Antivirals

Lecture 20
Biology 3310/4310
Virology
Spring 2017

You can't go back and you can't stand still. If the thunder don't get you, then the lightning will.
Jerry Garcia
The Wheel (lyrics by Robert Hunter)

Vaccines can prevent viral disease

- But they have modest or no therapeutic effect if an individual is already infected (exception?)
- Our second arm of antiviral defense is antivirals
- Can stop infection once it has started

Despite 50 years of research, our arsenal of antiviral drugs remains dangerously small

Only about 100 antiviral drugs are available on the US market

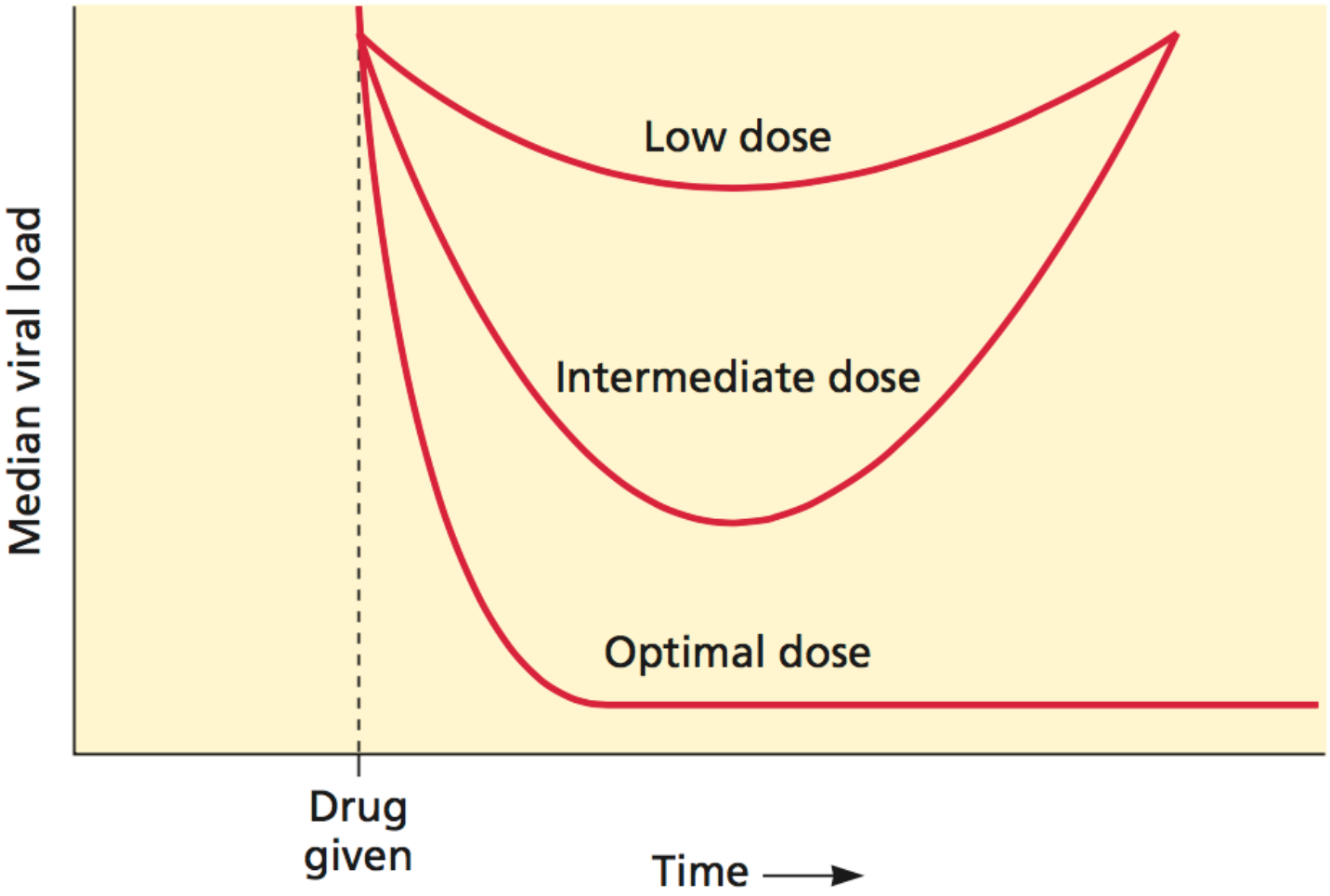
Most against HIV, HCV, herpesviruses - Persistent infections

Why are there so few antiviral drugs?

- Compounds interfering with virus growth can adversely affect the host cell
  - *Side effects are common (unacceptable)*
  - *Every step in viral life cycle engages host functions*
- Some medically important viruses can't be propagated, have no animal model, or are dangerous
  - *HBV, HPV*
  - *Smallpox*
  - *Ebola, Lassa*

An unappreciated third reason may be the most important

- A compound must block virus replication completely! It must be *potent*
- Many standard pharmaceuticals can be effective if enzyme activity is partially blocked
- Partial inhibition is not acceptable for an antiviral drug - resistant mutants will arise
- Makes drug discovery expensive



Principles of Virology, ASM Press

Another serious problem for antiviral discovery:

Many acute infections are of short duration

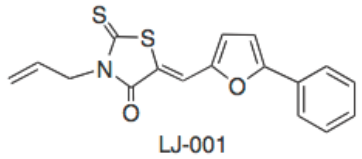
- By the time the patient feels ill, it is too late to impact clinical disease
- Antiviral drugs for these viruses must be given early in infection or *prophylactically* to populations at risk
  - *Safety issues; giving drugs to healthy people not wise (exception: PrEP)*
- No broad-spectrum antiviral agents are currently available
- Lack of rapid diagnostic reagents has hampered development of antiviral drugs

LJ1001, a broad spectrum antiviral

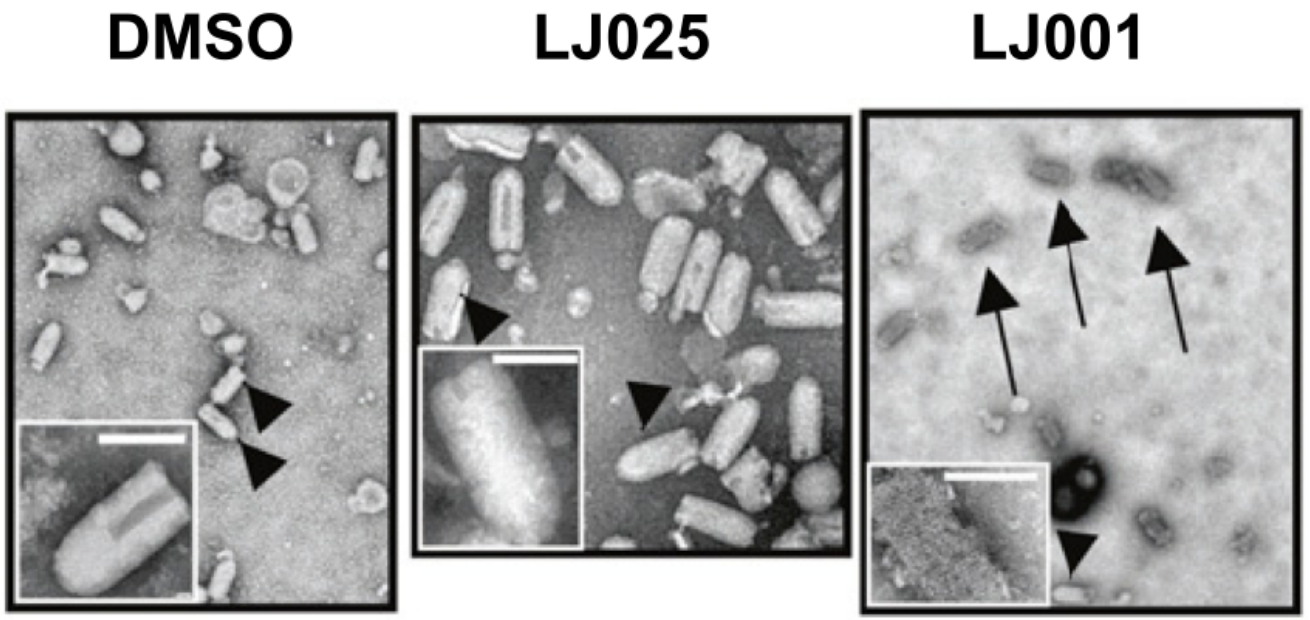
Virus	Family	Genome type	Envelope (yes/no)	Activity
Ebola[L] (cat A)	Filoviridae	ssRNA(−)	Y	++
Marburg[L] (cat A)	Filoviridae	ssRNA(−)	Y	++
Influenza A[L] (cat A)	Orthomyxoviridae	ssRNA(−)	Y	+++
Junín[L] (cat A)	Arenaviridae	ssRNA(−)	Y	++
Rift Valley fever[L] (cat A)	Bunyaviridae	ssRNA(−)	Y	+++
LaCrosse[L] (cat B)	Bunyaviridae	ssRNA(−)	Y	+++
Nipah[L,P] (cat C)	Paramyxoviridae	ssRNA(−)	Y	++
Omsk hemorrhagic fever[L] (cat C)	Flaviviridae	ssRNA(+)	Y	++
RSSE[L] (cat C)	Flaviviridae	ssRNA(+)	Y	++
PIV-5[L]	Paramyxoviridae	ssRNA(−)	Y	++
HPIV-3[L]	Paramyxoviridae	ssRNA(−)	Y	++
Newcastle disease[L] *	Paramyxoviridae	ssRNA(−)	Y	++
HIV-1[L,P] *	Retroviridae	ssRNA(−)RT	Y	++
Murine leukemia[L]	Retroviridae	ssRNA(−)RT	Y	++
Yellow fever[L]	Flaviviridae	ssRNA(+)	Y	+++
Hepatitis C[L]	Flaviviridae	ssRNA(+)	Y	+++
West Nile[L]	Flaviviridae	ssRNA(+)	Y	+++
Vesicular stomatitis[L,P]	Rhabdoviridae	ssRNA(−)	Y	++
Cowpox[L]	Poxviridae	dsDNA	Y	+
Vaccinia[L]	Poxviridae	dsDNA	Y	++
Adenovirus[L] **	Adenoviridae	dsDNA	N	−
Coxsackie B[L] **	Picornaviridae	ssRNA(+)	N	−
Reovirus[L]	Reoviridae	dsRNA	N	−

http://www.ncbi.nlm.nih.gov/pubmed/20133606

LJ1001, a broad spectrum antiviral



LJ-001



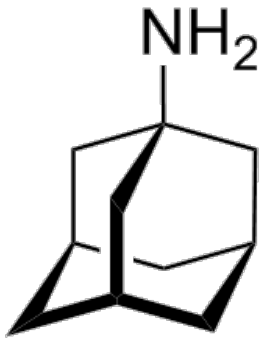
Antiviral history

- The first modest search for antiviral drugs occurred in the early 1950s
  - *Chemists looked at derivatives of the sulfonamide antibiotics*
  - *Synthesis of thiosemicarbazones active against poxviruses*
  - *Smallpox was still a major threat after WWII*
- 1960s and 1970s: “blind screening” programs to find chemicals with antiviral activity
  - *Spurred on by successes in the treatment of bacterial infections with antibiotics*

Blind screening

- No attempt to focus discovery on a virus or a virus-specific mechanism
- Random chemicals and natural product mixtures tested for ability to block replication of a variety of viruses in cell culture systems
- **Hits**, compounds or mixtures that block *in vitro* viral replication; purified and fractions tested in various cell culture and animal models for safety and efficacy
- Promising molecules called **leads** were modified systematically by medicinal chemists
  - *To reduce toxicity, increase solubility and bioavailability*
  - *To improve other pharmacokinetic properties*

Thousands of molecules were made and screened before a specific antiviral was even tested in humans

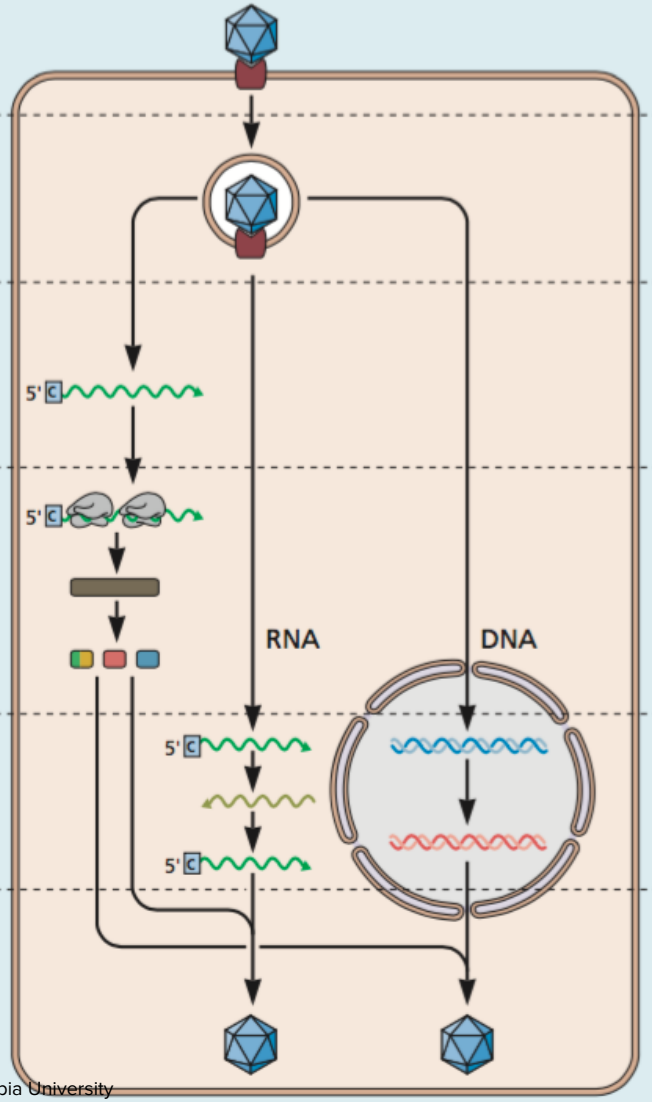


- Considerable effort, very little success
- One exception: Symmetrel (amantadine)
  - *Approved late 1960s for treatment of influenza A virus infections*
  - *One of three drugs now available for influenza*
- Mechanism of action was often unknown or speculative
  - *Mechanism of action of Symmetrel deduced early 1990s*

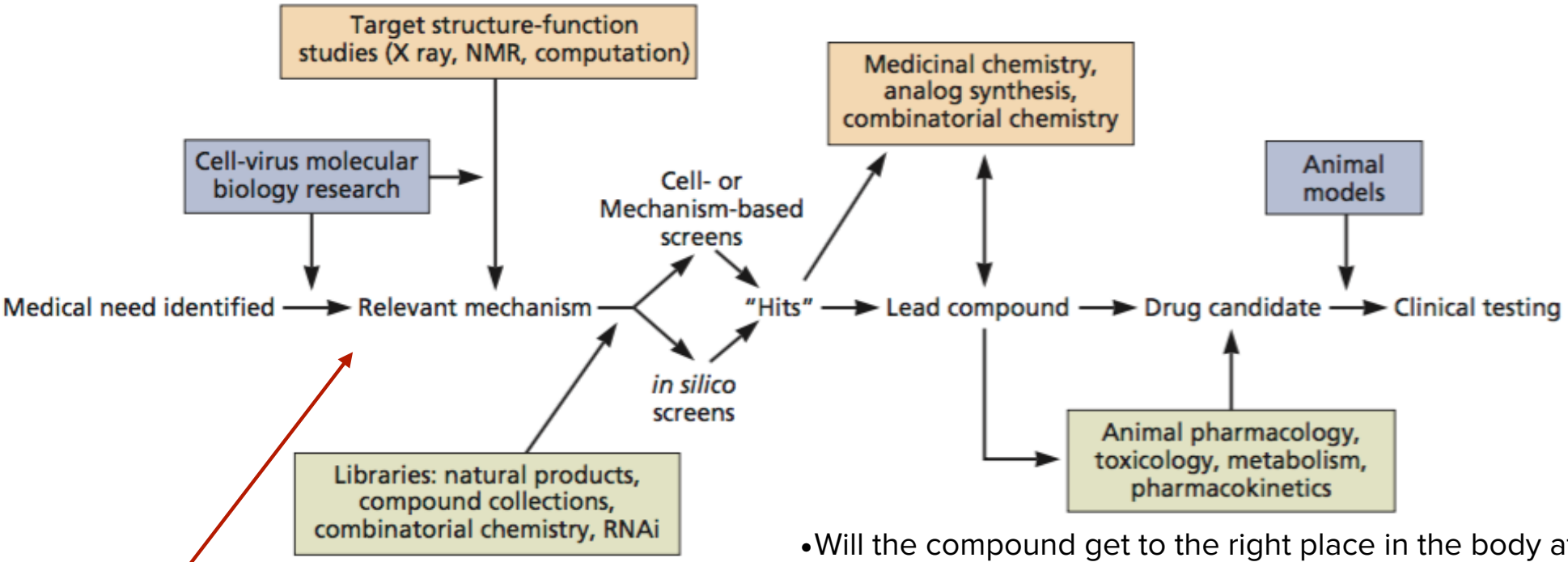
Antiviral discovery today

- Recombinant DNA technology & sophisticated chemistry make targeted discovery possible
- Essential viral genes cloned, expressed in genetically tractable organisms, purified, analyzed in atomic detail
- Life cycles of most viruses known, targets for intervention can be generalized
- Modern technology allows inhibitors to be found even for viruses that cannot be propagated in cell culture
- Blind screening procedures are dead

Function	Lead compound or example	Virus
Attachment	Peptide analogs of attachment protein	HIV
Penetration and uncoating	Dextran sulfate, heparin	HIV, herpes simplex virus
mRNA synthesis	Interferon	Hepatitis A, B, and C viruses; papillomavirus
	Antisense oligonucleotides	Papillomavirus, human cytomegalovirus
Protein synthesis/ Initiation	Interferon	Hepatitis A, B, and C viruses; papillomavirus
DNA/RNA replication	Nucleoside, nonnucleoside analogs	Herpesviruses, HIV, hepatitis B and C virus
Assembly	Peptidomimetics	HIV, herpes simplex virus



The path of drug discovery



Proof of principle

- Will the compound get to the right place in the body at the right concentration? (bioavailability)
- Will the compound persist in the body long enough to be effective? (pharmacokinetics)
- Will the compound be safe? (toxicity and specificity)

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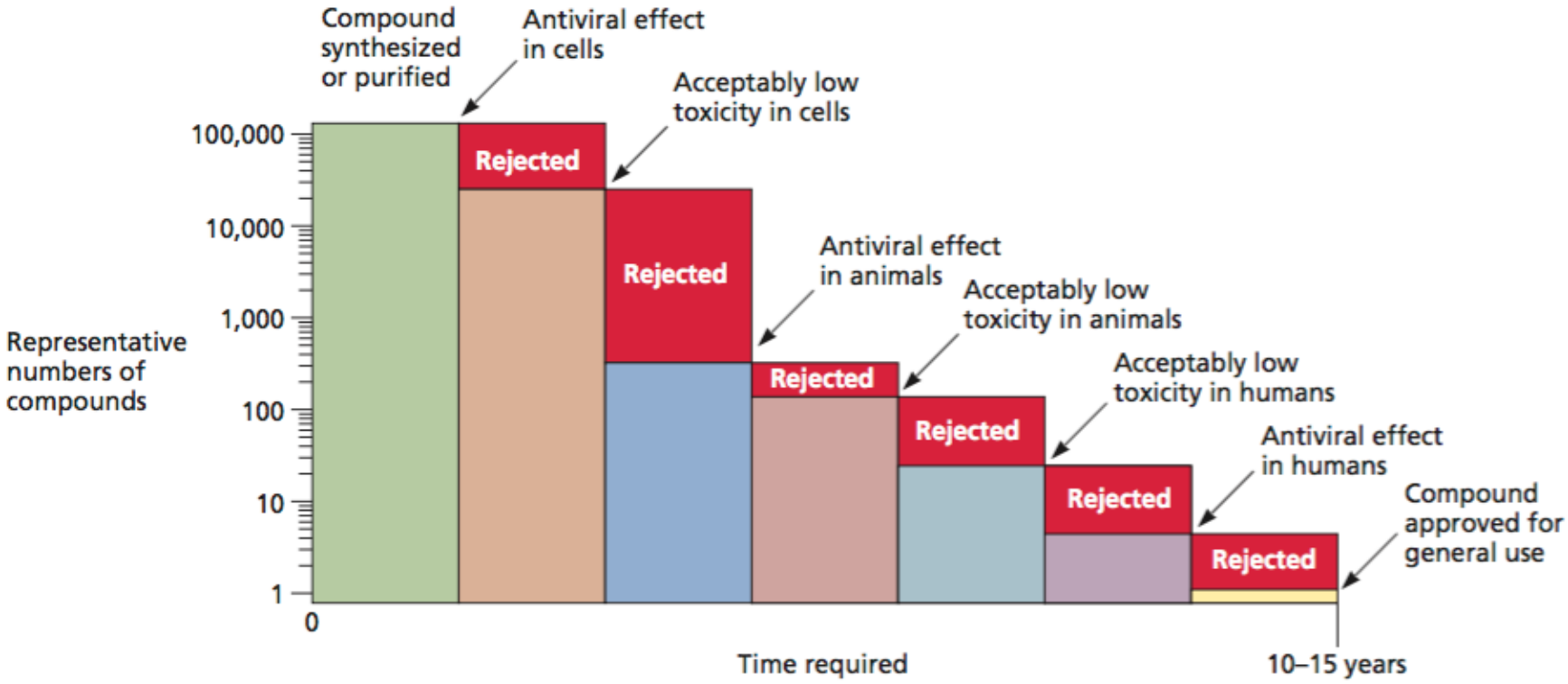
Significant hurdles stand in the way of finding effective antiviral drugs



It is not unusual for the cost to bring an antiviral drug to market to **exceed $100-200 million dollars!**

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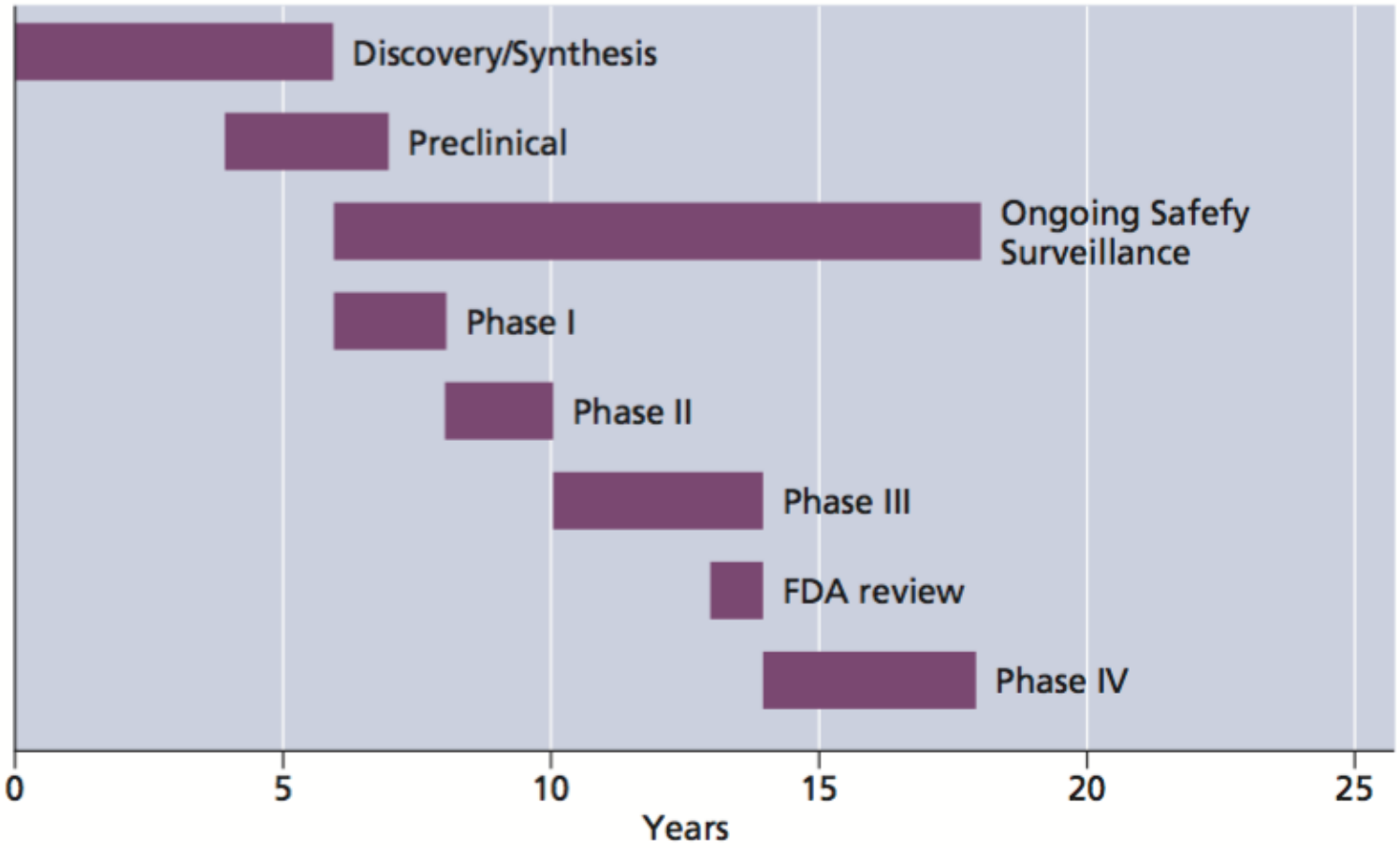
From drug discovery to the clinic



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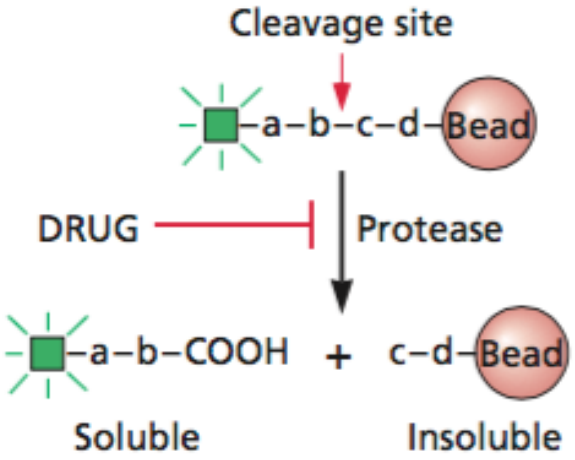
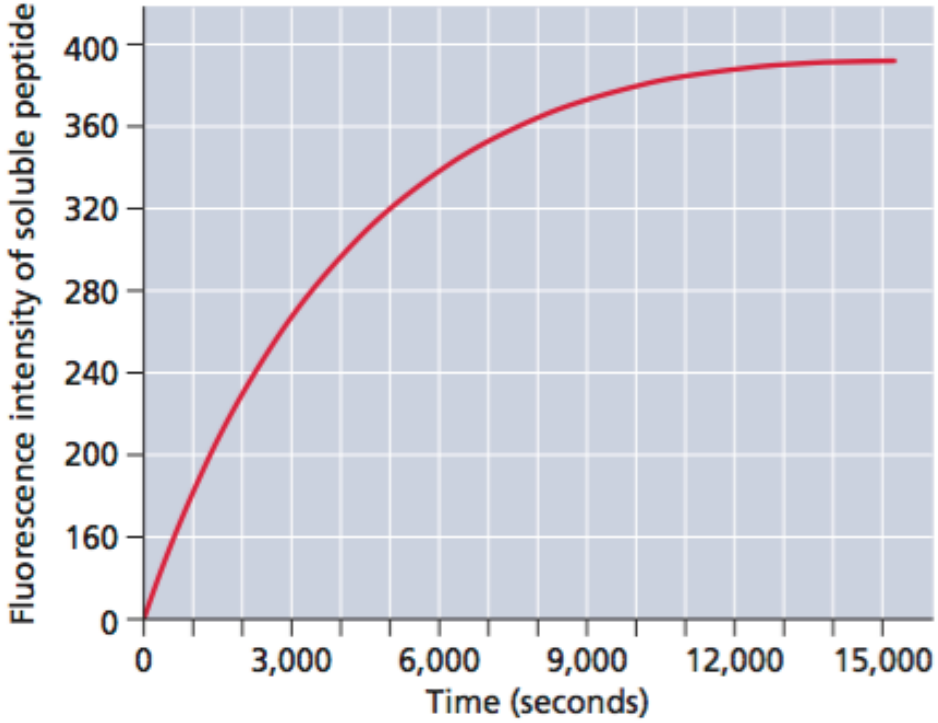
Mechanism-based screens





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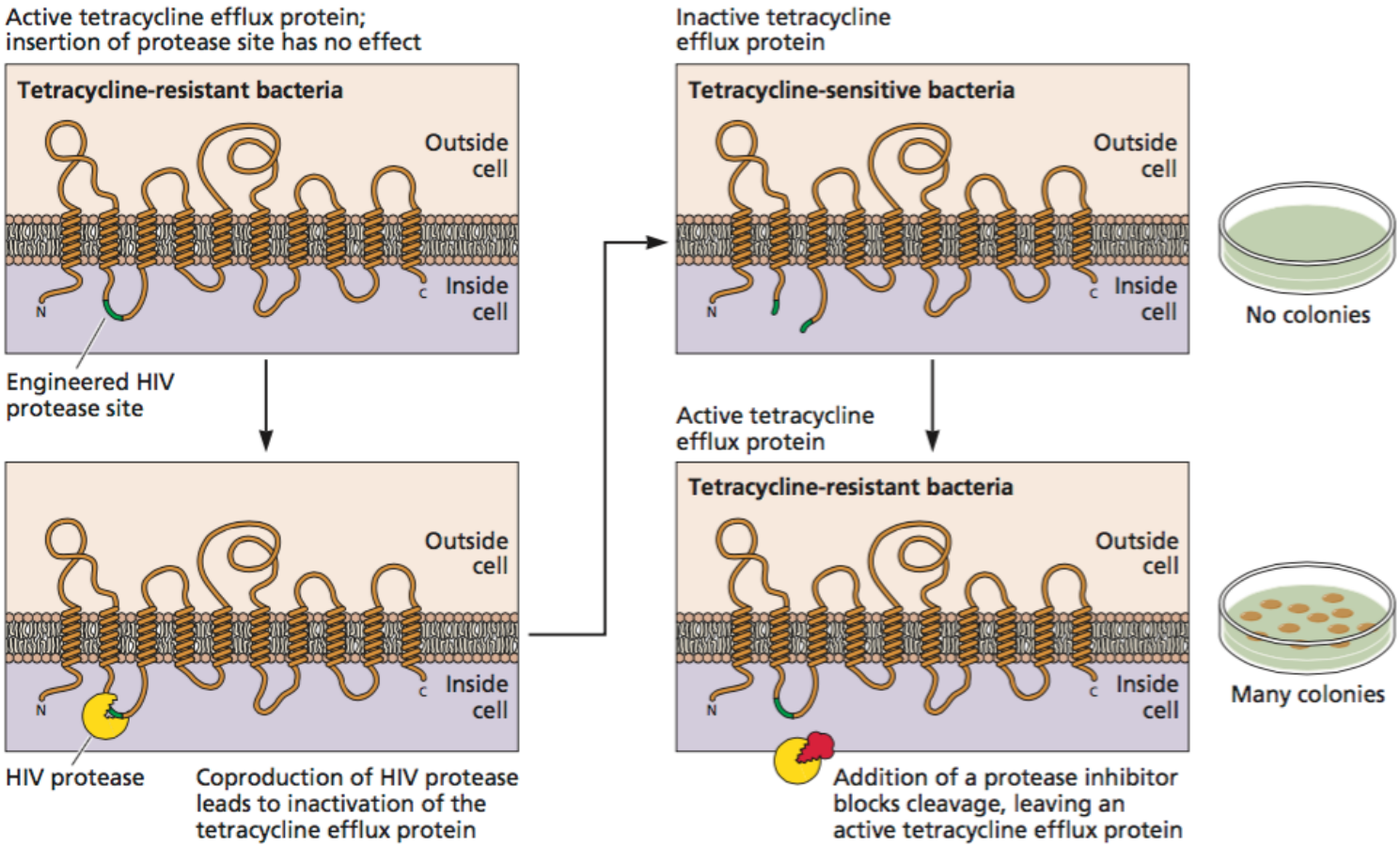
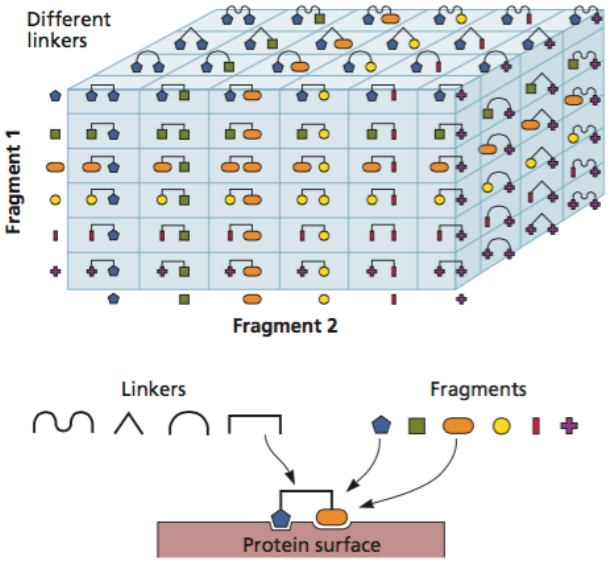
Cell-based screen

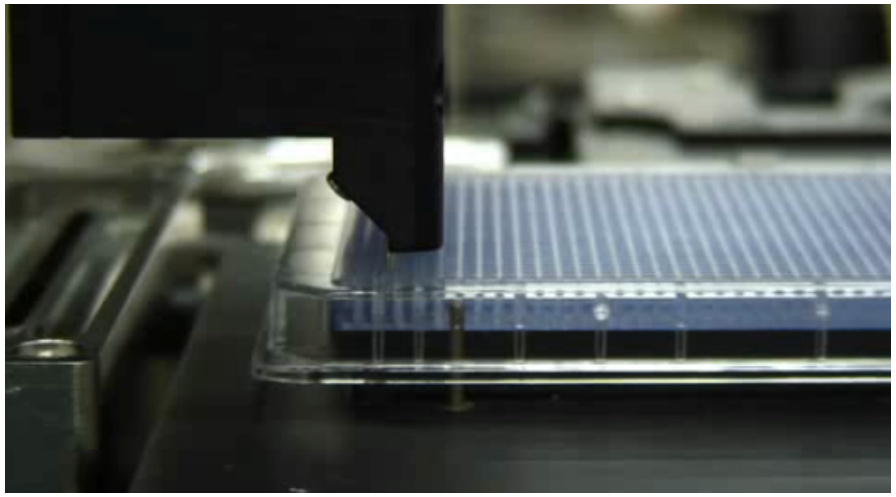


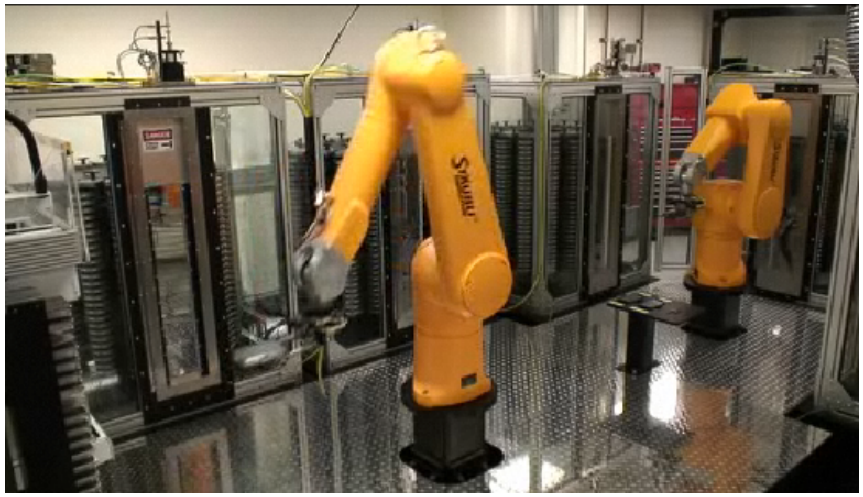
Antiviral screening

- High-throughput: 10,000 compounds/day
- Chemical libraries
- Natural products
- Combinatorial chemistry
- Structure-based design
- *In silico* screening



High throughput screening





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room number: virus

We have many antibiotics, but fewer antivirals. What is a reason for the difference?

A. Robotic screening is slow
B. There are few serious viral infections
C. Resistance is a problem
D. Antivirals must be potent
E. All of the above

Resistance to antiviral drugs

- Resistance to **any** antiviral drug must be anticipated
  - Viruses replicate efficiently
  - Modest to high mutation frequencies
- Special concern during extended therapy for chronic infections (HIV, HBV, HCV)
- Viral mutants resistant to every antiviral drug in arsenal have been detected
- Disconcerting because antiviral arsenal is small

Dangers of drug resistance

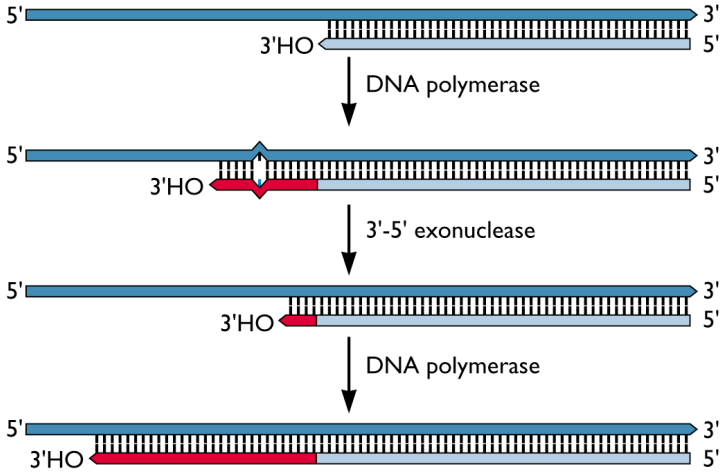
- Patient cannot be treated with same drug
- If no other drug is available, infection cannot be stopped
- Genetic analysis of resistance provides insight into antiviral mechanism
- May reveal new strategies to reduce or circumvent problem

Mechanisms of drug resistance

- RNA viruses: error prone RNA polymerase, no correction mechanism
- One misincorporation in 10^4 - 10^5 nucleotides polymerized (10^6 greater than host DNA genome)
- In RNA viral genome of 10 kb, this frequency leads to one mutation in 1-10 genomes

Mechanisms of drug resistance

- DNA viruses: most DNA polymerases can excise and replace misincorporated nucleotides
- DNA viruses evolve more slowly than RNA viruses because they have less diversity



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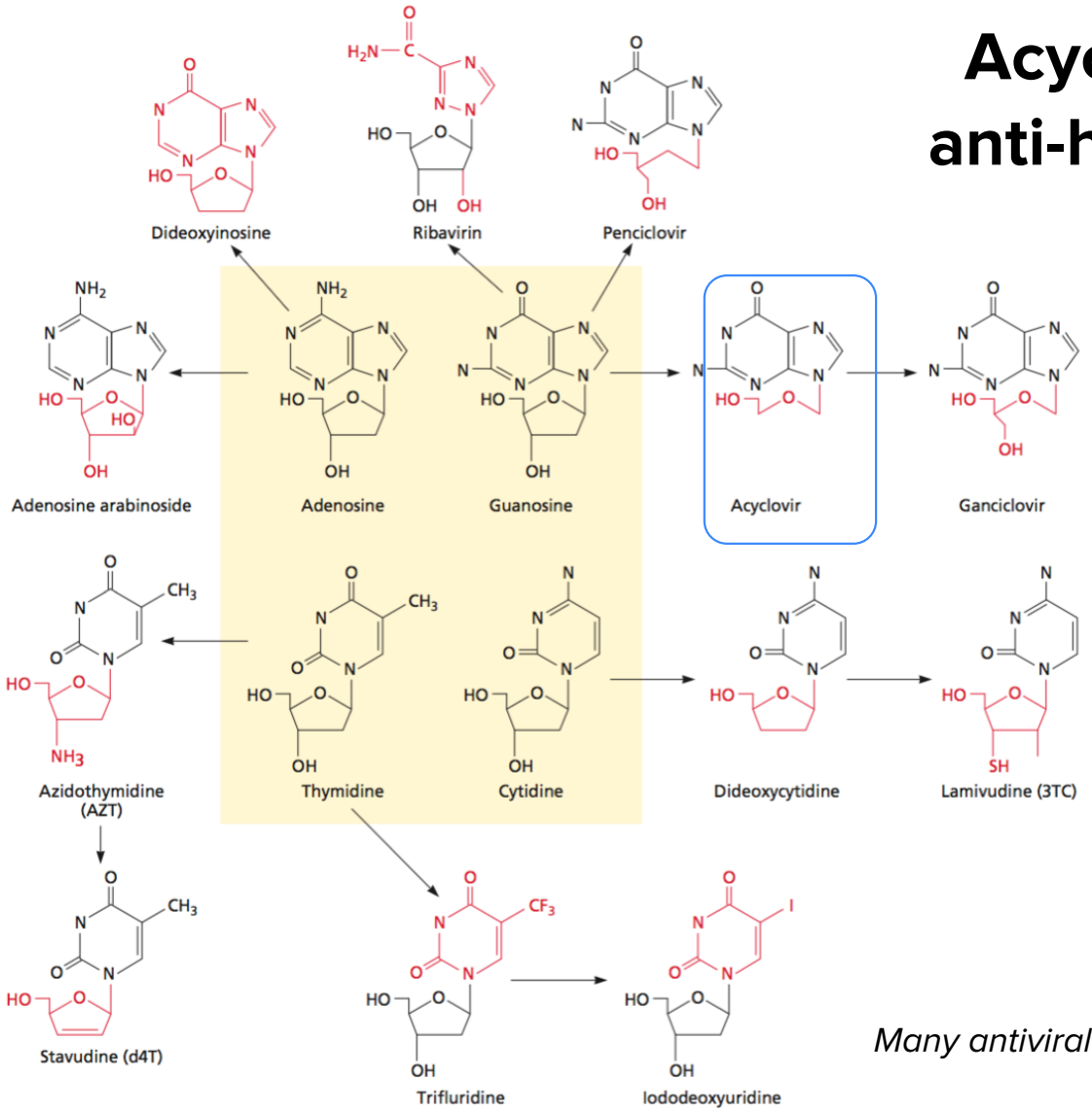
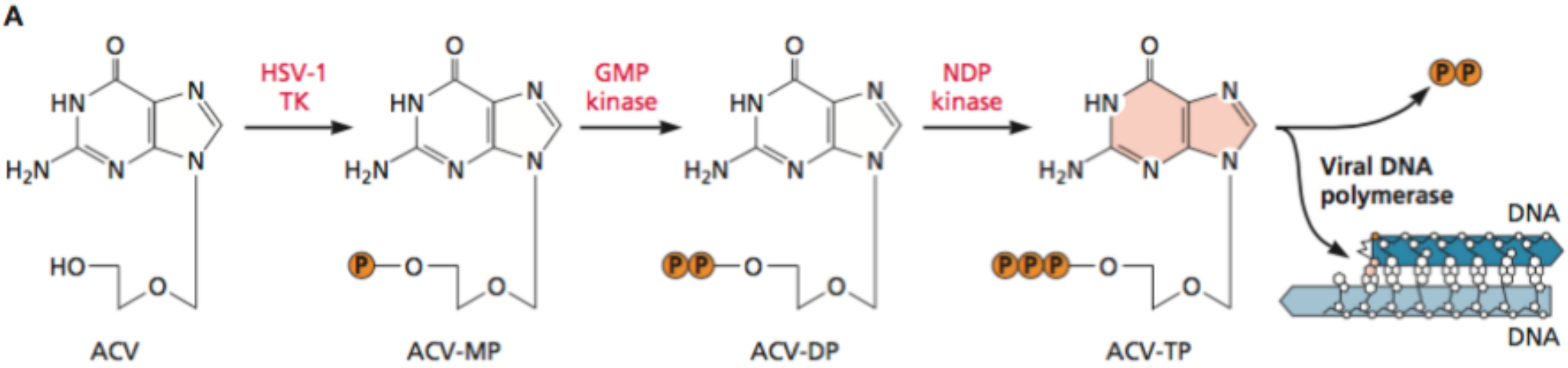
Acyclovir, a highly effective, anti-herpes simplex virus drug

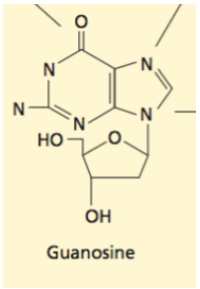
A *prodrug; a nucleoside analog*



Many antiviral compounds are nucleoside and nucleotide analogs

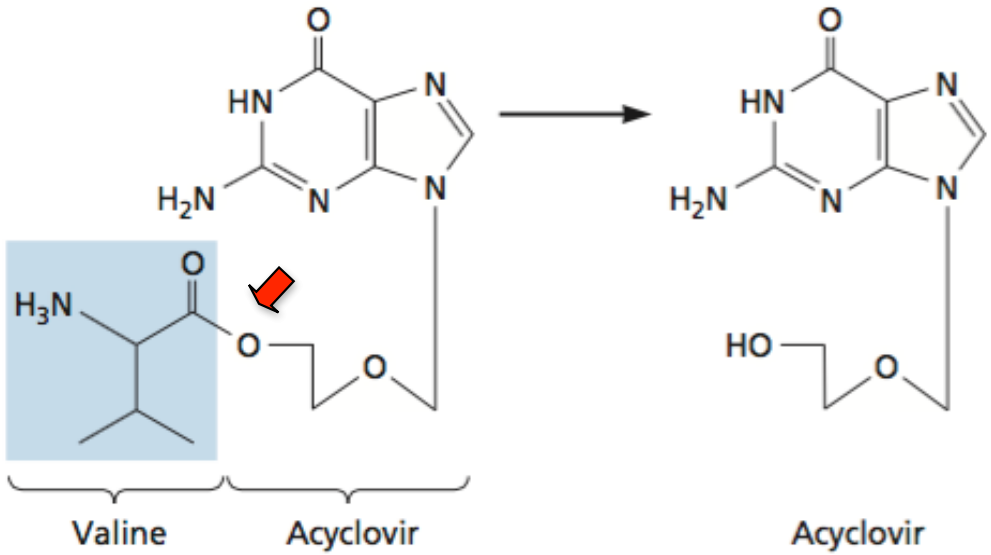
Acyclovir mechanism of action





Improving acyclovir

- Valacyclovir (valatrex), an L-valyl ester derivative of acyclovir, has markedly improved bioavailability
- Ester is taken up after oral administration, acyclovir is released when the ester is cleaved by cellular enzymes

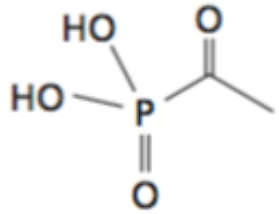


Principles of Virology, ASM Press

Acyclovir-resistant HSV

- Arise spontaneously during virus replication
- Some mutants cannot phosphorylate the pro-drug
  - *Mutations are in viral thymidine kinase gene*
- Some mutants cannot incorporate phosphorylated drug into DNA
  - *Mutations are in viral DNA polymerase gene*

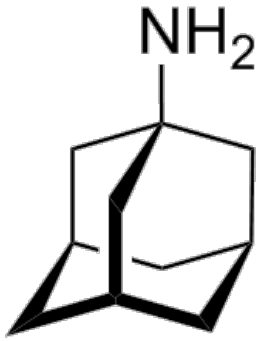
Acyclovir-resistant HSV



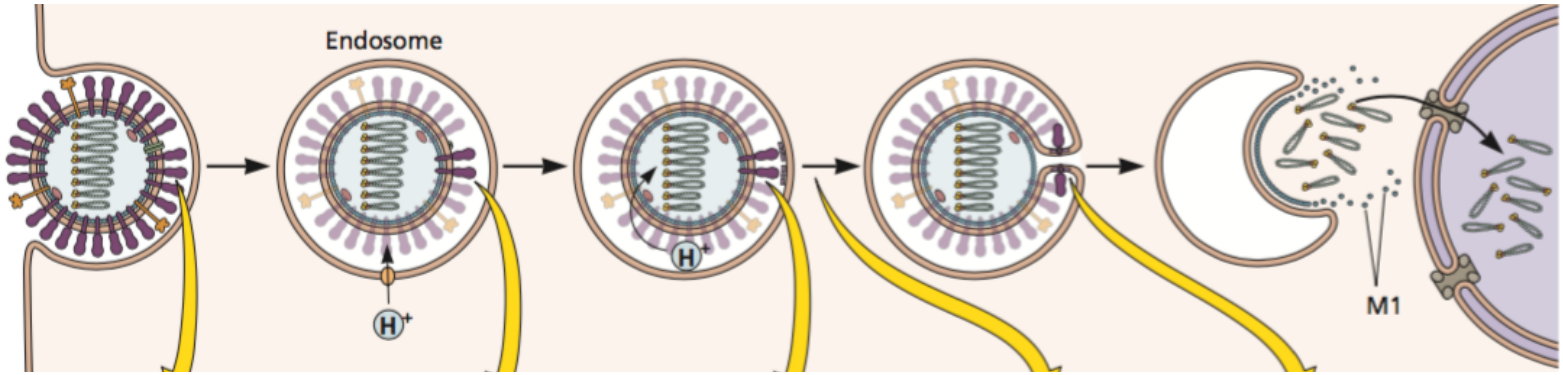
Foscarnet

- TK mutants can be devastating in AIDS patients
  - *May cause disseminated disease*
  - *Often resistant to other nucleoside analogs that require viral TK (cross-resistance)*
  - *Treat with Foscarnet, DNA polymerase inhibitor (side effects)*
- DNA polymerase mutants may also be resistant to Foscarnet: no treatment options left

Symmetrel (Amantadine)



- Interacts with influenza viral M2 protein (ion channel)
- Blocks entry of protons into virion, prevents uncoating



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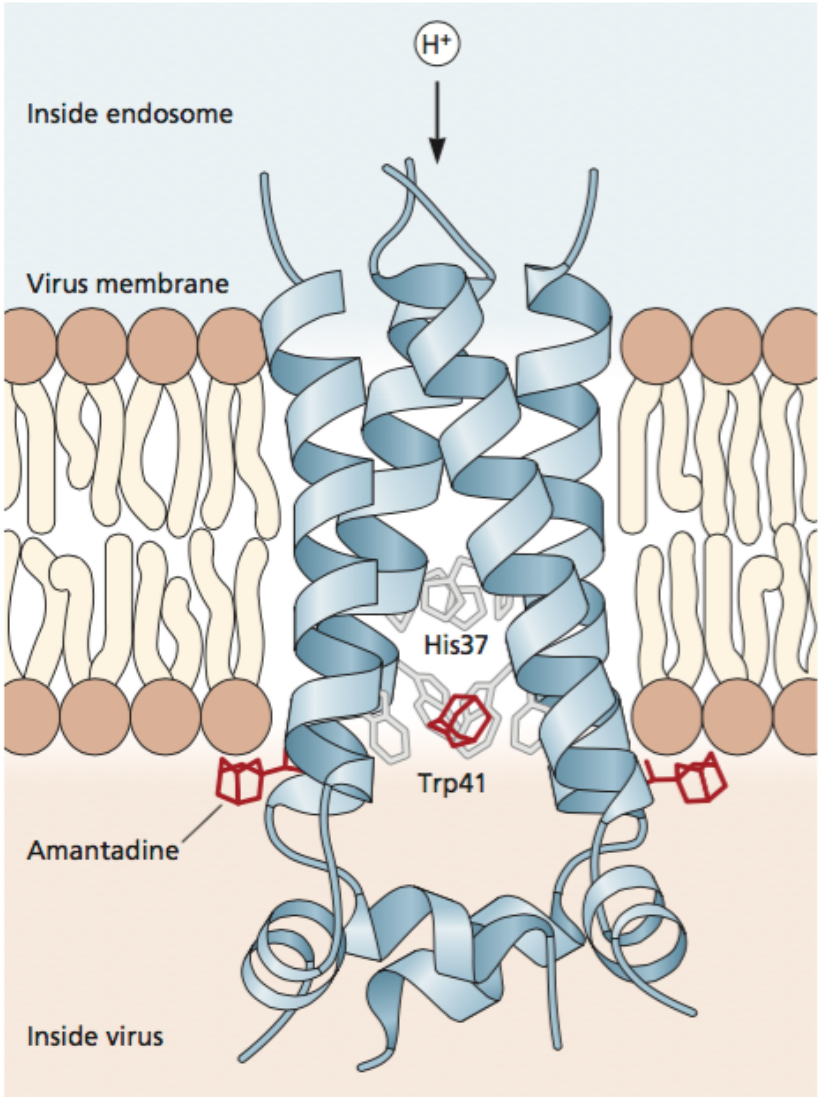

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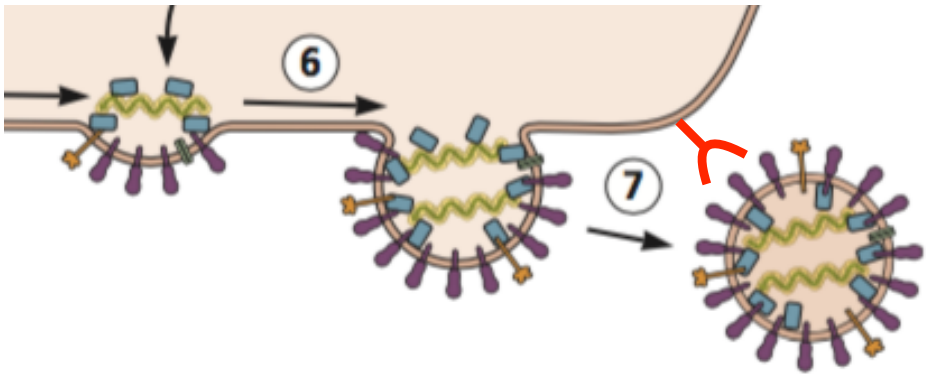
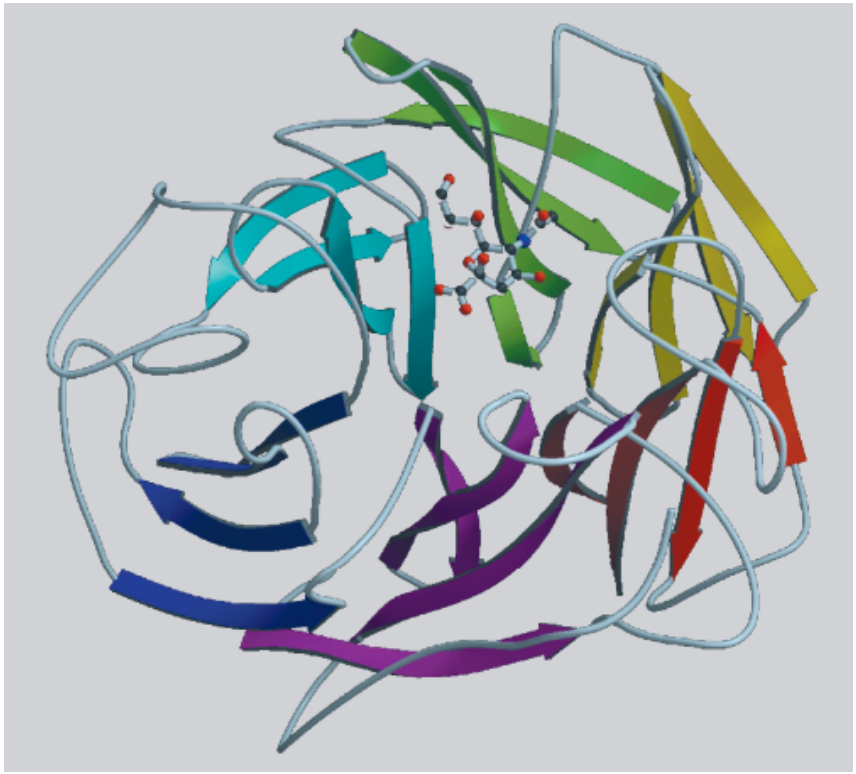
Resistance to which antiviral would involve amino acid changes in a viral enzyme?

A. Acyclovir
B. Amantadine
C. LJ001
D. Penicillin
E. All of the above

Influenza virus NA inhibitors





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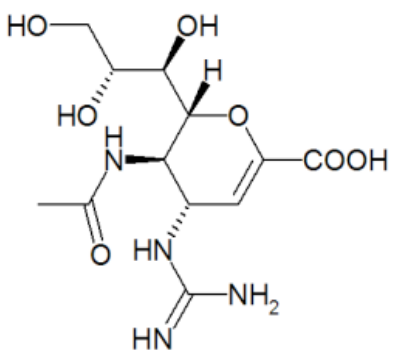
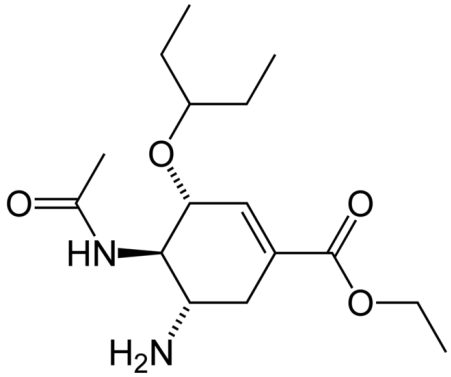
Influenza virus NA inhibitors



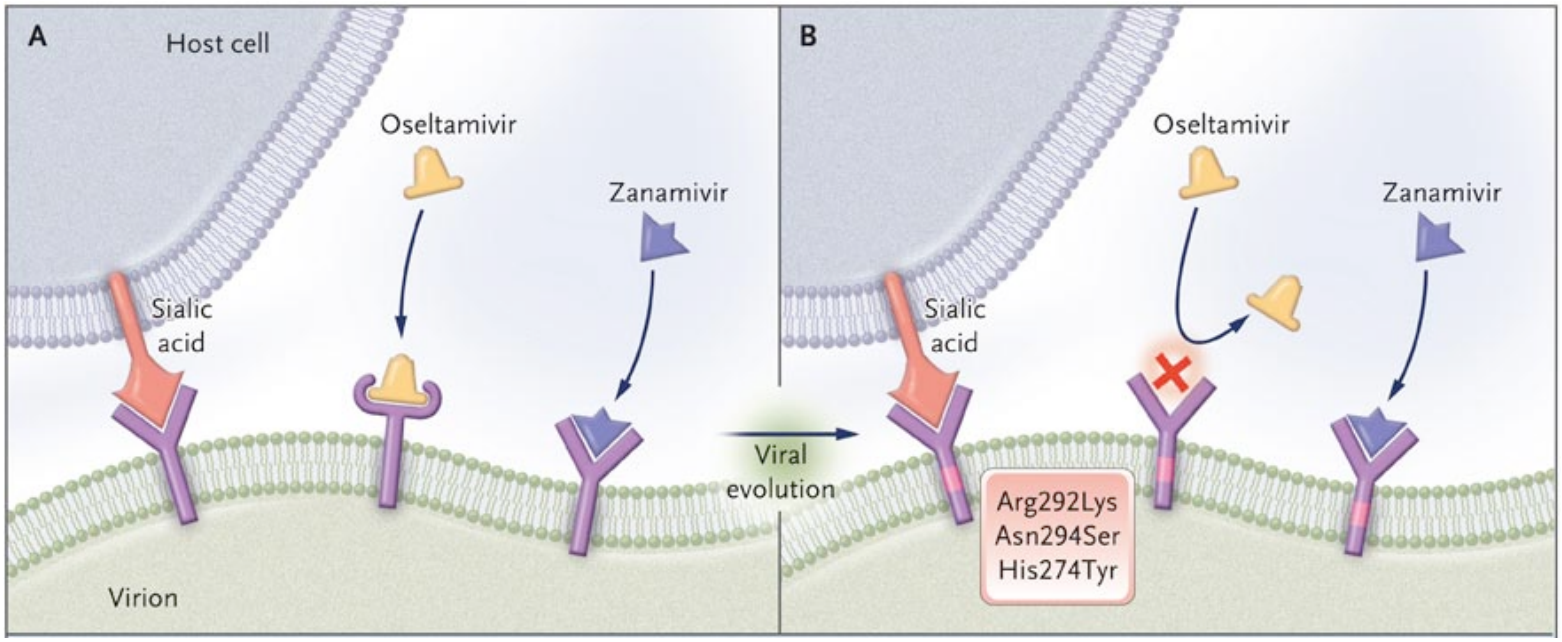
Zanamivir
(Relenza)



Oseltamivir
(Tamiflu)

- Designed to mimic natural ligand, sialic acid
- Closer inhibitor to natural compound, less likely target can change to avoid binding drug while maintaining viable function

How inhibitors of NA (Tamiflu, Relenza) work



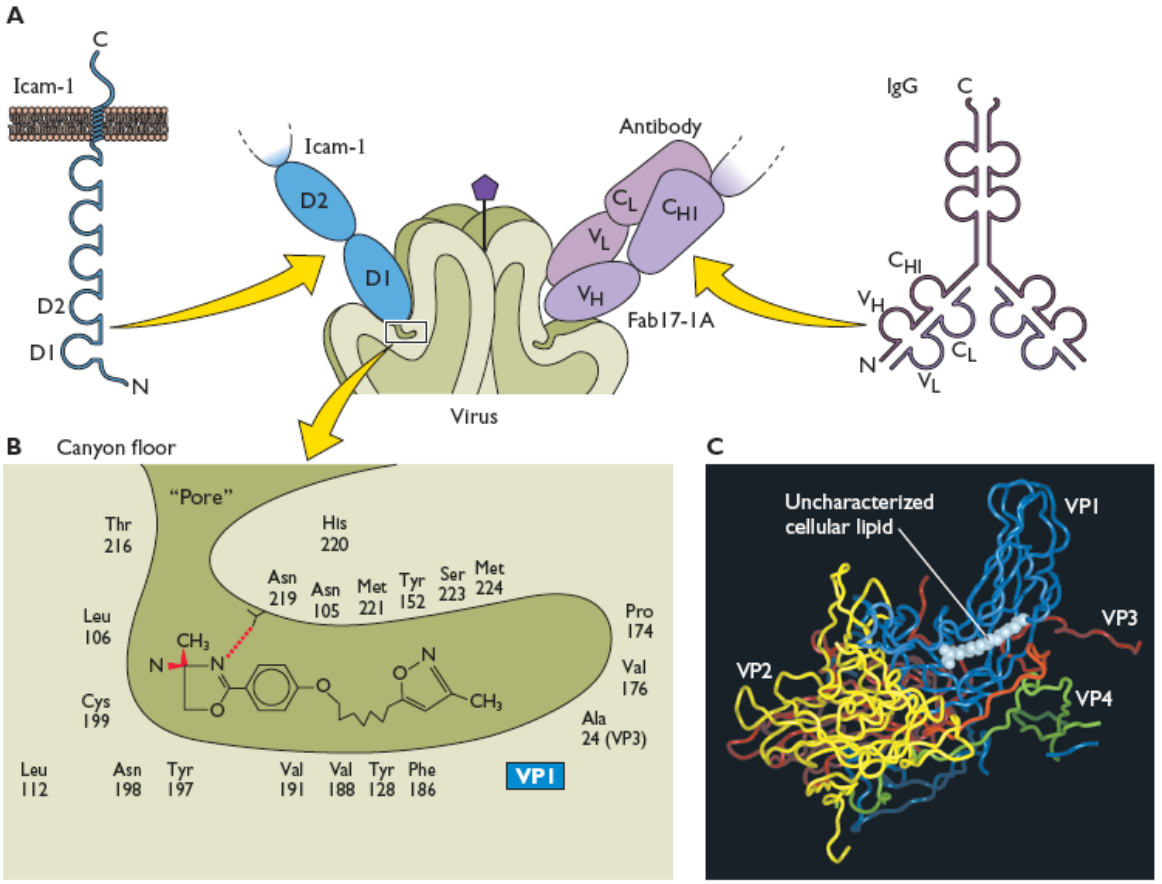
Neuraminidase Inhibitor Resistance Testing Results on Samples Collected Since October 1, 2016

	Oseltamivir		Zanamivir		Peramivir	
	Virus Samples tested (n)	Resistant Viruses, Number (%)	Virus Samples tested (n)	Resistant Viruses, Number (%)	Virus Samples tested (n)	Resistant Viruses, Number (%)
Influenza A (H1N1)pdm09	240	0 (0.0)	234	0 (0.0)	240	0 (0.0)
Influenza A (H3N2)	1,525	0 (0.0)	1,525	0 (0.0)	1,043	0 (0.0)
Influenza B	566	0 (0.0)	566	0 (0.0)	566	0 (0.0)

Circulating H1N1 and H3N2 viruses are largely resistant to Adamantanes, not recommended for use

http://www.cdc.gov/flu/weekly/index.htm

WIN compounds



Inhibitors of picornavirus uncoating

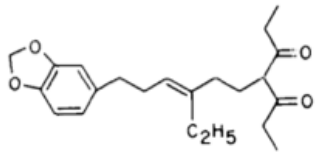


TABLE 1. In Vitro *Antiviral Activity*

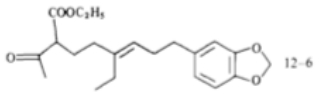
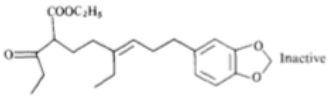
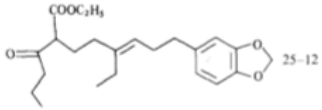
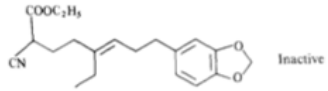
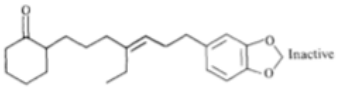
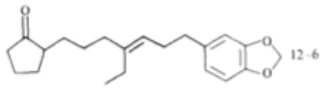
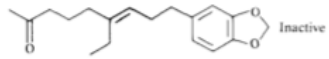
Structure	MIC μg/ml equine rhinovirus
	12–6
	Inactive
	25–12
	Inactive
	Inactive
	12–6
	Inactive

TABLE 2. In Vitro *Antiviral Activity*

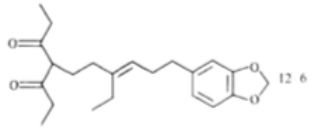
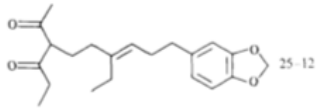
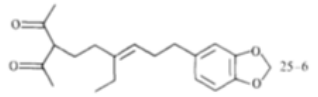
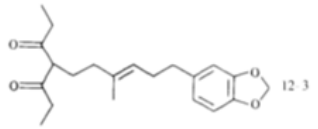
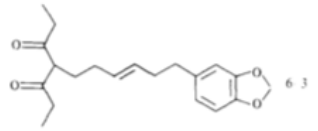
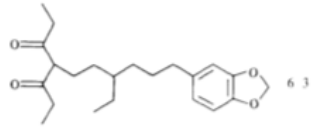
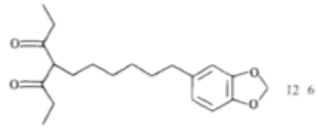
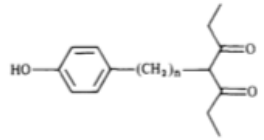
Structure	MIC μg/ml equine rhinovirus
	12–6
	25–12
	25–6
	12–3
	6–3
	6–3
	12–6

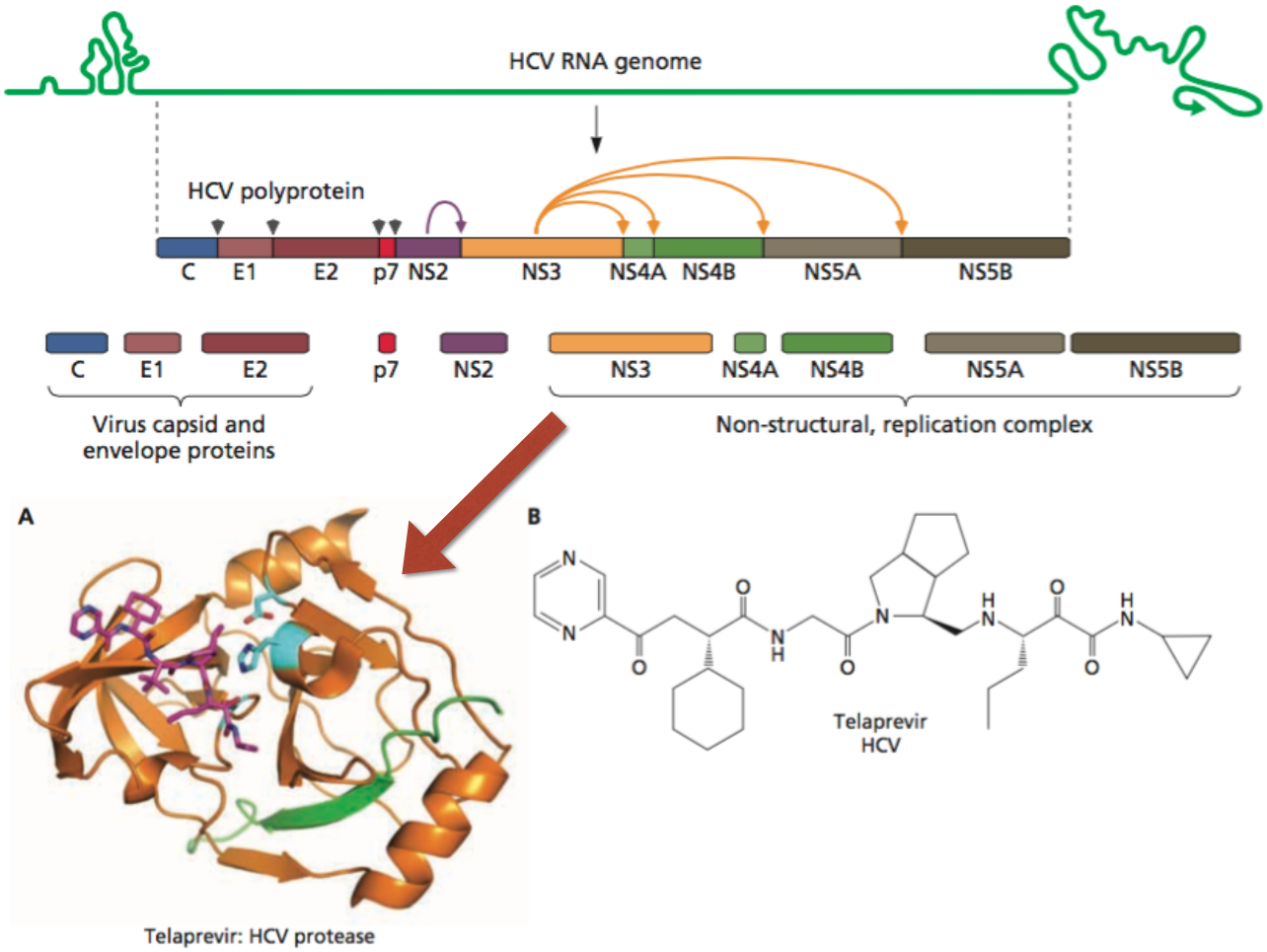
TABLE 3. In Vitro *Antiviral Activity*



n	MIC (μg/ml), equine rhinovirus
3	Inactive
4	25–6
5	25–12
6	6–3
7	12–6
8	6–3
9	Inactive
10	25–12

Pharmac. Ther. 29:287, 1985

New HCV drugs



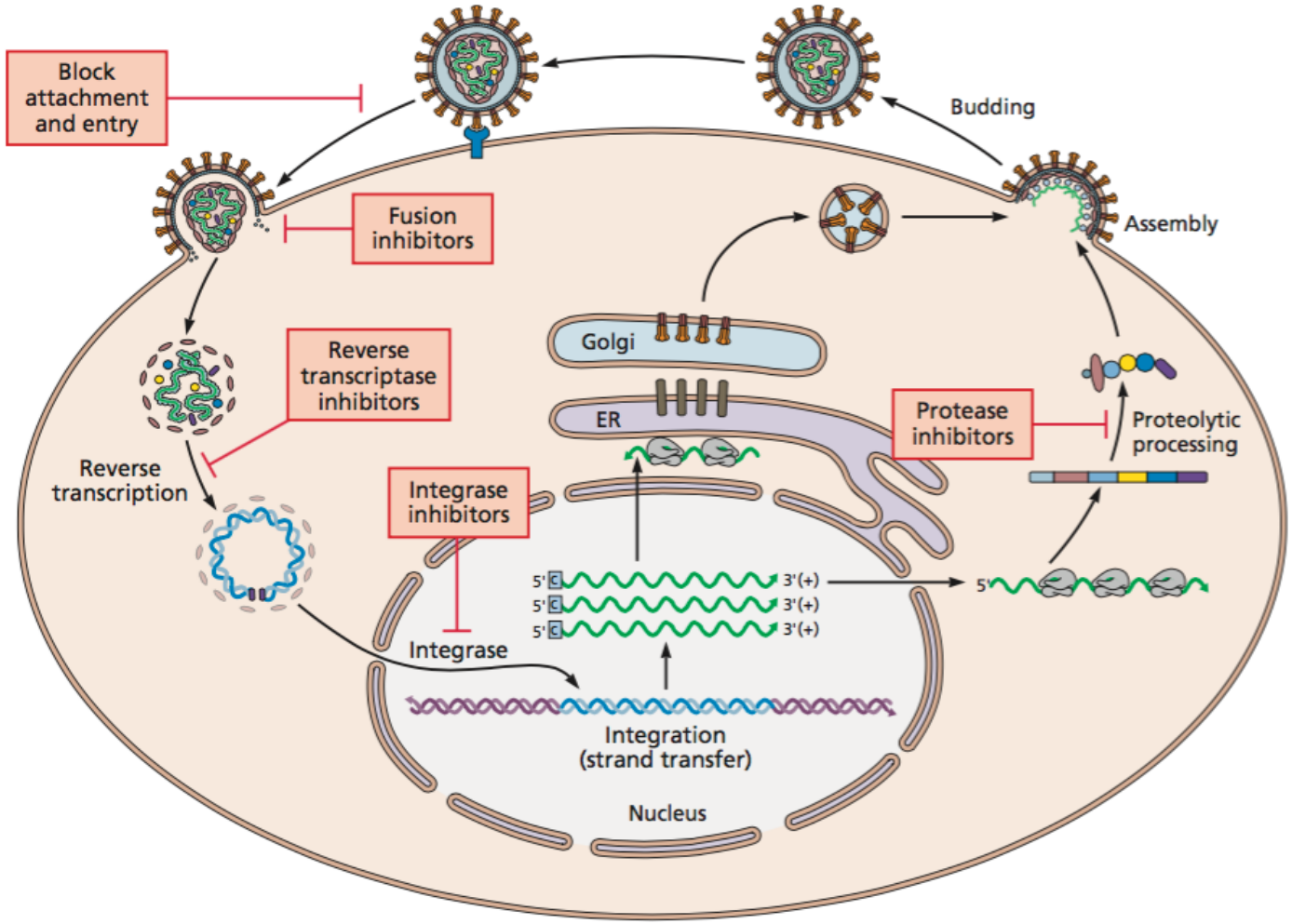
HCV new drug pipeline

Target	Generic name	Brand name	Developer	Date approved/ Trial phase
Polymerase (NS5B)	Sofosbuvir	Sovaldi	Gilead Sciences	2013
Nucleoside	Mericitabine		Roche	II
Nonnucleoside	Deleobuvir		Boehringer Ingelheim	III
	ABT-333		Abbott	III
RNA binding (NS5A)	Ledipasvir		Gilead Scienes	III (filed)
	Daclatasvir		Bristol-Myers Squibb	III
	ABT-267		Abbott	III
Protease (NS3/4A)	Telaprevir	Incivek	Vertex/Johnson & Johnson	2011
	Boceprevir	Victrelis	Merck	2011
	Simeprevir	Olysio	Janssen/Tibotec/Medivir	2013
	Faldaprevir		Boehringer Ingelheim	III
	Vaniprevir		Merck	III
	Samatasvir		Idenix	II
Combinations	Sofosbuvir + ledipasvir		Gilead Sciences	III
	Faldaprevir + deleobuvir		Boehringer Ingelheim	III
	Simeprevir + samatasvir + TMC647055/r		Janssen	II
	ABT-450/r + ABT-267 and ABT-333		Abbott	II
	MK-8742 + MK-5172		Merck	II

http://www.hcvdrugs.com/

Principles of Virology, ASM Press

Targets for intervention: HIV replication

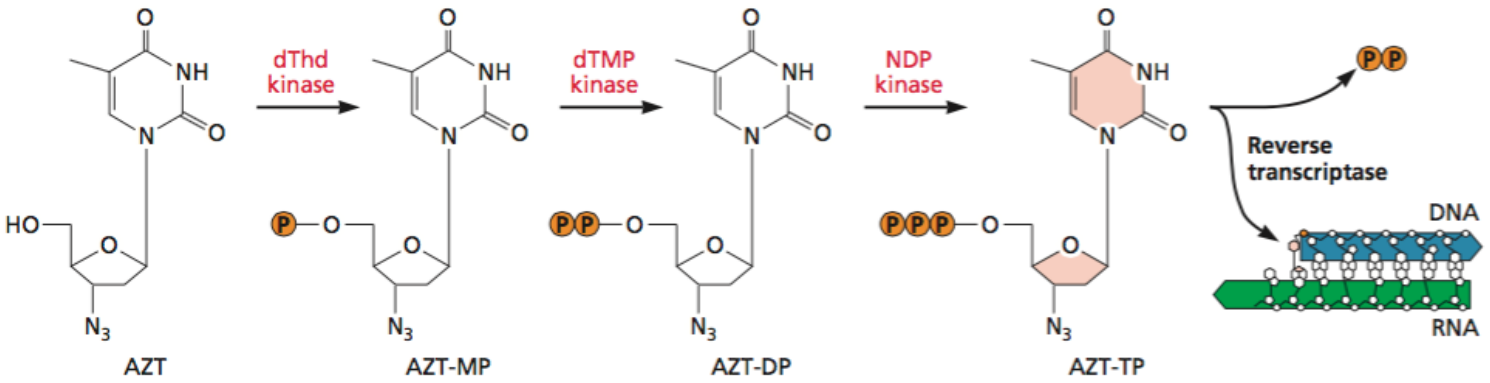


Principles of Virology, ASM Press

The problem with AIDS therapy: relentless viral replication for years

Azido-deoxythymidine (AZT) - first HIV drug

- Initially discovered during screens for anti-tumor cell compounds
- Phosphorylated to active form by cellular kinases
- Chain terminator
- Not good substrate for most cellular polymerases, better for HIV RT



Principles of Virology, ASM Press

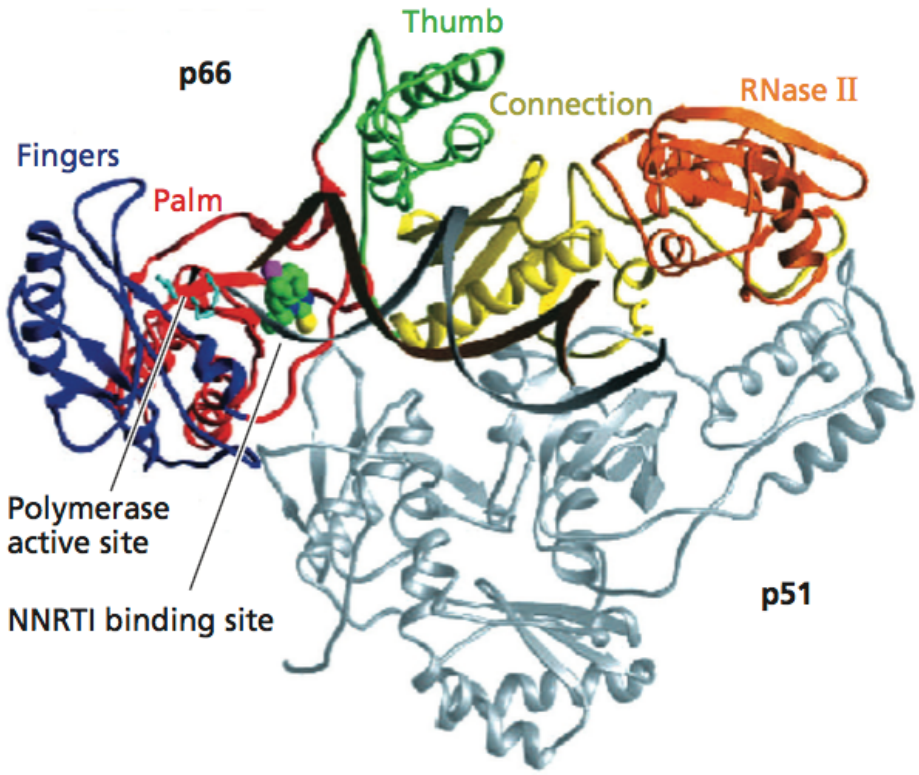
AZT

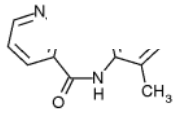
- Substantial side effects (unlike acyclovir)
- Can be given orally, is absorbed rapidly, but half-life is ~1 hr (degraded by liver enzymes)
- Consequently patients dosed 2-3x daily
- Short half-life, multiple dose regimen problematic: resistant mutants **will** be selected

Resistance to AZT

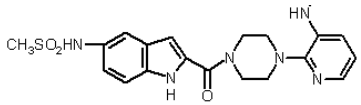
- Mutants resistant to AZT arose immediately after drug was licensed
- Single aa changes at one of four sites in RT
- Altered RT do not bind phosphorylated AZT
- New nucleoside analogs developed: Didanosine (ddI), Zalcitabine (ddC), Stavudine (d4T), Lamivudine (3TC)
- This lead to combination therapy, use of two antiviral drugs to combat resistance
- Mutants resistant to two drugs arose <1 yr

Non-nucleoside RT inhibitors (NNRTI)

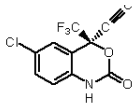




nevirapine (Viramune)



delavirdine



efavirenz

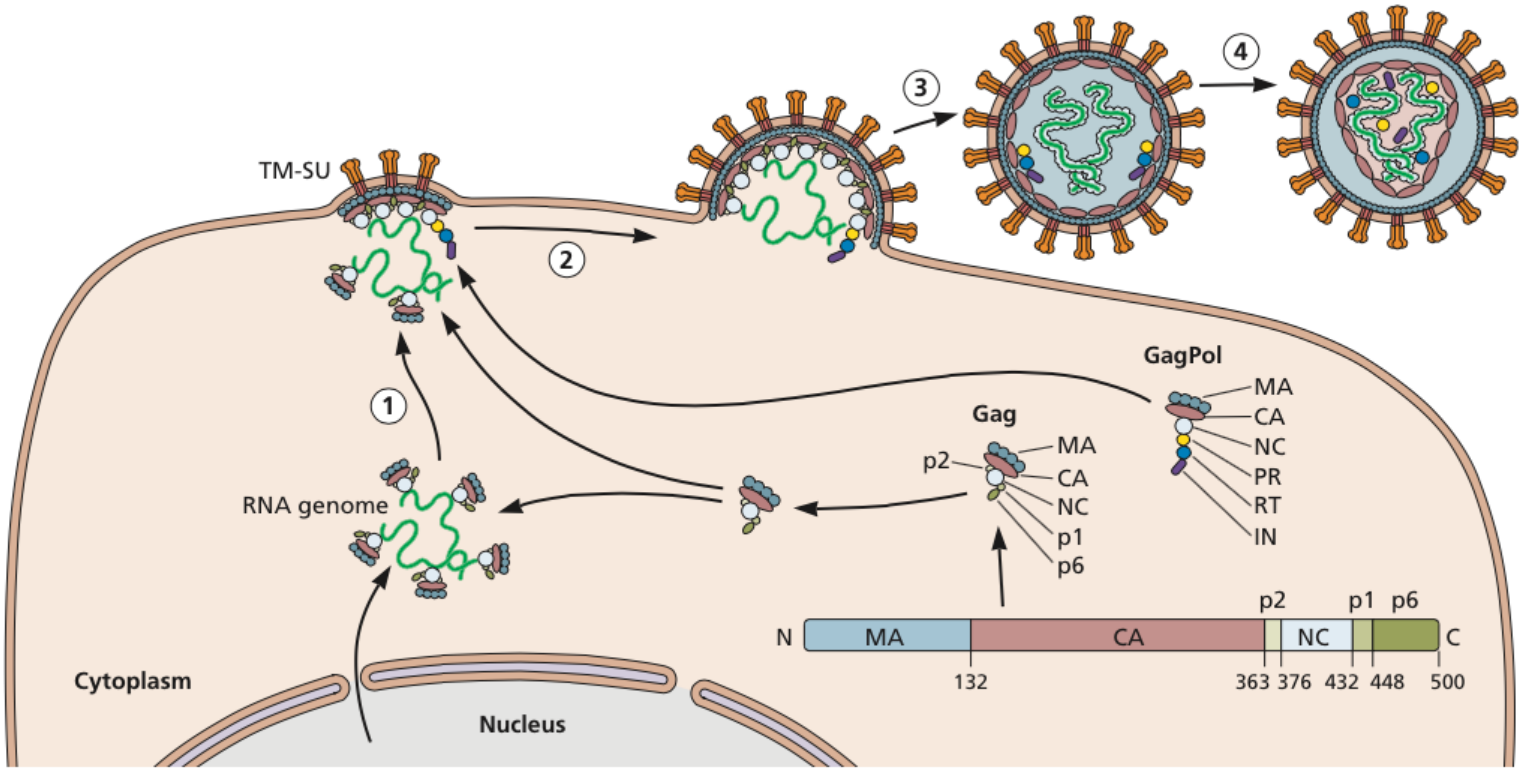
11-cyclopropyl-4-methyl-5,11-dihydro-6*H*- dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one

Resistance to NNRTIs

- Resistant mutants are selected rapidly
- Amino acid substitutions in any of seven residues that line binding sites on enzyme confer resistance
- Cannot be used alone for treatment of AIDS
- Now used largely in combination therapy

Antiviral drugs that target HIV protease

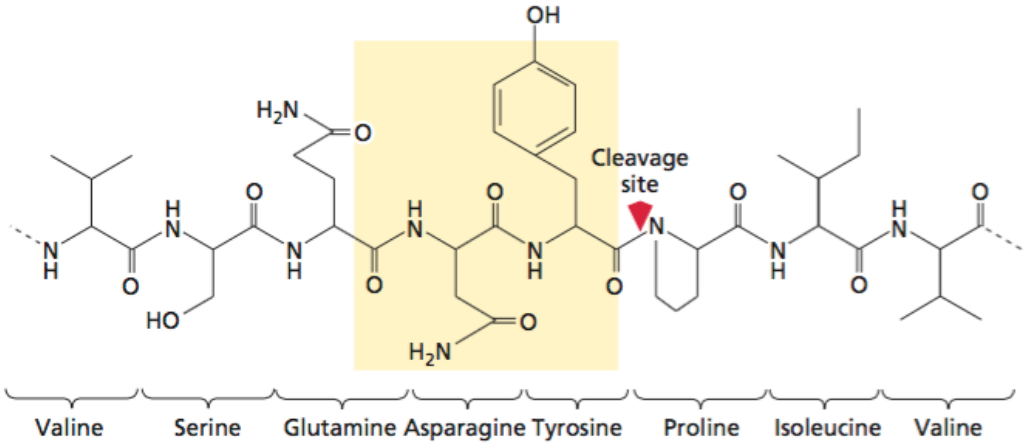
HIV protease absolutely required for production of infectious virions

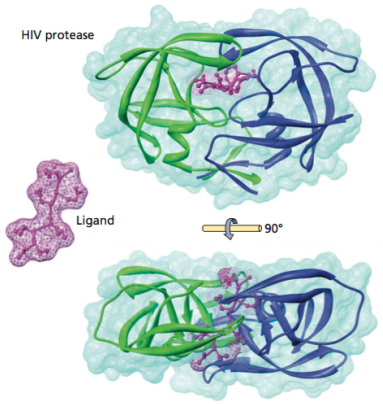


Antiviral drugs that target HIV protease

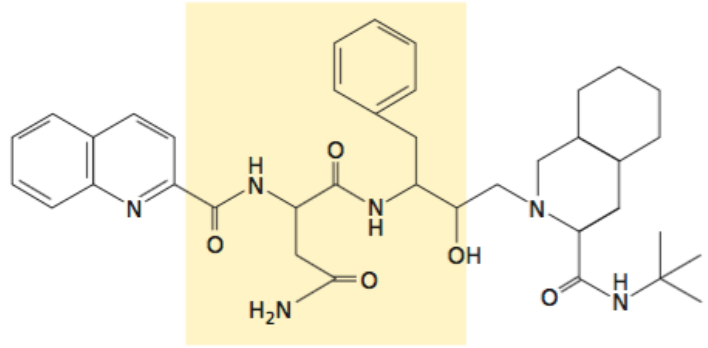
Key finding: HIV protease recognizes and cleaves small synthetic peptides

A Natural substrate of the HIV-1 protease

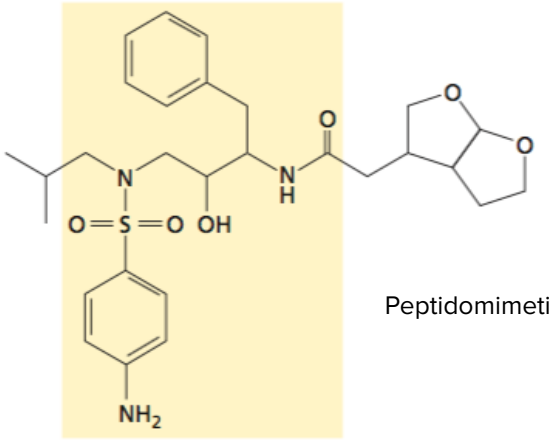




B Saquinavir

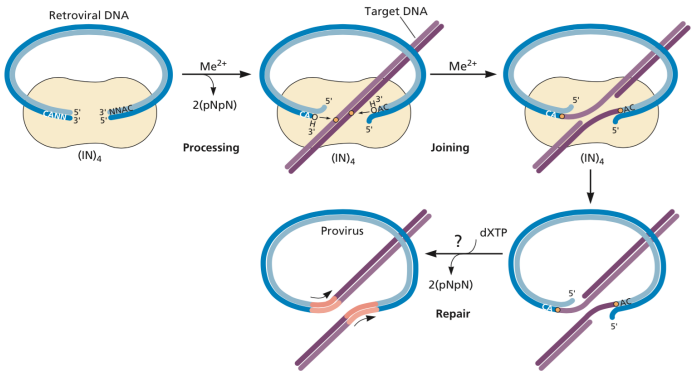


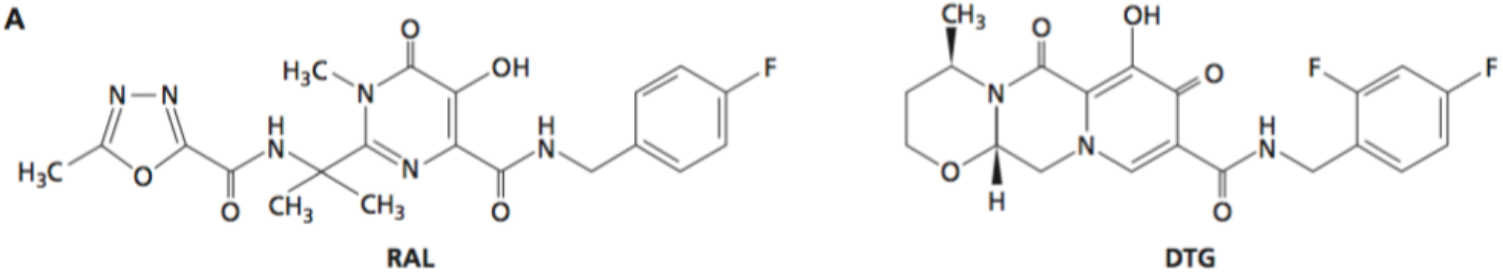
C Darunavir

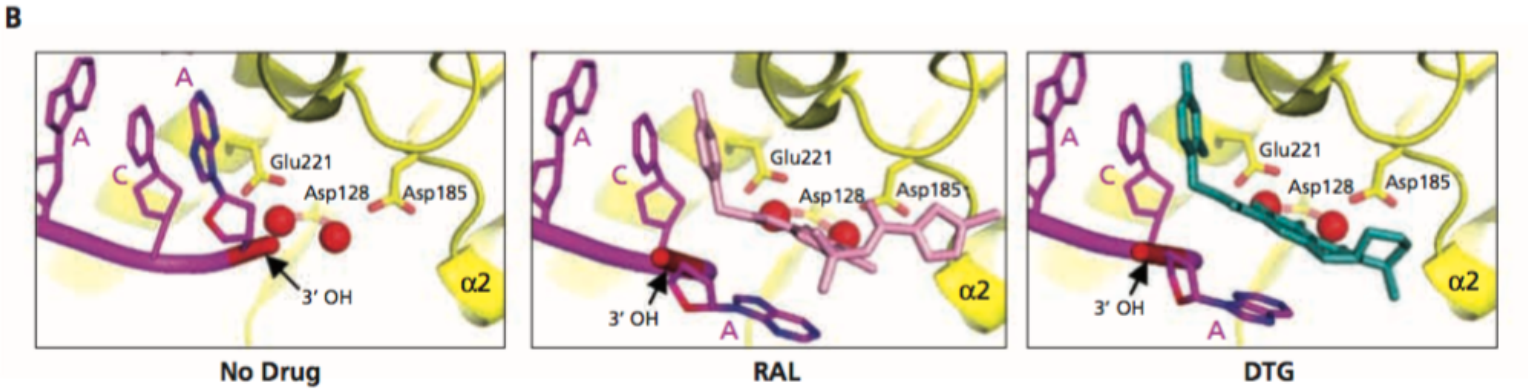


Peptidomimetic

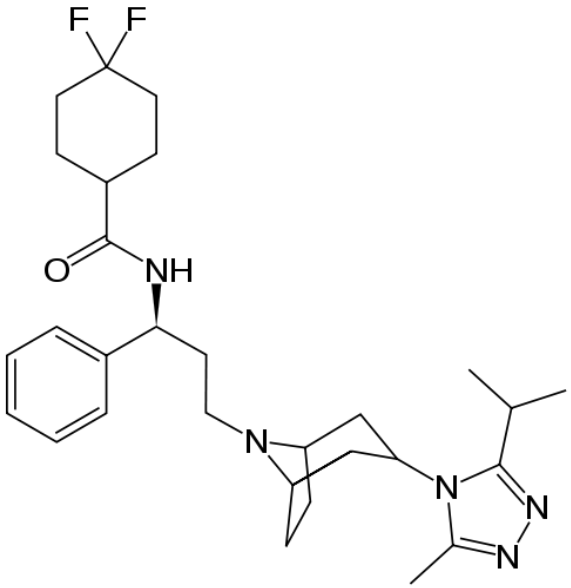
IN inhibitors

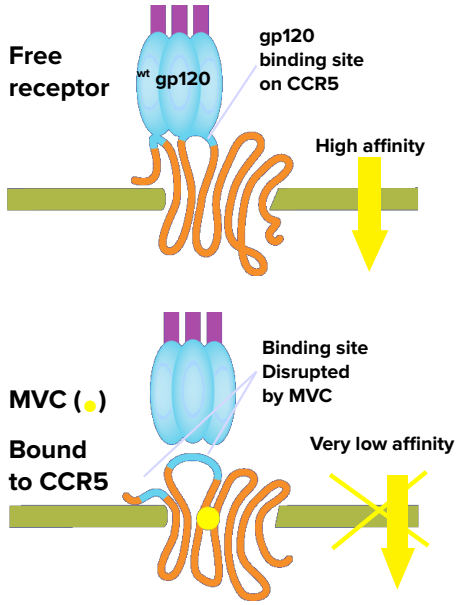






Maraviroc: CCR5 inhibitor





Go to:

b.socrative.com/login
room number: virus

Which of the following HIV antivirals inhibits the earliest stage of infection?

A. Nucleoside inhibitors
B. NNRTIs
C. CCR5 inhibitors
D. Integrase inhibitors
E. Fusion inhibitors

Combination therapy

- HAART: HIV can be treated as a chronic disease
- Target different mechanisms
- One pill containing three inhibitors

Mathematics of drug resistance

- Assume one mutation needed for drug resistance
- Mutation rate 1 every 10^4 bases polymerized
- Each base is substituted in every 10^4 viruses
- Each person makes 10^{10} new viruses/day
- $10^{10}/10^4 = 10^6$ viruses will be produced each day with resistance to one drug

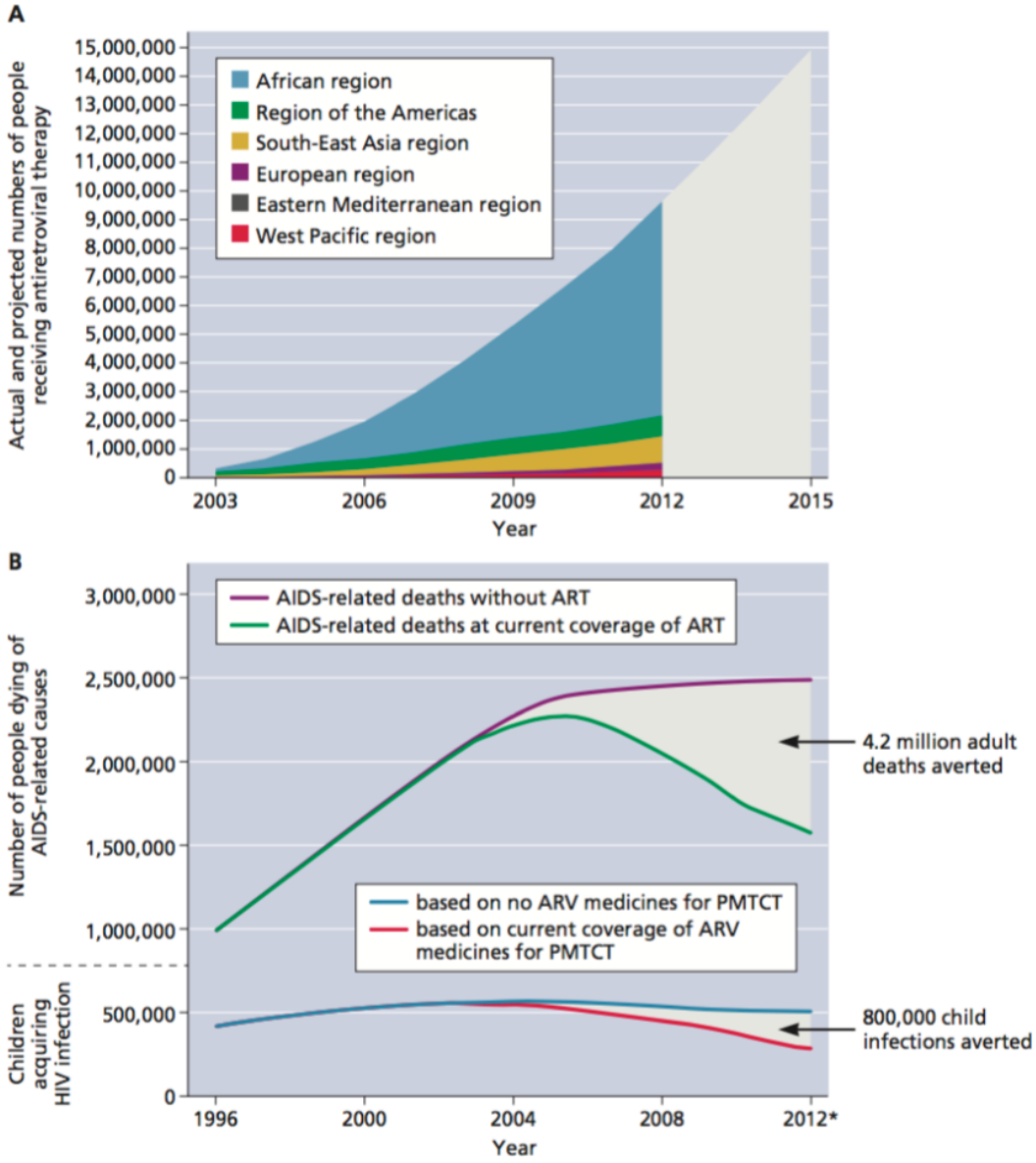
Mathematics of drug resistance

- Developing resistance to two drugs: $10^4 \times 10^4 = 10^8$
- $10^{10}/10^8 = 100$ viruses resistant to two drugs per day
- Resistance to three drugs: $10^4 \times 10^4 \times 10^4 = 10^{12}$ viruses needed
- Remember replication is suppressed by drugs

Brand name	Generic name(s)	Manufacturer name	Approval date	Time to approval
Nucleoside reverse transcriptase inhibitors (NRTIs)[a,b]				
Retrovir	Zidovudine, azidothymidine, AZT, ZDV	GlaxoSmithKline (original sponsor Burroughs-Wellcome)	19 March 1987	3.5 months
Videx	Didanosine, dideoxyinosine, ddI	Bristol Myers-Squibb	9 October 1991	6 months
Hivid	Zalcitabine, dideoxycytidine, ddC (no longer marked as of December 31, 2006)	Hoffmann-La Roche	19 June 1992	7.6 months
Zerit	Stavudine, d4T	Bristol Myers-Squibb	24 June 1994	5.9 months
Epivir	Lamivudine, 3TC	GlaxoSmithKline	17 November 1995	4.4 months
Combivir	Lamivudine and zidovudine	GlaxoSmithKline	27 September 1997	3.9 months
Ziagen	Abacavir sulfate, ABC	GlaxoSmithKline	17 December 1998	5.8 months
Videx EC	Enteric coated didanosine, ddI EC	Bristol Myers-Squibb	31 October 2000	9 months
Trizivir	Abacavir, zidovudine, and lamivudine	GlaxoSmithKline	14 November 2000	10.9 months
Viread	Tenofovir disoproxil fumarate, TDF	Gilead Sciences	26 October 2001	5.9 months
Emtriva	Emtricitabine, FTC	Gilead Sciences	02 July 2003	10 months
Epzicom	Abacavir and lamivudine	GlaxoSmithKline	02 August 2004	10 months
Truvada	Tenofovir disoproxil fumarate and emtricitabine	Gilead Sciences	02 August 2004	5 months
Nonnucleoside reverse transcriptase inhibitors (NNRTIs)[c]				
Viramune	Nevirapine, NVP	Boehringer Ingelheim	21 June 1996	3.9 months
Rescriptor	Delavirdine, DLV	Pfizer	4 April 1997	8.7 months
Sustiva	Efavirenz, EFV	Bristol Myers-Squibb	17 September 1998	3.2 months
Intelence	Etravirine	Tibotec Therapeutics	18 Jane 2008	6 months
Protease inhibitors (PIs)				
Invirase	Saquinavir mesylate, SQV	Hoffmann-La Roche	6 December 1995	3.2 months
Norvir	Ritonavir, RTV	Abbott Laboratories	1 March 1996	2.3 months
Crixivan	Indinavir, IDV,	Merck	13 March 1996	1.4 months
Viracept	Nelfinavir mesylate, NFV	Agouron Pharmaceuticals	14 March 1997	2.6 months
Fortovase	Saquinavir (no longer marketed)	Hoffmann-La Roche	7 November 1997	5.9 months
Agenerase	Amprenavir, APV	GlaxoSmithKline	15 April 1999	6 months
Kaletra	Lopinavir and ritonavir, LPV/RTV	Abbott Laboratories	15 September 2000	3.5 months
Reyataz	Atazanavir sulfate, ATV	Bristol-Myers Squibb	20 June 2003	6 months
Lexiva	Fosamprenavir calcium, FOS-APV	GlaxoSmithKline	20 October 2003	10 months
Aptivus	Tipranavir, TPV	Boehringer Ingelheim	22 June 2005	6 months
Prezista	Darunavir	Tibotec, Inc.	23 June 2006	6 months
Fusion inhibitors				
Fuzeon	Enfuvirtide, T-20	Hoffmann-La Roche and Trimeris	13 March 2003	6 months
Entry inhibitors—CCR5 co-receptor antagonists				
Selzentry	Maraviroc	Pfizer	06 August 2007	8 months
HIV integrase strand transfer inhibitors				
Isentress	Raltegravir	Merck & Co., Inc.	12 October 2007	6 months
Multi-class combination products				
Atripla	Efavirenz, emtricitabine and tenofovir disoproxil fumarate	Bristol-Myers Squibb and Gilead Sciences	12 July 2006	2.5 months

Complera (Efavirenz, emtricitabine, rilpivirine) 2011, Stribild (Efavirenz, emtricitabine, cobicistat) 2012

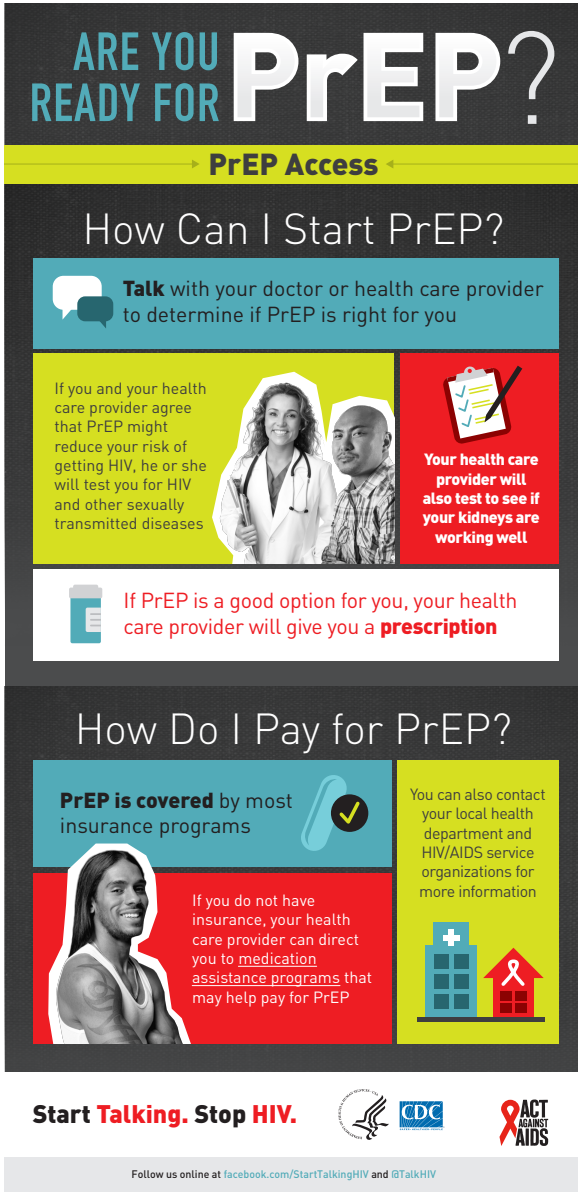
ART saves lives



Principles of Virology, ASM Press

Pre-exposure prophylaxis (PrEP)

- Daily double therapy (tenofovir and emtricitabine) for those at high risk for HIV infection
- Reduces risk of sexual transmission of HIV-1 by >90%
- Reduces risk of transmission by IVDU by >70%
- No resistance in trials, but real world?
- https://www.ncbi.nlm.nih.gov/pubmed/27391094



There are 10^{16} HIV genomes on the planet today

With this number of genomes, it is highly probable that HIV genomes exist that are resistant to every one of the antiviral drugs that we have now, or EVER WILL HAVE!