A case of super-infection with two highly divergent HIV-1 strains: implications for the latent reservoir

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none to declare

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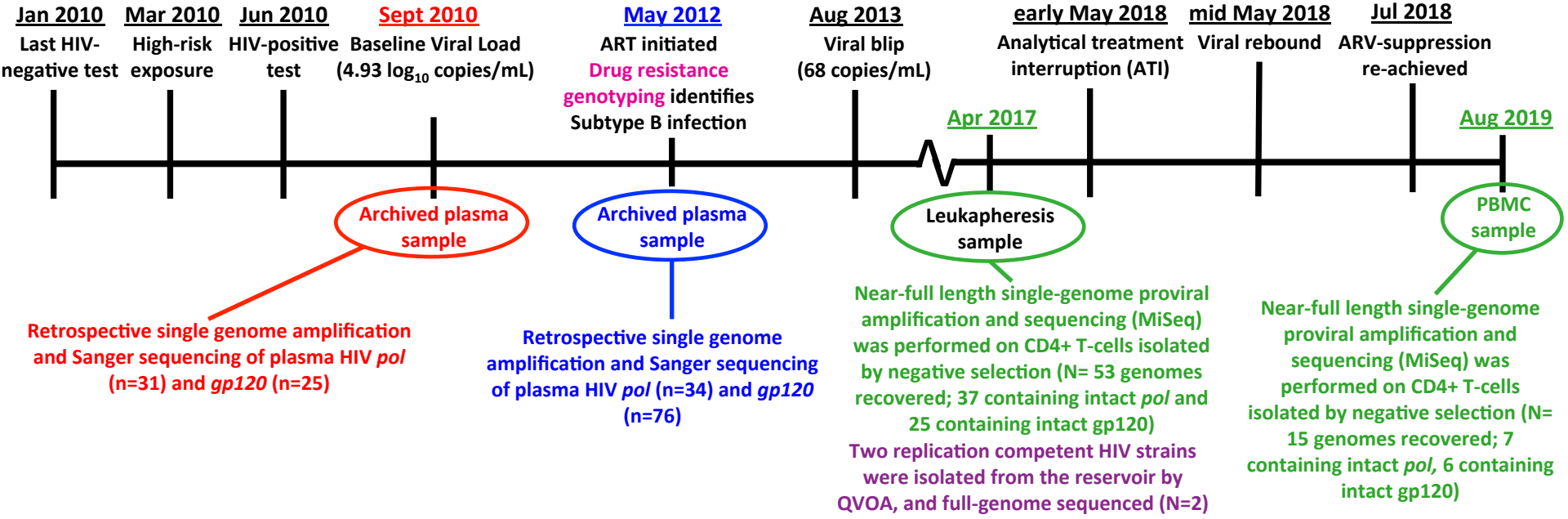
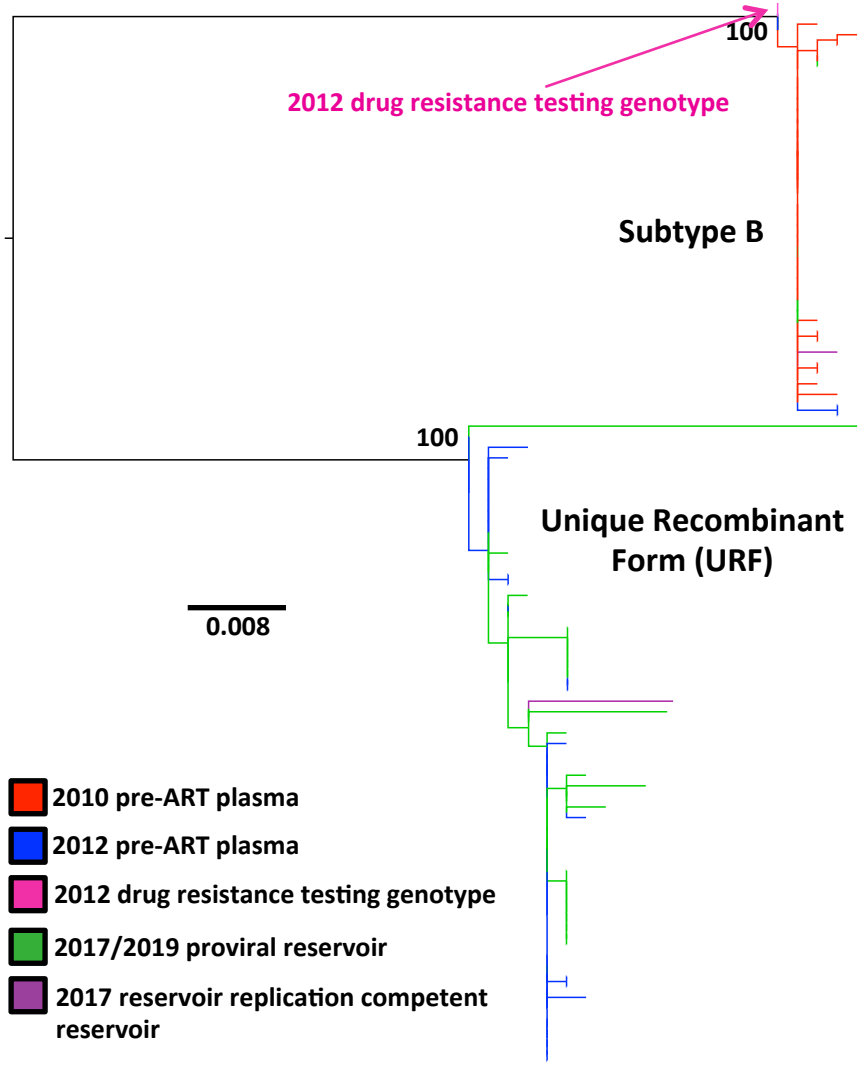
BACKGROUND and OBJECTIVE:

The dynamics of HIV co-/super-infections with multiple subtypes are poorly understood, particularly as these relate to reservoir seeding and persistence. We describe a unique case of initial subtype B infection followed by super-infection by a unique recombinant form (URF) in a participant of an HIV cohort study in Canada.

STUDY PARTICIPANT CLINICAL AND SAMPLING HISTORY:

OM5346 is a 51-year old male of African decent living in Toronto, ON. Diagnosed with HIV in June 2010, he initiated ART in May 2012 and clinical HIV drug resistance genotyping identified a subtype B infection. He maintained viral suppression until May 2018 (aside from a small viral blip in August 2013) when he underwent Analytical Treatment Interruption (ATI). Viral rebound occurred ~2 weeks after treatment cessation and ART was re-initiated (suppression achieved July 2018). Sample timepoints, type and sequences recovered are indicated below.





RESULTS: OM5346 is infected with both subtype B and a Unique Recombinant Form (URF)

OM5346 was originally clinically genotyped (2012) as infected with subtype B.

However, additional retrospective single-genome *pol* and *gp120* amplification and sequencing from 2010 and 2012 pre-ART plasma samples, as well as full genome amplification and sequencing from the proviral (2017, 2019) and replication competent (2017) reservoir revealed OM5346 to be super-infected with both a subtype B and a non-B strain.

Figure 1 (left): Maximum likelihood phylogenetic tree of *pol* sequences from OM5346. Two distinct clades are observed: one subtype B (top) and the other non-B (bottom). *Pol* was excised from full genome sequences when present using GeneCutter (HIV LANL). Scale= nucleotide substitutions/ site. Bootstrap values are indicated below nodes.

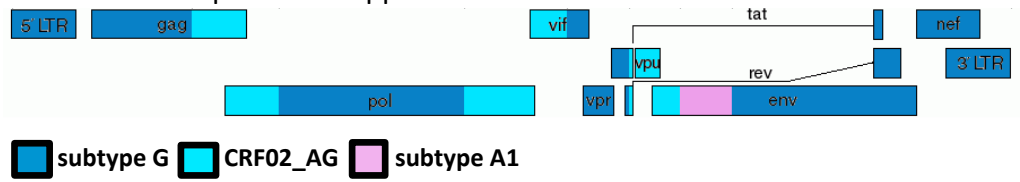
Notably, both viral strains (as proviruses and replication competent outgrowth viruses) were represented in the reservoir.

gp120 phylogeny (not shown) exhibited a similar structure.

No within-host recombinants (between B and non-B strains) were observed.

RIP 3.0 analysis of full genome sequences of the non-B strain revealed it to be a previously un-described Unique Recombinant Form (URF) comprised of subtype A1, G and CRF02_AG

Figure 2 (below): Schematic of Unique Recombinant Form (URF) infecting OM5346. Breakpoints are approximate.



RESULTS 2: Super-infection occurred between 2010-2012

Figure 3 (left): OM5346 maximum likelihood *pol* phylogenies, separated by subtype.

All Pre-ART sequences sampled from plasma in 2010 are subtype B (red sequences only observed in subtype B tree, not in URF tree), while those sampled from 2012 were both subtype B and URF (blue sequences are in both subtype B and URF trees), though subtype B sequences were relatively infrequent at this timepoint.

This indicates that OM5346 was initially infected with subtype B in 2010 and subsequently super-infected with the URF, which then dominated untreated infection before ART was initiated in May 2012.

RESULTS 3: OM5346 reservoir subtype distribution resembles that of plasma RNA immediately pre-ART (2012)

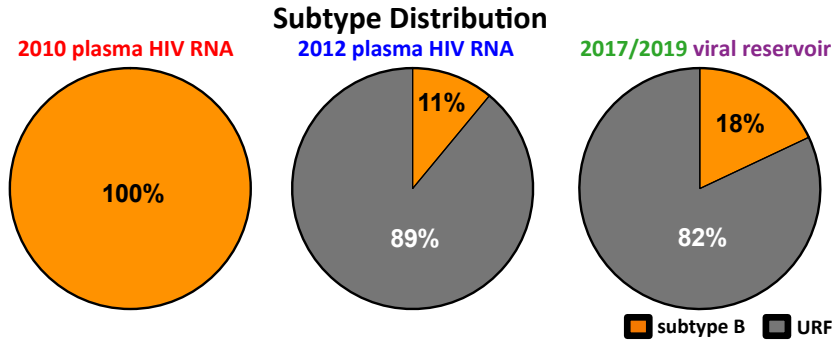


Figure 4 (above): Subtype distribution of HIV sequences in 2010 and 2012 plasma RNA and 2017/2019 reservoir. Subtype B and URF sequences were observed at similar frequencies in 2012 plasma HIV RNA and viral reservoir sampled in 2017/2019, where the URF dominated both time points.

SUMMARY:

Retrospective pre-ART plasma sampling identified a case of super-infection with highly divergent HIV strains in an individual recruited to a HIV cohort Study in Toronto, Ontario.

Initial infection occurred with a subtype B strain in 2010. Later, super-infection occurred with a previously un-described unique recombinant form (URF), comprising subtype G, A1 and CRF02_AG. By the time ART was initiated in 2012, the URF had become the dominant circulating strain.

Subtype distribution in the HIV reservoir measured 5 and 7 years following ART initiation reflected pre-ART subtype distribution, with replication competent viruses of both infecting strains recovered by QVOA.

No within-host recombinants were observed.

CONCLUSIONS:

Super-/co-infection with multiple HIV strains yields a genetically complex, replication competent HIV reservoir comprising highly distinct HIV quasispecies.

Treatment interruption carries the risk of *de novo* within-host recombination events.

Such cases of extreme within-host HIV diversity may complicate HIV remission strategies.

Future studies should be conducted to investigate reservoir size and composition in additional individuals infected with multiple HIV subtypes.

We thank the study participant, without whom this research would not be possible.

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