Overview of Flavivirus Therapeutics

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Outline

• Antiviral strategy
• Compounds tested in dengue clinical trials
• Repurposing strategy for Zika therapeutics
• Current status of dengue direct antiviral agents
• Knowledge gaps for Zika virus therapeutics
Antiviral strategy

- Targeting **viral proteins**
  - Polymerase/reverse transcriptase inhibitors
  - HIV and HCV protease inhibitors
  - HIV integrase inhibitors
  - HCV NS5A inhibitors
  - Attachment and entry inhibitors: small molecule, peptide, antibody
- Targeting **host proteins** required for viral life cycle
  - HIV CCR5 co-receptor inhibitor (Mavaviroc)
- Stimulating **immune system**
  - Interferon
  - Immune modulators: RIG-I, MDA5, Sting, and TLR modulators
- Targeting **molecular pathways** that lead to diseases
  - Molecular pathways that lead to pathogenic diseases should be clearly defined for therapeutic intervention.
Flavivirus antiviral approach

**Target-based approach**

Viral target with or without enzymatic activity

1. Enzyme activity-based HTS
2. Fragment-based screening
3. Structure-based rational design
4. Virtual screening

Hits and leads

Clinical candidate section

**Cell-based phenotypic approach**

Assay development:
1. Virus infection-based
2. Replicon-based
3. Luciferase-reporting virus
4. High-content image infection assay

HTS

Hits and leads

Target deconvolution: Host and viral targets
Compounds tested in dengue clinical trials: Repurposing strategy

- **Inhibit viral targets**
  - Balapiravir (J Infect Dis 2013 207(9):1442): excess cytokine production triggered by dengue virus infection prevented the conversion of the balapiravir prodrug to its active form (JVI 83:1740)

- **Inhibit host targets** that are essential for viral infection cycle
  - Celgosivir and iminosugar: α-glucosidase inhibitor (Lancet Infect Dis 2014 14:706)

- **Block the pathological pathways** that lead to severe dengue diseases (DHF/DSS)
  - Corticosteroid: inflammation inhibitor (Clin Infect Dis 2012 55:1216)
  - Ketotifen: antihistamine mast cell stabilizer to treat vascular leakage (ongoing)

*No bona fide* inhibitor specifically designed for dengue has ever been tested in clinics.
Repurposing strategy for Zika therapy

- Screening clinically approved drugs for potential Zika virus therapy should be performed.

- For any compounds active against Zika virus, does the compound exposure reach efficacious concentration (e.g., >EC\textsubscript{90}) in humans?
  - Human pharmacokinetic data are usually available for clinical compounds.

- Is the repurposed compound fast acting?
  - Fast-acting inhibitors are required to treat acute infections.

- Does pre-infection of Zika virus in patients affect compound potency?
  - Viral infection changes cell physiology that may affect compound potency, especially when the compound is a pro-drug that requires host enzyme metabolism.

- Can the compound reach viral replication sites in humans? What are the cell types that Zika virus infects?
  - Is the compound’s tissue/organ distribution known?
Flavivirus antiviral targets

- Capsid
- Envelope
- Pr peptide
- Protease-Helicase
- Methyltransferase
- Polymerase

5′ UTR

Structural

C  prM  E  NS1  2A  2B  NS3  4A  4B  NS5

Nonstructural

ORF

Furin

Protease Helicase NTPase 5′-RTPase

Polymerase Methyltransferase
Dengue: direct antiviral agents (I)

- **Capsid**: compound ST148; submicromolar EC$_{50}$; active in dengue AG129 mice (AAC 2013 57:15)
  - Issues: poor solubility and physical chemical property (e.g., solubility); poor formulation and bioavailability

- **Envelope**
  - Therapeutic antibodies: pan-serotype activity, resistance, and cost of therapy in developing countries
  - Small molecules: target a pocket between domain-I and domain-II; submicromolar EC$_{50}$ poor solubility (e.g., compound 6); no in vivo activity (AAC 2009 53:1823)
  - Peptide inhibitors: block envelope stem region folding-back (similar to Enfuvirtide/T20 in HIV); however, flavivirus fusion occurs in endosome, so the peptides need enter cells before exerting antiviral activity
Dengue: direct antiviral agents (II)

- **Protease**: flat substrate-binding surface with two positively charged P1 and P2 residues, making rational design of inhibitors challenging; protease inhibitors with low activity, especially in cell culture

- **Helicase**: flat substrate binding surface, making it challenging for identifying antiviral inhibitors

- **NS4B**: the most common viral target with hits identified from cell-based phenotypic screens. Some compounds have good drug-like properties and in vivo activity (e.g., compound 14a).
  
  - Issues: 1. Lack pan-serotype activity for compounds with good drug-like properties (JVI 2015 89:8233); 2. Lack drug-like properties for compounds with pan-serotype activities (JVI 2011, 85:11183)
Dengue: direct antiviral agents (III)

- **Methyltransferase**: selective inhibitors of flavivirus methyltransferase (e.g., compound 10) could be achieved through rational design, but these inhibitors lack cell permeability (JBC 2011 286:6233)

- **Polymerase**
  - **Non-nucleoside inhibitors**: compounds with nanomolar IC$_{50}$ against dengue polymerase have been achieved; weak cellular activity due to low cell permeability (JBC 2016 in press)
  - **Nucleoside inhibitors**: NITD008 showed cell culture and mouse model activities, but failed in preclinical safety (PNAS 2009 106:26435).

T-705 (Favipiravir) is active against yellow fever virus in cell culture and in animal model (AAC 2009 53:202).
Knowledge gaps for Zika virus therapeutics

- Understand Zika diseases
  - Replication sites in humans (organ, tissue, and cell types) will determine (i) cell culture system that should be used for drug discovery; (ii) compound distribution in vivo during drug discovery
  - Kinetics of viral infection and disease development in patients determine the window of treatment.

- Point-of-care diagnostics to differentiate infections between Zika, dengue, and Chikungunya viruses.

- Good animal model that recapitulates Zika diseases in humans.

- How to develop antiviral compounds in pregnant patients?