

PROVIRUS FROM EARLY-TREATED HIV REVEALS EARLIER VARIANTS TARGETED BY INITIAL NEUTRALIZING ANTIBODIES

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BACKGROUND.

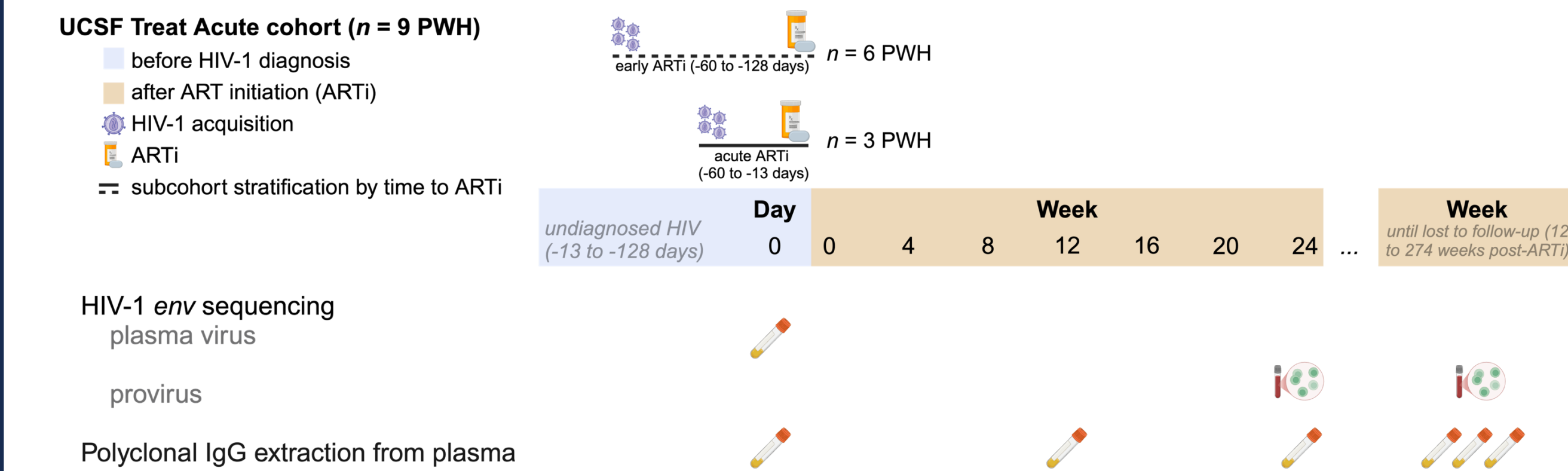
Early antiretroviral therapy (ART) restricts the size and diversity of the proviral reservoir and limits immune dysfunction, potentially offering an intervenable clinical juncture for immunotherapeutic cure efforts.

We recently sequenced early plasma HIV-1 *envs* and characterized the neutralizing antibody responses after acute and early ART initiation (ARTi). We found that ARTi during early (60–128 days after acquisition) but not acute (<60 days) disease allows sufficient viral replication to induce initial autologous neutralizing antibodies (anAbs). These anAbs then gain potency and breadth during the subsequent months of suppressive ART [1,2].

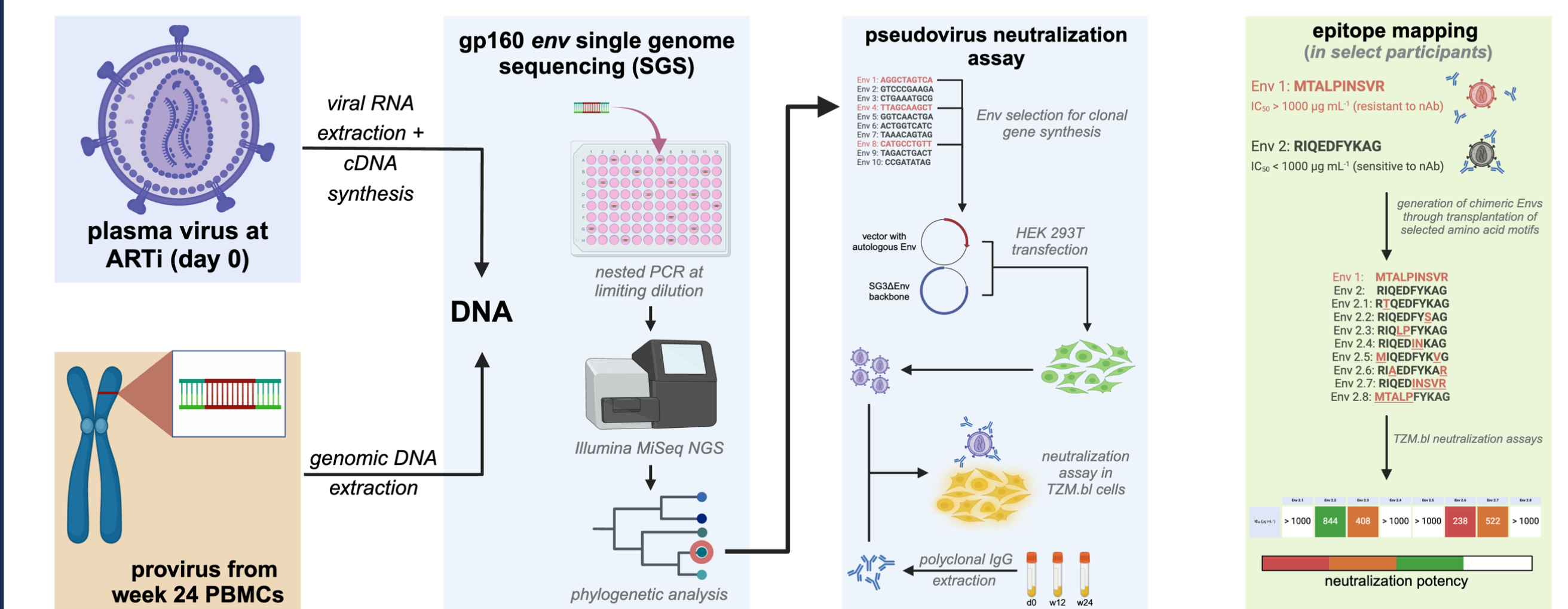
Here, we build on this work by sequencing HIV-1 provirus from PBMCs collected after 6 months of ART. We compared plasma Envs phylogenetically and phenotypically, asking if the archived reservoir could permit inference of transmitted/founder (TF) virus(es) and earlier virus species that may be sensitive to initial anAbs.

METHODS.

UCSF Treat Acute cohort [3]

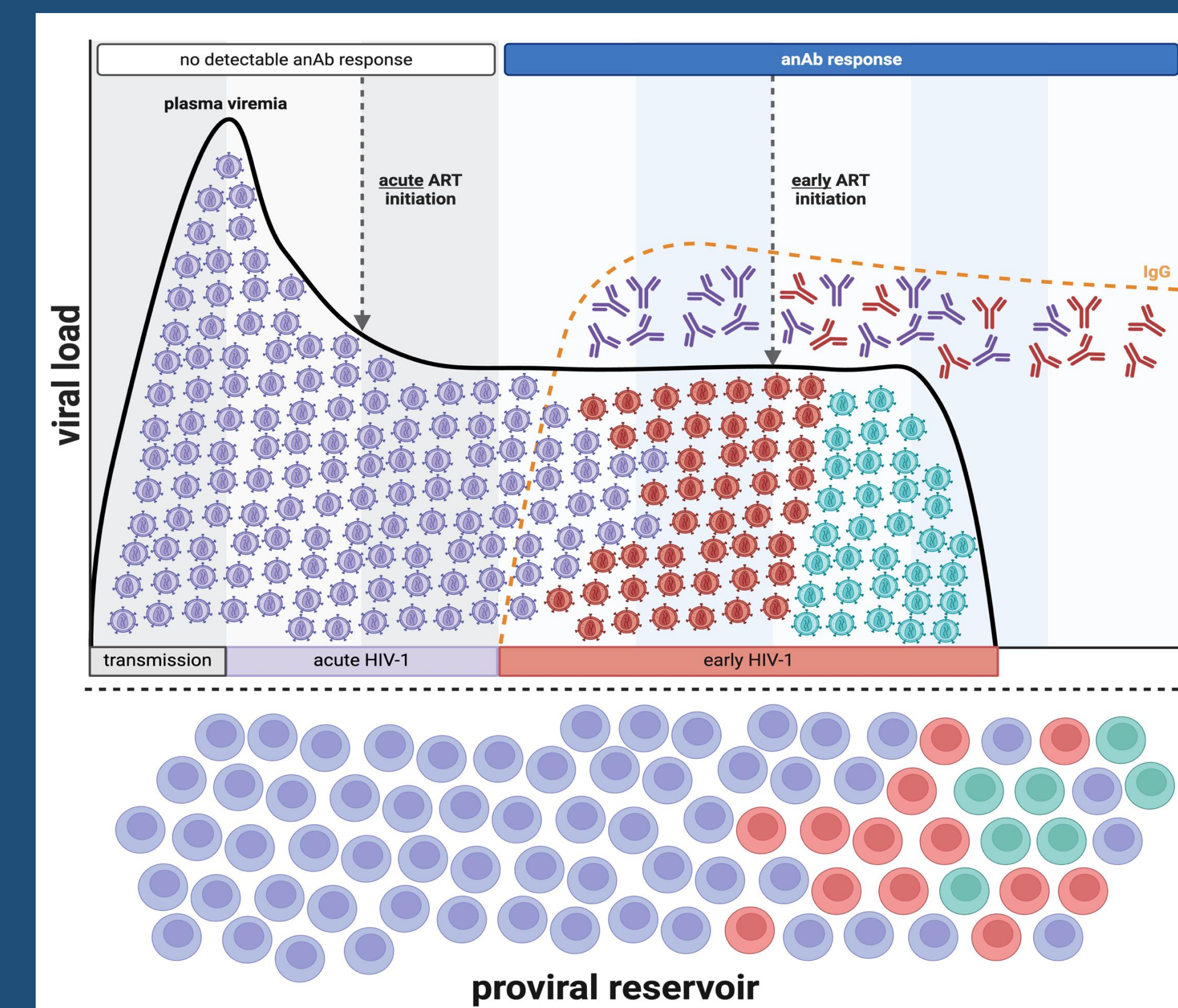


Participants ($n = 9$ PWH [3 treated acutely, 6 treated early; all male]) were recruited at HIV-1 diagnosis and linked to care for ARTi (day 0). They were then followed longitudinally for peripheral blood draws to isolate peripheral blood mononuclear cells (PBMCs) and immunoglobulin G (IgG).

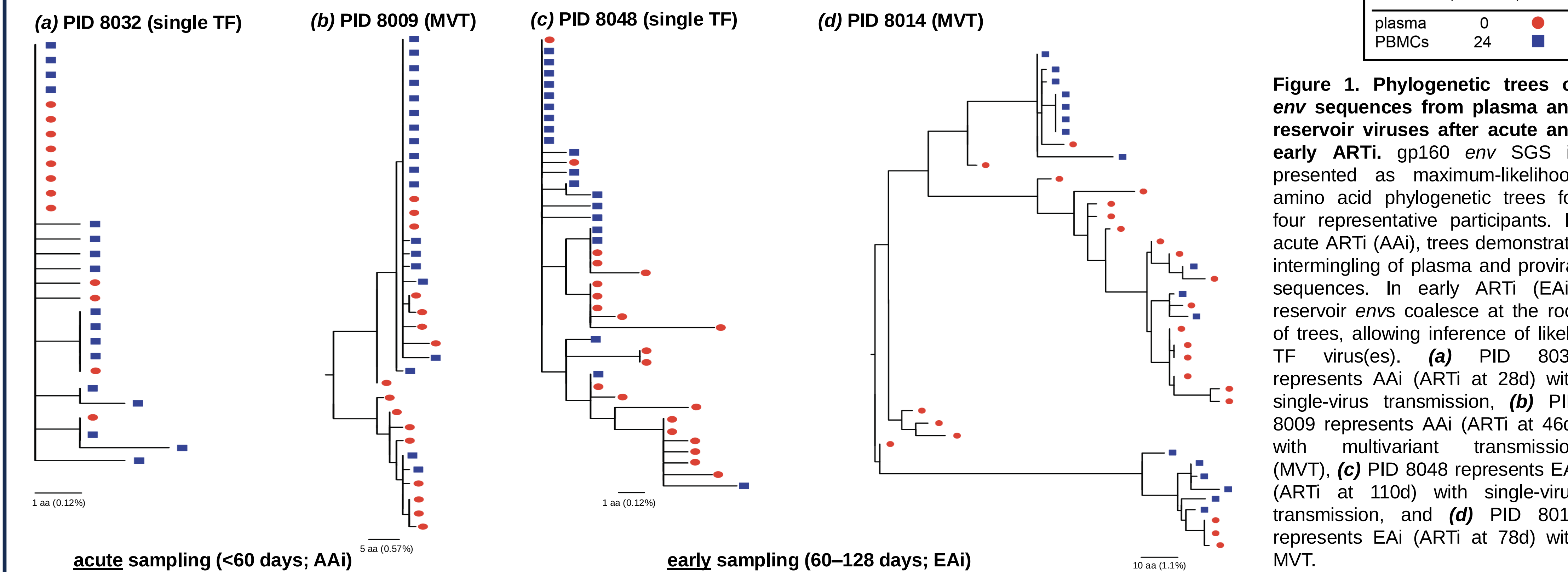


Single genome sequencing (SGS) of gp160 *env* from plasma at ARTi and PBMC provirus from 24 weeks of ART was performed; sequences were analyzed by maximum-likelihood amino acid phylogenetic trees (PhyML) on a per-participant basis. Select Envs were tested for neutralization sensitivity to longitudinal plasma IgG by TZM.bl assays [4]. Chimeric Envs encoding select regions were generated to map anAb responses at the molecular level.

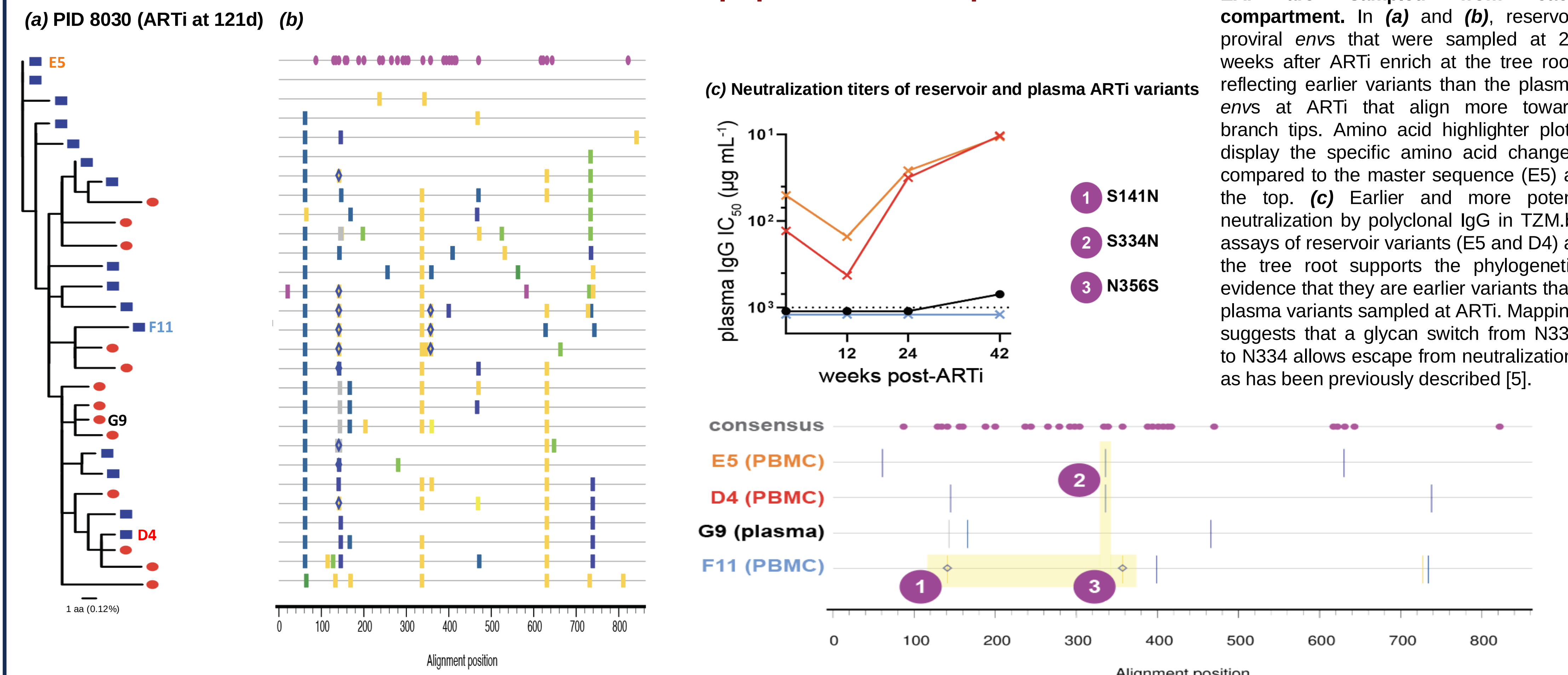
Phylogenetic and phenotypic analyses of paired plasma virus at ART initiation and archived provirus enable tracking of virus-host evolution



Reservoir (week 24 PBMC provirus) and plasma virus (at ART initiation) *env* sequencing.



EAI: archived reservoir *envs* reflect earlier virus populations than plasma *envs*.



In diverse virus populations from MVT, recombination, and anAb escape: archived reservoir and neutralization sensitivities inform the direction of viral evolution.

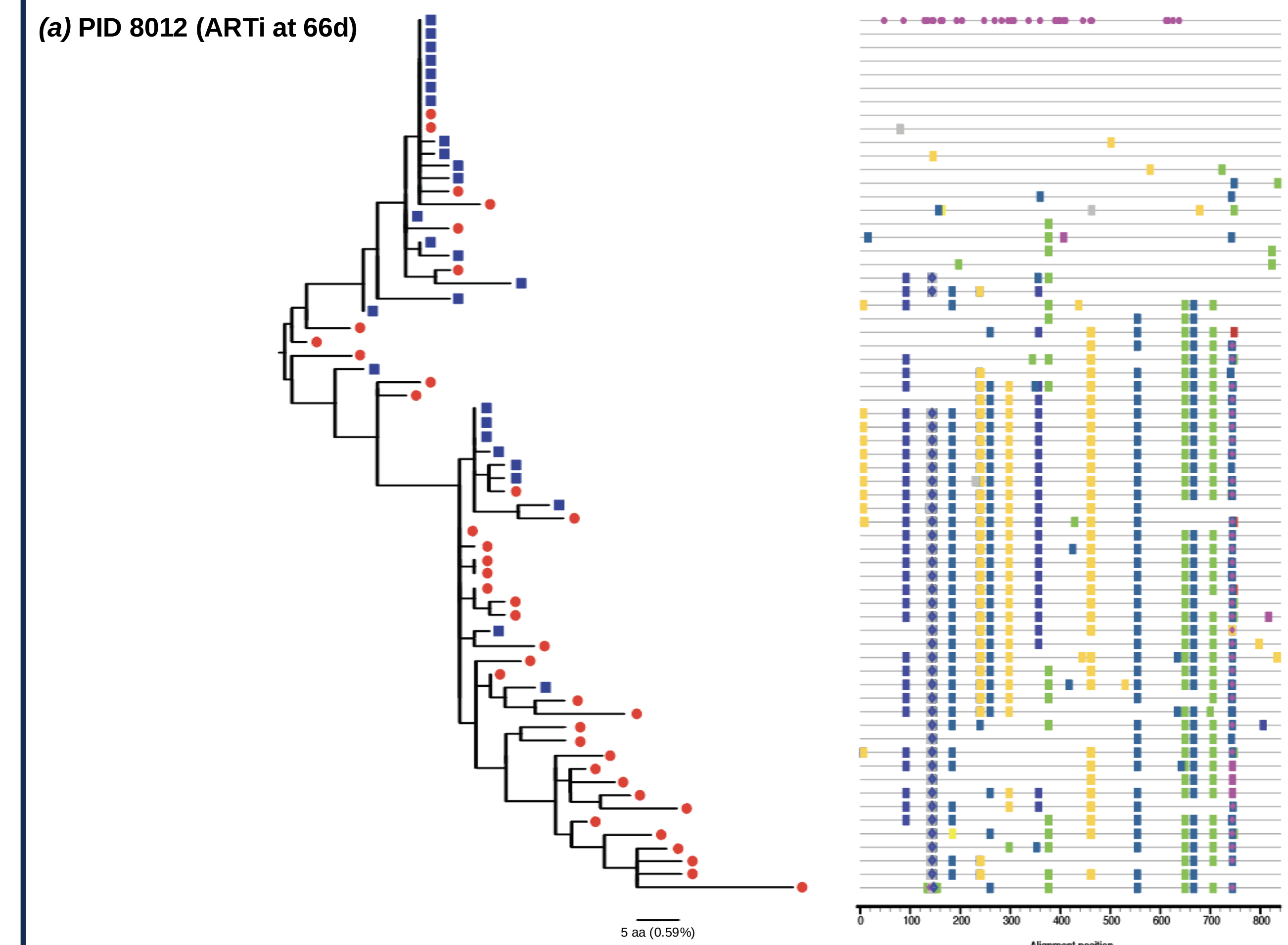


Figure 3. Phylogenetic and phenotypic features of plasma and reservoir viruses from participants with multivariant transmission. (a) PID 8012 initiated ART 66 days post-acquisition. *env* sequences sampled from the proviral compartment aided in rooting variants to depict the evolution of each lineage. Ongoing neutralization data suggest that initial anAbs target a V1 epitope with an N141 glycan whereas later variants feature mutations herein and demonstrate anAb resistance. (b) PID 8035 initiated ART 118 days post-acquisition. Phylogeny shows the major lineage (top) established by the likely TF (depicted as the master sequence) with early reservoir variants and plasma viruses derived from this TF featuring extensive V1V2 mutations. Preliminary neutralization data with epitope mapping suggest that the initial anAb response targets gp120 C4 with resistance conferred through V1V2 mutations, distinguishing between anAb-sensitive and -resistant variants.

CONCLUSIONS AND IMPLICATIONS.

Phylogenetic and phenotypic comparisons of plasma viruses replicating at early ARTi and those archived in the reservoir 6 months post-ARTi indicate:

- With **acute** ARTi, reservoir and ARTi plasma viruses are nearly identical.
- With **early** ARTi, plasma virus shows evidence of escape from the initial anAb response with greater phylogenetic diversity and resistance to initial anAb responses. The archived reservoir, however, contains a large proportion of earlier, anAb-sensitive variants.
- Results suggest that proviral sampling can serve as a periscope to characterize earlier variants and support inference of TF virus(es). Similarly, relatively superficial reservoir sampling may not fully sample minor variants (e.g., later, anAb-resistant) that are more likely to be rebound-competent [6,7].

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