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[1-6]
- **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

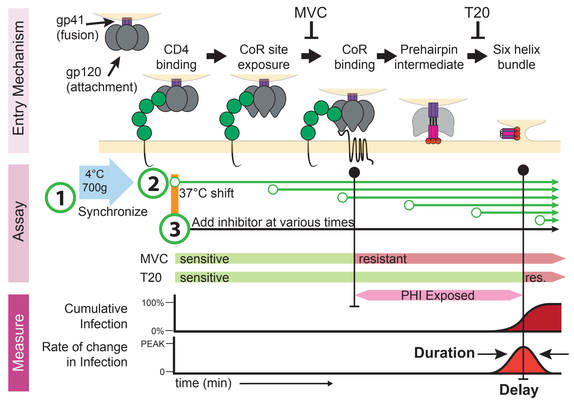
- Time of addition kinetics assay, optimized for reproducibility
- Measure kinetics of six helix bundle formation using T20
- Measure kinetics of co-receptor (CoR) engagement using CCR5 inhibitor maraviroc (MVC)
- Structurally diverse and transmission-associated isolates
  (255 HIV Env isolates characterized)
  - Standard R5-using M and T-tropic isolates (JR-CSF, BaL)
  - 10 isolates from globally representative neutralization panel [7]
  - 14 acute T/F Envs (clade B)[8]
  - 107 early/longitudinal isolates from 5 adult transmission pairs (clade C)[9]
  - 14 mother-to-child (MTCT) transmitted Envs (Clade C, D, A, with BG505)[10]
  - 108 Zambian Maternal and Infant MTCT isolates associated with breast milk (BMT) and in utero (IUT) transmission (ZEBS, clade C)[11]

RESULTS & SIGNIFICANCE

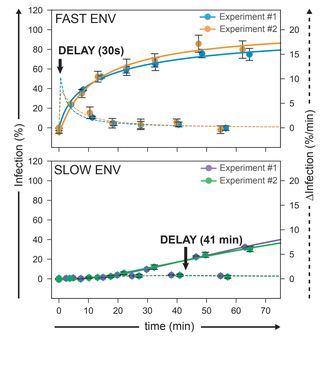
- Very well confined window of average T20 delay (4-16 min).
  This is only a small part of natural range (<1 min, >40 min).
- Extreme kinetics are rare members of quasispecies.
- Infant BMT isolates had some of the slowest extremes.
- Prehairpin is exposed during co-receptor engagement.
  Kinetic component of T20 sensitivy 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.

TIME OF ADDITION ASSAY

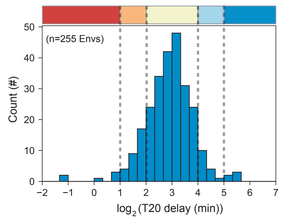


Delay is how long it takes to go from CD4-bound to either the co-receptor bound maraviroc (MVC) resistant state or the T20-resistant six helix bundle.



Lognormal distribution provides a highly accurate description of infection over time

PRIMARY HIV-1 ISOLATES EXHIBIT BROADLY DIVERSE KINETICS



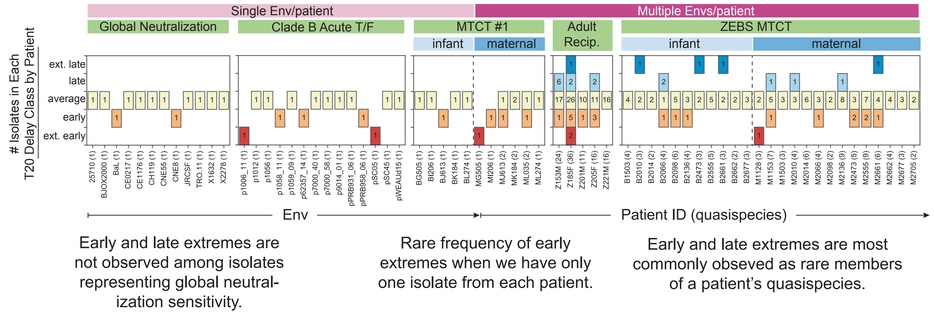
T20 delay classes:

extremely early	≤2 min
early	2-4 min
average	4-16 min
late	16-32 min
extremly late	≥32 min

255 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.

Breakdown of T20 kinetics by panel and patient

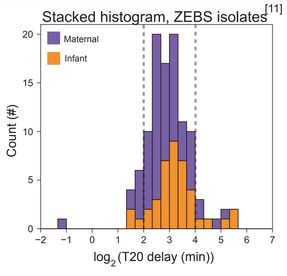


Early and late extremes are not observed among isolates representing global neutralization sensitivity.

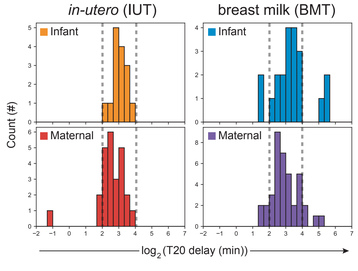
Rare frequency of early extremes when we have only one isolate from each patient.

Early and late extremes are most commonly obseved as rare members of a patient's quasispecies.

DIVERSE KINETICS LINKED TO BREAST MILK TRANSMISSION



Similar, diverse range of T20 kinetics for maternal and infant ZEBS isolates.



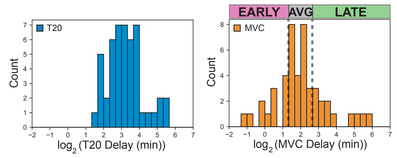
Envs associated with transmission *in utero* have very narrow range of kinetics.

Kinetic diversity primarily linked to Envs associated with breast milk transmission.

Both maternal and infant BMT Envs can have extremely late T20 delay.

Infant BMT Envs have the latest extremes.

GP41 EXPOSURE IS DRIVEN BY CO-RECEPTOR BINDING



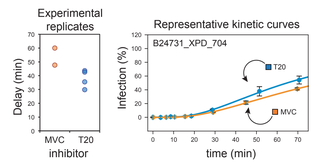
Measured MVC delay for subset of 48 Envs with diverse T20 delay.

MVC delay categorized by quartiles as early (Q1), average (Q2 + Q3) or late (Q4).

Standard model of entry says that

Co-receptor binding occurs before six helix bundle formation.

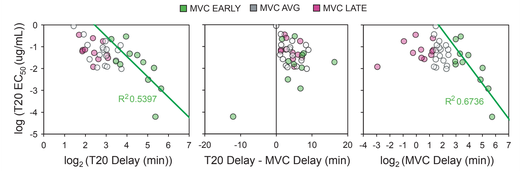
MVC delay should always be earlier than T20 delay



Infant BMT Env whose MVC delay is 12 minutes after T20

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

Longer prehairpin exposure means more sensitive to T20



T20 Delay should be related to T20 sensitivity, **and it is**

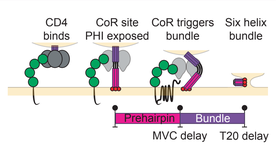
T20 Delay - MVC Delay should predict T20 sensitivity, **but does not**

MVC Delay should not predict T20 sensitivity, **but it does**

T20 sensitivity is associated with MVC and T20 delay for isolates with **late MVC delays**

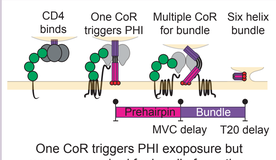
Our data support alternative models where prehairpin exposure is defined by co-receptor binding

CD4 triggers prehairpin exposure

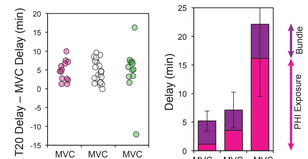


CD4 triggers exposure of CoR binding site and pre-hairpin, CoR binding triggers bundle formation.

Co-receptor stoichiometry



One CoR triggers PHI exposure but more are required for bundle formation.



Bundle can form before, during or after co-receptor binding.

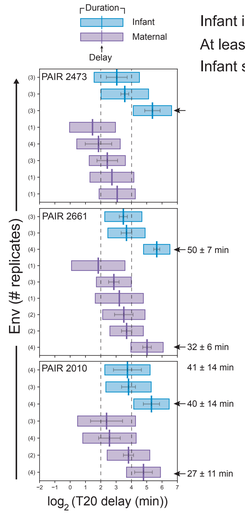
MVC delay marks the end of pre-hairpin exposure and **should predict T20 sensitivity**.

T20 - MVC delay is bundle formation and **should not predict T20 sensitivity**.

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

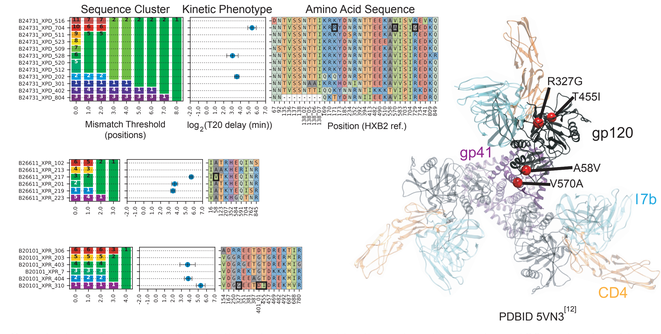
SEQUENCE DETERMINANTS OF INFANT BMT EXTREMES



Infant isolates associated with breast milk transmission had some of the most extreme late T20 delays.

At least one of these extreme isolates were found in 3/6 infants associated with breast milk transmission.

Infant sequences are highly conserved with few differences between average and extreme kinetics



Genotypic features associated with extreme slow kinetics primarily map to gp120 and not gp41.

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

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