Emerging Viruses

Part IIb
Follow Up from Part I
Vaccines and Inhibitors
Cellular Responses to Viral Invasion: “Restriction Factors”
Cells fight viral infection using a series of restriction factors.

A point of inhibition

**Restriction factors for Influenza infection**

*Viruses 2017, 9(12), 376; doi:10.3390/v9120376*
Some Other Restriction Factors
Nature of Interactions Within and Between Proteins
## Forces That Keep the Protein Structure Together

<table>
<thead>
<tr>
<th>Level of Structure</th>
<th>Interactions that stabilize the structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>Covalent bond (amide/peptide bond)</td>
</tr>
<tr>
<td>Secondary</td>
<td>Hydrogen bonds</td>
</tr>
<tr>
<td>Tertiary</td>
<td>Ionic bonds, disulfide bonds,</td>
</tr>
<tr>
<td></td>
<td>hydrophobic interactions, hydrogen</td>
</tr>
<tr>
<td></td>
<td>bonding</td>
</tr>
<tr>
<td>Quaternary</td>
<td>Ionic bonds, disulfide bonds,</td>
</tr>
<tr>
<td></td>
<td>hydrophobic interactions, hydrogen</td>
</tr>
<tr>
<td></td>
<td>bonding</td>
</tr>
</tbody>
</table>

Kahn Academy: The Chemistry of Protein Structure
Hydrophobic interactions (clustering of hydrophobic groups away from water) and van der Waals interactions

Hydrogen Bond

Polypeptide backbone

Disulfide bridge

Ionic bond
Protein Secondary Structures

- **Parallel β Sheet**
  - Unsymmetrical hydrogen bonding: One amino acid residue forms hydrogen bonds with two other amino acid residues.
  - 2 polypeptide chains

- **Antiparallel β Sheet**
  - Symmetrical 1:1 hydrogen bonding
  - 2 polypeptide chains

- Side chains (R) from the helix, side created by the R
- C=O of n-th amino acid
- N-H of (n+4)th amino acid

Kahn Academy: The Chemistry of Protein Structure
Electrostatic Interactions Help Stabilize Protein-Protein Interactions

Figure 1. Molecular representations of the C3d-CR2 interaction. (A) Surface representation of the C3d-CR2 interaction with C3d in gray and CR2 in green (PDB Code: 3QED). (B) Electrostatic potential surface projection for C3d (PDB Code: 1C3D). (C) Electrostatic potential surface projection for CR2 (PDB Code: 1LY2). The code for panels (B) and (C) are as follows: the color transitions from red – white – blue when going from negative ($-5 \text{ kT/e}$) – neutral (0 kT/e) – positive (+5 kT/e) electrostatic potential.

doi:10.1371/journal.pcbi.1002840.g001
Review of the Immune System
Immune System

- Tonsils
- Lymphatic Vessels
- Thymus
- Lymph Nodes
- Spleen
- Appendix
- Bone Marrow
Innate vs Adaptive Immunity

**Innate**
- Primitive: In all multicellular organisms
- Initiated within minutes
- Directed toward classes of molecules
- Effectors broadly reactive
- No anamnestic response
- Effectors = epithelial cells, phagocytes, endothelial cells, fibroblasts

**Adaptive**
- Only in vertebrates
- Activated over days
- Directed toward specific epitopes on molecules
- Effectors highly specific
- Memory persists
- Effectors = lymphocytes, Antigen Presenting Cells (APCs)
Adaptive Immunity Maturation

Bone Marrow

Lymphoblasts

Bone marrow maturation

B lymphocytes

Memory cells

Plasma cells

Antibodies

Humoral response

Thymus

Regulator T cells

Helper T cells

Suppressor T cells

Cytotoxic (killer) T cells

Effector T cells

Cellular (cell-mediated) response
Vaccines

Goal:
Generate a life-long immune response
(neutralizing antibody production)
Neutralizing Antibody Requirements

• Proteins require flexibility
• Binding changes conformation
• Successful Antibodies (or Antivirals) must hinder those changes
• Antibodies access only the surface of the virion or cell.
All Steps in the Viral Life Cycle are Targets for Intervention
The Power of Vaccines to Vanquish Disease

Meredith Wadman, and Jia You Science 2017;356:364-365

Published by AAAS
Vaccines are our proven best defense against viruses

- Vaccination mobilizes the host immune system to prevent virus infections
  - *Immune memory*
- Vaccination breaks the chain of transmission
Vaccines stimulate a protective immune response

- Initial immune response
  - Antibody prevalence and T cell number
  - Time (days): 7, 14, 21, 28, 35, 42
- Protective immunity
  - Time: 1, 2, 3, 4
- Immunological memory
  - Mild or inapparent reinfection

First infection
Requirements of an effective vaccine

- Safety: no disease, minimal side effects
- Induce protective immunity in the population
- Protection must be long-lasting
- Low cost (<$1, WHO); genetic stability; storage considerations; delivery (oral vs. needle)
Requirements of an effective vaccine

- Induction of an *appropriate immune response*
  - *Th1* vs *Th2* response
- Vaccinated individual must be *protected against disease* caused by a virulent form of the specific pathogen
  - *Just getting ‘a response’ is not enough* (e.g. producing antibodies)
How vaccines work in the real world

- Maintenance of a critical level of immunity
- Herd immunity
Herd Immunity

- Virus spread stops when the probability of infection drops below a critical threshold
- The threshold is virus and population specific
- Smallpox: 80 - 85%
- Measles: 93 - 95%
- No vaccine is 100% effective
- When 80% of population is immunized with measles, 76% of population is immune
Vaccine programs depend on public acceptance of their value

The senior pastor of Eagle Mountain International Church in Newark, Texas, had been critical of measles vaccination, and at least 12 people infected in the congregation did not receive the vaccine.

23 measles cases were reported in a largely unvaccinated Hare Krishna community in Stokes County, North Carolina.

All 58 measles cases reported in an outbreak in Brooklyn, New York, involved unvaccinated members of an Orthodox Jewish community.
Vaccines can be active or passive

- Active - instilling into the recipient a modified form of the pathogen or material derived from it that induces immunity to disease
  - Long term protection
- Passive - instilling the products of the immune response (antibodies or immune cells) into the recipient
  - Short term protection
A natural passive vaccine

Fraction of adult values

Months

Passively transferred maternal IgG

Serum immunoglobulin levels

Conception

Birth

Years

IgM

IgG

IgA

Adult
Passive therapy with convalescent serum

- Jordi Casals infected himself with Lassa virus at Yale in 1969
- Transfused with blood from nurse (Penny Pinneo) who had survived Lassa fever

- Used in some cases during last Ebola outbreak
Zmapp, the best known passive vaccine

- Raised in mice immunized with virus-like particles
- Chimerized into human IgG1 scaffold
- Produced in tobacco plants
Types of Active Vaccines

- **Attenuation**
  - Replication competent natural virus vaccine
  - (live-attenuated)

- **Inactivation**
  - Inactivated virus vaccine

- **Fractionation**
  - Nonrecombinant, purified subunit vaccine

- **Cloning**
  - Bacterial cell
  - Replication competent virus vector vaccine
  - DNA vaccine
  - Protein
  - Virus-like particle vaccine
  - Subunit vaccine

The virulent parental virus

(Virology Lectures 2017 - Prof. Vincent Racaniello - Columbia University)

Principles of Virology, ASM Press
Live attenuated vaccines are used to protect against:

- Measles, mumps, rubella (MMR combined vaccine)
- Rotavirus
- Smallpox
- Chickenpox
- Yellow fever
- Shingles

Inactivated vaccines are used to protect against:

- Hepatitis A
- Flu (shot only)
- Polio (shot only)
- Rabies

Inactivated vaccines are safer, but do not always elicit a strong immune response.
Subunit vaccines

- Break virus into components, immunize with purified components
- Clone viral gene, express in bacteria, yeast, insect cells, cell culture, purify protein
- Antigen usually a capsid or membrane protein
Some successful subunit vaccines

Cancer vaccine

- Hepatitis B virus (HBV) - HBsAg protein produced in yeast
- Assembles into empty particles

For these viruses, the proteins that antibodies target contain the budding function
These proteins retain their native conformations in the subunit particle
VLP Vaccine

Human papillomaviruses

- Agents of warts (>170 types)
- Some are transmitted sexually, most common STD in USA
- Some cause low risk genital warts
- Others are high risk for cancers: cervix, vagina, penis, anus, oropharynx (31,000/yr; mostly 16, 18)
- Nearly half of Americans infected with genital HPV (18-59)
Human papillomavirus vaccines

Cancer vaccines

- *Gardasil* (Merck): types 6, 11, 16, 18 produced in *S. cerevisiae*
- *Gardasil-9* (Merck): types 6, 11, 16, 18, 31, 33, 45, 52, 58
- *Cervarix* (GlaxoSmithKline): types 16, 18 produced in insect cells
- Should be given before becoming sexually active

Should be given to BOTH females AND males
VLP Vaccines

Substitute Surface Proteins from Virus of Interest
(Including T-cell stimulating epitopes)
# Viral vaccines licensed in the US

<table>
<thead>
<tr>
<th>Disease or virus</th>
<th>Type of vaccine</th>
<th>Indications for use</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Attenuated, oral</td>
<td>Military recruits</td>
<td>One dose</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>Inactivated whole virus</td>
<td>Travelers, other high-risk groups</td>
<td>0, 1, and 6 mo</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>Yeast-produced recombinant surface protein</td>
<td>Universal in children, exposure to blood, sexual promiscuity</td>
<td>0, 1, 6, and 12 mo</td>
</tr>
<tr>
<td>Influenza</td>
<td>Inactivated viral subunits</td>
<td>Elderly and other high-risk groups</td>
<td>One dose seasonally</td>
</tr>
<tr>
<td>Influenza</td>
<td>Recombinant proteins</td>
<td>Elderly; those with egg allergies</td>
<td>One dose seasonally</td>
</tr>
<tr>
<td>Influenza</td>
<td>Attenuated</td>
<td>Children 2–8 yr old, not previously vaccinated with influenza vaccine</td>
<td>Two doses at least 1 mo apart</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Children 2–8 yr old, previously vaccinated with influenza vaccine</td>
<td>One dose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Children, adolescents, and adults 9–49 yr old (e.g., FluMist, FluB60)</td>
<td>One dose</td>
</tr>
<tr>
<td>Japanese encephalitis</td>
<td>Inactivated whole virus</td>
<td>Travelers to or inhabitants of high-risk areas in Asia</td>
<td>0, 7, and 30 days</td>
</tr>
<tr>
<td>Measles</td>
<td>Attenuated</td>
<td>Universal vaccination of infants</td>
<td>12 mo of age; 2nd dose, 6 to 12 yr of age</td>
</tr>
<tr>
<td>Mumps</td>
<td>Attenuated</td>
<td>Universal vaccination of infants</td>
<td>Same as measles, given as MMR</td>
</tr>
<tr>
<td>Papilloma (human)</td>
<td>Yeast- or SF9-produced virus-like particles</td>
<td>Females 9–26 yr old</td>
<td>Three doses</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>Reassortant</td>
<td>Healthy infants</td>
<td>2, 3, and 6 mo or 2 and 4 mo of age depending on vaccine</td>
</tr>
<tr>
<td>Rubella</td>
<td>Attenuated</td>
<td>Universal vaccination of infants</td>
<td>Same as measles, given as MMR</td>
</tr>
<tr>
<td>Polio (inactivated)</td>
<td>Inactivated whole viruses of types 1, 2, and 3</td>
<td>Changing; commonly used for immunosuppressed where live vaccine cannot be used</td>
<td>2, 4, and 12–18 mo of age, then 4 to 6 yr of age</td>
</tr>
<tr>
<td>Polio (attenuated)</td>
<td>Attenuated, oral mixture of types 1, 2, and 3</td>
<td>Universal vaccination; no longer used in United States</td>
<td>2, 4, and 6–18 mo of age</td>
</tr>
<tr>
<td>Rabies</td>
<td>Inactivated whole virus</td>
<td>Exposure to rabies, actual or prospective</td>
<td>0, 3, 7, 14, and 28 days postexposure</td>
</tr>
<tr>
<td>Smallpox</td>
<td>Vaccinia virus</td>
<td>Certain laboratory workers</td>
<td>One dose</td>
</tr>
<tr>
<td>Varicella</td>
<td>Attenuated</td>
<td>Universal vaccination of infants</td>
<td>12 to 18 mo of age</td>
</tr>
<tr>
<td>Varicella-zoster</td>
<td>Attenuated</td>
<td>Adults 60 yr old and older</td>
<td>One dose</td>
</tr>
<tr>
<td>Yellow fever</td>
<td>Attenuated</td>
<td>Travel to areas where infection is common</td>
<td>One dose every 10 yr</td>
</tr>
</tbody>
</table>
Can viral diseases be eradicated?

- Smallpox eradication program launched 1967, eradicated 1978
- Two features essential for eradication:
  - Replication in only one host
  - Vaccination induces lifelong immunity

- Current target for eradication by vaccine = polio
Questions?
Antivirals: The Second Arm of Antiviral Defense
HIV env: Why Neutralizing Antibodies are so Difficult to Produce

A common strategy among enveloped viruses
All Steps in the Viral Life Cycle are Targets for Intervention

The HIV Life Cycle
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
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
The path of drug discovery

- Target structure-function studies (X-ray, NMR, computation)
- Cell-virus molecular biology research
- Medical need identified
- Relevant mechanism
- “Hits”
- Lead compound
- Drug candidate
- Clinical testing
- Animal models
- Animal pharmacology, toxicology, metabolism, pharmacokinetics

Libraries: natural products, compound collections, combinatorial chemistry, RNAi

Cell- or Mechanism-based screens

in silico screens

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)
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
Antiviral screening

- High-throughput: 10,000 compounds/day
- Chemical libraries
- Natural products
- Combinatorial chemistry
- Structure-based design
- *In silico* screening
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!
From drug discovery to the clinic
Compounds that have been identified

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

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
Anti-HIV Antiviral Development

A Sharp Learning Curve
HIV Env: Strategies for Immune Evasion

Need to reach the sheltered portions of Env to inhibit
But years of relentless replication makes antiviral escape likely
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

- Substantial side effects (unlike acyclovir)
- Can be given orally, is absorbed rapidly, but half-life is \(\sim 1\) 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 (ddl), 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)
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

Valine Serine Glutamine Asparagine Tyrosine Proline Isoleucine Valine

B Saquinavir

C Darunavir

Peptidomimetic
IN inhibitors

A

RAL

DTG

B

No Drug

RAL

DTG
Maraviroc: CCR5 inhibitor
(block binding to fusion-triggering receptor)
Enfuvertude: Fusion inhibitor

- C-helix peptide
- Binds N-helix
- Prevents fold-back for fusion
<table>
<thead>
<tr>
<th>Brand name</th>
<th>Generic name(s)</th>
<th>Manufacturer name</th>
<th>Approval date</th>
<th>Time to approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrovir</td>
<td>Zidovudine, azidothymidine, AZT, ZDV</td>
<td>GlaxoSmithKline (original sponsor)</td>
<td>19 March 1987</td>
<td>3.5 months</td>
</tr>
<tr>
<td>Videx</td>
<td>Didanosine, dideoxynosine, ddI</td>
<td>Bristol Myers-Squibb</td>
<td>9 October 1991</td>
<td>6 months</td>
</tr>
<tr>
<td>Hivid</td>
<td>Zalcitabine, dideoxyxycytidine, ddC (no longer marked as of December 31, 2006)</td>
<td>Hoffman-La Roche</td>
<td>19 June 1992</td>
<td>7.6 months</td>
</tr>
<tr>
<td>Zerit</td>
<td>Stavudine, d4T</td>
<td>Bristol Myers-Squibb</td>
<td>24 June 1994</td>
<td>5.9 months</td>
</tr>
<tr>
<td>Epivir</td>
<td>Lamivudine, 3TC</td>
<td>GlaxoSmithKline</td>
<td>17 November 1995</td>
<td>4.4 months</td>
</tr>
<tr>
<td>Combivir</td>
<td>Lamivudine and zidovudine</td>
<td>GlaxoSmithKline</td>
<td>27 September 1997</td>
<td>3.9 months</td>
</tr>
<tr>
<td>Ziagen</td>
<td>Abacavir sulfate, ABC</td>
<td>GlaxoSmithKline</td>
<td>17 December 1998</td>
<td>5.8 months</td>
</tr>
<tr>
<td>Videx EC</td>
<td>Enteric coated didanosine, ddI EC</td>
<td>Bristol Myers-Squibb</td>
<td>31 October 2000</td>
<td>9 months</td>
</tr>
<tr>
<td>Trizivir</td>
<td>Abacavir, zidovudine, and lamivudine</td>
<td>GlaxoSmithKline</td>
<td>14 November 2000</td>
<td>10.9 months</td>
</tr>
<tr>
<td>Viread</td>
<td>Tenofovir disoproxil fumarate, TDF</td>
<td>Gilead Sciences</td>
<td>26 October 2001</td>
<td>5.9 months</td>
</tr>
<tr>
<td>Emtriva</td>
<td>Emtricitabine, FTC</td>
<td>Gilead Sciences</td>
<td>02 July 2003</td>
<td>10 months</td>
</tr>
<tr>
<td>Eproica</td>
<td>Abacavir and lamivudine</td>
<td>GlaxoSmithKline</td>
<td>02 August 2004</td>
<td>10 months</td>
</tr>
<tr>
<td>Truvada</td>
<td>Tenofovir disoproxil fumarate and emtricitabine</td>
<td>Gilead Sciences</td>
<td>02 August 2004</td>
<td>5 months</td>
</tr>
</tbody>
</table>

**Nonnucleoside reverse transcriptase inhibitors (NNRTIs)**

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Generic name(s)</th>
<th>Manufacturer name</th>
<th>Approval date</th>
<th>Time to approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viramune</td>
<td>Nevirapine, NVP</td>
<td>Boehringer Ingelheim</td>
<td>21 June 1996</td>
<td>2.9 months</td>
</tr>
<tr>
<td>Rescriptor</td>
<td>Delavirdine, DELV</td>
<td>Pfizer</td>
<td>4 April 1997</td>
<td>8.7 months</td>
</tr>
<tr>
<td>Sustiva</td>
<td>Efavirenz, EFV</td>
<td>Bristol Myers-Squibb</td>
<td>17 September 1998</td>
<td>3.2 months</td>
</tr>
<tr>
<td>Intellence</td>
<td>Etravirine</td>
<td>Tibotec Therapeutics</td>
<td>18 June 2000</td>
<td>6 months</td>
</tr>
</tbody>
</table>

**Protease inhibitors (PIs)**

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Generic name(s)</th>
<th>Manufacturer name</th>
<th>Approval date</th>
<th>Time to approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invirase</td>
<td>Saquinavir mesylate, SQV</td>
<td>Hoffman-La Roche</td>
<td>6 December 1995</td>
<td>3.2 months</td>
</tr>
<tr>
<td>Norvir</td>
<td>Ritonavir, RTV</td>
<td>Abbott Laboratories</td>
<td>1 March 1996</td>
<td>2.3 months</td>
</tr>
<tr>
<td>Crixivan</td>
<td>Indinavir, IDV</td>
<td>Merck</td>
<td>13 March 1995</td>
<td>1.4 months</td>
</tr>
<tr>
<td>Viracept</td>
<td>Nelfinavir mesylate, NFV</td>
<td>Agouron Pharmaceuticals</td>
<td>14 March 1997</td>
<td>2.6 months</td>
</tr>
<tr>
<td>Fortovase</td>
<td>Saquinavir (no longer marketed)</td>
<td>Hoffman-La Roche</td>
<td>7 November 1997</td>
<td>5.9 months</td>
</tr>
<tr>
<td>Agenrasa</td>
<td>Amprenavir, APV</td>
<td>GlaxoSmithKline</td>
<td>15 April 1999</td>
<td>6 months</td>
</tr>
<tr>
<td>Kaletra</td>
<td>Lopinavir and ritonavir, LPV/RTV</td>
<td>Abbott Laboratories</td>
<td>15 September 2000</td>
<td>3.5 months</td>
</tr>
<tr>
<td>Reyataz</td>
<td>Atazanavir sulfate, ATV</td>
<td>Bristol Myers-Squibb</td>
<td>20 June 2003</td>
<td>6 months</td>
</tr>
<tr>
<td>Lexiva</td>
<td>Fosamprenavir calcium, FOS-APV</td>
<td>GlaxoSmithKline</td>
<td>20 October 2003</td>
<td>10 months</td>
</tr>
<tr>
<td>Aplisys</td>
<td>Tipranavir, TPV</td>
<td>Boehringer Ingelheim</td>
<td>22 June 2005</td>
<td>6 months</td>
</tr>
<tr>
<td>Prezista</td>
<td>Darunavir</td>
<td>Tibotec, Inc.</td>
<td>23 June 2006</td>
<td>6 months</td>
</tr>
</tbody>
</table>

**Fusion inhibitors**

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Generic name(s)</th>
<th>Manufacturer name</th>
<th>Approval date</th>
<th>Time to approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuzion</td>
<td>Enfuvirtide, T-20</td>
<td>Hoffman-La Roche and Trimeris</td>
<td>13 March 2003</td>
<td>6 months</td>
</tr>
</tbody>
</table>

**Entry inhibitors—CCR5 co-receptor antagonists**

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Generic name(s)</th>
<th>Manufacturer name</th>
<th>Approval date</th>
<th>Time to approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selentreny</td>
<td>Maraviroc</td>
<td>Pfizer</td>
<td>06 August 2007</td>
<td>8 months</td>
</tr>
</tbody>
</table>

**HIV integrase strand transfer inhibitors**

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Generic name(s)</th>
<th>Manufacturer name</th>
<th>Approval date</th>
<th>Time to approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isentress</td>
<td>Kalegevir</td>
<td>Merck &amp; Co., Inc.</td>
<td>12 October 2007</td>
<td>6 months</td>
</tr>
</tbody>
</table>

**Multi-class combination products**

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Generic name(s)</th>
<th>Manufacturer name</th>
<th>Approval date</th>
<th>Time to approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atripla</td>
<td>Efavirenz, emtricitabine and tenofovir disoproxil fumarate</td>
<td>Bristol-Myers Squibb and Gilead Sciences</td>
<td>12 July 2006</td>
<td>2.5 months</td>
</tr>
</tbody>
</table>
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
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
Combination therapy

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

And still no cure!

The virus is hiding out in quiescent cells. Current efforts aimed at activating infected, quiescent cells so drugs can work on them.
Hepatitis C – A Success Story?
### HBV vs HIV vs HCV

<table>
<thead>
<tr>
<th></th>
<th>HIV</th>
<th>HCV</th>
<th>HBV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>U.S. Prevalence</strong></td>
<td>1 million</td>
<td>3 million</td>
<td>1.3–3 million</td>
</tr>
<tr>
<td><strong>Worldwide Prevalence</strong></td>
<td>35 million</td>
<td>160 million</td>
<td>350 million</td>
</tr>
<tr>
<td><strong>Percent Diagnosed in U.S.</strong></td>
<td>80%</td>
<td>50%</td>
<td>30%</td>
</tr>
<tr>
<td><strong>Percent Diagnosed Who Are Treated in U.S.</strong></td>
<td>70%</td>
<td>33%</td>
<td>6-10%</td>
</tr>
<tr>
<td><strong>Nature</strong></td>
<td>RNA retrovirus</td>
<td>RNA virus</td>
<td>DNA virus</td>
</tr>
<tr>
<td><strong>Virions Produced per Day</strong></td>
<td>$10^{10}$</td>
<td>$10^{12}$</td>
<td>$10^{13}$</td>
</tr>
<tr>
<td><strong>Enzyme Targets for Therapy</strong></td>
<td>Multiple</td>
<td>Multiple</td>
<td>One</td>
</tr>
<tr>
<td><strong>Curable?</strong></td>
<td>Unclear, lifelong suppression with HAART therapy</td>
<td>Yes</td>
<td>Unclear, lifelong suppression with Nuc therapy</td>
</tr>
<tr>
<td><strong>Why Easy / Difficult?</strong></td>
<td>Proviral DNA integrated into host genome, difficult to eliminate</td>
<td>RNA virus existing in the host cytoplasm, can eradicate with cocktail of small molecules DAAAs</td>
<td>cccDNA inside the nucleus, also integrated into host genome, difficult to eliminate</td>
</tr>
<tr>
<td><strong>Need Immune Component in Therapeutic Regimen for Cure?</strong></td>
<td>Maybe</td>
<td>No</td>
<td>Maybe</td>
</tr>
<tr>
<td><strong>Transmission</strong></td>
<td>Infected blood/needles, sex</td>
<td>Infected blood/needles, sex</td>
<td>Infected blood/needles, sex</td>
</tr>
<tr>
<td><strong>Vertical Transmission</strong></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Vaccine</strong></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>2015 U.S. Sales</strong></td>
<td>$9.3 billion</td>
<td>$13.3 billion</td>
<td>$700 million</td>
</tr>
</tbody>
</table>

**HCV:** No intracellular reservoir in the cellular genome
Growing Burden of Mortality Associated with Viral Hepatitis in the US (1999-2007)


- 73% of HCV and 59% of HBV-related deaths in persons aged 45-64

- Co-morbidities associated with increased odds ratio of mortality
  - Chronic Liver Disease (32.1; HCV and 34.4; HBV)
  - co-infection with other hepatitis virus (29.9; HCV and 31.5; HBV)
  - Alcohol related (4.6; HCV and 3.7; HBV)
  - HIV co-infection (1.8; HCV and 4.0; HBV)

- Mortality rates of HBV, HCV, and HIV; United States 1999-2007

Holmberg SD, et al. 62nd AASLD; San Francisco, CA; November 4-8, 2011. Abst. 243
Hepatitis C Virus:
Morphology and Characteristics

- Nucleic Acid: 9.6 kb ssRNA(+)
- Classification: *Flaviviridae, Hepacivirus*
- Genotypes: 1 to 6
- Enveloped
- No known viral reservoir
- Does not integrate into host genome
The Hepatitis C Virus

- Error-prone RNA-dependent, RNA polymerase
  - poor proofreading function
  - high replication rate \emph{in vivo}
~9.6 kb genome: 0.1-1 error per RNA synthesized

Key Target Areas of Drug Discovery Focus and Key Drugs

Adapted from: Liver International
pages 69–78, 23 DEC 2013 DOI: 10.1111/liv.12423
New HCV drugs

HCV RNA genome

HCV polyprotein

C E1 E2 p7 NS2 NS3 NS4A NS4B NS5A NS5B

Virus capsid and envelope proteins

Non-structural, replication complex

Telaprevir: HCV protease

Telaprevir HCV
HCV NS5B (Polymerase) Inhibitors and their Binding Sites

Prevent genome synthesis
Mechanism of Inhibition of HCV NS5A (binding protein – coordinates the interaction of RNA and the Polymerase, NS5B)

Prevent RNA Attachment for Initiation of Genome Synthesis by NS5B
Telaprevir (NS3)
Sofosbuvir (NS5B)
Sofosbuvir (NS5B) + Ledipasvir (NS5A)
# HCV new drug pipeline

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

*Harvoni*
HCV Curative Therapy Today

• IFN-Free curative therapies are a reality

• Simple oral fixed-dose and short duration therapies

• >95% cure rates across multiple genotypes

• High cure rates in difficult to treat patient populations

• Patient access is the issue

• HCV can become a rare disease in the future
Hepatitis C Drug News for 2018 – No Pipeline
Questions?