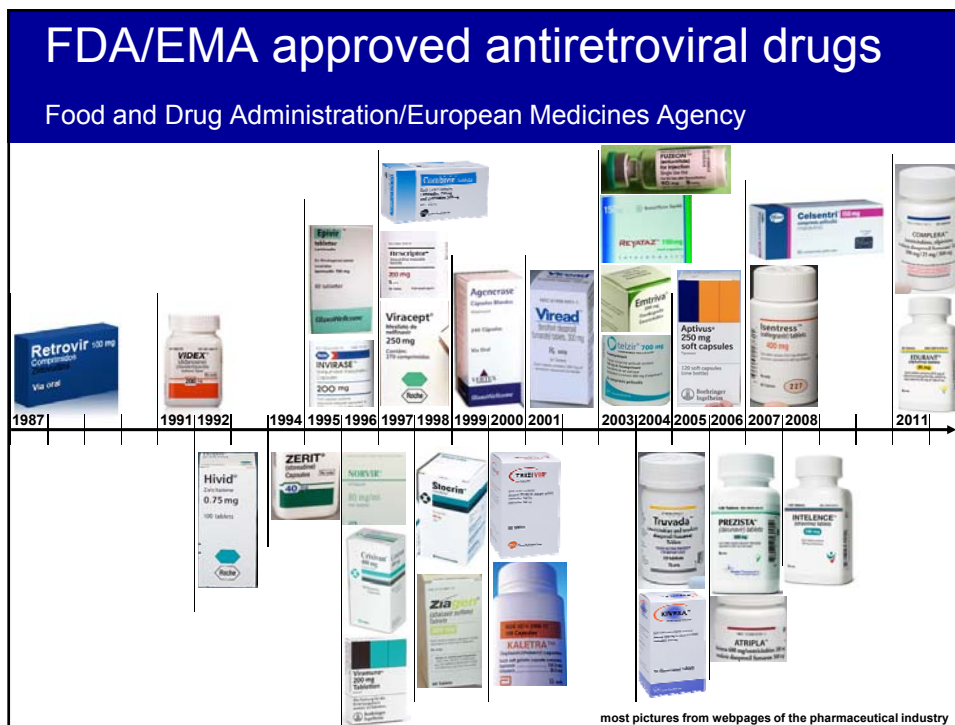
NW Cummins & AD Badley, Cell Death & Disease 2010

## HIV pathogenesis and some outstanding questions



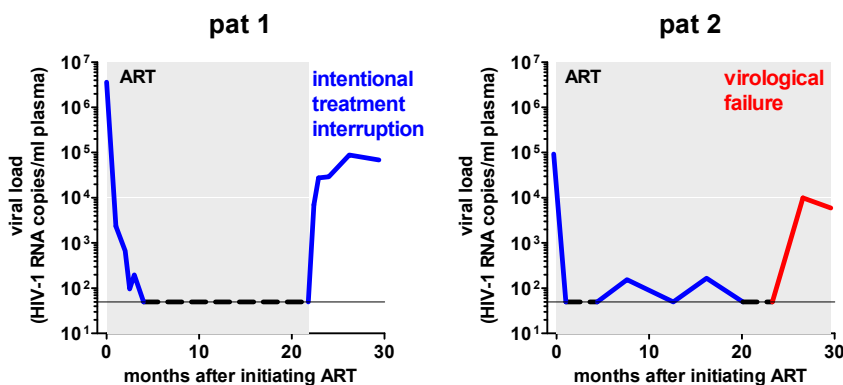
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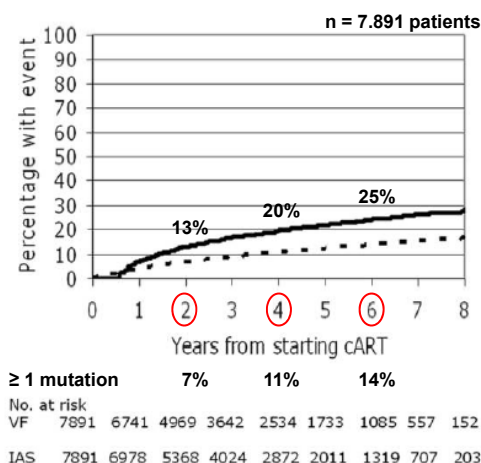


## Challenges of current ART and on the way to the eradication of HIV

- viral reservoir (latent infection)
- drug resistance (ongoing replication)



## Rate of virologic failure and development of drug resistance



The UK Collaborative Group, CID 2010

## resistere = hold back

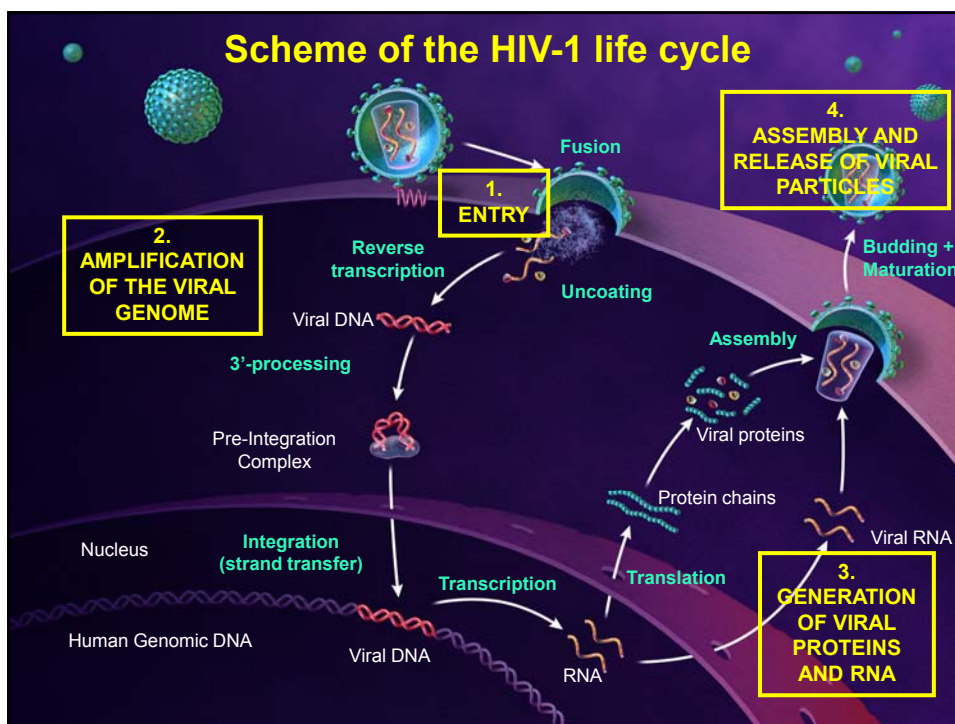
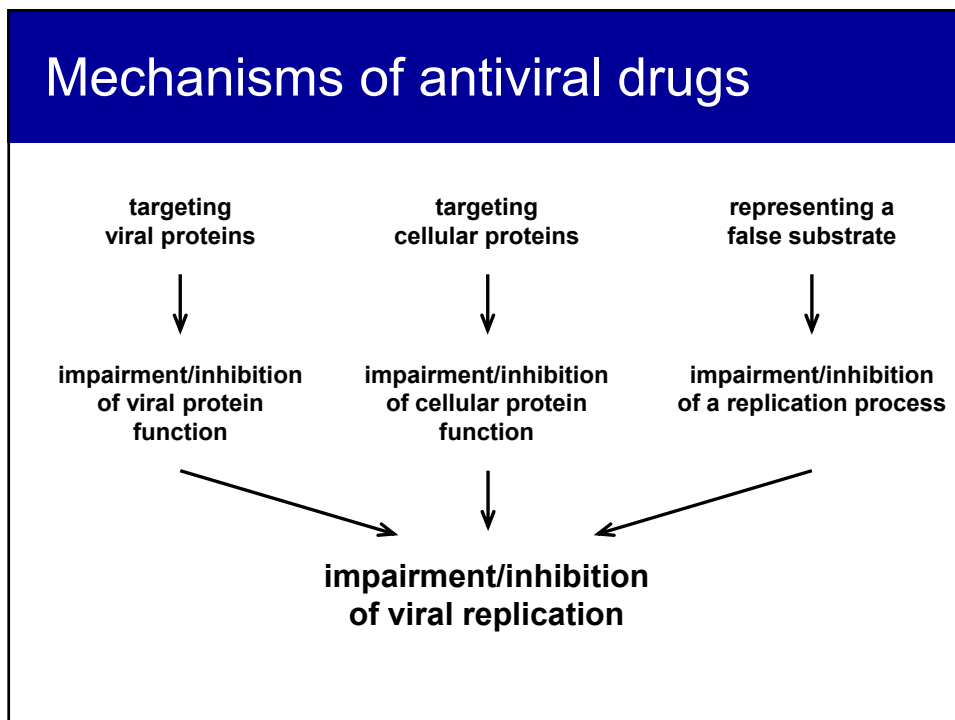
### resistance

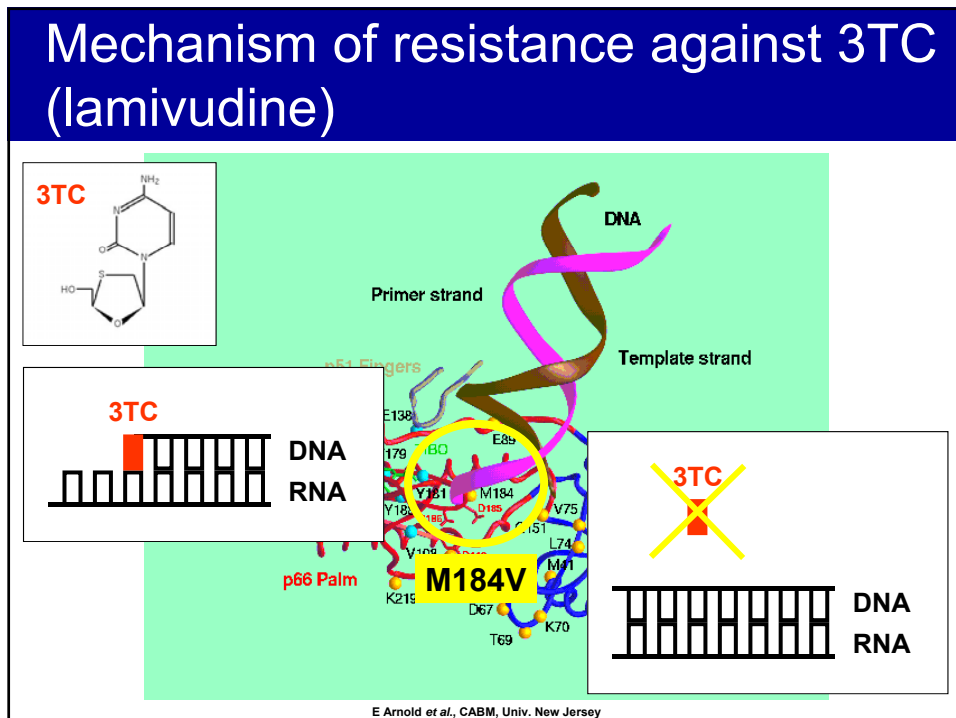
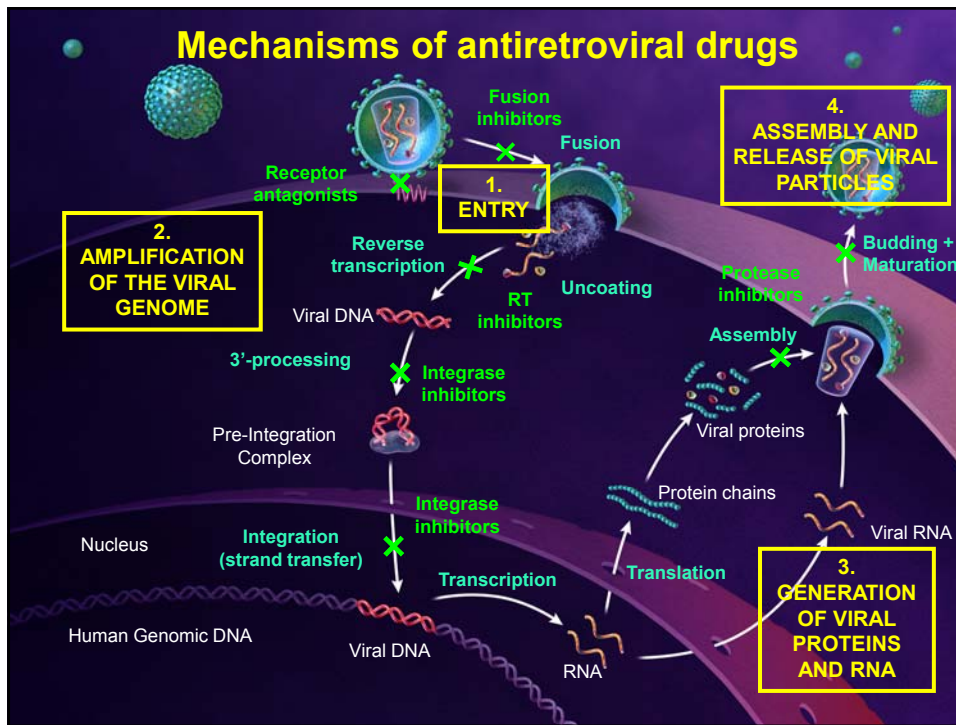
Pronunciation: /rɪ'zɪst(ə)ns /

noun

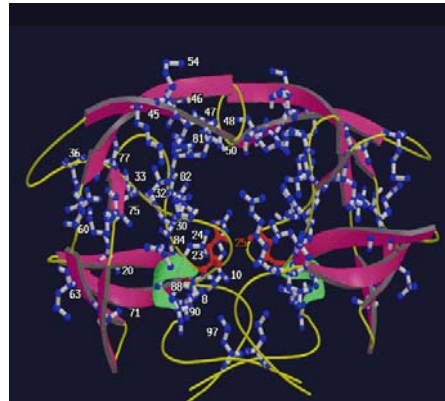
- [mass noun] the refusal to accept or comply with something:  
*they displayed a narrow-minded resistance to change*
  - the use of force or violence to oppose someone or something:  
*government forces were unable to crush guerrilla-style resistance*  
*she put up no resistance to being led away*
  - (also resistance movement) a secret organization resisting authority, especially in an occupied country
  - (the Resistance) the underground movement formed in France during the Second World War to fight the German occupying forces and the Vichy government. Also called *Maquis*
- the ability not to be affected by something, especially adversely:  
*some of us have a lower resistance to cold than others*
  - [mass noun] *Medicine & Biology* lack of sensitivity to a drug, insecticide, etc especially as a result of continued exposure or genetic change
- the impeding or stopping effect exerted by one material thing on another:  
*air resistance was reduced by streamlining*
- the degree to which a substance or device opposes the passage of an electric current causing energy dissipation. By Ohm's law resistance

Oxford Dictionaries  
The world's most trusted dictionaries





## HIV-1: Resistance against protease inhibitors



up to 25% of  
all amino  
acids can be  
mutated in a  
PI-resistant  
virus (and the  
protease still  
functions)

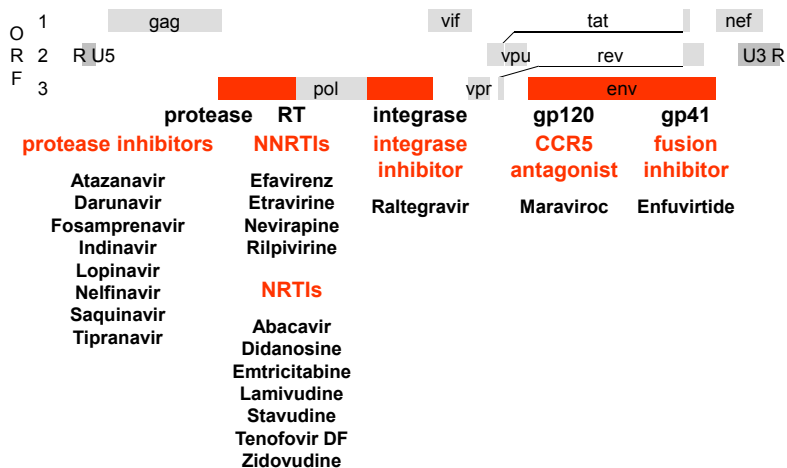
hiv-web.lanl.gov

## Resistance mutations in the HIV-1 protease

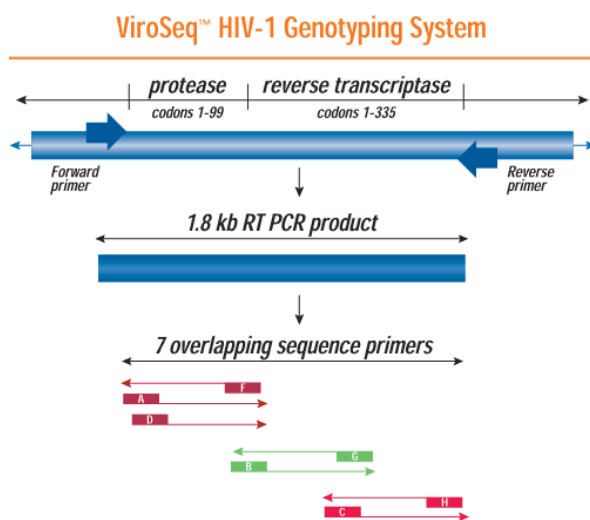
Atazanavir +/− ritonavir*	L	G	K	L	V	L	F	M	M	G	I	F	I	D	I	I	A	G	V	I	N	L	I	
	10	16	20	24	32	33	34	36	46	48	50	53	54	60	62	64	71	73	82	84	85	88	90	93
	I	E	B	I	I	I	Q	I	I	V	L	L	L	E	V	L	V	C	A	V	V	S	M	L
	F	C	T	V	V	V	L	V	L	V	M	I	A	I	L	A	I	I	I	I	I	I	I	I
Darunavir/ ritonavir*	V				V	L			I	I	I							T	L	I	L			
	11				32	33			47	50	54							74	76	84	89			
	I				I	F			V	V	M							P	V	V	V			
Fosamprenavir/ ritonavir*	L				V				M	I	I	I					G	L	V	I	L			
	10				21				46	47	50	54					72	76	82	84	90			
	F				I				I	V	V	L	V				S	V	A	V	V	M		
	S				V				L	V	M						A	P	S	T				
Indinavir/ ritonavir*	L	K	L	Y	M				M	I	I					A	G	L	V	I	L			
	10	20	24	32	36				46	47	50	54				71	73	76	77	82	84	90		
	I	M	I	I	I				I	V	V	V	I			V	S	V	I	A	V	M		
	I	B	V						L							T	A	I	I					
Lopinavir/ ritonavir*	L	K	L	V	L				M	I	I	F	I	L	A	G	L	V	I	L				
	10	20	24	32	33				46	47	50	53	54	63	71	73	76	82	84	90				
	F	M	I	I	F				I	V	V	L	V	P	V	V	S	V	A	V	M			
	I	B	V						L	A	L	A	M	S					A	I	T	S		
Nelfinavir*	L				D				M		M						A	V	V	I	N	L		
	10				30				36		46						71	77	82	84	88	90		
	F				N				I		L						V	T	I	A	V	S	M	
	I																T	I	I					
Saquinavir/ ritonavir*	L				L						G	I	I	I	A	G	V	V	I	L				
	10				24						48	54	62	71	73	77	82	84	90					
	I				I						V	V	V	T	S	I	A	V	M					
	I																							
Tipranavir/ ritonavir*	L				L	M			K	M	I	I	Q	H	T	V	N	I	L					
	10				23	36			43	46	47	54	58	69	74	82	83	84	89					
	V				F	I			T	L	V	A	E	K	P	L	D	V	I	M				

VA Johnson et al., Top. HIV Med. 2010

## Genotypic HIV drug resistance testing

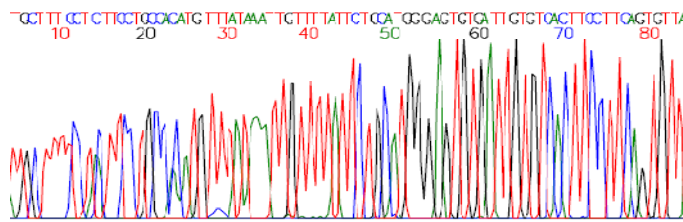


## HIV-1 resistance testing: Genotype (bulk sequencing)



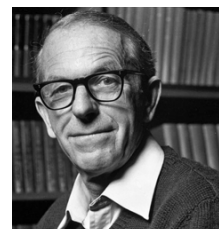
[www.abbottmolecular.com/ViroSeqHIV1GenotypingSystem\\_1079.aspx](http://www.abbottmolecular.com/ViroSeqHIV1GenotypingSystem_1079.aspx)

## Sanger ddNTP chain termination sequencing

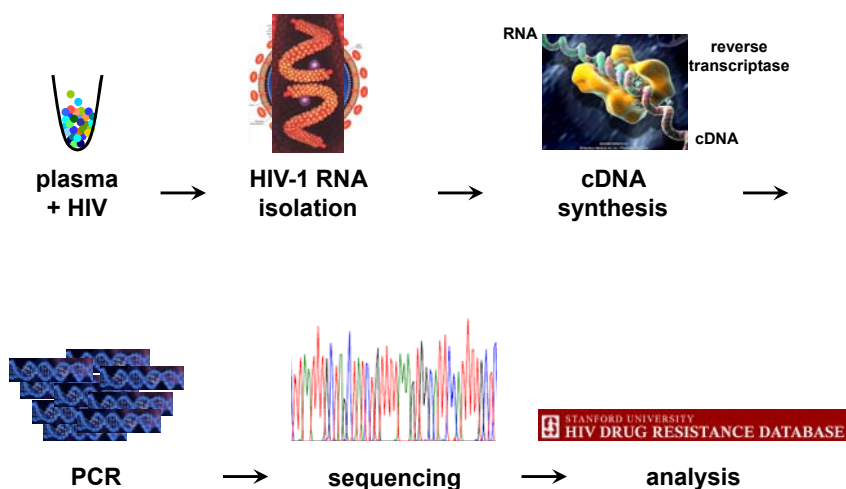


Frederick Sanger

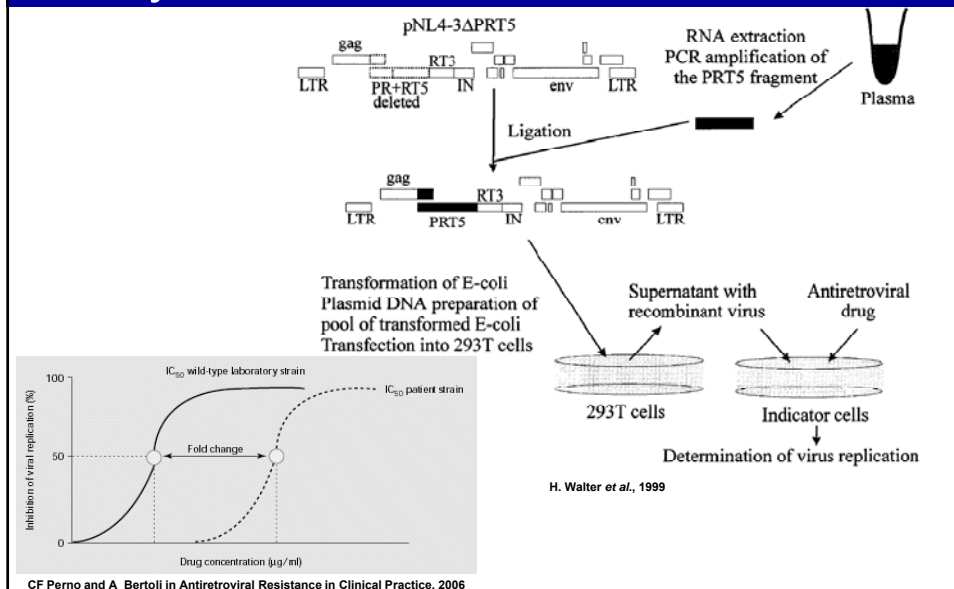
2. Nobel Prize in Chemistry in 1980  
for the determination of base sequences in nucleic acids  
1. Nobel price in Chemistry in 1958  
for his work on the structure of proteins, especially that of insulin



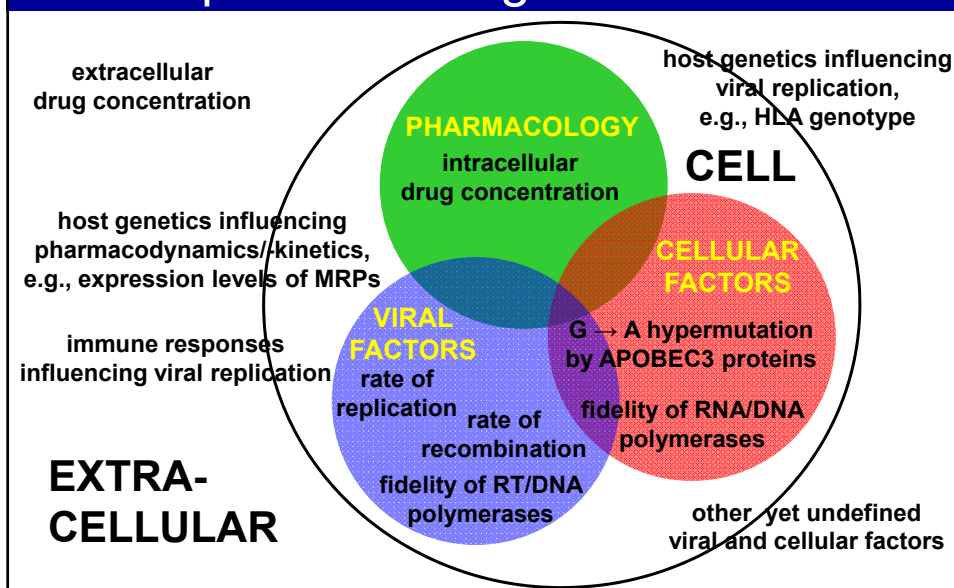
## Plasma sample to genotype



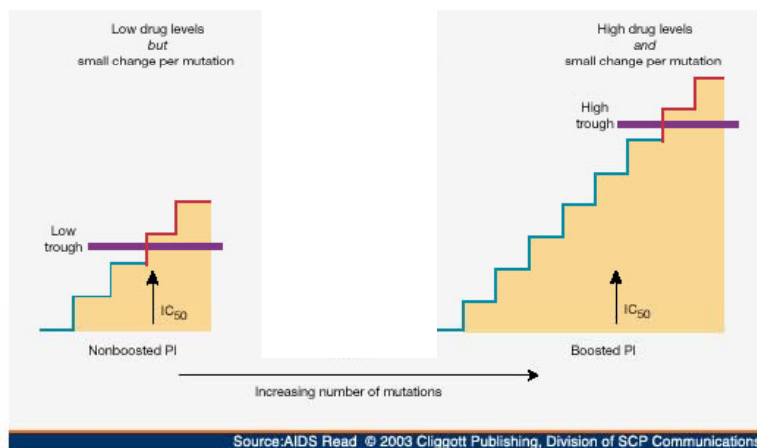
## HIV-1 resistance testing: Phenotype assay



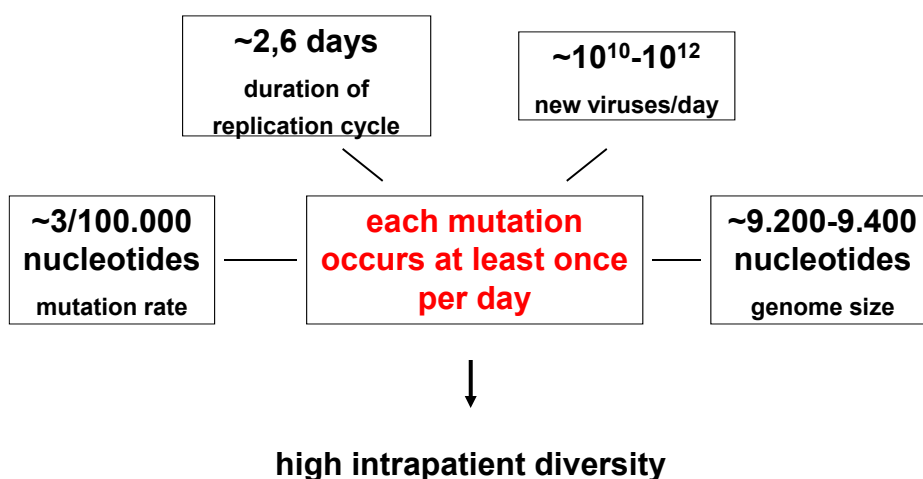
## Some factors influencing the development of drug resistance



## Potency of treatment is inversely associated with the development of drug resistance



## Evolution and diversity of HIV



## Summary

- current forth generation screening tests are highly specific and sensitive
  - CAVE: window period
- pathogenesis of HIV is mainly driven by virus replication, CD4<sup>+</sup> T cell depletion, and chronic immune activation
- development of resistance depends mainly on efficacies of drugs, genetic barriers, and mutation rate of the virus