ENTRY KINETICS OF GLOBALLY REPRESENTATIVE AND VERTICALLY TRANSMITTED HIV

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INTRODUCTION
- HIV entry is an organized process that depends on transient states
- Transiently exposed regions in the fusion protein gp41 are highly conserved, attractive vaccine targets
- MPER is targeted by bNAbs with wide breadth (e.g., 2F5, 4E10, 10E8)
- HR1 is targeted by T20 (Fuzeon)

Understanding the kinetics of transient states may shed light on new transmission associated phenotypes and lead to new avenues of vaccine development

BACKGROUND
- A variety of techniques are available for measuring kinetics
- Time of addition allows multiple stages to be measured with a single, standardized protocol
- Previous kinetic studies are limited to only a few isolates
- Breadth of kinetics among circulating isolates is not well characterized

GOAL
- Our goal is to characterize the breadth of HIV-1 entry kinetics among circulating HIV Envelope (Env) isolates and isolates associated with transmission

APPROACH
- Time of addition kinetics assay, optimized for reproducibility
- Measure kinetics of six helix bundle formation using T20
- Measure kinetics of co-receptor (CoR) engagement using CCR5 inhibitor maraviroc (MVC)

RESULTS & SIGNIFICANCE
- Very well confined window of average T20 delay (4-16 min).
  - This is only a small part of natural range (<7 min, >40 min).
- Extreme kinetics are rare members of quasiespecies.
- Infant BMT isolates had some of the slowest extremes.
- Prehairpin is exposed during co-receptor engagement.
- Kinetic component of T20 sensitivity linked to co-receptor kinetics.
- Only a few amino acids lie between average and extreme kinetics.
- Kinetic extremes might be part of a normal phenotypic landscape

HIV-1 exhibits a broad and dynamic landscape of kinetic phenotypes that link co-receptor binding to gp41 exposure and sensitivity to gp41-targeting inhibitors.

PRIMARY HIV-1 ISOLATES EXHIBIT BROADLY DIVERSE KINETICS
- 285 primary HIV Env isolates
- Well-formed distribution of T20 delays centered about 4-16 minutes with broad range of extremes from as early as <30 seconds to as late as >32 minutes.

DIVERSE KINETICS LINKED TO BREAST MILK TRANSMISSION
- Similar, diverse range of T20 kinetics for maternal and infant ZEBS isolates.
- Kinetic diversity primarily linked to Env associated with breast milk transmission.
- Both maternal and infant BMT Env can have extremely late T20 delays.
- Infant BMT Env have the latest extremes.

SEQUENCE DETERMINANTS OF INFANT BMT EXTREMES
- Infant isolates associated with breast milk transmission had some of the most extreme T20 delays.
- All at least one of these extreme isolates were found in 3/6 infants associated with breast milk transmission.
- Infant sequences are highly conserved between averages and extreme kinetics

GP41 EXPOSURE IS Driven BY CO-RECEPTOR BINDING
- Measured MVC delay for subset of 48 Envs with diverse T20 delay.
- MVC delay categorized by quartiles as early (Q1), average (Q2 or Q3) or late (Q4).

During the prehairpin exposure occurs after co-receptor binding and before six helix bundle formation.

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Supports the role of co-receptor binding in prehairpin exposure kinetics.

References

For our data support alternative models where prehairpin exposure is defined by co-receptor binding
- Bundle can form before, during or after co-receptor binding.
- MVC delay marks the end of prehairpin exposure and should predict T20 sensitivity.
- T20 - MVC delay should be less than 12 minutes.
- Only isolates with late MVC delays spend most of their time in the prehairpin exposed state.

Prehairpin exposure and T20 sensitivity is driven by co-receptor binding kinetics.

GP41 triggers prehairpin exposure
- One CoR triggers PH bundle formation and should not predict T20 sensitivity.
- T20 - MVC delay should not predict T20 sensitivity.

Co-receptor mechanism
- Standard model of entry says that

MVC delay should be <32 minutes and does not sensitivity, but does

Prehairpin exposure occurs after co-receptor binding and before six helix bundle formation.

Longer prehairpin exposure means more sensitivity to T20.

MVC Delay should not predict T20 sensitivity

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Co-receptor mechanism

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