Antivirals

Session 20

Virology Live

Fall 2021

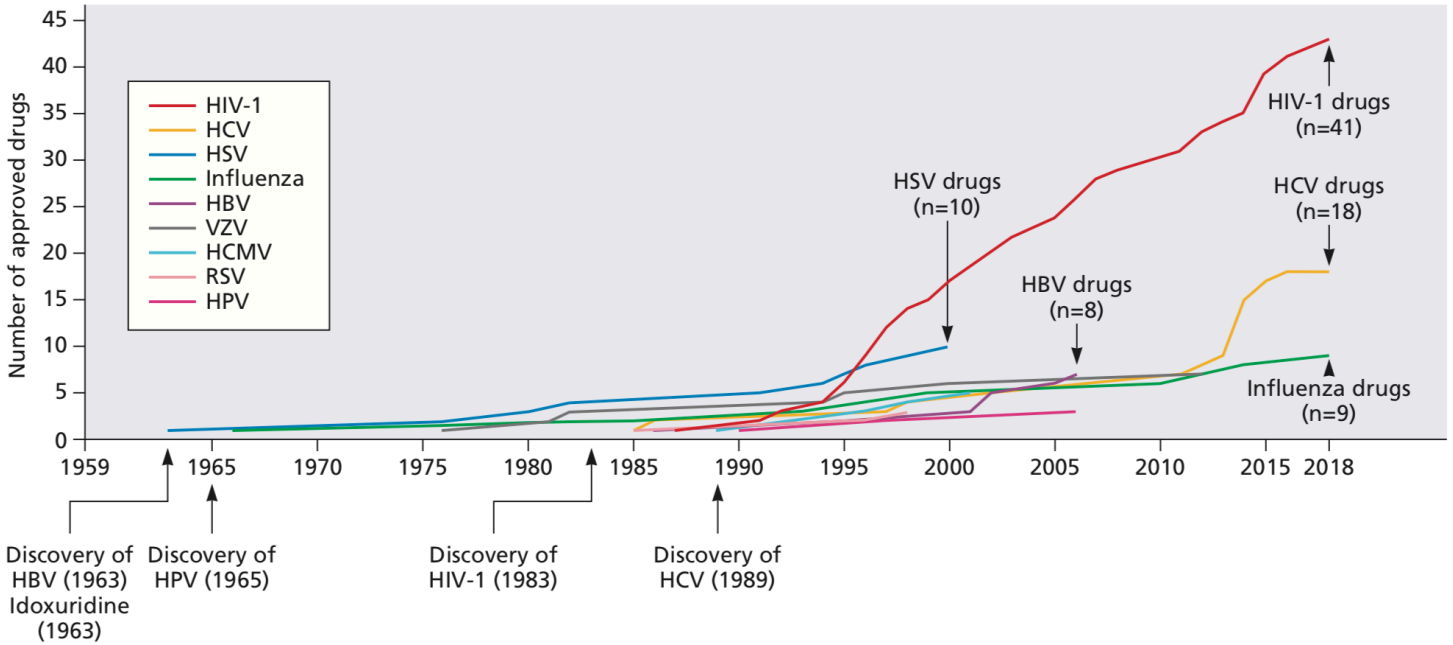
Though the doctors treated him, let his blood, and gave him medications to drink, he nevertheless recovered

Leo Tolstoy

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 60 years of research,
our arsenal of antiviral drugs remains dangerously small

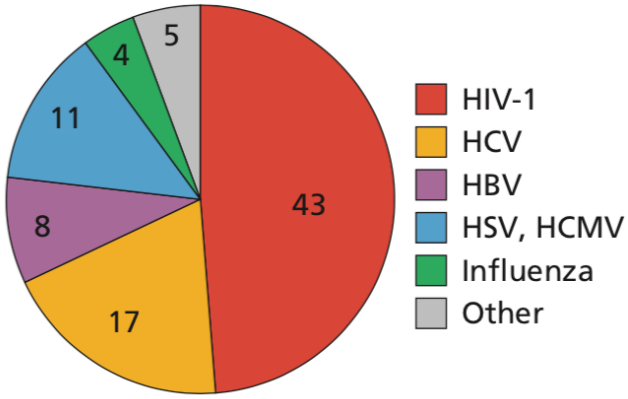


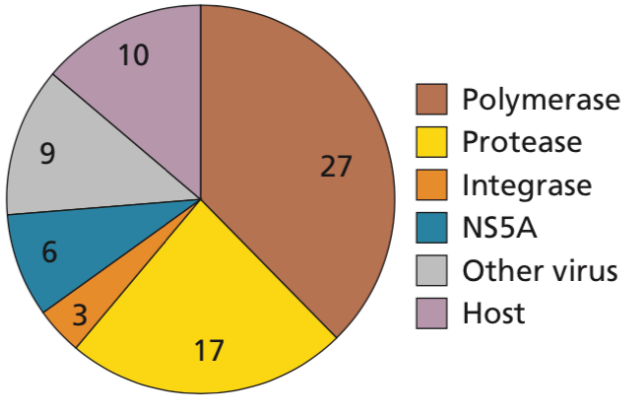
Only about 100 antiviral drugs are available on the US market

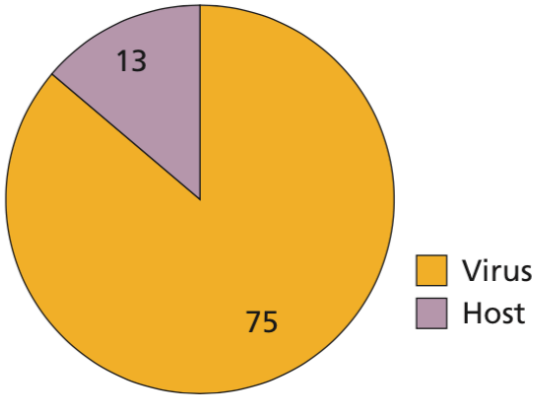
Most against HIV, HCV, herpesviruses - Persistent infections

Principles of Virology, ASM Press

Antiviral drugs by virus and target



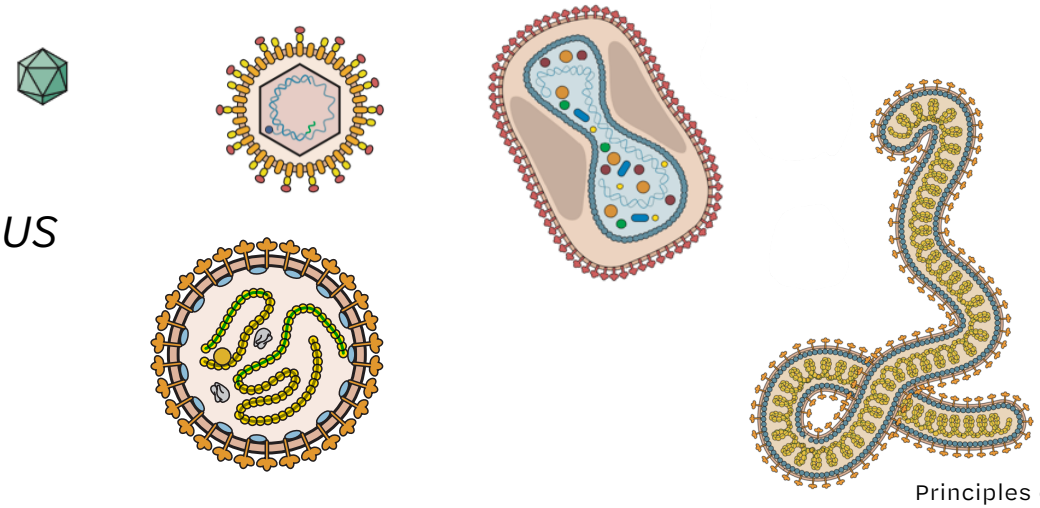




Principles of Virology, ASM Press

Why are there so few antiviral drugs?

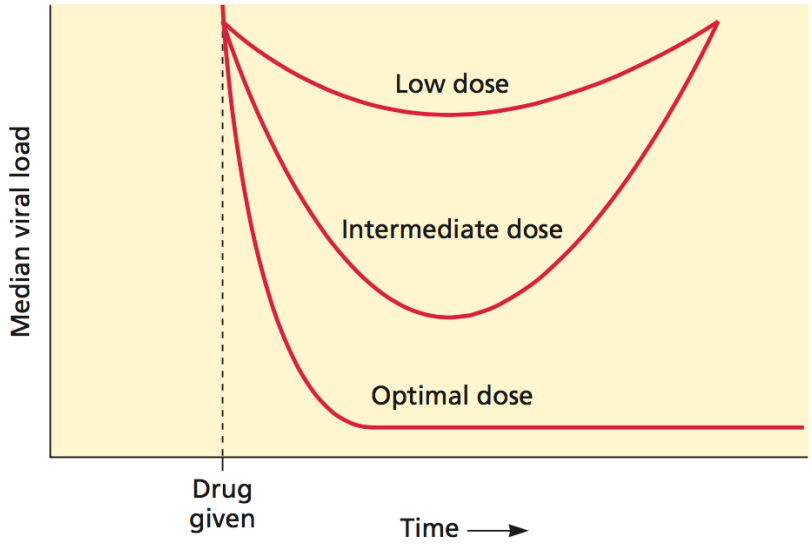
- Compounds interfering with virus growth can adversely affect the host cell
  - *Side effects are common (unacceptable)*
  - *Every step in viral reproduction cycle engages host functions*
- Some medically important viruses can't be propagated, have no animal model, or are dangerous
  - *HBV, HPV*
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



Principles of Virology, ASM Press

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:

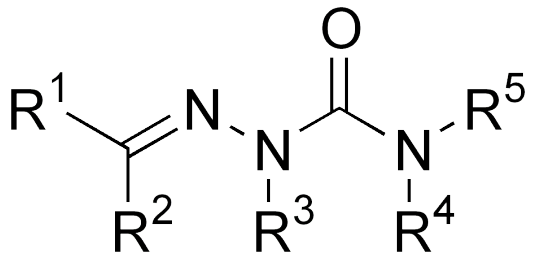
Many acute infections are of short duration

- 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

Principles of Virology, ASM Press

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*

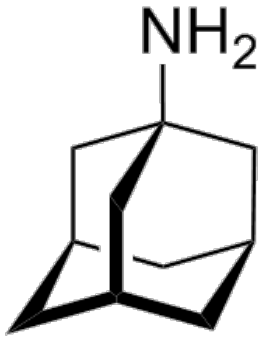


- 1960s and 1970s: “blind screening” programs to find chemicals with antiviral activity
  - *Spurred on by successes in the treatment of bacterial infections with antibiotics*

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*

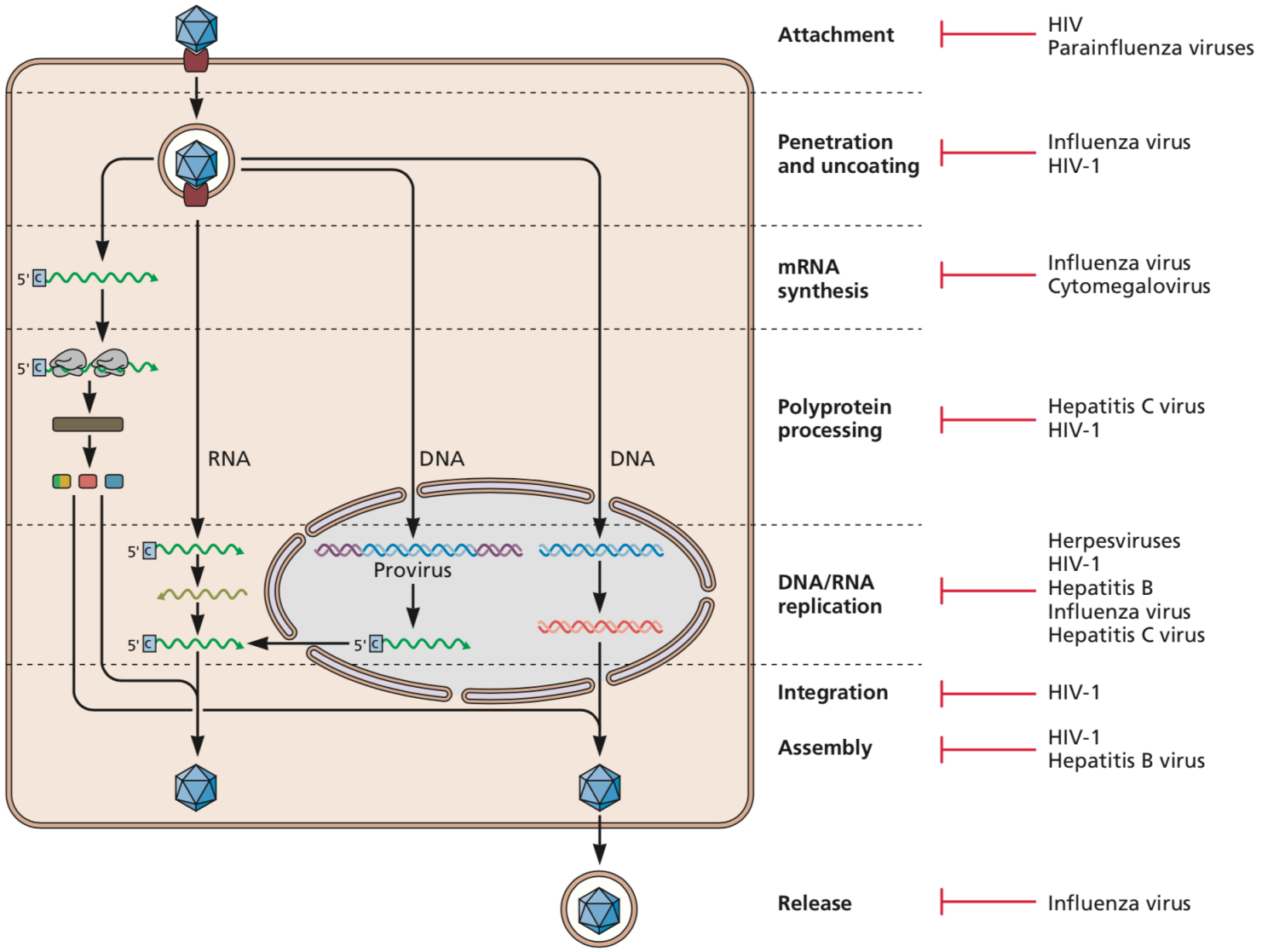
Thousands of molecules were made and screened before a specific antiviral was even tested in humans



- 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
- Reproduction 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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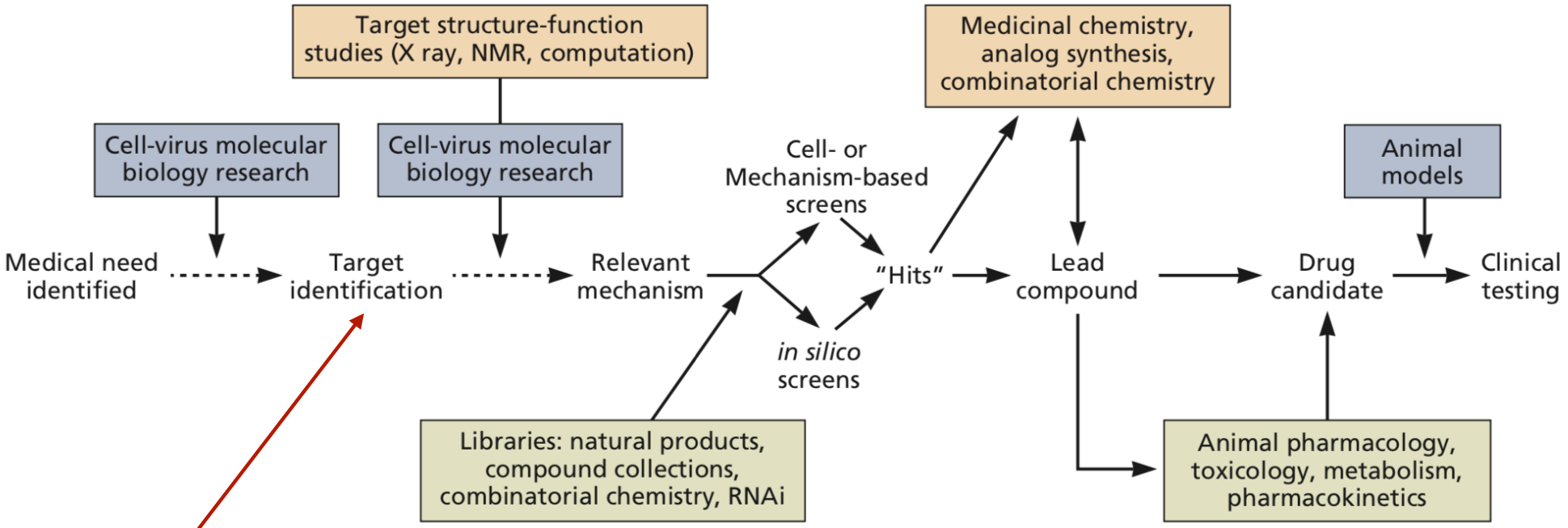
HIV-1
RNA
DNA
DNA
Provirus
DNA/RNA replication
Herpesviruses
HIV-1
Hepatitis B
Influenza virus
Hepatitis C virus
Integration
HIV-1
Assembly
HIV-1
Hepatitis B virus
Release
Influenza virus

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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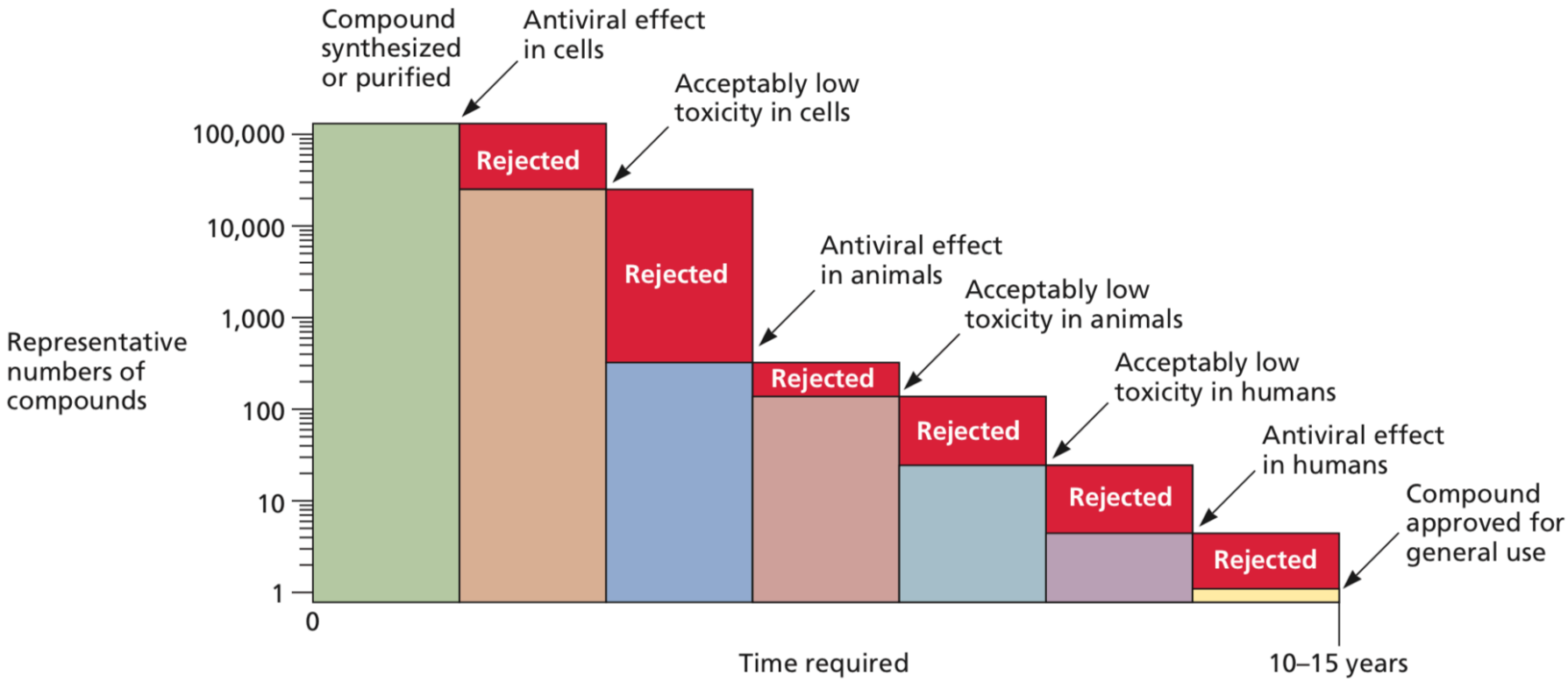
Significant hurdles stand in the way of finding effective antiviral drugs



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

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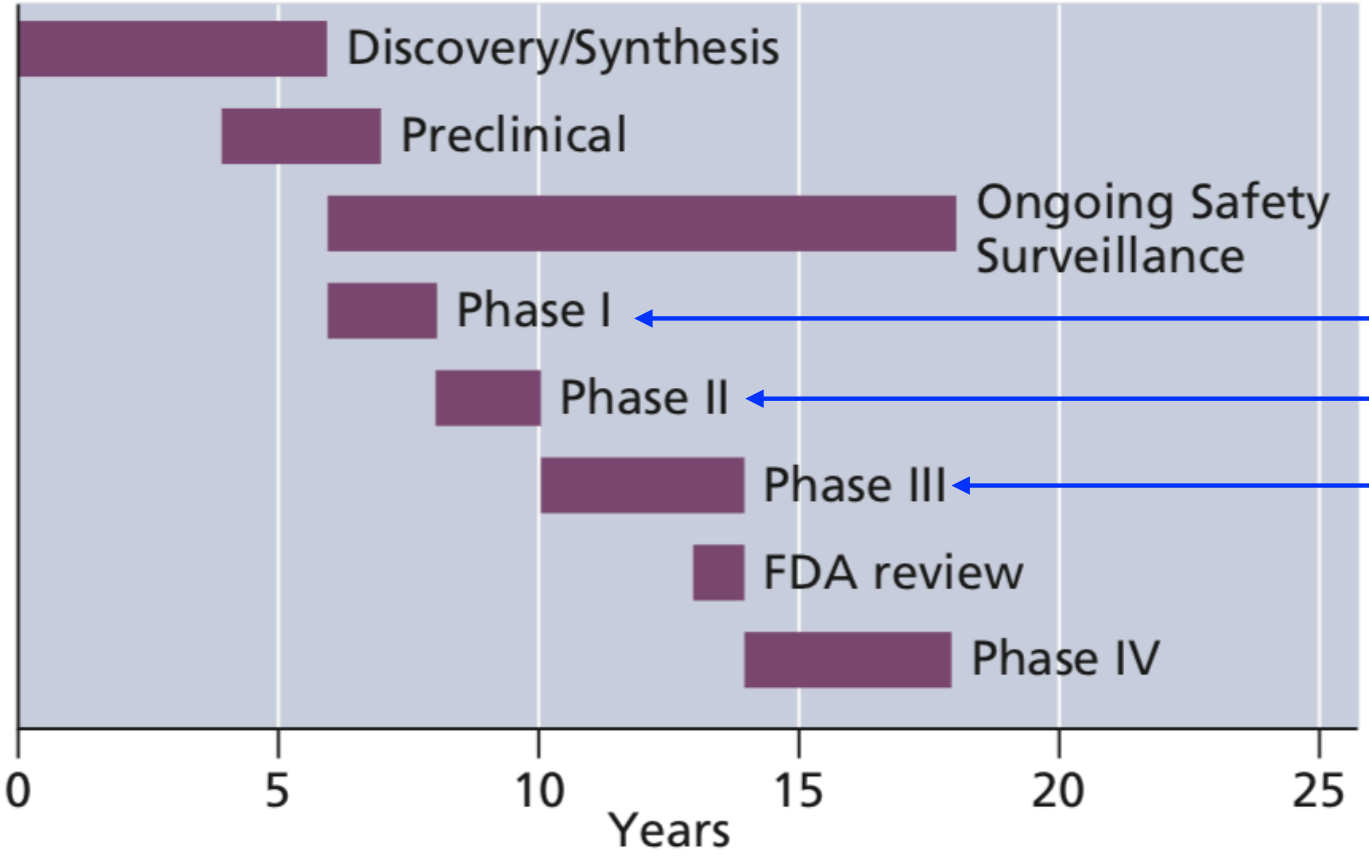
From drug discovery to the clinic

Safety is the overriding concern



Safety (20-80 healthy subjects)

Efficacy (100-300 subjects)

Efficacy, side effects, superiority (100-1000s subjects)

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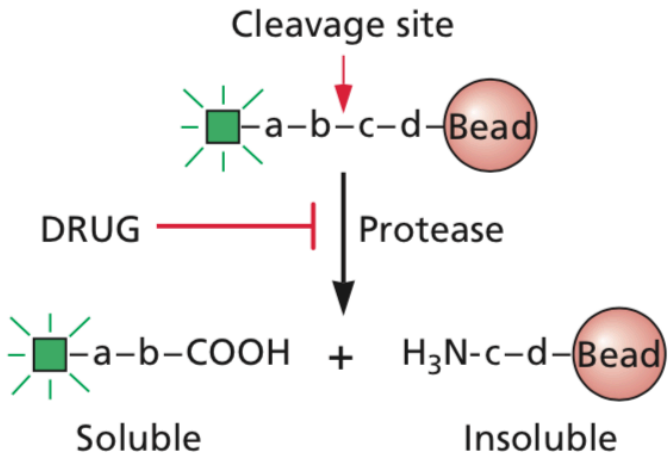
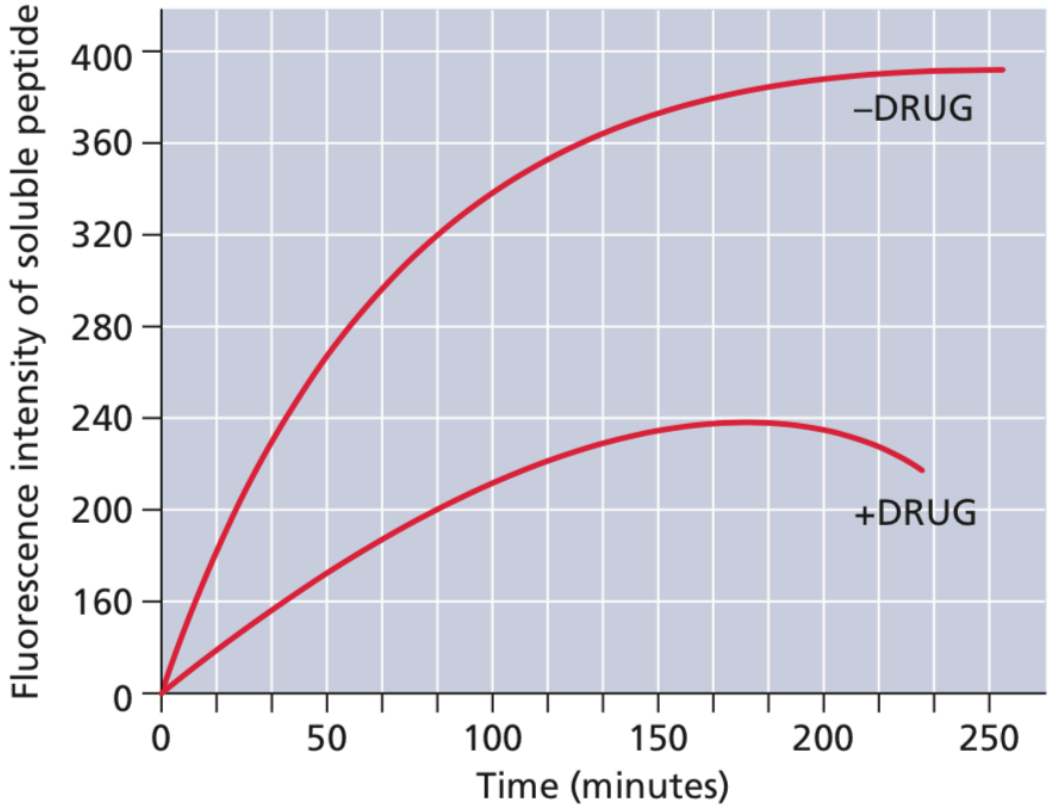
Mechanism-based screens





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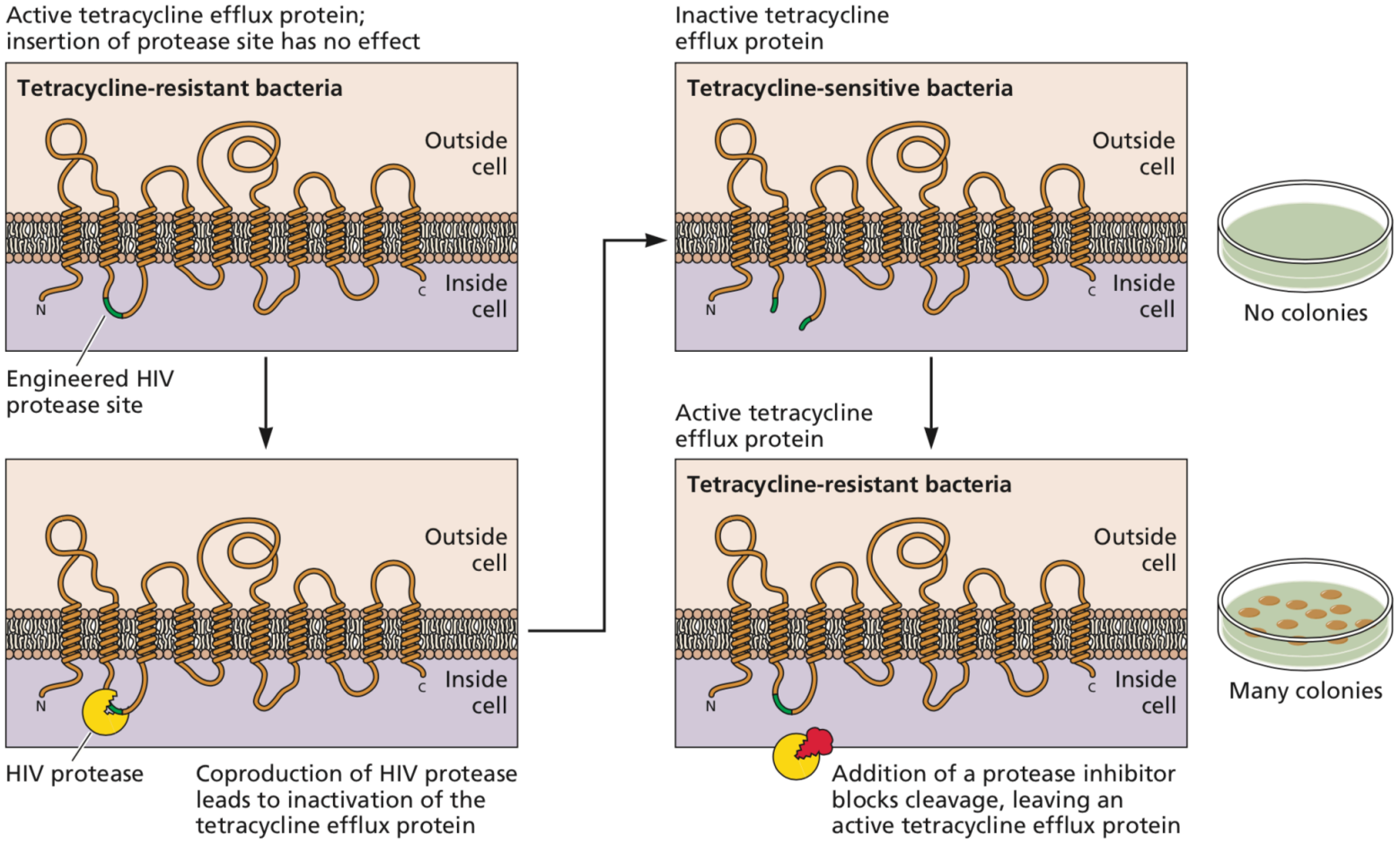
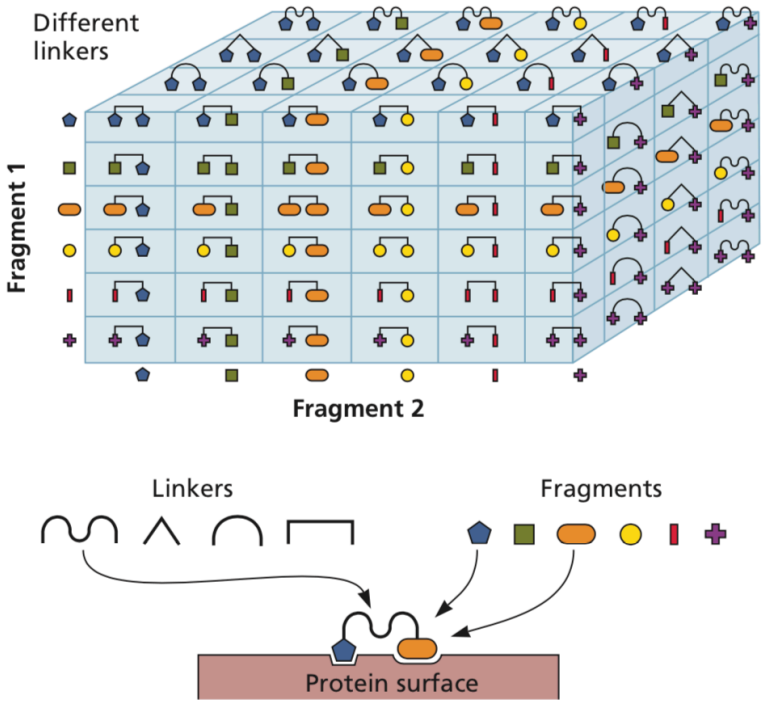
Cell-based screen



Antiviral screening

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



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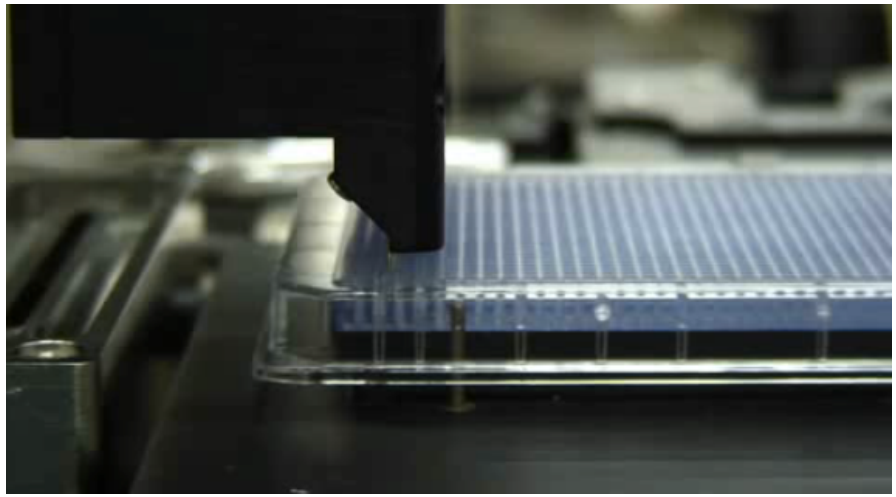
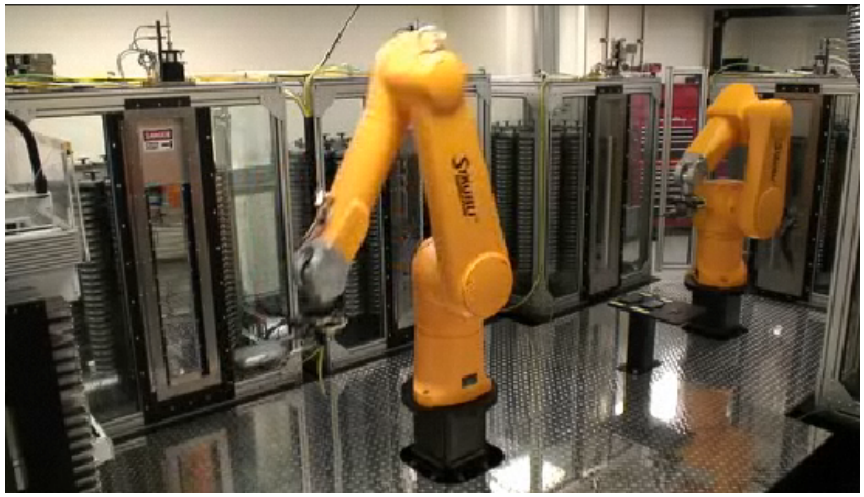
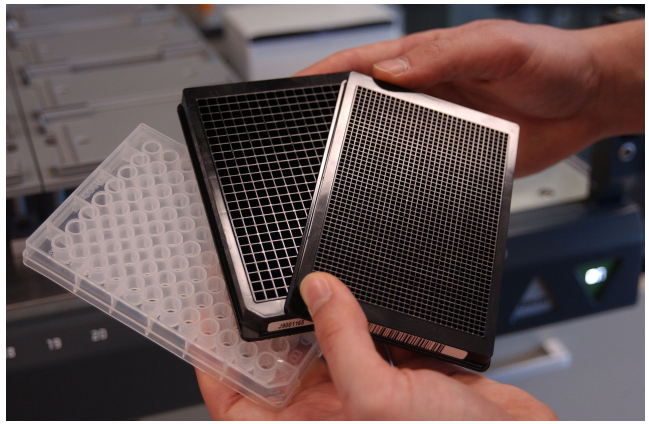
High throughput screening







Microtiter plates with 96, 384 and 1536 wells

Go to:

b.socrative.com/login/student

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

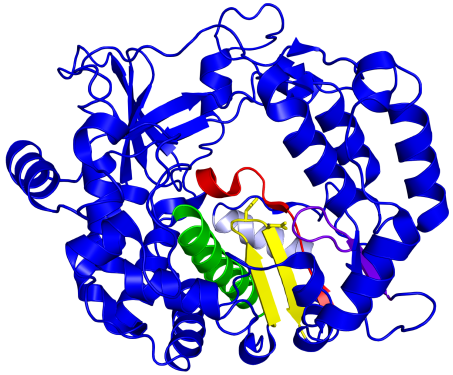
Resistance to antiviral drugs

- 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

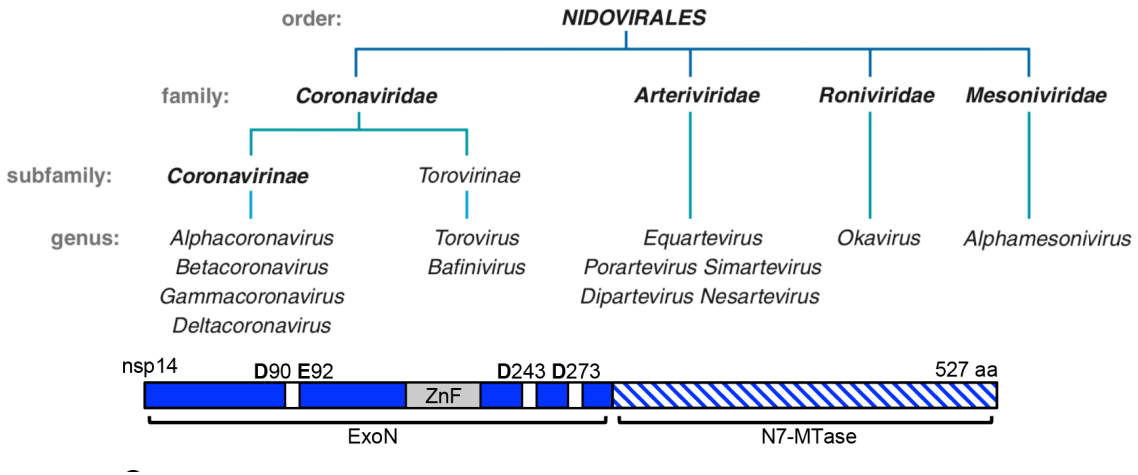
- Patient cannot be treated with same drug
- If no other drug is available, infection cannot be stopped
- Genetic analysis of resistance provides insight into antiviral mechanism
- May reveal new strategies to reduce or circumvent problem

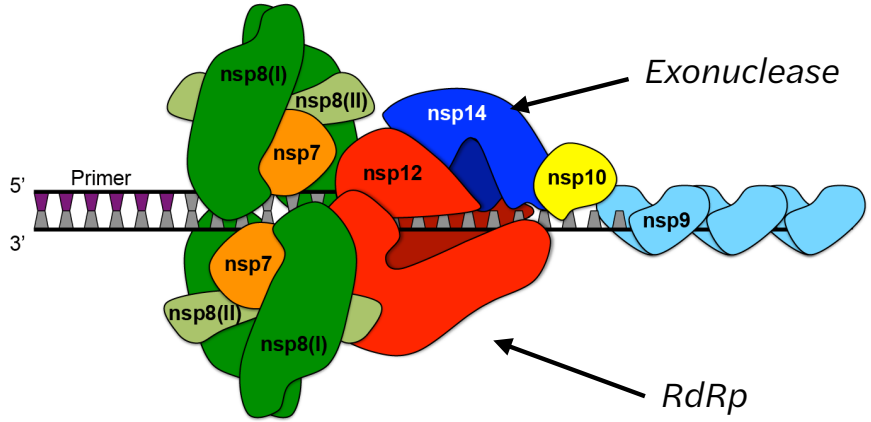
Mechanisms of drug resistance



- 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

Nidoviral genomes encode a proofreading exonuclease

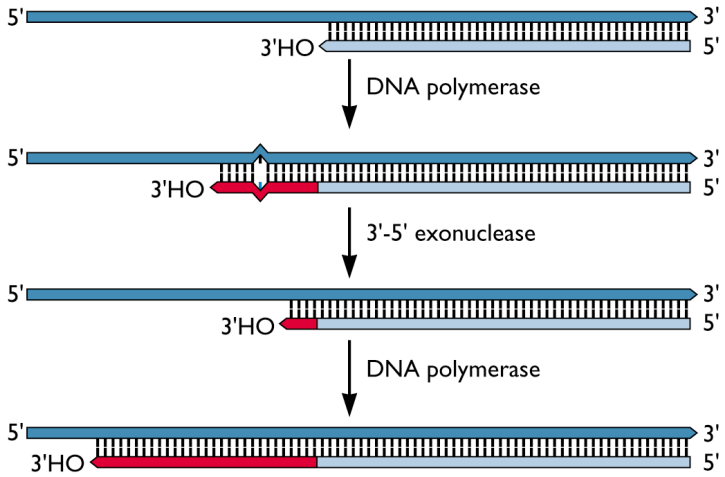




https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1003760

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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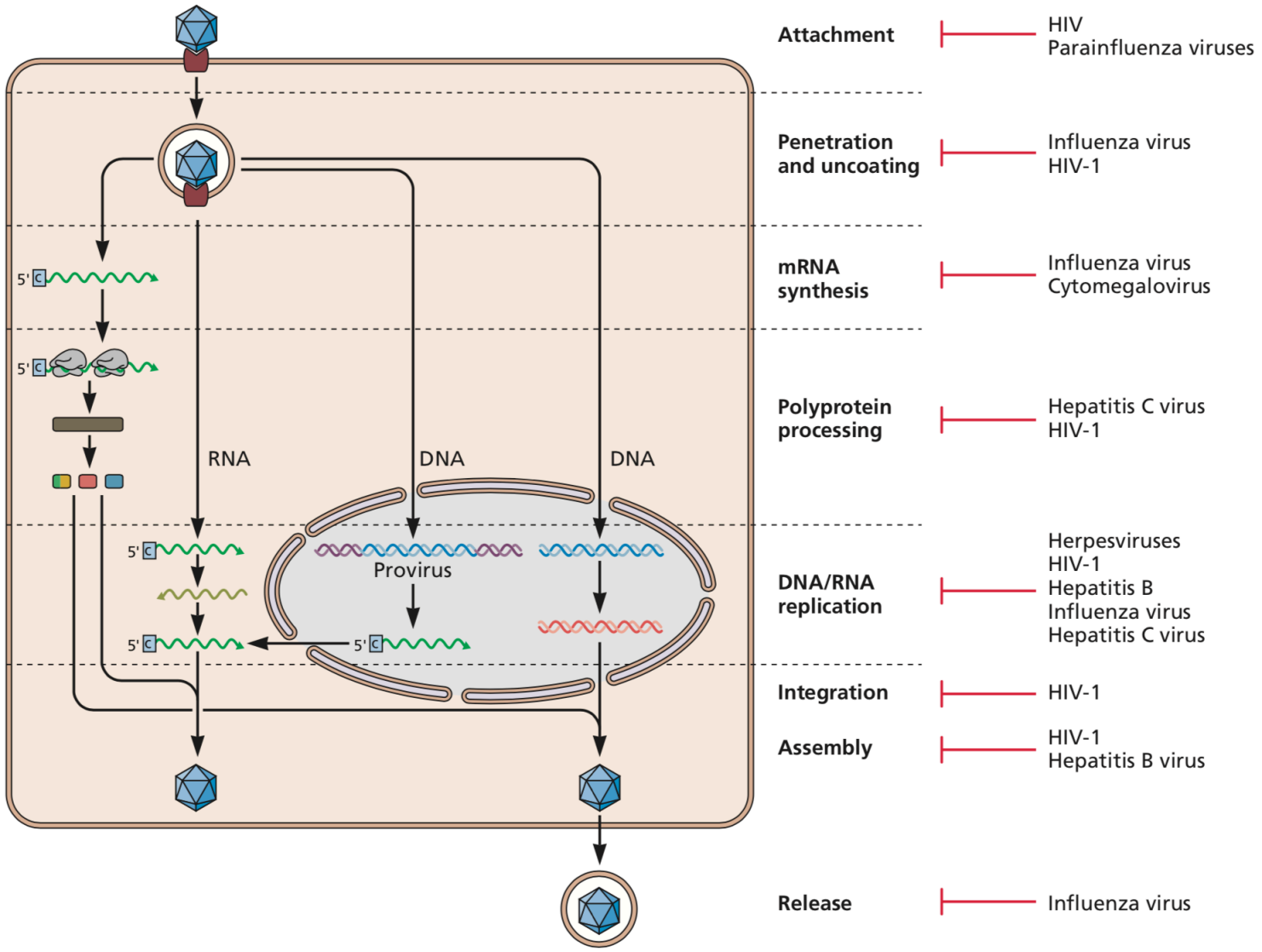
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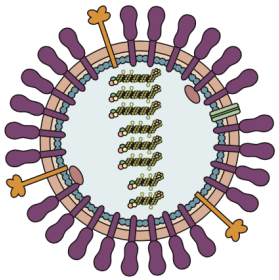
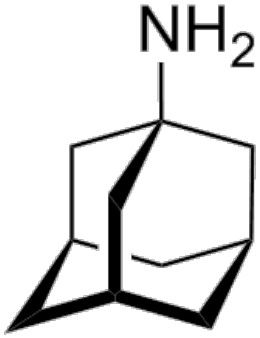
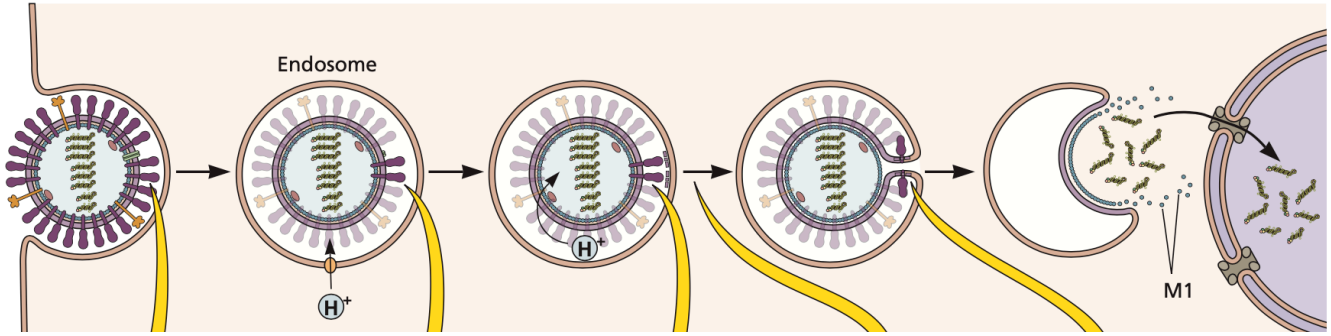
Entry Inhibitor

Symmetrel (Amantadine)





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



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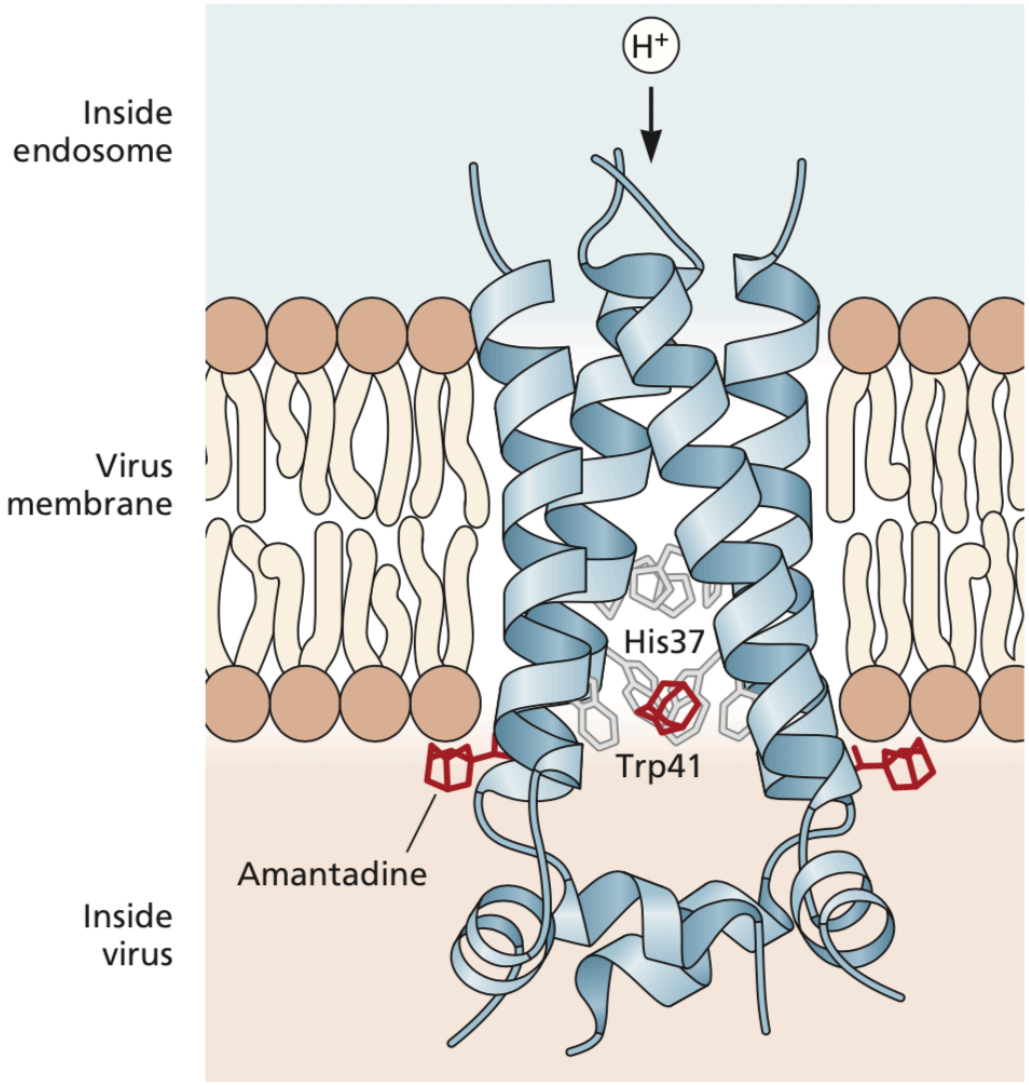

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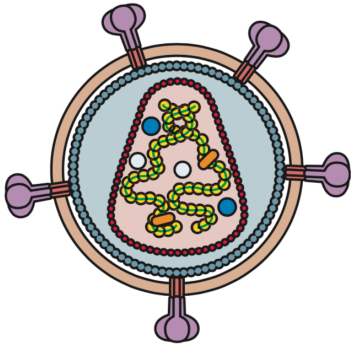
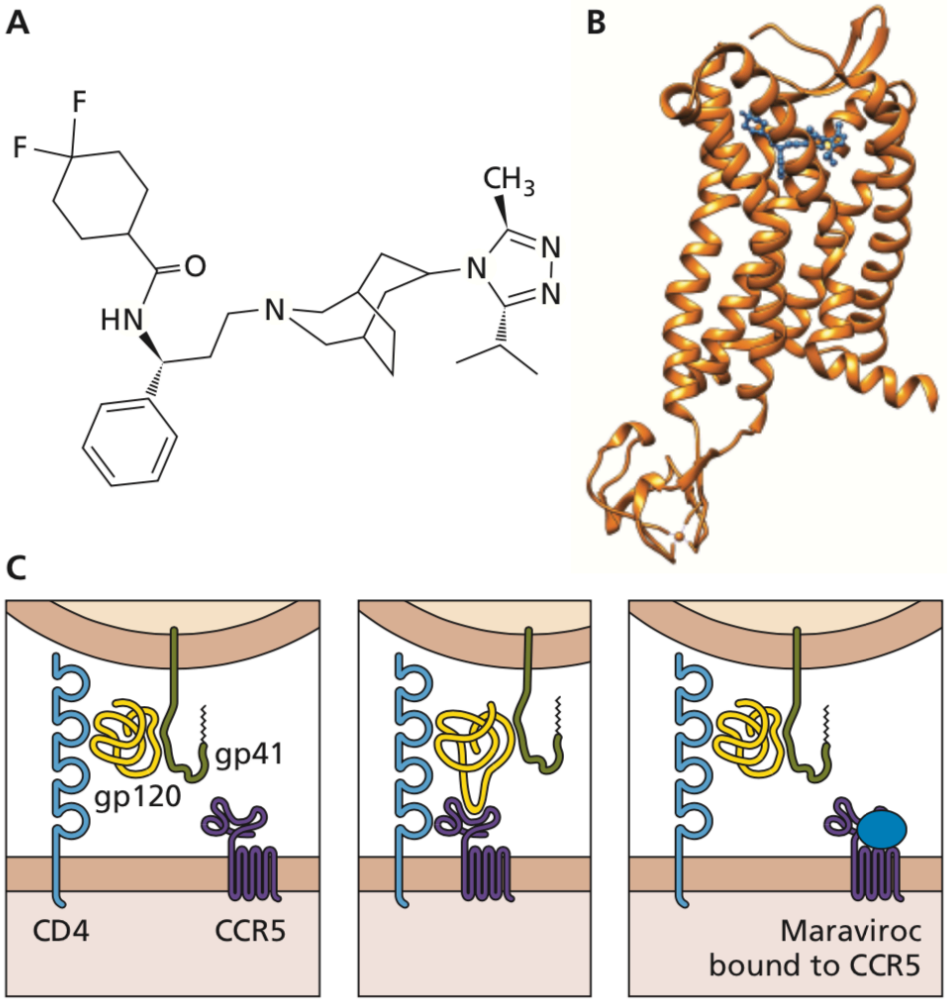
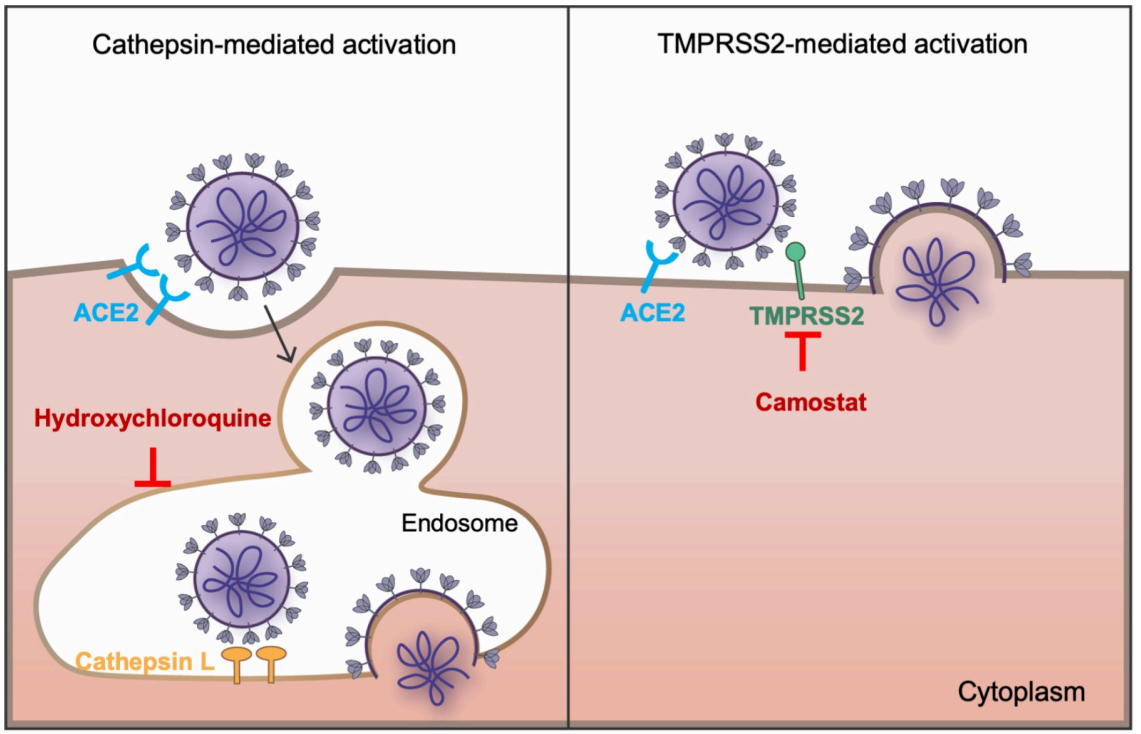
Maraviroc: CCR5 inhibitor





Why hydroxycholorquine failed

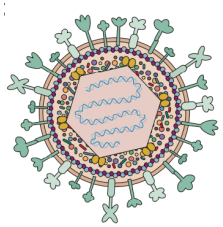
- HCQ known to inhibit infection by multiple viruses by inhibiting endosome acdification
- Found to inhibit reproduction of SARS-CoV-2 in cells in culture
- Given EUA in US

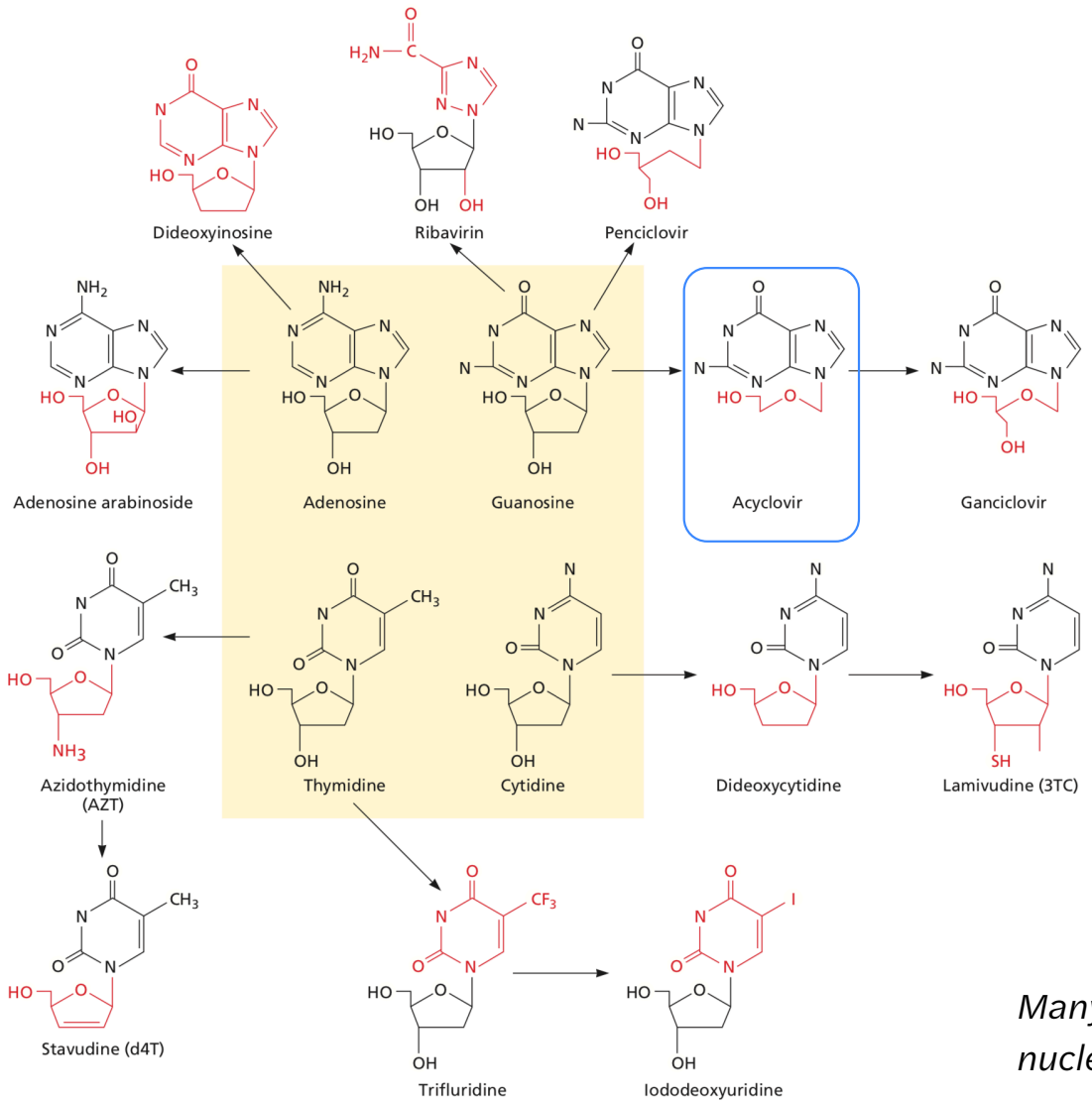


Polymerase Inhibitors

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

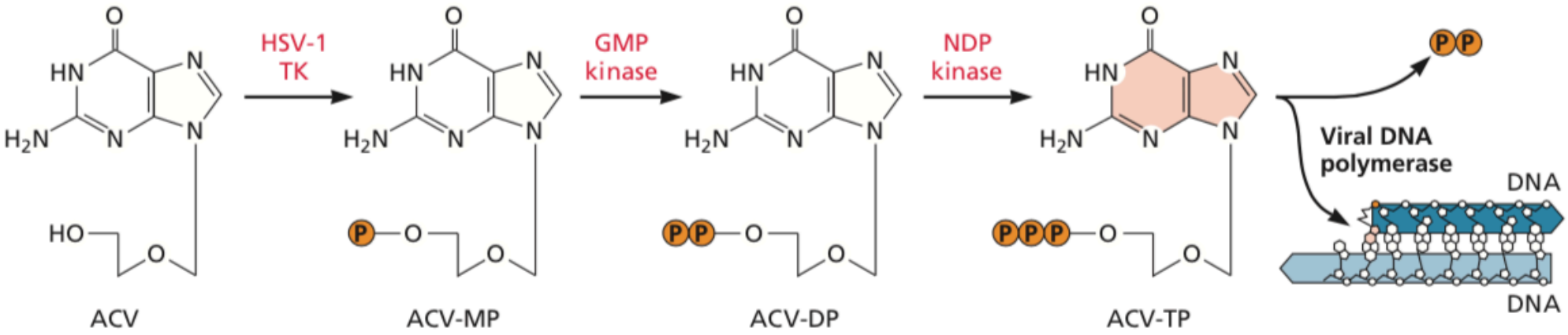
A prodrug; a nucleoside analog

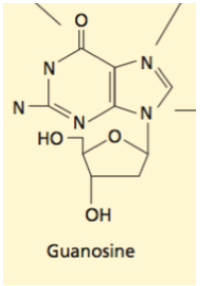




Many antiviral compounds are nucleoside (no P) and nucleotide (1-3 P) analogs

Acyclovir mechanism of action





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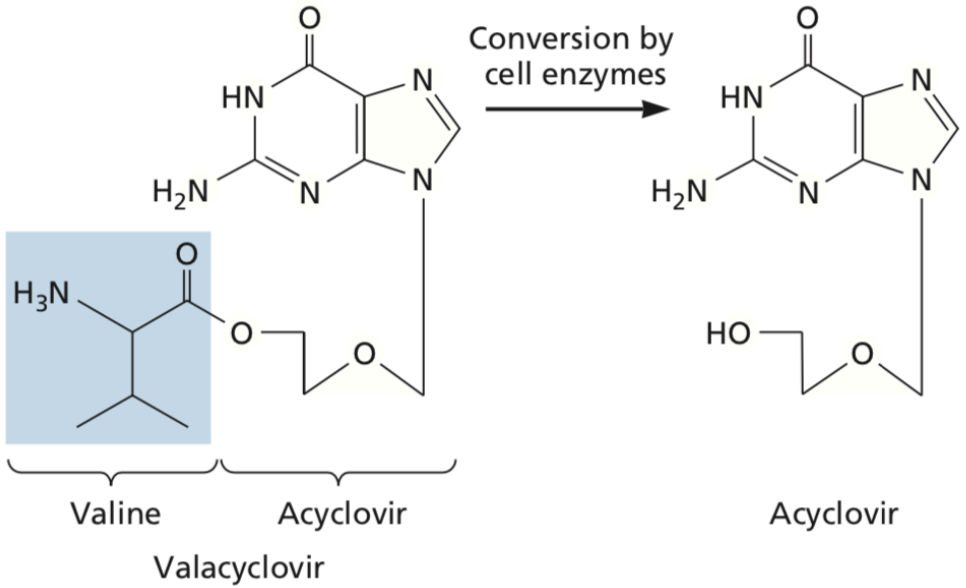
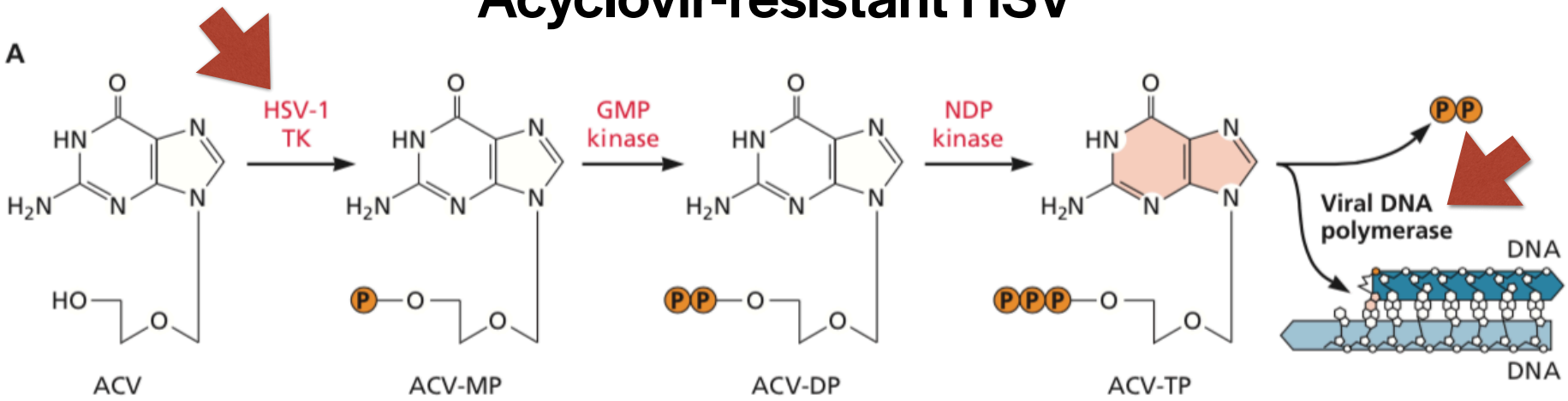
Improving acyclovir

- 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



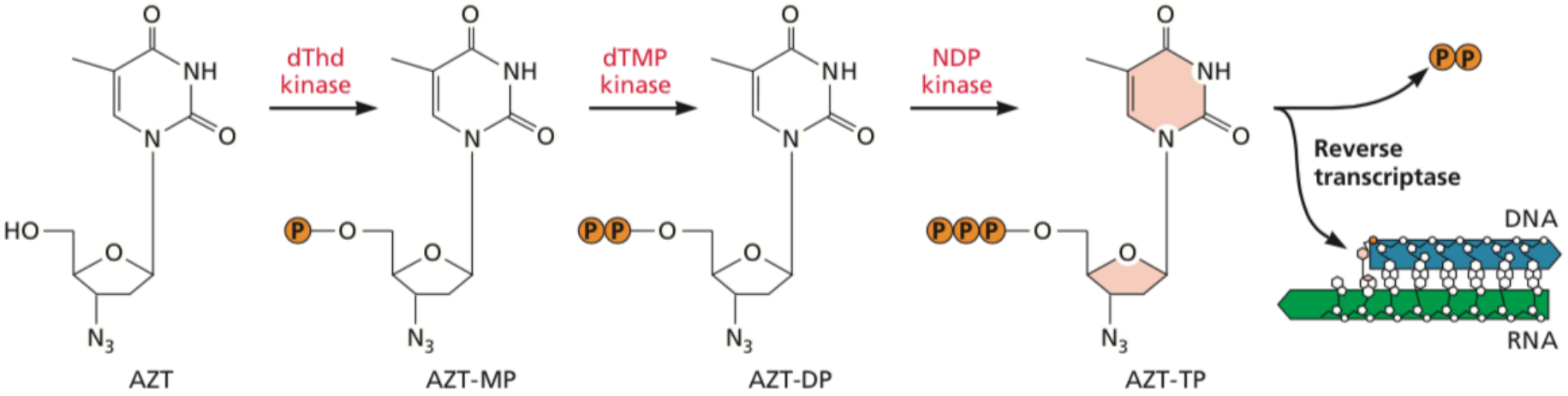
Acyclovir-resistant HSV



- 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*

Azido-deoxythymidine (AZT) - first HIV-1 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-1 RT



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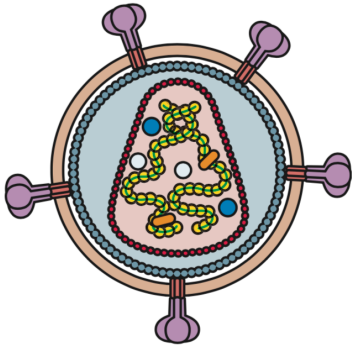
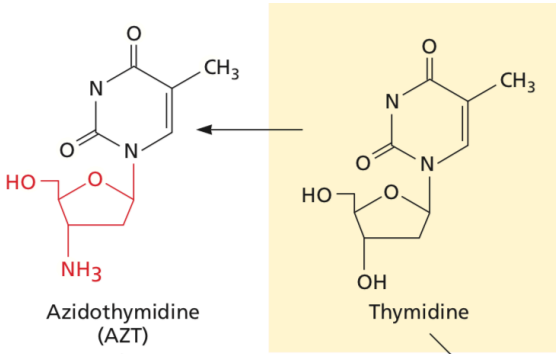
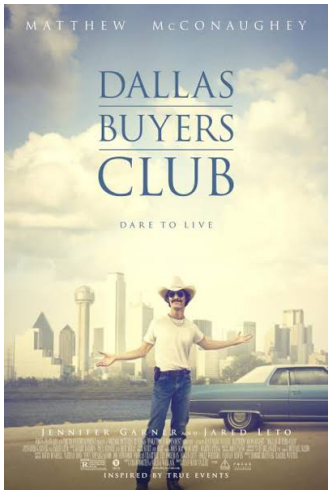


AZT



- 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



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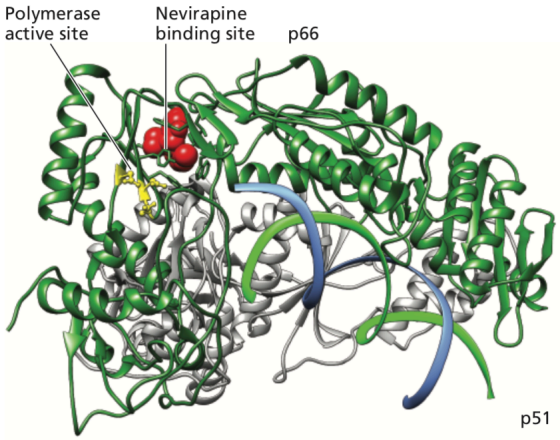
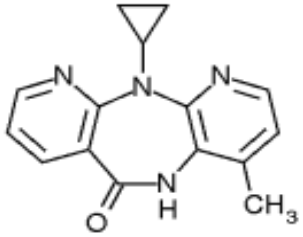
Resistance to AZT

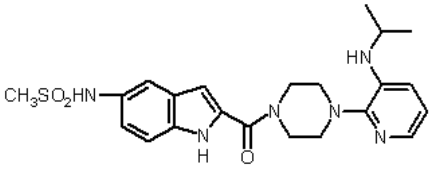
- Mutants resistant to AZT arose immediately after drug was licensed
- Single aa changes at one of four sites in RT
- Altered RT do not bind phosphorylated AZT
- New nucleoside analogs developed: Didanosine (ddI), Zalcitabine (ddC), Stavudine (d4T), Lamivudine (3TC)
- This lead to combination therapy,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



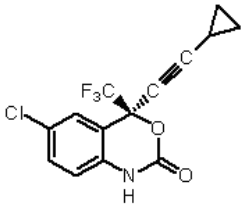
Non-nucleoside HIV-1 RT inhibitors (NNRTI)



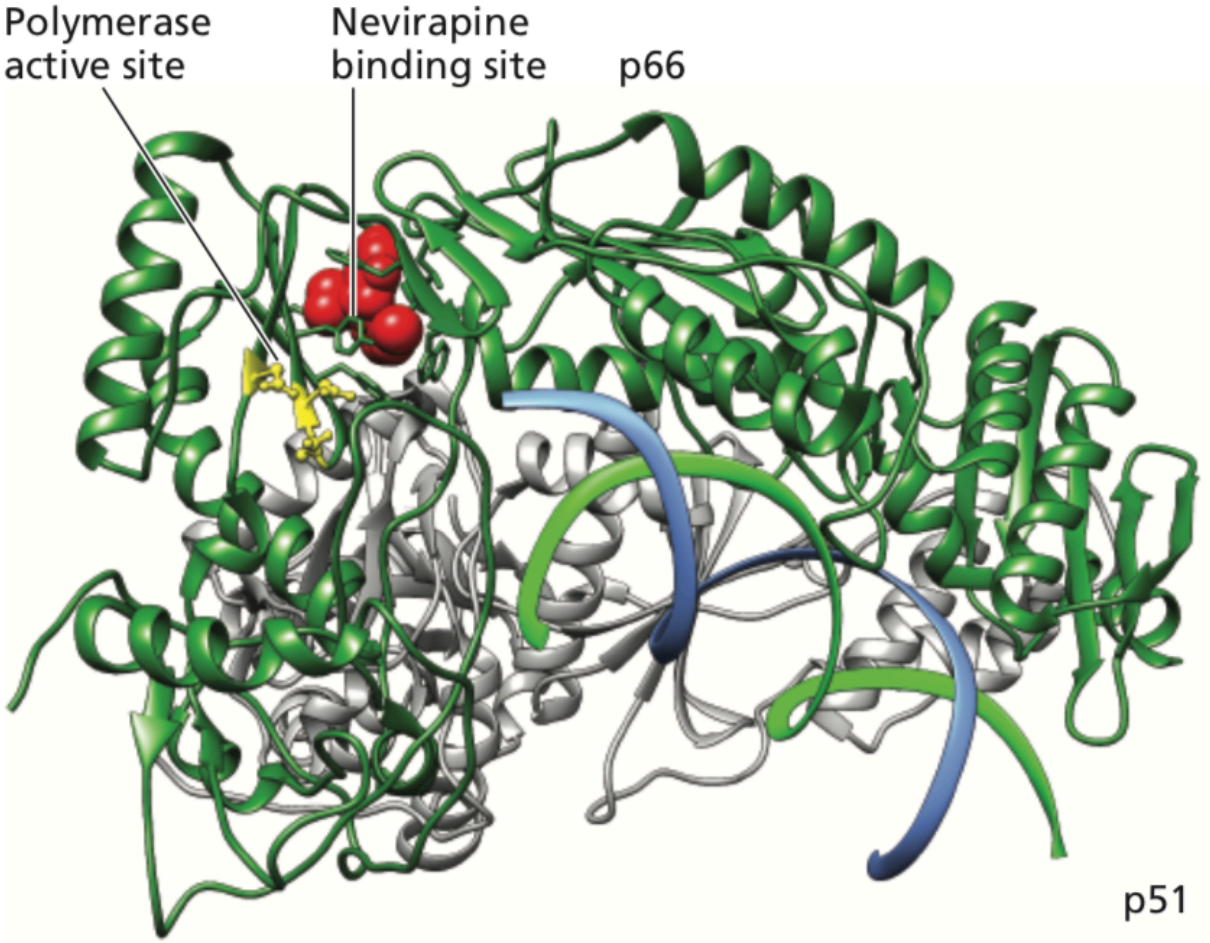
Nevirapine (Viramune)



Delavirdine (Rescriptor)



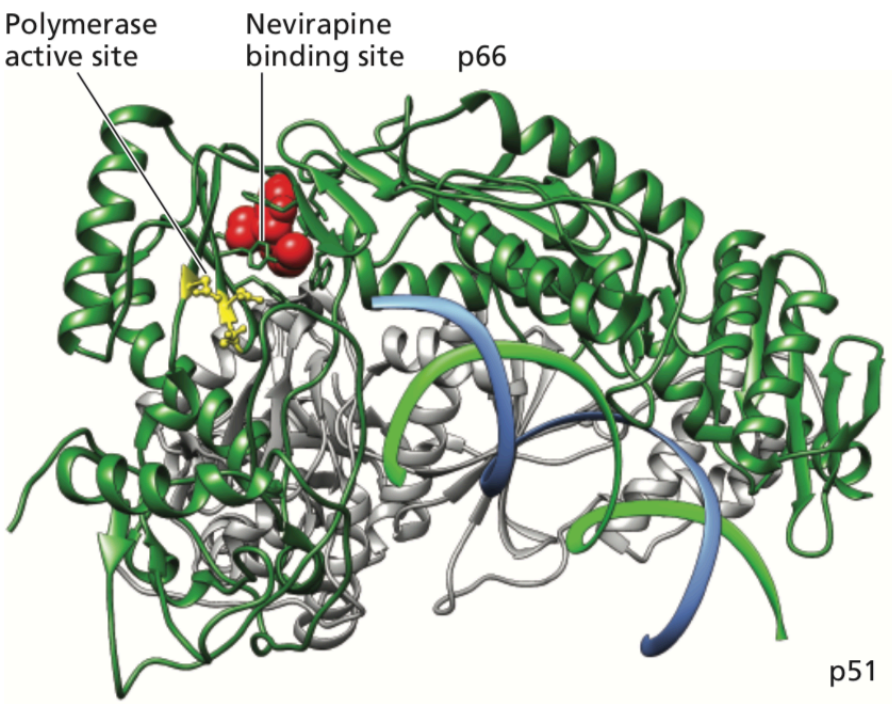
Efavirenz (Sustiva)



Principles of Virology, ASM Press

Resistance to NNRTIs

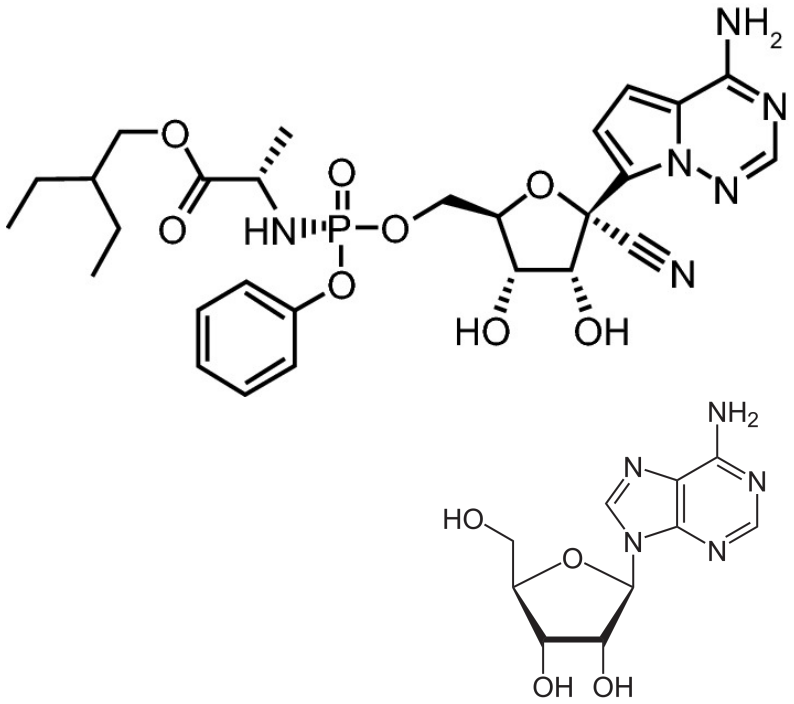
- Resistant mutants are selected rapidly
- Amino acid substitutions in any of seven residues that line binding sites on enzyme confer resistance
- Cannot be used alone for treatment of AIDS
- Now used largely in combination therapy



Principles of Virology, ASM Press

SARS-CoV-2 nucleoside analogs

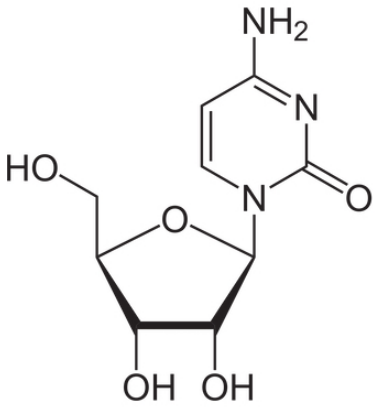
Remdesivir



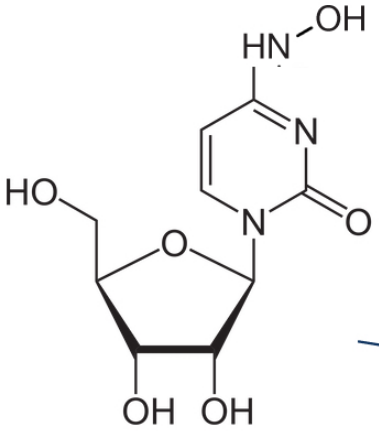
- Prodrug of adenosine nucleoside analog - chain terminator
- Developed for W. African Ebolavirus outbreak, 2013
- Inhibits replication of multiple RNA viruses by chain termination
- Found to inhibit SARS-CoV-2 replication in cells
- Received EUA in US after phase 3 trials
- Must be delivered intravenously
- No effect on hospitalized COVID-19

SARS-CoV-2 nucleoside analogs

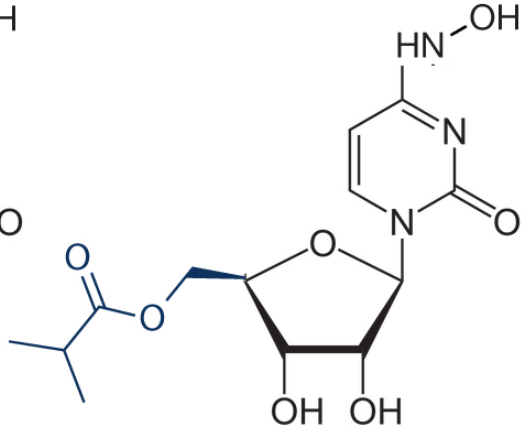
Molnupiravir



Cytidine



N4-hydroxycytidine (EDD-1931)



EIDD-2801

- Prodrug of cytidine nucleoside analog (2015)
- Templates as U
- Inhibits replication of multiple RNA viruses in cell culture by mutagenesis
- Found to inhibit SARS-CoV-2 replication in cells and in mice
- Inhibits replication and transmission in ferrets
- Orally bioavailable
- EUA in US

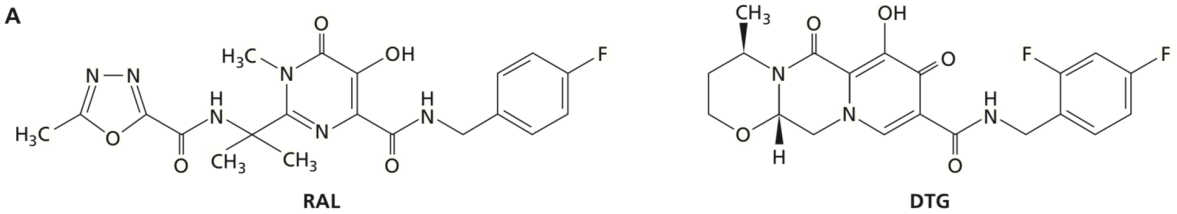
Go to:

b.socrative.com/login/student
room number: virus

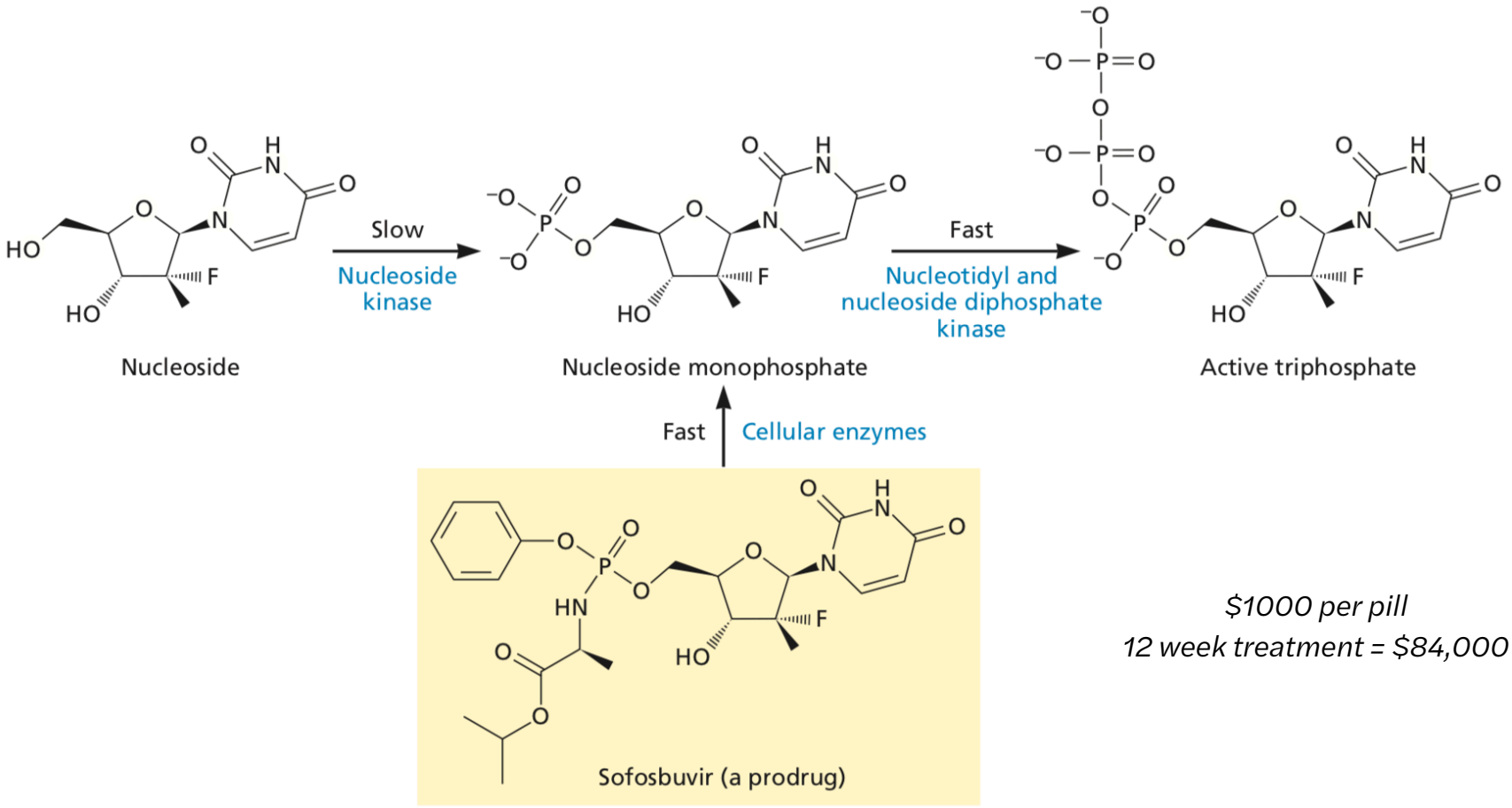
Resistance to which antiviral would involve amino acid changes in a viral enzyme?

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

IN inhibitors



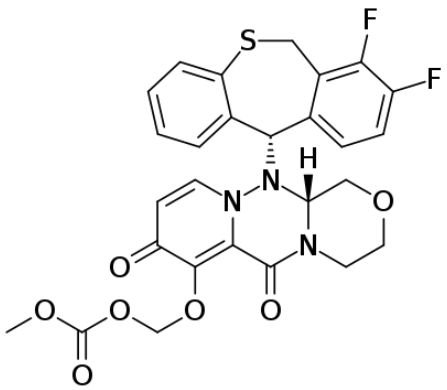
Hepatitis C virus RNA polymerase inhibitor

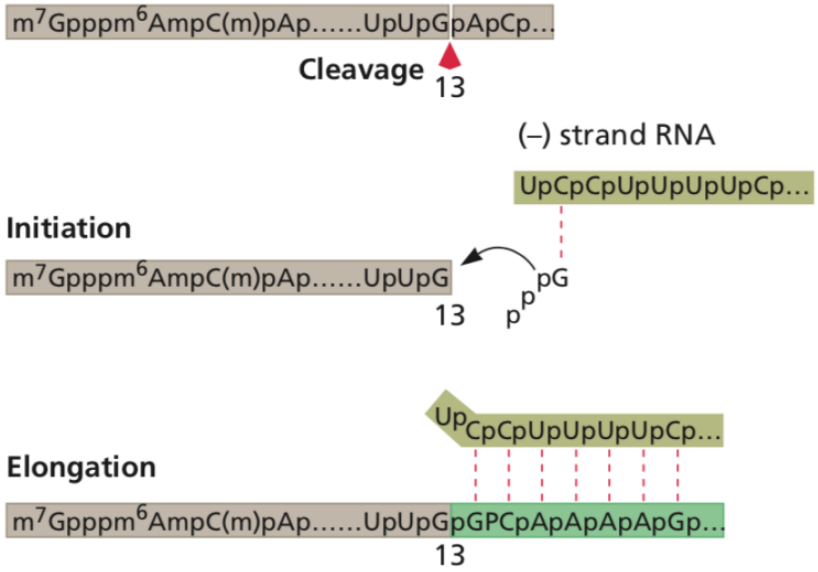


$1000 per pill
12 week treatment = $84,000

Baloxavir: A new influenza virus antiviral

- Approved by FDA October 2018 for treatment of acute uncomplicated influenza in people 12 years of age and older who have been symptomatic for no more than 48 hours
- Inhibitor of influenza endonuclease

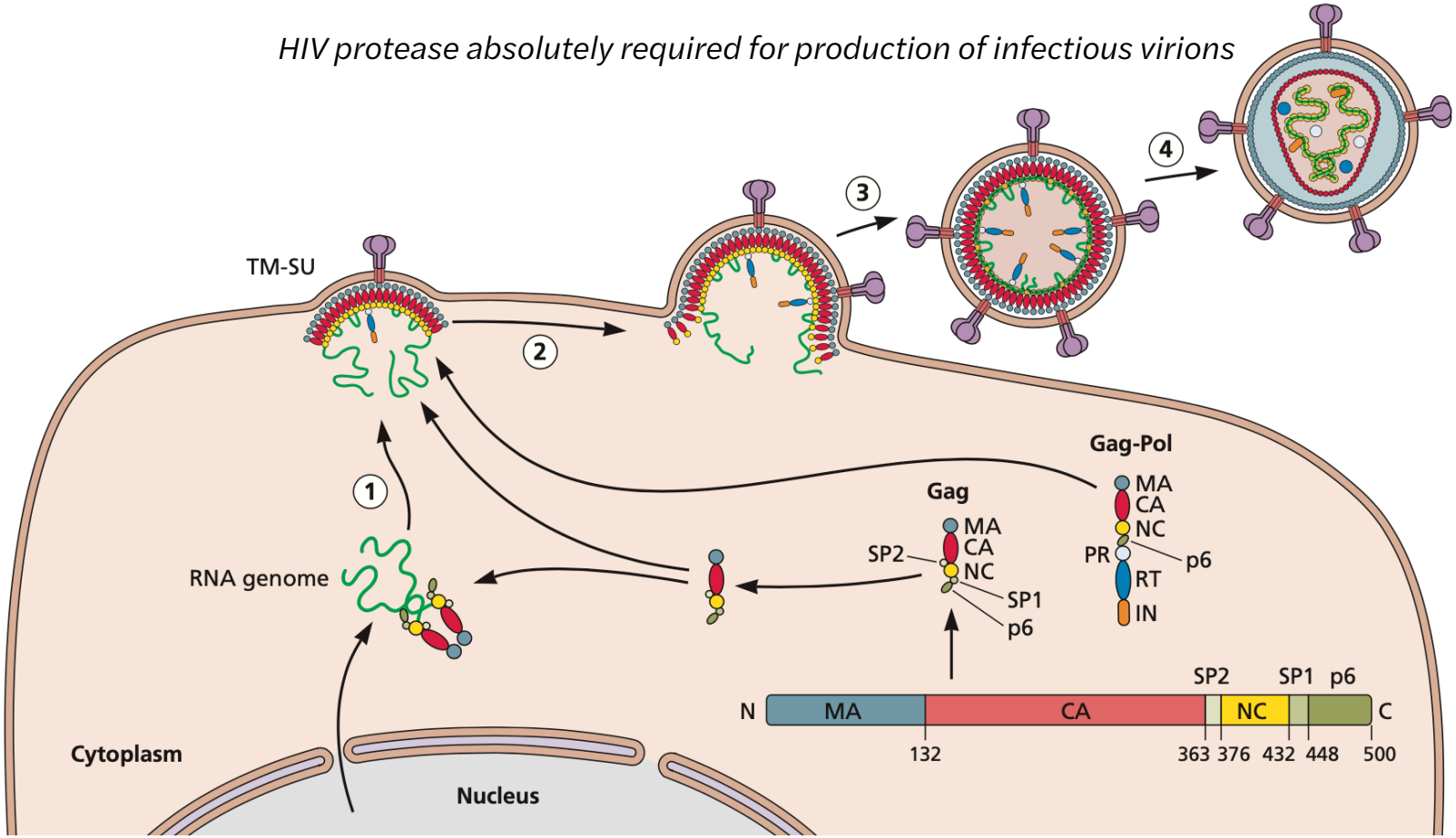




Principles of Virology, ASM Press

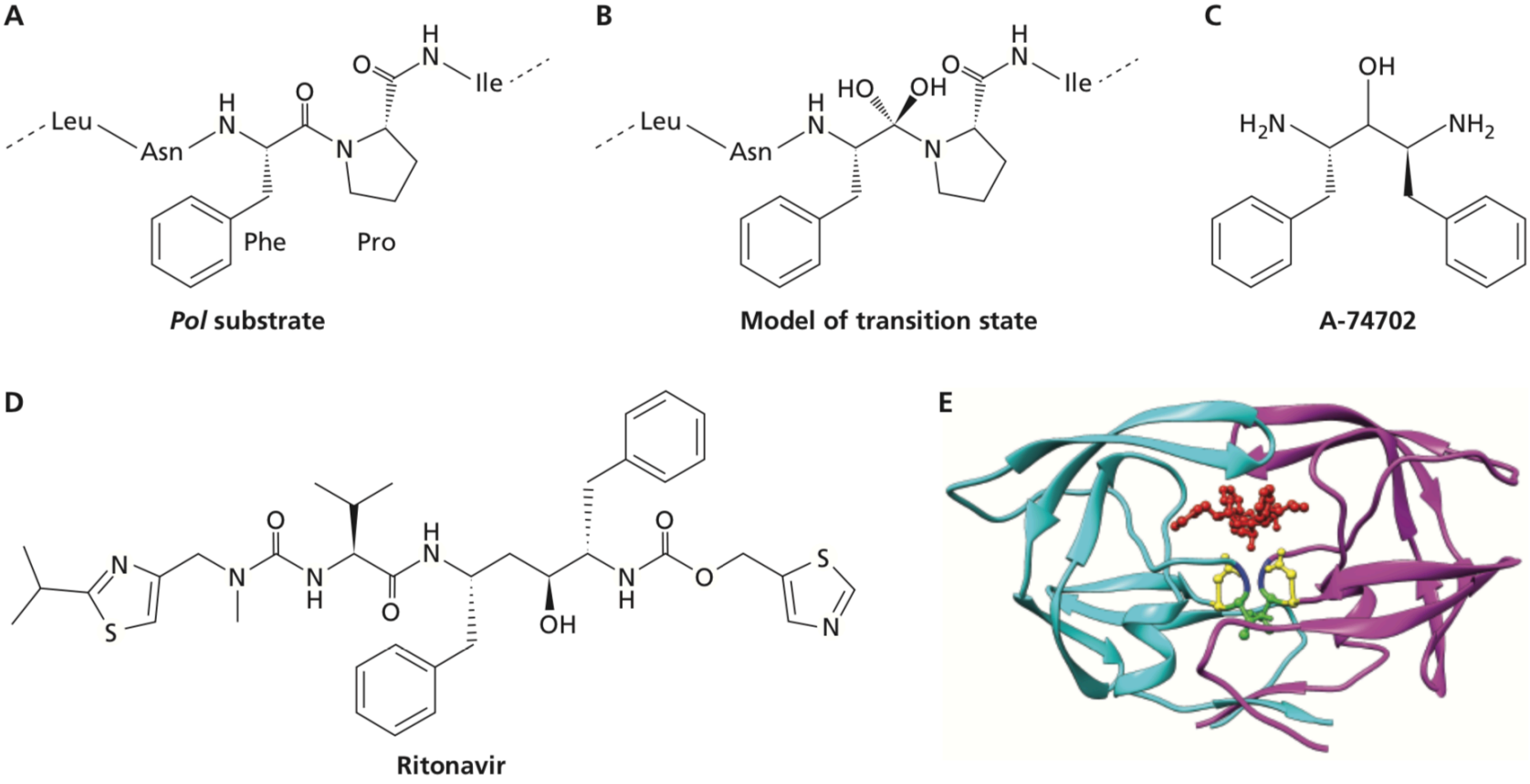
Protease Inhibitors

HIV protease absolutely required for production of infectious virions

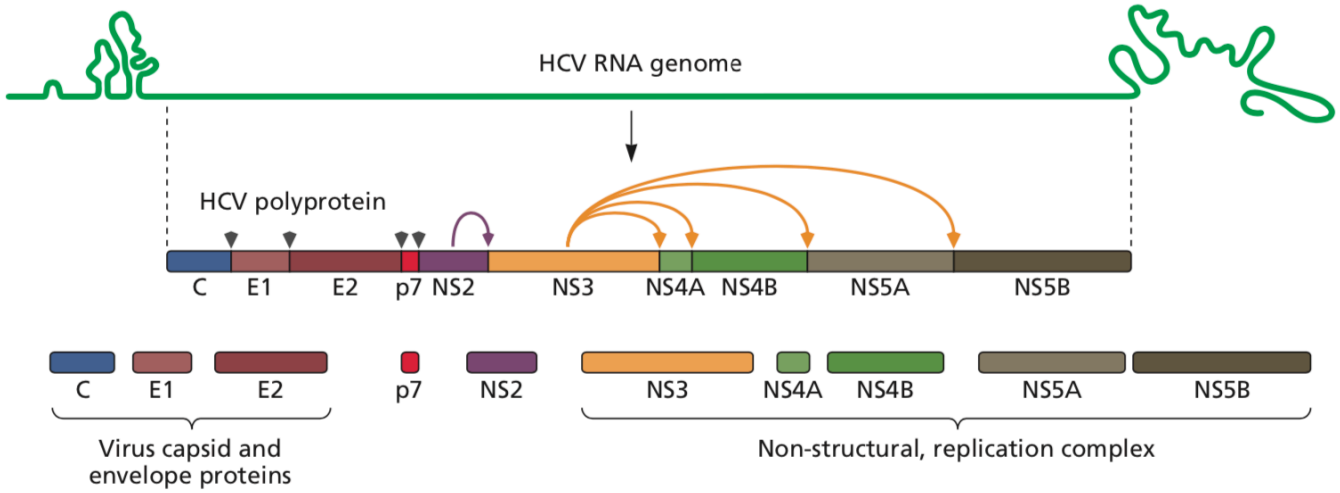


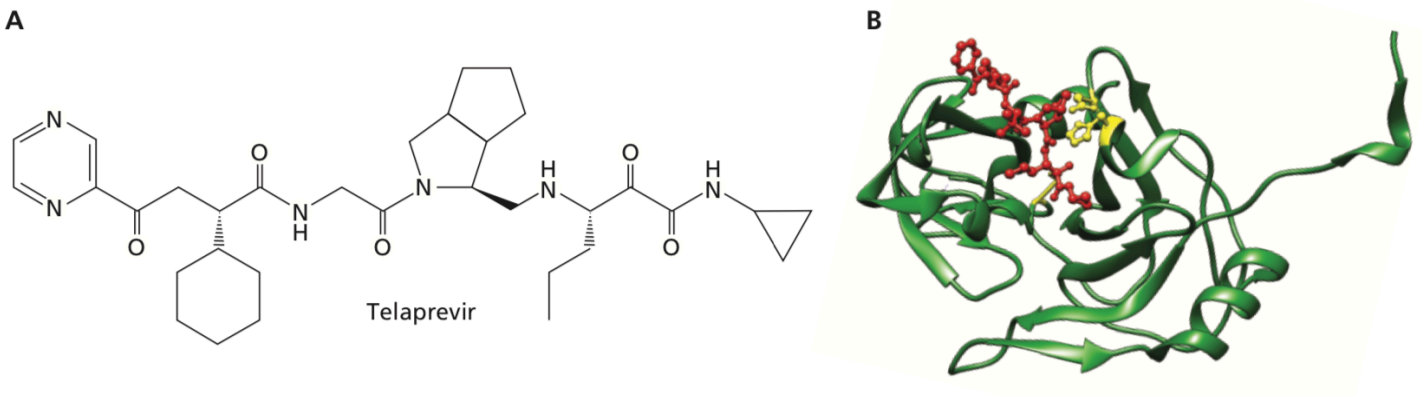
Antiviral drugs that target HIV protease

Development of Ritonavir, a peptidomimetic

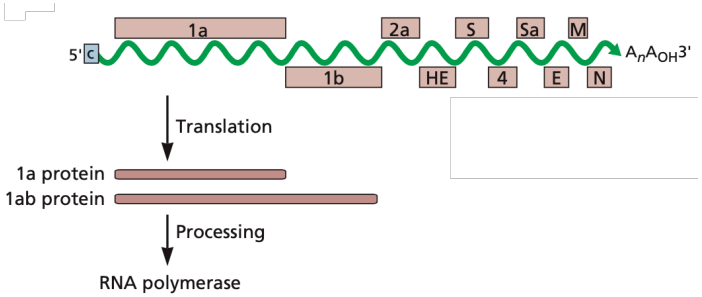


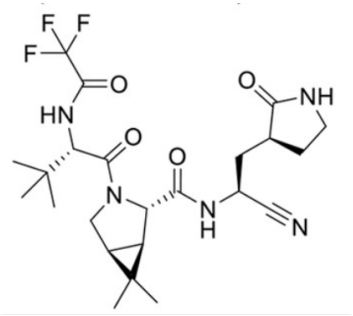
Hepatitis C virus protease inhibitor

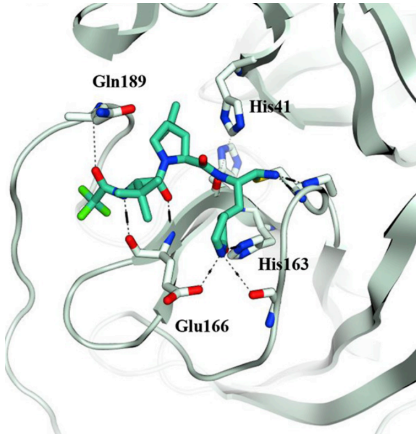


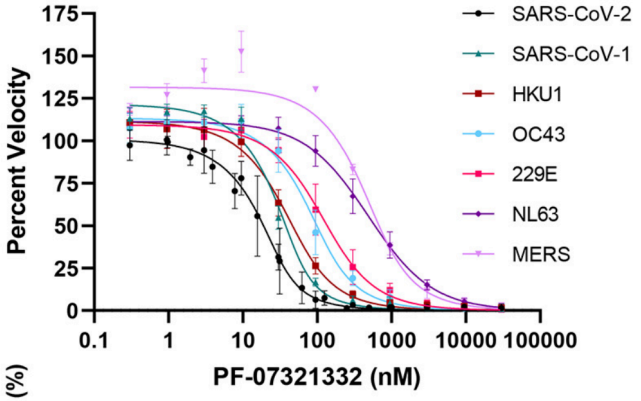


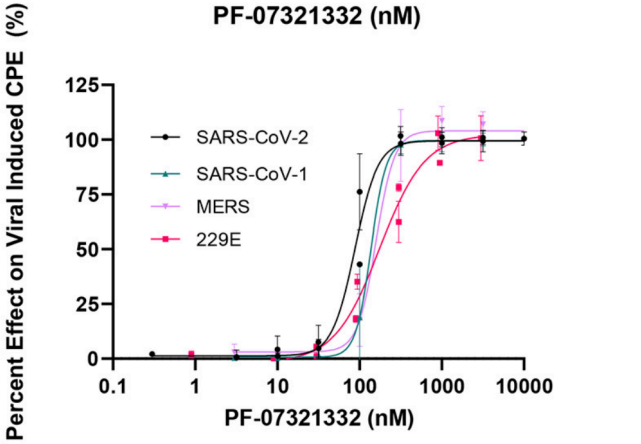
Paxlovid - SARS-CoV-2 M^{pro} inhibitor





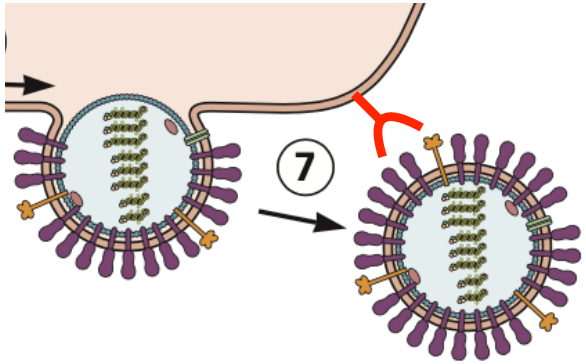


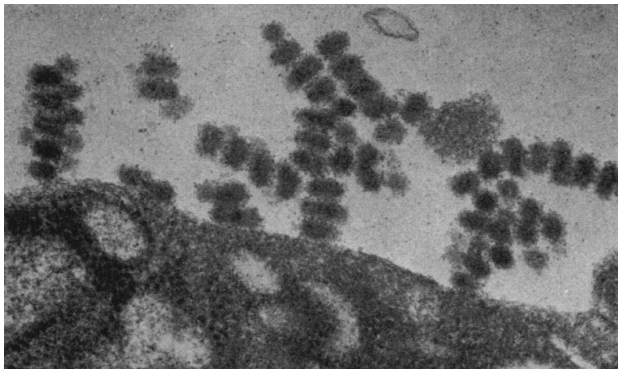




https://doi.org/10.1126/science.abl4784

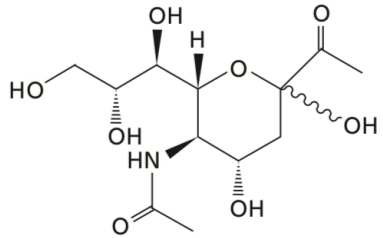
Influenza virus NA inhibitors





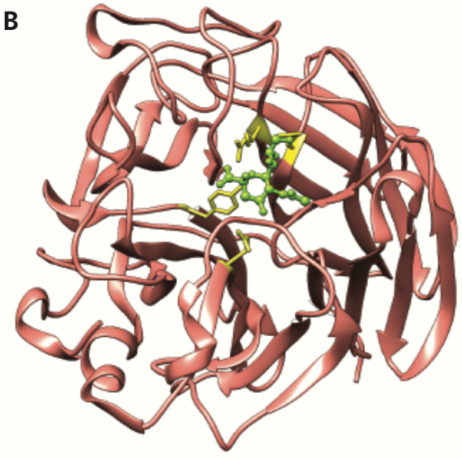
J Gen Virol. 1976 Oct;33(1):159-63.

A

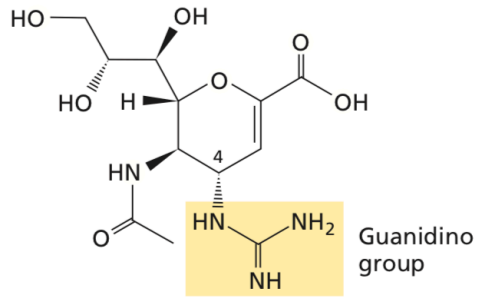


Sialic acid
(*N*-acetyl neuraminic acid)

B

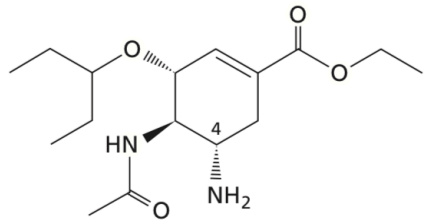


C



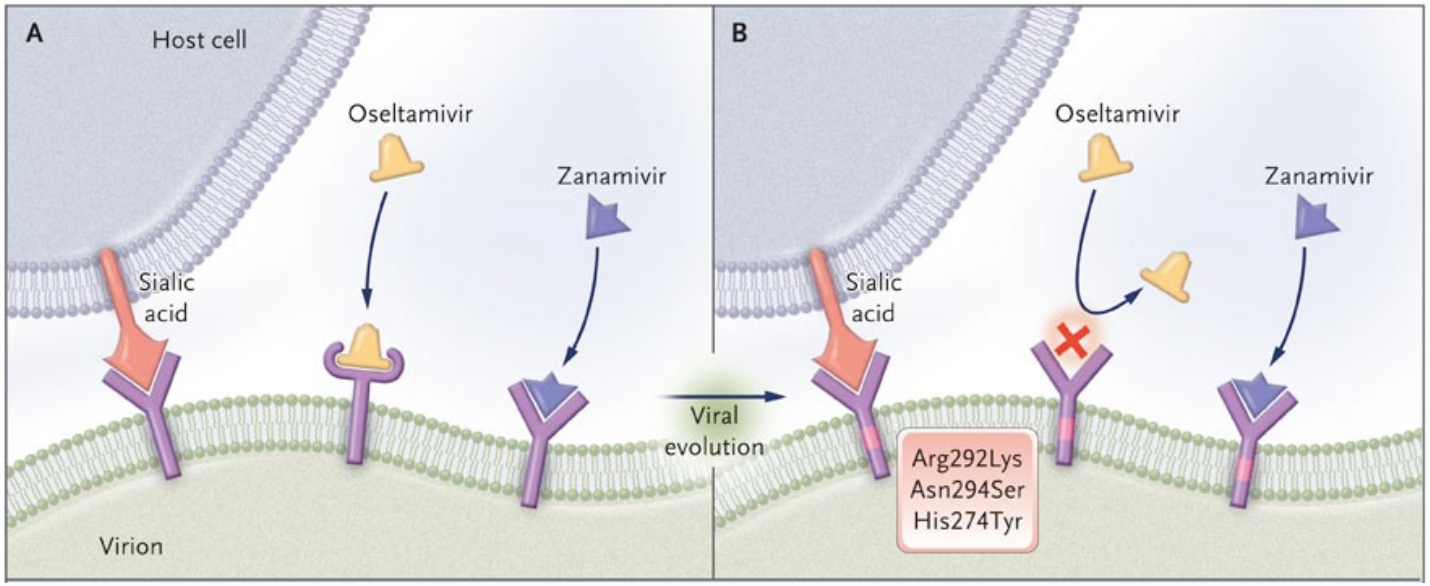
Guanidino group

Zanamivir
"Relenza"



Oseltamivir
"Tamiflu"

Influenza virus NA inhibitors



- 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

Neuraminidase Inhibitor Resistance Testing Results on Samples Collected Since September 2019

Antiviral Medication			Total Viruses*	A/H1	A/H3	B/Victoria	B/Yamagata
Neuraminidase Inhibitors	Oseltamivir	Viruses Tested	2,433	885	502	954	92
		Reduced Inhibition	1 (0.04%)	(0.0%)	(0.0%)	1 (0.1%)	(0.0%)
		Highly Reduced Inhibition	4 (0.2%)	4 (0.5%)	(0.0%)	(0.0%)	(0.0%)
	Peramivir	Viruses Tested	2,433	885	502	954	92
		Reduced Inhibition	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
		Highly Reduced Inhibition	5 (0.2%)	4 (0.5%)	(0.0%)	1 (0.1%)	(0.0%)
	Zanamivir	Viruses Tested	2,433	885	502	954	92
		Reduced Inhibition	2 (0.1%)	(0.0%)	(0.0%)	2 (0.2%)	(0.0%)
		Highly Reduced Inhibition	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)
PA Endonuclease Inhibitor	Baloxavir	Viruses Tested	2,541	884	584	978	95
		Reduced Susceptibility	(0.0%)	(0.0%)	(0.0%)	(0.0%)	(0.0%)

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

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

Go to:

b.socrative.com/login/student

room number: virus

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

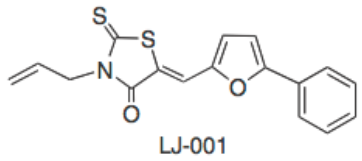
A. Nucleoside inhibitors
B. NNRTIs
C. CCR5 inhibitors
D. Integrase inhibitors
E. Fusion inhibitors

Are broad spectrum antivirals possible? LJ001

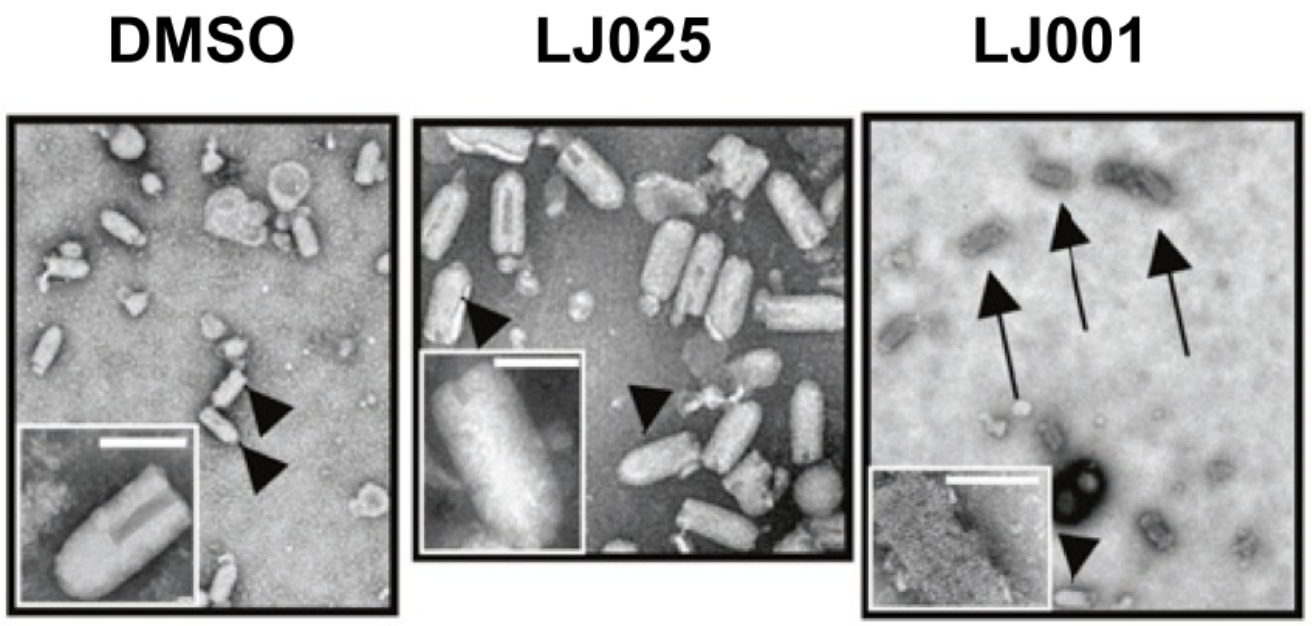
Virus	Family	Genome type	Envelope (yes/no)	Activity
Ebola[L] (cat A)	Filoviridae	ssRNA(−)	Y	++
Marburg[L] (cat A)	Filoviridae	ssRNA(−)	Y	++
Influenza A[L] (cat A)	Orthomyxoviridae	ssRNA(−)	Y	+++
Junín[L] (cat A)	Arenaviridae	ssRNA(−)	Y	++
Rift Valley fever[L] (cat A)	Bunyaviridae	ssRNA(−)	Y	+++
LaCrosse[L] (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

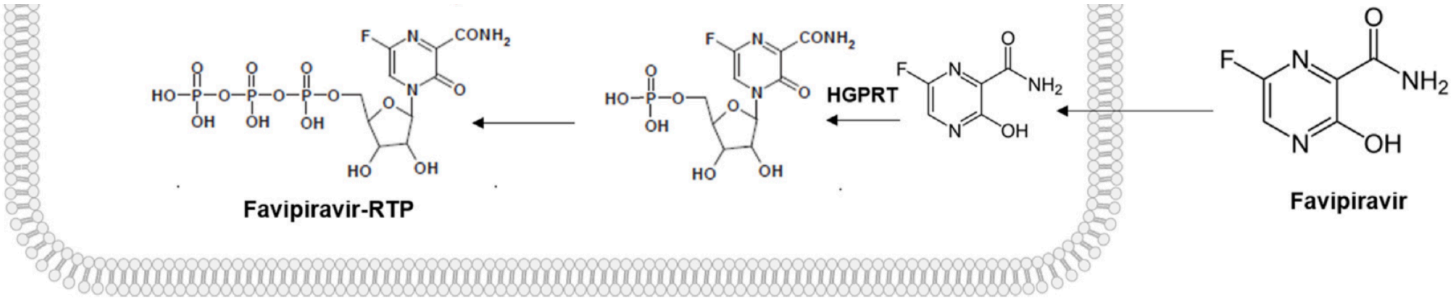
LJ1001, a broad spectrum antiviral



LJ-001



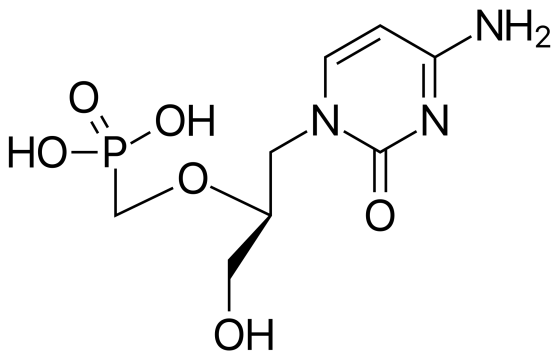
Favipiravir (Avigan)



- Broad-spectrum inhibitor of RNA viruses
- Target: RdRp, a nucleoside analog
- (+) RNA: WNV, YFV, ZIKV, WEEV, CHIKV, picornaviruses, norovirus, CoV
- (-) RNA: Lassa virus, EBOV, Rabies virus, measles virus, Pichinde, Junin, Rift Valley fever virus, Hantaviruses, Respiratory syncytial virus, parainfluenza virus
- Licensed in Japan to treat influenza

https://doi.org/10.1016/j.antiviral.2018.03.003

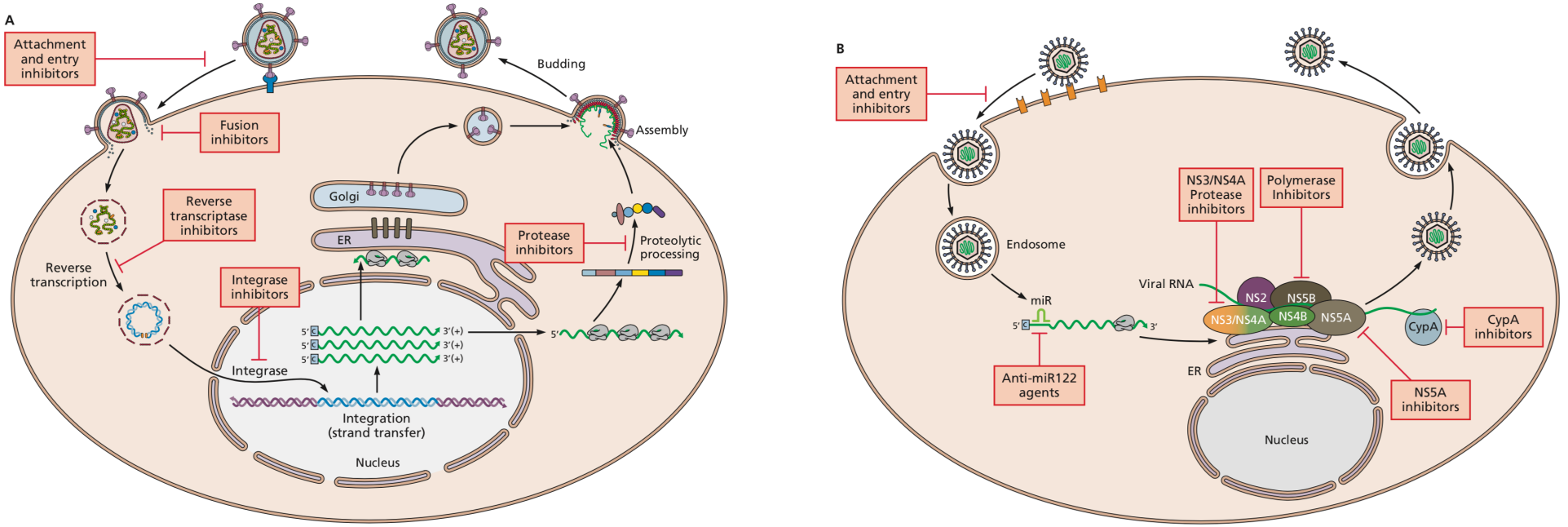
Cidofovir (Vistide)



- Broad-spectrum inhibitor of DNA viruses (adenovirus, poxvirus, herpes simplex virus, polyomavirus, papillomavirus)
- Acyclic cytosine phosphonate
- Phosphate group makes it a mimic of deoxycytidine monophosphate
- Diphosphorylated by host cell enzymes
- Diphosphorylated form has higher affinity for viral DNA polymerases than host, a property of acyclic nucleotide analogues

Two Stories of Antiviral Success

Combination Therapy for AIDS and Hepatitis C



Key to drug development: Life-long persistent infections

Combination therapy

- HAART: HIV can be treated as a chronic disease
- Target different mechanisms
- One pill containing three inhibitors
- Does not cure infection! Latent reservoir remains

Mathematics of drug resistance

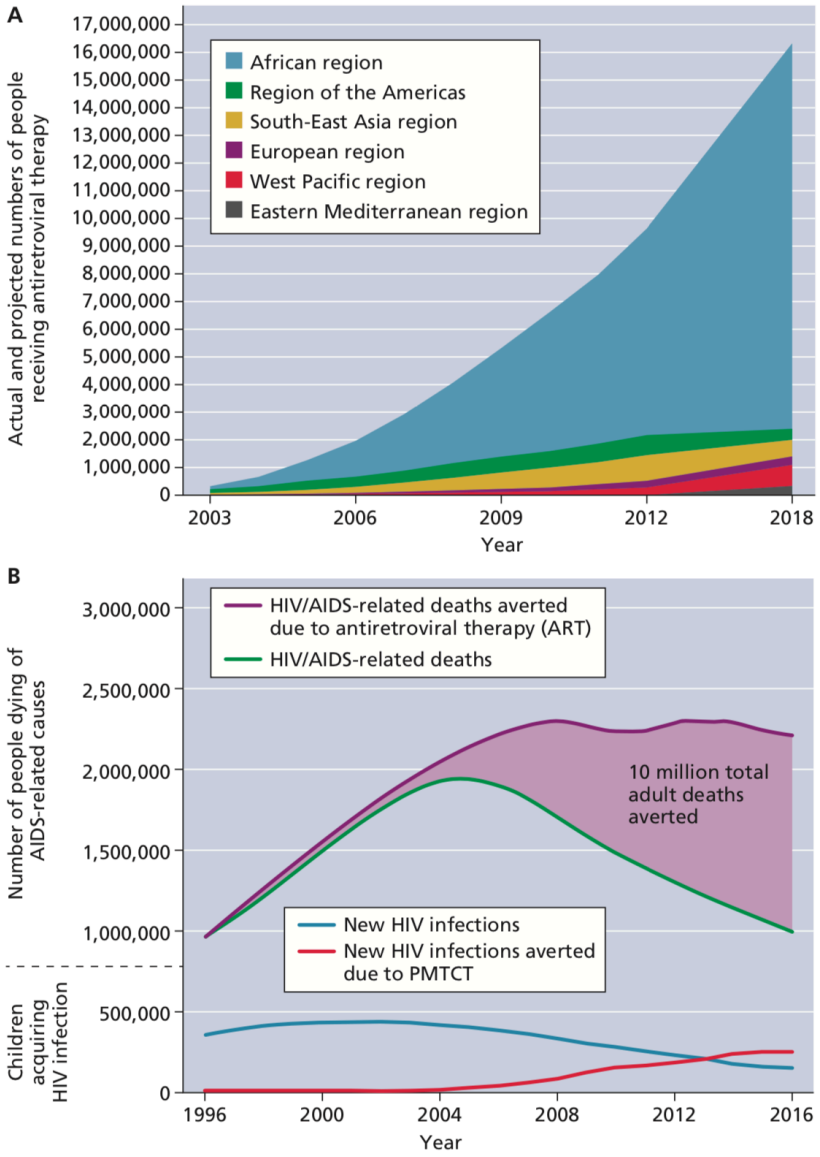
- Assume one mutation needed for drug resistance
- Mutation rate 1 every 10^4 bases polymerized
- Each base is substituted in every 10^4 viruses
- Each person makes 10^{10} new viruses/day
- $10^{10}/10^4 = 10^6$ viruses will be produced each day with resistance to one drug

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

Target	Generic name	Brand name	Manufacturer	Year
Reverse transcriptase	Zidovudine (AZT)	Retrovir	GlaxoSmithKline	1987
Nucleos(t)ide inhibitors	Didanosine (ddI)	Videx	Bristol-Myers Squibb	1991
	Zalcitabine (ddC)	Hivid	Hoffmann-La Roche	1992
	Stavudine (d4T)	Zerit	Bristol-Myers Squibb	1994
	Lamivudine (3TC)	Epivir	GlaxoSmithKline	1995
	Abacavir (ABC)	Ziagen	GlaxoSmithKline	1998
	Tenofovir (TDF)	Viread	Gilead Sciences	2001
	Emtricitabine (FTC)	Emtriva	Bristol-Myers Squibb	2003
Nonnucleoside inhibitors	Nevirapine (NVP)	Viramune	Roxane	1996
	Delavirdine (DLV)	Rescriptor	Pfizer	1997
	Efavirenz (EFV)	Sustiva	DuPont	1998
	Etravirine (ETR)	Intelence	Tibotec	2008
	Rilpivirine (RPV)	Edurant	Tibotec	2011
Protease	Saquinavir (SQV)	Invirase	Hoffmann-La Roche	1995
	Ritonavir (RTV)	Norvir	Abbott	1996
	Indinavir (IDV)	Crixivan	Merck	1996
	Nelfinavir (NFV)	Viracept	Agouron	1997
	Amprenavir (APV)	Agenerase	GlaxoSmithKline	1999
	Lopinavir/RTV	Kaletra	Abbott	2000
	Atazanavir (ATV)	Revataz	Bristol-Myers Squibb	2003
	Fosamprenavir (FPV)	Lexia	ViiV	2003
	Tipranavir (TPV)	Aptivus	Boehringer Ingelheim	2005
	Darunavir (DRV)	Prezista	Tibotec	2006
Integrase	Raltegravir (RAL)	Isentress	Merck	2007
	Elvitegravir (EVG)	Vitekta	Gilead Sciences	2012
	Dolutegravir (DTG)	Tivicay	GlaxoSmithKline	2013
Entry	Enfuvirtide (T20)	Fuzeon	Genentech	2003
	Maraviroc (MVC)	Selzentry	Pfizer	2007
Combinations	3TC/AZT	Combivir	ViiV	1997
	ABC/3TC/AZT	Trizivir	ViiV	2000
	TDF/FTC	Truvada	Gilead Sciences	2004
	DRV/cobicistat (COBI)	Prezcobix	Janssen	2006
	TDF/FTC/EFV	Atripla	Bristol-Myers Squibb/ Gilead Sciences	2006
	TDF/FTC/RPV	Complera	Gilead Sciences	2011
	TDF/FTC/EVG/COBI	Stribild	Gilead Sciences	2012
	DTG/ABC/3TC	Triumeq	Gilead Sciences	2014
	RAL/3TC	Dutrebis	Merck	2015
	ATV/COBI	Evotaz	ViiV	2015
	TAF[a]/COBI/FTC/EVG	Genvoya	Gilead Sciences	2015
	TAF/RPV/FTC	Odefsey	Gilead Sciences	2016
	TAF/FTC	Descovy	Gilead Sciences	2016
	DTG/RPV	Juluca	ViiV	2017
	Bictegravir/FTC/TAF	Biktarvy	Gilead Sciences	2018

ART saves lives

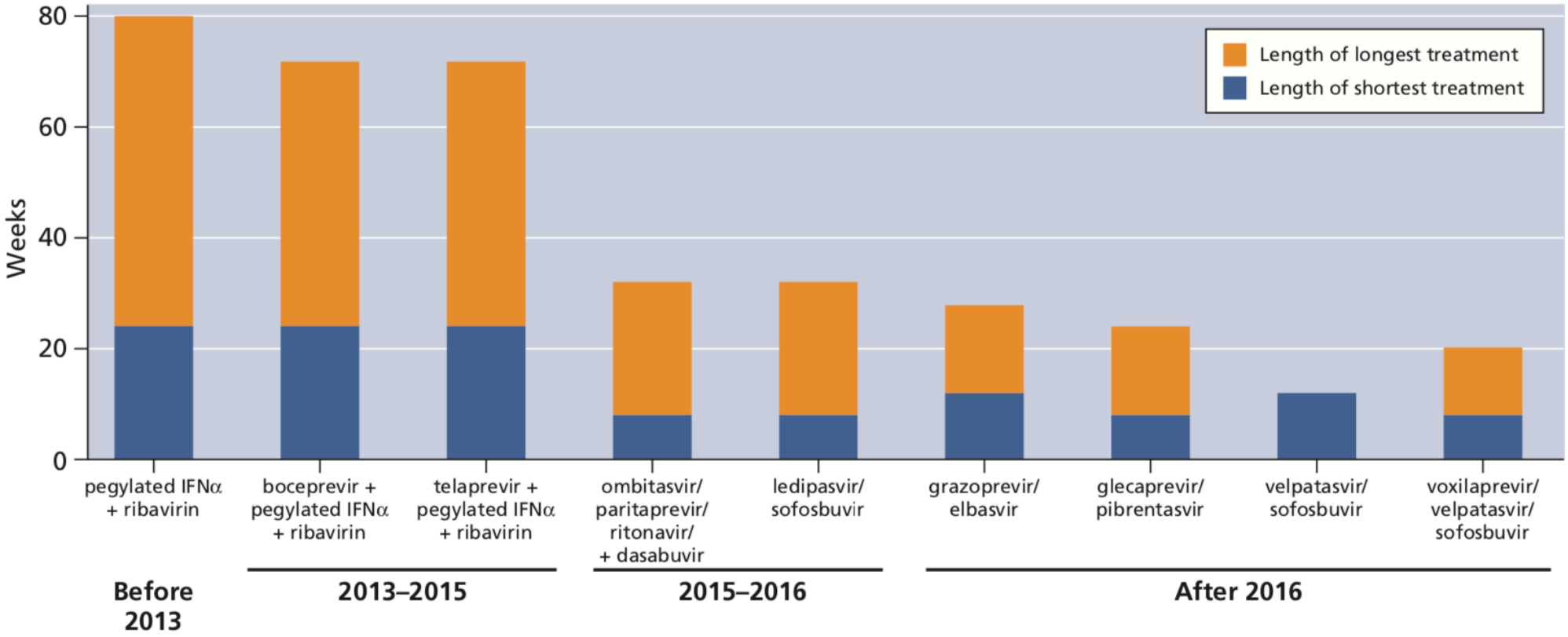


Principles of Virology, ASM Press

Pre-exposure prophylaxis (PrEP vs PEP)

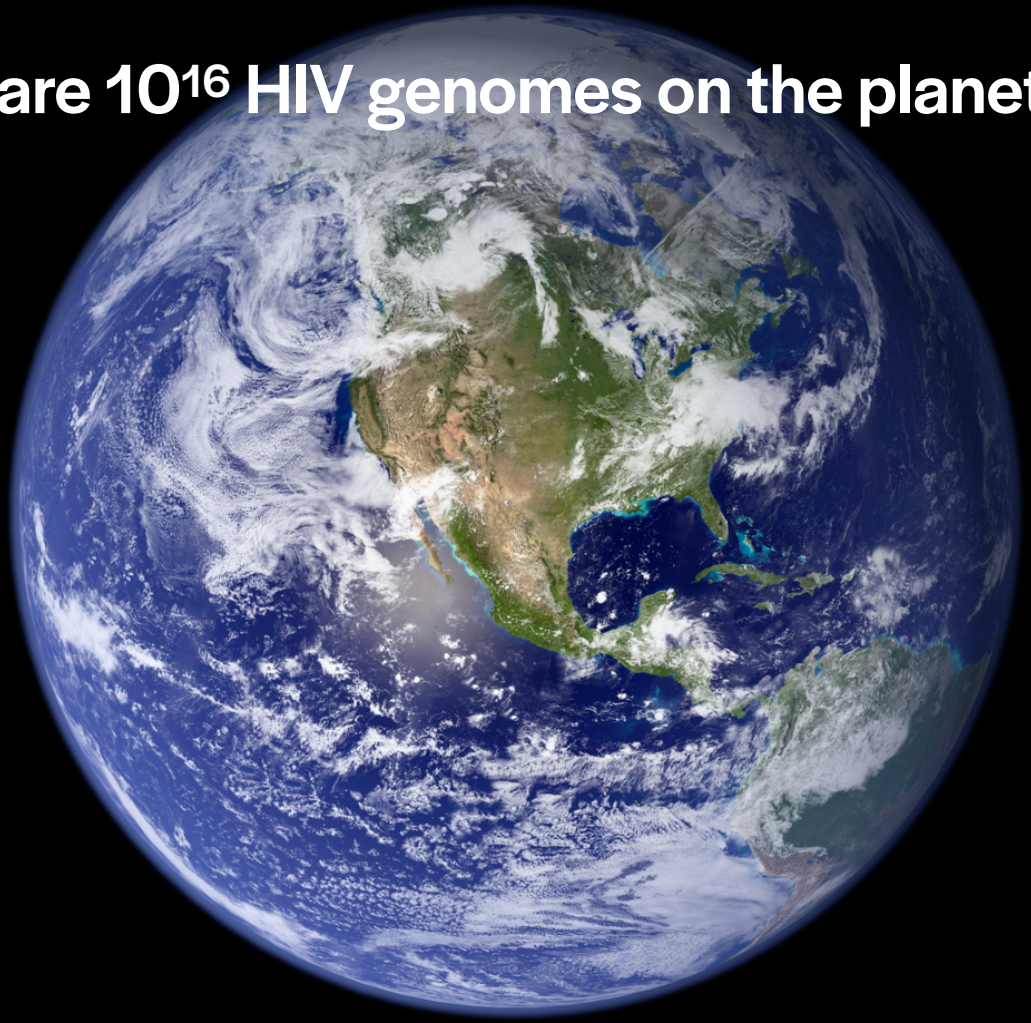
- Daily double therapy (tenofovir and emtricitabine) for those at high risk for HIV-1 infection
- Reduces risk of sexual transmission of HIV-1 by >90%
- Reduces risk of transmission by IVDU by >70%

Decreasing length of treatment regimens for hepatitis C



Principles of Virology, ASM Press

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!

Next time: Evolution