Part I
INTRODUCTION
STRUCTURE OF HIV SURFACE PROTEINS
HIV ENTRY INTO HOST CELLS
ENTRY INHIBITORS
Introduction

- Viral entry = complex multi-step process with coordinated, sequential interactions between proteins on virion and host cell surface
  - → multiple molecular interactions can be inhibited

- human immunodeficiency virus (HIV) → two classes of antiretroviral drugs: 
  - fusion inhibitors and chemokine receptor antagonists
Introduction

- Surface glycoprotein gp120 and transmembrane protein gp41
  - only viral proteins on extracellular surface of intact virion
  - encoded by viral envelope gene env
  - both originate as single polyprotein gp160
- Glycoproteins associate as homodimer of non-covalently linked gp120-gp41 heterodimers → form Env spike (120-140 Å)
- Env = HIV-1 envelope glycoprotein complex
- Each virion displays 8-14 spikes
Structure of the HIV surface proteins - gp120

- Heavily glycosylated protein
  - forms distal portion of Env spike
  - responsible for binding to cellular receptors
- Contains 5 conserved domains (C1-5) and 5 variable domains (V1-5)
  - conserved regions form core of protein
  - variable regions form distally positioned loops
- Many conserved N-linked glycosylation sites
- Three key structural regions
Structure of the HIV surface proteins - gp120

- **Inner domain**
  - conserved between HIV stains
  - undergoes conformational changes

- **Outer domain**
  - heavily glycosylated
  - contains variable region V3-5
  - presenting varying and cloaked surface to humoral immune response

- **Bridging sheet**
  - heavily glycosylated
  - contains variable region V1-2
  - contribution to CD4 and coreceptor binding site, masking CD4 binding site
Structure of the HIV surface proteins - gp41

● Proximal transmembrane portion of Env spike
  ○ anchoring complex in viral membrane
  ○ mediation of fusion

● Three distinct regions
  ○ C-terminal cytoplasmic tail
  ○ membrane spanning domain (transmembrane anchor TM)
  ○ large ectodomain
Structure of the HIV surface proteins - gp41

- Large ectodomain with four major structures:
  - Membrane-proximal external region (MPER)
    - highly conserved
    - essential for viral fusion and infection
  - Two heptad-repeat regions (HR1 and HR2)
    - α-helical leucine zipper-like motifs
    - drive membrane fusion by forming six-helix bundle
  - Amino-terminal fusion peptide (FP)
HIV entry into host cells

1. Attachment of viral surface Env gp120 protein to CD4 receptor on target cell
2. Subsequent interaction of Env-CD4 complex with coreceptor
3. Virus-host cell membrane fusion mediated by Env transmembrane gp41 protein
HIV entry into host cells - Attachment

- Initial interactions between HIV and target cell
  - nonspecific electrostatic interactions between positively charged domains on gp120 and negatively charged proteoglycans on host cell surface
  - specific interactions between host proteins incorporated in viral membrane and their ligands on target cell
  - interaction between α4β7 Integrin and gp120 might also promote HIV infection
HIV entry into host cells - CD4 binding

- CD4 makes contact with CD4 binding site of gp120
- Upon CD4 binding, conformational changes in gp120 occur
  - bridging sheet domain becomes ordered 4-stranded β-sheet structure (altering position and flexibility of V1 and V2 loop)
  - V3 loop extends and projects away from virion spike
  - bridging sheet and V3 loop are positioned towards host membrane
  - creating and exposing coreceptor binding site
HIV entry into host cells - Coreceptor binding

- Coreceptors used for HIV entry: CCR5 and CXCR4
- Binding of gp120 to CCR5 involves two interactions:
  - N-terminus of CCR5 binds to bridging sheet and V3 loop of gp120 → conformational change of V3 loop (flexible loop to rigid β-hairpin)
  - second interaction between extracellular loops of CCR5 and tip of V3 loop
HIV entry into host cells - Fusion

- Binding of gp120 to coreceptor triggers conformational change in receptor
  - exposure of gp41 fusion peptide and insertion in host cell membrane
  - heptad repeat regions HR1 and HR2 rearrangement into energetically favourable six-helix bundle (HR2 domains pack in antiparallel manner into grooves of inner HR1 3-coil core)
  - cellular and viral membranes in close proximity → initiation of fusion pore → entry of viral capsid
Entry inhibitors

- HIV entry → complex multi-step process
  → large number of potential targets to inhibit entry
- Entry inhibitors are heterogenous group of compounds
- Focus on compounds that are or have been tested in clinical trials
Drugs blocking gp120-CD4 interaction

- recombinant, soluble CD4 ($sCD4$) molecules as molecular decoys for cell associated CD4
- highly conserved CD4-binding site of gp120 → development of small molecular inhibitors to interrupt interaction
Drugs blocking gp120-CD4 interaction

- **BMS-378806**
  - interference with conformational changes in gp120
  - shown to successfully block infection when used as topical microbicide

- **BMS-626529** and prodrug **BMS-663068**
  - stabilisation of gp120 conformation that is incapable of binding to CD4
  - reduction of plasma HIV RNA levels

- **Monoclonal antibody Ibalizumab**
  - binds to CD4, not immunosuppressive
  - “post-attachment” inhibitor → does not prevent binding, blocks subsequent interactions with coreceptor
Drugs blocking gp120-coreceptor interaction

- Natural ligands for CCR5
- Chemokines have dual effect on CCR5 receptor:
  - induction of internalization of receptor shortly after exposure
  - CCR5 recycled to surface over time
  - chemokines act as competitive antagonists for gp120
Drugs blocking gp120-coreceptor interaction

- Number of derivatives for chemokine RANTES
- Small molecular inhibitors for CCR5
  - bind in hydrophobic pocket in transmembrane domains → allosteric inhibitors
  - conformational changes extracellular loops
- Maraviroc
  - effective in low nanomolar range against HIV stains that use CCR5 as coreceptor
  - one of two approved entry inhibitor drugs for treatment of HIV-infected patients in USA and Europe
- Currently no approved drug for gp120/CXCR4 interactions
Drugs blocking gp41-mediated membrane fusion

- Synthetic peptides corresponding with HR1/HR2 domains of gp41
  - potent antiviral effects
  - interference with packing of HR2 domains into grooves of HR1 coil
  - no formation of six-helix bundle driving membrane fusion
Drugs blocking gp41-mediated membrane fusion

- **Fusion inhibitor** Enfuvirtide (**Fuzeon**) first entry inhibitor approved for HIV infection management
  - linear, 36 amino acid synthetic peptide based on HR2 sequence
  - disrupts formation of six-helix bundle by competing with HR2 for binding to HR1

- **Sifuvirtide**
  - enhanced potency against wide range of HIV stains

- **Peptide from human blood (VIRIP)** potently blocked HIV infection
  - entry blocking by binding to fusion peptide and preventing insertion in host membrane
  - optimized dipeptide derivative (**VIR-576**) currently tested
Part II

CLINICAL CONSIDERATIONS

PATIENT IDENTIFICATION FOR TREATMENT

VIRAL RESISTANCE

ENTRY INHIBITORS IN COMBINATION THERAPY

MULTIPLE ENTRY INHIBITOR SYNERGY

FUTURE DIRECTIONS
Introduction

● Entry inhibitors are diverse
  ○ Target multiple stages of HIV entry
  ○ Good judgement in clinic required

● Entry inhibitors considerable potential
  ○ Resistance to RT, protease, & integrase inhibitors

● Maraviroc (CCR5 antagonist) and
● Enfuvirtide (Fuzeon; gp41 binder)
Patient Identification for Treatment

- Susceptibility to EIs
- CD+ T-cell count
  - Maraviroc > 50 CD+Tcells/mm³ > Fuzeon
- Disease state related to coreceptor use
  - CCR5 in transmission cases - optimal early
  - CXCR4 in advanced disease - Maraviroc less suitable
- Alternative coreceptors
  - Tropism testing (Trofile Assay)
- Different resistance pathways
Viral Resistance

- Maraviroc and Fuzeon orthogonal
  - Target different stages of HIV entry mechanism

**FIGURE 1. Schematic of the gp41 ectodomain.** HR1 and HR2 are represented as cylinders, and position 38 in HR1 is indicated. Residues Gln-142, Asn-145, Glu-146, and Leu-149, which interact with residue 38, are underlined in the HR2 sequence. HR2-based peptide fusion inhibitors are shown underneat. Mutations introduced in T1249mut and T2635mut are bold and underlined. Numbering is based on the sequence of HXB2 gp41.
Resistance to Maraviroc

- Outgrowth of pre-existing CXCR4-tropic HIV isolates

- Emergence of CCR5-tropic viruses
  - Drug-bound receptor non-competitive mechanism
  - Competitive mechanism = IC50 shift, no max. Reduction

- Resistance mapping
  - gp120 V3 loop and gp41
  - Pathway influenced by *env* gene & CCR5 antagonist used
  - Altered interactions with CCR5 at critical entry residues

- Many cases unclear
  - Coreceptor switching to CXCR4 and
  - Mutations that confer reduced drug susceptibility
Viral Resistance

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# Resistance to Enfuvirtide (Fuzeon)

- **Signature resistance mutations:**
  - HR1 domain
  - *Env* mutations outside HR1 little role in resistance

- **Result in:**
  - Reduced fusion efficiency
  - Enhanced antibody susceptibility

- **Compensatory HR2 mutations**
  - Can restore viral fusion
  - Maintain resistance

- **Patients retain CD4+ T cell increase**
  - Antiviral effects
  - Incomplete virologic suppression

## Illustration

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<tr>
<th>Pre-fusion</th>
<th>Post-fusion</th>
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<td>T20-sensitive GIV-SNY</td>
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FIGURE 6. **Four mechanisms of resistance to fusion inhibitors.** Molecular models (A) and schematic representation (B) of four different mechanisms of fusion inhibitor resistance. Small amino acids (Ala/Gly/Ser) create a hole in the contact site, whereas large residues (Phe/His/Met/Trp/Tyr) form a bulk that causes steric repulsion. Negatively charged residues (Asp/Glu) cause electrostatic repulsion, whereas positively charged amino acids (Lys/Arg) can form a salt bridge with the inhibitory peptide, possibly causing non-optimal helix packing and/or docking.
Viral Consequences to Resistance

- Env protein mutations impact fitness and tropism
- Antagonist-resistant Envs
  - Incomplete inhibition in presence of drug
  - Drug-bound CCR5 less efficient than native
- Drug-bound preference adaption
  - Improved entry efficiency and kinetics
  - Fitness cost to resistance mutation in drug absence
- CCR5-tropic resistant to CCR5 antagonist
  - Can have altered tropism on primary CD4+ T cells
  - Resistance consequence complex
Entry Inhibitors in Combination Therapy

- CT reduces drug resistance
  - Traditionally 2 RT inhibitors and 3\textsuperscript{rd} from another class

- Entry Inhibitor inclusion
  - Expands treatment options
  - Reduces toxicity (drug-sparing)
  - Combat MDR (salvage therapy)

- Maraviroc studied in sparing and salvage regimes
Multiple Entry Inhibitor Synergy

- Fuzeon resistance mutations can alter kinetics of HIV entry process and increase drug or antibody potency

- Number of resistance mutations required for more approved EIs may significantly impact viral fitness

- Synergy demonstrated between coreceptor antagonists, enfuvirtide, and monoclonal antibodies
Future Directions

- Preventative medicine
  - Microbicide development
- Large number of EI in development
- RANTES derivatives & Maraviroc
  - Potent inhibition of viral transmission
  - Resistance through coreceptor switching to CXCR4
- Alternative strategies
  - Genetic modification of hematopoietic stem cells or
  - CD4+ T cells to disrupt ccr5 or cxcr4 genes using Zinc fingers or TALEN
References


Balzarini, J. and Van Damme, L. Intravaginal and intrarectal microbicides to prevent HIV infection, 2005 February 15; 172(4) 461-464

Splettstoesser, T. Cartoon representation of the zinc-finger motif of proteins. The zinc ion (green) is coordinated by two histidine and two cysteine amino acid residues. Based on the X-ray structure of PDB 1A1L, 2017 November 17, www.scistyle.com, viewed on 21 November 2019
QUESTIONS?

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