Antivirals

Lecture 20
Biology W3310/4310
Virology
Spring 2014

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 30 antiviral drugs are available on the US market.

Most against HIV and herpesviruses - Persistent infections.
<table>
<thead>
<tr>
<th>Targets</th>
<th>Viruses</th>
<th>Examples of compounds approved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virion uncoating</td>
<td>Influenza A</td>
<td>Amantadine, rimantadine Tamiflu, Relenza</td>
</tr>
<tr>
<td>DNA polymerase</td>
<td>Herpesviruses (HSV-1, HSV-2, VZV, CMV, EBV, HHV-6, HHV-7, HHV-8)</td>
<td>Nucleosides: acyclovir, valacyclovir, ganciclovir, valganciclovir, penciclovir famiclovir, brivudin, foscarin, Acyclic nucleoside phosphonates: cidofovir, tenofovir</td>
</tr>
<tr>
<td></td>
<td>Herpesvirus (CMV)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIV</td>
<td></td>
</tr>
<tr>
<td>Reverse transcriptase</td>
<td>HIV</td>
<td>Nucleosides: zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, Nonnucleosides: nevirapine, delavirdine, efavirenz</td>
</tr>
<tr>
<td>Viral protease</td>
<td>HIV</td>
<td>Saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, Zanamivir, oseltamivir</td>
</tr>
<tr>
<td>Viral neuraminidase</td>
<td>Influenza A and B virus</td>
<td></td>
</tr>
<tr>
<td>Inosine monophosphate dehydrogenase</td>
<td>HCV, RSV</td>
<td>Ribavirin, Telaprevir, Boceprevir</td>
</tr>
</tbody>
</table>
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
- Many medically important viruses can’t be propagated, have no animal model, or are dangerous
  - HBV, HPV, norovirus
  - Smallpox
  - Ebola, Lassa, smallpox
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
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*
- No broad-spectrum antiviral agents are currently available
- Lack of rapid diagnostic reagents has hampered development of antiviral drugs
### LJ1001, a broad spectrum antiviral

<table>
<thead>
<tr>
<th>Virus</th>
<th>Family</th>
<th>Genome Type</th>
<th>Envelope (yes/no)</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ebola (cat A)</td>
<td>Filoviridae</td>
<td>ssRNA(-)</td>
<td>Y</td>
<td>+++</td>
</tr>
<tr>
<td>Marburg (cat A)</td>
<td>Filoviridae</td>
<td>ssRNA(-)</td>
<td>Y</td>
<td>++</td>
</tr>
<tr>
<td>Influenza A (cat A)</td>
<td>Orthomyxoviridae</td>
<td>ssRNA(-)</td>
<td>Y</td>
<td>+++</td>
</tr>
<tr>
<td>Junin (cat A)</td>
<td>Arenaviridae</td>
<td>ssRNA(-)</td>
<td>Y</td>
<td>++</td>
</tr>
<tr>
<td>Rift Valley fever (cat A)</td>
<td>Bunyaviridae</td>
<td>ssRNA(-)</td>
<td>Y</td>
<td>+++</td>
</tr>
<tr>
<td>LaCrosse (cat B)</td>
<td>Bunyaviridae</td>
<td>ssRNA(-)</td>
<td>Y</td>
<td>+++</td>
</tr>
<tr>
<td>Nipah (cat C)</td>
<td>Paramyxoviridae</td>
<td>ssRNA(-)</td>
<td>Y</td>
<td>++</td>
</tr>
<tr>
<td>Omsk hemorrhagic fever (cat C)</td>
<td>Flaviviridae</td>
<td>ssRNA(+)</td>
<td>Y</td>
<td>++</td>
</tr>
<tr>
<td>RSSE (cat C)</td>
<td>Flaviviridae</td>
<td>ssRNA(+)</td>
<td>Y</td>
<td>++</td>
</tr>
<tr>
<td>PIV-5</td>
<td>Paramyxoviridae</td>
<td>ssRNA(-)</td>
<td>Y</td>
<td>++</td>
</tr>
<tr>
<td>HPIV-3</td>
<td>Paramyxoviridae</td>
<td>ssRNA(-)</td>
<td>Y</td>
<td>++</td>
</tr>
<tr>
<td>Newcastle disease *</td>
<td>Paramyxoviridae</td>
<td>ssRNA(-)</td>
<td>Y</td>
<td>++</td>
</tr>
<tr>
<td>HIV-1 (cat C)</td>
<td>Retroviridae</td>
<td>ssRNA(-)RT</td>
<td>Y</td>
<td>++</td>
</tr>
<tr>
<td>Murine leukemia</td>
<td>Retroviridae</td>
<td>ssRNA(-)RT</td>
<td>Y</td>
<td>++</td>
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<tr>
<td>Yellow fever</td>
<td>Flaviviridae</td>
<td>ssRNA(+)</td>
<td>Y</td>
<td>+++</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>Flaviviridae</td>
<td>ssRNA(+)</td>
<td>Y</td>
<td>+++</td>
</tr>
<tr>
<td>West Nile</td>
<td>Flaviviridae</td>
<td>ssRNA(+)</td>
<td>Y</td>
<td>+++</td>
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<tr>
<td>Vesicular stomatitis P</td>
<td>Rhabdoviridae</td>
<td>ssRNA(-)</td>
<td>Y</td>
<td>++</td>
</tr>
<tr>
<td>Cowpox</td>
<td>Poxviridae</td>
<td>dsDNA</td>
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<td>+</td>
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<tr>
<td>Vaccinia</td>
<td>Poxviridae</td>
<td>dsDNA</td>
<td>Y</td>
<td>++</td>
</tr>
<tr>
<td>Adenovirus **</td>
<td>Adenoviridae</td>
<td>dsDNA</td>
<td>N</td>
<td>–</td>
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<tr>
<td>Coxsackie B **</td>
<td>Picornaviridae</td>
<td>ssRNA(+)</td>
<td>N</td>
<td>–</td>
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<tr>
<td>Reovirus</td>
<td>Reoviridae</td>
<td>dsRNA</td>
<td>N</td>
<td>–</td>
</tr>
</tbody>
</table>

LJ1001, a broad spectrum antiviral

![Chemical structure of LJ-001](image)

**Images**
- DMSO
- LJ025
- LJ001
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
Some targets for antiviral drug discovery

Fusion inhibitors (HIV)
Entry inhibitor (influenza)

Interferon (hepatitis C)

Nucleoside, non-nucleoside analogs (HIV, herpesvirus)

Protease inhibitors (HIV, HCV)

NA inhibitors (influenza)
The path of drug discovery

- 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)
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!
Mechanism-based screens

B

Cleavage site

Protease

Soluble

Insoluble

Fluorescence intensity of soluble peptide

Time (seconds)
Cell-based screen

Active tetracycline efflux protein; insertion of protease site has no effect

Engineered HIV protease site

HIV protease leads to inactivation of the tetracycline efflux protein

Inactive tetracycline efflux protein
Antiviral screening

- High-throughput: 10,000 compounds/day
- Chemical libraries
- Natural products
- Combinatorial chemistry
- Structure-based design
- *In silico* screening
High throughput screening
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.
Consider Acyclovir, the highly effective, anti-herpes simplex virus drug – a prodrug; a nucleoside analog

Many antiviral compounds are nucleoside and nucleotide analogs

Principles of Virology, ASM Press
Acyclovir mechanism of action

A

ACV

HSV-I TK

ACV-TP

GMP kinase

Viral DNA polymerase

NDP kinase

DNA
Improving acyclovir

- Valacyclovir (valtrex), 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
Acyclovir-resistant HSV

- 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
Influenza virus NA inhibitors
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
How inhibitors of NA (Tamiflu, Relenza) work
Neuraminidase Inhibitor Resistance Testing Results on Samples Collected Since October 1, 2013

<table>
<thead>
<tr>
<th>Virus Type</th>
<th>Oseltamivir</th>
<th>Zanamivir</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Virus Samples tested (n)</td>
<td>Resistant Viruses, Number (%)</td>
</tr>
<tr>
<td>Influenza A (H3N2)</td>
<td>381</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Influenza B</td>
<td>247</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>2009 H1N1</td>
<td>4,817*</td>
<td>56 (1.2)</td>
</tr>
</tbody>
</table>

*Includes specimens tested in national surveillance and additional specimens tested at public health laboratories in 19 states (AZ, CA, CO, DE, FL, GA, HI, ID, MA, ME, MD, MI, MN, NY, PA, TX, UT, WA, and WI) who share testing results with CDC.

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

http://www.cdc.gov/flu/weekly/index.htm
Inhibitors of picornavirus uncoating

Table 1. In Vitro Antiviral Activity

<table>
<thead>
<tr>
<th>Structure</th>
<th>MIC μg/ml equine rhinovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>COOC$_2$H$_5$</td>
<td>12-6</td>
</tr>
<tr>
<td>O =</td>
<td>12-6</td>
</tr>
<tr>
<td>COOC$_2$H$_5$</td>
<td>Inactive</td>
</tr>
<tr>
<td>O =</td>
<td>25-12</td>
</tr>
<tr>
<td>COOC$_2$H$_5$</td>
<td>Inactive</td>
</tr>
<tr>
<td>O =</td>
<td>12-3</td>
</tr>
<tr>
<td>CN</td>
<td>6-3</td>
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</table>

Table 2. In Vitro Antiviral Activity

<table>
<thead>
<tr>
<th>Structure</th>
<th>MIC μg/ml equine rhinovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>O =</td>
<td>Inactive</td>
</tr>
<tr>
<td>O =</td>
<td>Inactive</td>
</tr>
<tr>
<td>12-6</td>
<td></td>
</tr>
<tr>
<td>O =</td>
<td>Inactive</td>
</tr>
<tr>
<td>O =</td>
<td>Inactive</td>
</tr>
<tr>
<td>12-6</td>
<td></td>
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</tbody>
</table>

Table 3. In Vitro Antiviral Activity

<table>
<thead>
<tr>
<th>n</th>
<th>MIC (μg/ml), equine rhinovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Inactive</td>
</tr>
<tr>
<td>4</td>
<td>25-6</td>
</tr>
<tr>
<td>5</td>
<td>25-12</td>
</tr>
<tr>
<td>6</td>
<td>6-3</td>
</tr>
<tr>
<td>7</td>
<td>12-6</td>
</tr>
<tr>
<td>8</td>
<td>6-3</td>
</tr>
<tr>
<td>9</td>
<td>Inactive</td>
</tr>
<tr>
<td>10</td>
<td>25-12</td>
</tr>
</tbody>
</table>

Pharmac. Ther. 29:287, 1985
WIN compounds
New protease targets

Boceprevir

Telaprevir
miRNA target: HCV

## HCV new drug pipeline

<table>
<thead>
<tr>
<th>COMPANY</th>
<th>DRUG</th>
<th>Research</th>
<th>Preclin</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>NDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertex trial status</td>
<td>Telaprevir (VX-950) Protease Inhibitor plus VX-222 Non-nucleoside Polymerase inhibitor w/o Ribavirin and peginterferon</td>
<td></td>
<td></td>
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<tr>
<td>Bristol-Myers Squibb trial status - more</td>
<td>BMS-790052 NS5A Inhibitor plus BMS-650032 Protease Inhibitor w/o Ribavirin and peginterferon</td>
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<tr>
<td>Gilead trial status</td>
<td>GS 9190 NN Protease Inhibitor plus GS 9256 Protease Inhibitor w/o Ribavirin and peginterferon</td>
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<td>Bristol-Myers Squibb / Pharmasset</td>
<td>BMS-790052 NS5A inhibitor plus PSI-7992 Nucleotide Polymerase inhib. w/o Ribavirin</td>
<td></td>
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<tr>
<td>Boehringer Ingelheim trial status</td>
<td>BI 201336 Protease Inhibitor plus BI 207127 Polymerase inhib. w/o Ribavirin</td>
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<tr>
<td>Idenix trial status</td>
<td>IDX184 Nucleoside Polymerase Inhibitor plus IDX320 Protease Inhibitor</td>
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<td></td>
</tr>
<tr>
<td>Hoffman-La Roche trial status</td>
<td>R7128 (RO5024048) Nucleoside Polymerase Inhibitor plus ITMN-191 R7227 (RO5190591) Protease Inhibitor</td>
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<tr>
<td>Pharmasset</td>
<td>PSI-7977 a pyrimidine and PSI-938 a purine nucleotide analog polymerase inhibitors</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

http://www.hcvdrugs.com/
Targets for intervention: HIV replication

- Cell attachment, fusion, and entry
  - CD4 derivatives
  - Chemokine analogs
  - SU/V3 loop inhibitors

- Reverse transcription
  - Nucleoside analogs
  - Nonnucleoside inhibitors

- Integration
  - Integrase inhibitors

- Transcription and posttranscriptional processing
  - Tat inhibitors

- Virion assembly, release
  - Protease inhibitors
The problem with AIDS therapy: relentless viral replication for years
Azido-deoxythymididine (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
AZT

- AZT has 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)

A

p66

fingers

polymerase active site

palm

RNase H

connection

NNRTI binding site

p51

B

11-cyclopropyl-4-methyl-5,11-dihydro-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one (Viramune)

nevirapine

delavirdine

efavirenz
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 Protease inhibitor Ro 31-8959

Peptidomimetic
Mechanism of IN inhibitors

Raltegravir (Merck)
Licensed October 2007
Maraviroc: CCR5 inhibitor

Maraviroc is a CCR5 inhibitor that blocks the gp120 binding site on CCR5. In the presence of Maraviroc (MVC), the binding site is disrupted, leading to very low affinity for gp120.

- **Free receptor**: gp120 binding site on CCR5, high affinity.
- **Bound to CCR5**: Binding site disrupted by MVC, very low affinity.
HIV fusion inhibitors

- Fuzeon, licensed March 2003
- 36 amino acid synthetic peptide:
  
- Binds to TM subunit of viral glycoprotein, blocks transition into fusion active conformation
- Expensive ($25,000/yr), must be injected
- Resistance: aa changes in peptide binding site on TM
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
Do the math

- 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
Eviplera: emtricitabine, rilpivirine, tenofovir
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!