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BACKGROUND. Early antiretroviral therapy initiation (ARTi) in HIV-1 restricts reservoir size and diversity while preserving immune function, potentially improving opportunities for immunotherapeutic cure strategies. For antibody-based cure approaches, the development of autologous neutralizing antibodies (anAb) after acute/early ARTi is relevant, but poorly understood.

METHODS. We characterize antibody responses in a cohort of 23 participants following ARTi in acute HIV (<60 days after infection) and early HIV (60-128 days after infection).

RESULTS. Plasma virus sequences at the time of ARTi revealed evidence of escape from anAbs after early, but not acute, ARTi. HIV-1 Envs representing the transmitted/founder virus(es) (acute ARTi) or escape variants (early ARTi) were tested for sensitivity to longitudinal plasma IgG. After acute ARTi, no anAb responses developed over months to years of suppressive ART. In two of the three acute ARTi participants who experienced viremia after ARTi, however, anAbs arose shortly thereafter. After early ARTi, anAbs targeting those early variants developed between 12 and 42 weeks of ART and continued to increase in breadth and potency thereafter.

CONCLUSIONS. Results indicate a threshold of virus replication (~60 days) required to induce anAbs, after which they continue to expand on […]

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ABSTRACT

Background. Early antiretroviral therapy initiation (ARTi) in HIV-1 restricts reservoir size and diversity while preserving immune function, potentially improving opportunities for immunotherapeutic cure strategies. For antibody-based cure approaches, the development of autologous neutralizing antibodies (anAb) after acute/early ARTi is relevant, but poorly understood.

Methods. We characterize antibody responses in a cohort of 23 participants following ARTi in acute HIV (<60 days after infection) and early HIV (60-128 days after infection).

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Conclusions. Results indicate a threshold of virus replication (~60 days) required to induce anAbs, after which they continue to expand on suppressive ART to better target the range of reservoir variants.

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INTRODUCTION

HIV-1 cure strategies aim to eradicate the proviral reservoir and/or enhance immune-mediated virus suppression. Thus, understanding HIV-specific cellular and humoral immunity is central to the cure research agenda (1). Early antiretroviral therapy initiation (ARTi) has been shown to restrict reservoir size and diversity (2-7) while preserving immune function (8), providing an optimal setting for immunologic cure interventions. Furthermore, immediate ARTi at time of HIV-1 diagnosis limits transmission (9, 10) and disease progression (11, 12) in people with HIV (PWH). This early viral suppression, however, may also abrogate development of autologous neutralizing antibodies (anAbs). NAb responses against HIV develop slowly, often arising after three or more months of persistent virus replication (13). Other adaptive responses, such as HIV-specific cytotoxic T cells (CTLs) (14) and non-neutralizing antibodies (used in diagnostic testing algorithms and potentially mediating antibody-dependent cellular virus inhibition (ADCVI)), (15) arise during the first two to four weeks of acute disease. The kinetics of CTL (16) and effector antibody responses (17) after early ARTi have been described recently, but less is known about the timing, magnitude, and durability of host nAb development in this setting. Here, we aimed to characterize the kinetics and determinants of anAb development following acute and early HIV acquisition and immediate ARTi in the University of California, San Francisco (UCSF) Treat Acute HIV cohort (18).

Delayed development of anAbs distinguishes HIV from other viral infections, including SARS-CoV2 (19), influenza (20), and herpes viruses (21), against which potent nAbs develop within days of disease onset. Anti-HIV-1 neutralizing antibodies are classically defined as those that bind the functional HIV-1 envelope (Env) trimer and prevent infection of new target cells by sterically blocking access to cellular receptors, inhibiting necessary energy states, or impeding

conformational transitions (22). HIV-1 evades effective humoral immunity and delays nAb development by impairing CD4 T cell help and concealing neutralizing epitopes through dense glycosylation and conformational masking (23-26). Furthermore, the HIV virion displays many non-functional 'decoy' antigens such as gp41-gp120 monomers and gp41 stumps (27) with relatively few functional Env trimers (28). Most binding antibodies recognizing monomeric Env or gp41 antigens do not neutralize plasma virus (29, 30). Early anAbs are directed to target epitopes in the highly polymorphic 'variable' regions of Env (31), allowing for virus escape facilitated by the high error rate of HIV-1 replication (32). As a result, the earliest anAbs lack breadth and are highly strain specific for transmitted/founder (TF) virus(es), the virus(es) that establish productive infection following a transmission event (23, 33).

Early nAb responses can be quite potent but are insufficient to control HIV replication due to rapid immune escape. In untreated early infection, plasma virus evolves to escape activity of contemporaneous circulating anAbs as they arise (33). As untreated HIV progresses, successive cycles of viral escape from ongoing anAb responses continue, though escape may be less complete in chronic infection (34). NAbs with activity against a broad range of isolates can be identified in between 5-30% of chronically infected individuals, but these broadly neutralizing antibodies (bnAbs) arise only after many cycles of antibody development and viral escape over years of viremia, and are infrequently associated with virus control (35, 36). Furthermore, active HIV replication disrupts cellular immunity, inducing dysfunctional B cell (37) and T follicular helper cell (24) responses that impair effective Ab responses. Paradoxically, ongoing viral replication both provides the antigen exposure necessary for development and honing of anAb activity, while simultaneously impairing cellular immunity and allowing viral escape.

Early ARTi has complex implications for anAb development. By inhibiting viral replication, ART helps preserve cellular immunity and prevents further viral evolution, but also dramatically decreases the amount of Env antigen encountered by immune cells. Halting viral replication, however, does not eliminate Env expression. Initially upon ARTi, virus is expressed by productively infected cells which exhibit a range of half-lives (38, 39). During steady-state ART suppression, HIV Env exists via expression of provirus from reservoir cells (40) or surface recycling of complement-bound virions captured within follicular dendritic cell (FDC) endosomes (41, 42). Intact virions may remain archived within FDC networks for months to years on ART (42, 43). Similarly, vaccination studies in non-human primate models have shown that long-lived germinal centers facilitate persistent development of nAb responses over nine months after the last immunogen dose (44). Thus, even in the absence of viremia, sufficient antigen may be present to facilitate ongoing maturation of humoral responses.

Longitudinal cohort studies suggest that this low level of HIV antigen exposure during ART-mediated viral suppression may drive functional humoral immune responses. HIV-specific binding (i.e. non-neutralizing) antibodies appear to wane over long-term ART (45), and some participants with acute ARTi experience complete seroreversion (46, 47). Neutralizing antibodies, in contrast, appear to persist for years (48) and may even increase in potency over time (49, 50). Two recent studies of acute and early treatment cohorts inform our understanding of antibody development after early ARTi. A study of participants with acute and early ARTi (stage I through V) from the Military HIV Research Program (MHRP) by Mitchell and colleagues describe the induction and persistence of HIV-specific binding antibodies with effector functionality following ARTi in acute HIV at stage III disease or later. Despite ongoing germinal center activity, however, these participants did not develop neutralizing antibodies during the first year of ART (17).

Esmaeilzadeh and colleagues describe twelve individuals with early ARTi, some of whom demonstrated broadening of anAb titers over years of ART against autologous virus sampled at ARTi. This study also suggested the potential for those anAbs to restrict some reservoir virus populations from rebound at analytical treatment interruption (ATI) (51). These reports suggest differences between anAb responses after acute vs. early ARTi, as well as the potential for ongoing HIV-specific humoral immunity on suppressive ART.

Cure strategies harnessing anAbs, such as therapeutic vaccination, may differ substantially whether the desired outcome is to boost, broaden, or foster de novo anAb responses. To better inform future cure strategies, we aimed to characterize the kinetics of longitudinal HIV-specific anAb development after acute and early ARTi, rooting our assessment to autologous plasma virus populations sequenced at the time of ARTi. Our results suggest a that "threshold" of viral replication prior to, or after, ARTi is required to induce nascent anAb responses that then continue to mature during long-term suppressive ART.

RESULTS

Study cohort. Twenty-three participants from the UCSF Treat Acute HIV cohort (18) diagnosed with acute (<60 days) or early (>60 days) HIV and initiating immediate ART were included in this study. Participant demographics are described in Table 1. Time to ARTi ranged from 13 to 128 days after estimated date of detectable HIV infection (EDDI) (52). Median followup was 42 weeks (range 12-274 weeks) from date of ART initiation. Plasma from 24 weeks post-ARTi was available from all but one participant. All participants were cisgender men of age 21 to 45 years (median = 28), the majority of whom (20/23; 87%) identified as men who have sex with men (MSM). The cohort was racially diverse, with participants self-identifying as Latino

(7/23; 30.4%), White (5/23; 21.7%), Asian (4/23; 17.4%), Black (3/23; 13.0%), Pacific islander (1/23; 4.3%), mixed White/Latino (2/23; 8.7%), and mixed Latino/Native American (1/23; 4.3%). All participants provided written informed consent, and the institutional review boards of UCSF and the University of Pennsylvania approved the research.

Time to ARTi determination and participant stratification. Time to ARTi was determined by EDDI algorithm (https://tools.incidence-estimation.org/idt/), which uses clinical testing results to estimate a date at which a viral load of 1 copy/mL would theoretically be detectable (52). Thus by 'time to ARTi' we are referring to estimated time between initial productive viral replication and ARTi, and not time between virus acquisition and ART initiation as this would include the length of an unknown eclipse period ranging 1-7 days (53). Participants were stratified by pre-test probability of possessing existing anAb responses at ART initiation. Those initiating ART within 60 days of EDDI were considered 'Acute ART initiators' (AAi, N=15) and participants initiating ART between 60-128 days were considered 'Early ART initiators' (EAi, N=8). This differentiates participants with fewer than 2 months of viremia prior to ARTi in whom baseline anAbs are likely absent (AAi) and those with 2-4 months of viremia in whom baseline anAbs may be present (EAi).

Viral load kinetics. After ARTi, plasma virus undergoes multiphasic decay resulting from graded attrition of infected cells with variable half-lives (38, 39). ARTi at the earliest stages of disease is associated with longer times to virus suppression under clinical assay detection limits, possibly due to greater magnitude of cell infection during peak viremia of acute HIV (54). In our cohort, viral loads at ARTi were significantly higher in the AAi group (4,142 to >10,000,000 copies/mL, median of 2,026,349 copies/mL), compared to the EAi group (9,525 to 297,362

copies/mL, median of 66,503 copies/mL) ($p = 0.02$, two-tailed Mann-Whitney). Accordingly, first undetectable viral load (<40 copies/mL) was observed between 2 and 50 weeks after ARTi (median of 5 weeks) and trended later in AAi (median = 8 weeks) than in EAi (median = 4 weeks) ($p = 0.08$, two-tailed Mann-Whitney) (Table 1, Figure 1).

Persistent or intermittent low levels of detectable viremia after ARTi occur via several mechanisms, including incomplete drug adherence enabling ongoing viral replication, full suppression of viral replication with continued virus production from longer-lived infected cells, and stochastic expression of provirus from activated reservoir cells (40). Whether this low-level viremia on ART can contribute to anAb development is unknown, but we note several participants with detectable viremia after 24 weeks of ART. Participant 8043 initiated ART during acute HIV, and subsequently experienced a prolonged time to viral suppression. Following rapid reduction in viremia from >10,000,000 to 422 copies/mL in the first 4 weeks on ART, participant 8043 then maintained low-level viremia through 50 weeks of ART (Figure S1). After achieving virus suppression on ART, a total of seven participants demonstrated a subsequent detectable viral load (>40 copies/mL by commercial assay, Table 1, Figure S1). Three of these participants (8016, 8028, and 8043) initiated ART during acute disease, achieved suppression, then had an episode of measured viremia >1,000 copies/mL (Figure 1B, red). No planned interruptions in therapy were guided by the participants' clinicians, but participant report and drug level testing confirmed ART nonadherence during these rebound viremic episodes (data not shown). Virus rebound after analytical treatment interruption (ATI) has been shown to boost anAb responses and elicit anAbs in those without detectable titers (34, 55), thus we considered these three participants as a distinct group from those in whom AAi was followed by continuous virus suppression.

Gp120 binding antibodies. The earliest HIV Env-specific antibody responses are non-neutralizing. Cross-reactive responses to gp41 arise in the first 2 weeks of viremia and de novo gp120-specific binding responses are detectable within 4 weeks (30, 56-58). As they represent the earliest de novo Env-specific antibody responses, we measured gp120-specific plasma binding antibodies at ARTi in all participants. Binding Ab were present at ARTi in 16 of 23 (70%) participants, and magnitude correlated with time to ARTi (Spearman r = 0.81, p <0.0001, Figure 2A). Participants with negative 3rd generation clinical ELISA testing (EDDI of < 20 days) had low or no detectable baseline gp120 binding responses. In participants initiating ART between 20 to 90 days after EDDI, baseline binding Ab levels increased with longer pre-ART viremic period, until reaching a plateau in participants with viremia for 90 days or more prior to ARTi.

After ARTi, binding antibody levels did not rise significantly in either AAi or EAi participants who maintained virus suppression (Figure 2B), but responses were more dynamic in the initial weeks of HIV infection. As shown in Figure 2C, binding antibodies rose over the first 12 weeks on ART in most participants with AAi (9/15, 60%) and none with EAi (0/8, 0%) (p=0.015, two-tailed Fisher Exact test, Figure 2C). Change in binding antibodies correlated positively with markers of acute infection, including prolonged time to first undetectable viral load (Spearman r = 0.424, p <0.5, Figure S2A) and higher viral load at ARTi (Spearman r = 0.6475, p<0.001, Figure S2B). After the initial weeks of ART, binding antibodies stabilized or decreased in 22/23 (96%) participants in the ensuing 12-24 weeks (Figure 2C). Only participant 8048, who experienced new low-level detectable viremia (528 copies/mL) at week 20, showed increased binding Ab during this period. In AAi, only those participants with subsequent rebound showed a significant increase in binding Abs between ARTi and the final timepoint (Figure 2B). Together,

data suggest binding antibody development is determined by viremia during the first 8-12 weeks of infection, and continued viremia following AAi continues to drive these responses.

Tier 1 neutralizing antibody responses. Tier 1 HIV viruses, such as MN and SF162, are laboratory-adapted strains that possess open, non-native Env conformations rendering them highly sensitive to neutralization by a broad range of antibodies (59, 60). Antibodies that neutralize Tier 1 viruses can be elicited by monomeric gp120 protein but are not protective against HIV virus in vivo (61, 62). Distinct from binding Ab and anAbs, emergence of Tier 1 neutralizing antibodies (T1nAb) may represent an intermediate stage in the progression of these Env-specific humoral responses, indicating de novo responses to immunogenic epitopes of the Env trimer that are well-shielded in the 'functional' trimeric Env of primary virus isolates. We assessed for plasma T1nAbs at ARTi and longitudinal timepoints.

At ARTi, T1nAb were absent in AAi and present only in later EAi (>90 days), correlating positively with time to ARTi (MN.3 Spearman $r = -0.76$, $p < 0.0001$; SF162 Spearman $r = -0.67$, $p = 0.0005$, Figure 2D, F). In contrast to binding Ab, T1nAb did not subsequently increase on suppressive ART in AAi. In the three participants with AAi and rebound, however, T1nAb did develop (Figure 2E, G). T1nAb were detectable in the majority of EAi participants (6/8, 75%) at ARTi and did not change significantly over ART (Figure 2E, G). Like the AAi participants, the two EAi participants with absent T1nAb at ARTi, 8022 and 8012, did not later develop T1nAb on ART.

Sequence characterization of early virus lineages. To assess the multiplicity of infection and extent of virus evolution that had occurred prior to ARTi, we characterized plasma virus at the

time of HIV diagnosis and ARTi. Single genome sequencing (SGS)-derived gp160 Env from plasma virus at ARTi in all 23 participants (n = 425 total sequences, median 18 per participant) are displayed in maximum-likelihood phylogenies (Figure S3-6; representative examples in Figure 3A). Of the sequences generated, 40/425 (9.4%) contained nonsense or frameshift mutations, which were included in nucleotide phylogenies but discarded from amino acid phylogenies. Using visual inspection and a validated model of random virus evolution (63), wherein sequences conform to star-like phylogeny (SLP) in the absence of either multivariant transmission or adaptation to selective pressure, we enumerated the multiplicity of infection.

Among the AAi participants, eight of the fifteen participants' sequences demonstrated a single, low-diversity lineage that conformed to SLP or near-SLP, indicating productive infection with a single virus (Figure S3). Molecular clock estimates from these sequences predicting the time since infection aligned closely with clinical estimates (Figure 3B), though these estimates may be less accurate in populations not conforming to SLP (63). The other seven AAi participants' sequences did not conform to SLP but demonstrated two or more distinct low-diversity lineages suggesting acquisition of multiple related but genetically distinct TF viruses, heretofore referred to as multivariant transmission (MVT) (Figure S4). Within these AAi MVT participants, each distinct lineage conformed to SLP or near-SLP and matched clinical timing estimates despite far greater diversity within the overall sequence alignment (Figure 3B). Lineages exhibiting SLP in these AAi participants allowed for inference of the transmitted/founder (TF) sequence as the common ancestor of that population. Inferred TF viruses for 14/15 AAi participants were cloned to be tested phenotypically (Figure S3, S4). In MVT participants, the TF representing the inferred dominant clade (representing <50% of sequences) was cloned. We additionally attempted to clone

minor clade TF viruses, but some Envs lacked infectivity in functional assays thus only dominant clade virus was tested for participants 8061, 8017, and 8009 (Figure S4).

In the EAi participants, sequences demonstrated greater diversity and defied SLP, suggesting virus adaptation to immune pressure prior to ARTi (Figure 3C). While cytotoxic T lymphocytes can exert potent pressure on viral populations in acute infection, anAbs function as the primary selective pressure at the Env locus (32, 64, 65). Five of the eight EAi participants had relatively low-diversity sequence populations, consistent with single virus transmission and early virus adaptation (e.g., Figure 3A, participant 8048). Given sequence evidence of virus adaptation, the TF virus could not be inferred, but one or more representative 'Early' Envs, suspected to be immune escape variants, were cloned (Figure S5). Three EAi participants demonstrated high levels of sequence diversity suggesting MVT with subsequent diversification via selection and recombination (e.g., Fig 3A, participant 8014). In these participants, Envs representing different regions of the phylogeny were cloned for testing (Figure S6). In total, twenty-nine infectious pseudoviruses were generated. Across the entire cohort, MVT was identified in ten of the twenty-three participants (43%).

Autologous neutralizing antibody responses. Using the inferred TF or Early (likely anAb escape) variants, we measured anAb responses in twenty-two participants (Figure 4). At the time of ARTi, we expected no plasma neutralization of contemporaneous Envs, because either 1) anAbs had not yet developed, or 2) contemporaneous virus will have escaped from nascent anAbs. As expected, week 0 plasma IgG did not neutralize autologous virus in any participant (Figure 4A). After ARTi, AAi participants with continuous ART suppression (n=11) failed to develop anAbs at any longitudinal timepoint when followed from 12 to 274 weeks (Figure 4A).

277 In contrast, anAbs did develop in two of three (66%) AAi participants with rebound viremic
278 episode after ARTi (8028 and 8043). In these two participants, anAbs were detected either at
279 (8043) or after (8028) a rebound viremic timepoint (Figure 4B), though it is unclear from clinical
280 data the duration of viremia prior to sampling.
281 AnAbs developed in seven of eight (88%) EAi participants after ARTi. AnAbs were first
282 detected at week 12 in four participants (8012, 8035, 8048, 8068), week 24 in 2 participants (8014,
283 8038), and week 42 in 1 participant (8030) (Figure 4C). Notably, potency continued to rise over
284 longitudinal timepoints in four of the seven who developed anAbs, indicating continued evolution
285 of the anAb response after many months or years of ART suppression. In the other three
286 participants, anAbs were detected at final timepoint and plasma from later timepoints was not
287 available.
288
289 ***Rebound virus populations in AAi participants.*** In the three participants with AAi and
290 subsequent rebound, plasma virus Env sequences from rebound viremic timepoint were sequenced
291 (n=28, median 11 per participant) and are displayed with acute plasma virus (Figure 5). Due to
292 intermittent sampling, we do not know the exact timing, duration or magnitude of rebound viremia
293 apart from sampling timepoints.
294 Rebound was detected in participant 8016 at week 103, after more than a year of virus
295 suppression. Rebound virus was largely identical to one of the two TF lineages identified during
296 acute infection (Figure 5), demonstrating lack of virus evolution from acute viremia. Binding Ab
297 and T1nAb to MN but not SF162 transiently increased prior to detection of rebound but did not
298 continue to rise thereafter (Figure S7A). Plasma antibodies from prior, during, and after rebound
299 failed neutralize either rebound or TF lineages (Figure 5, S9). This lack of anAb development,

300 combined with absence of sequence evolution in rebound plasma virus, suggests a limited duration
301 of rebound viremia.

302 Participant 8028 had detectable rebound at week 15, shortly after their first undetectable
303 viral load post-ARTi, and then developed low-level anAbs at week 24 (Figure 4). Rebound
304 sequences were identical or nearly identical to the single TF virus lineage (Figure 5). No
305 neutralizing activity was detected in rebound timepoint plasma IgG, though binding Ab and T1nAb
306 were increased from prior measures (Figure S7A). The minimal diversity contained in the rebound
307 Envs included 1-2 amino acid substitutions, including two shared mutational motifs (sites: K178N
308 and alteration of potential N-linked glycosylation site (PNGS) at position 88: V89I, N88K). Envs
309 containing the K178N mutation were similarly neutralized by week 24 plasma, while the loss of
310 the PNGS at position 88 conferred a modest increase in resistance to plasma anAbs (IC50: 926
311 ug/mL vs 598 ug/mL), suggesting possible early escape (Figure 5).

312 Participant 8043 demonstrated virus suppression at week 50 after a prolonged period of
313 low-level viremia during the first 24 weeks of ART. When next sampled at week 102, anAb
314 responses neutralizing the largest of 3 TF lineages had developed (Figure 4). Rebound virus
315 aligned with minimal diversification to this dominant lineage, as well as with two other lineages
316 reflecting recombinants or previously unsampled lineages (Figure 5). The dominant clade
317 represented a smaller proportion of the rebound population (six of twelve sequences, 50%)
318 compared to the pre-ARTi population (fourteen of nineteen sequences, 74%). Within the dominant
319 clade, two sequences shared an I294V substitution that did not confer resistance to anAbs when
320 tested in vitro (IC50 = 155 ug/mL). Both minor lineages (sampled only pre-ARTi) and the
321 recombinant lineage (sampled only at rebound) were resistant to anAbs (IC50 >1,000 ug/mL).
322 Three mutations in the gp120 region of this recombinant lineage differed from dominant clade TF

323 virus: N362K in C3 region, S411N in V4, and I491V in C5. To our knowledge these mutations
324 have not previously been described in early anAb or bnAb escape. Of the three, we note that
325 N362K leads to PNGS loss at this site adjacent to the CD4 binding site (CD4bs) and thus may
326 affect CD4 binding kinetics or sensitivity to neutralization by antibodies targeting CD4bs and
327 CD4-induced epitopes (66-69). The shift in lineage frequencies suggests anAbs may elicit
328 pressure, but persistence of the sensitive lineage within the circulating plasma population indicates
329 that anAbs of this titer are insufficiently potent to block replication completely.
330
331 ***AnAb responses in EAi participants with multivariant transmission***. Three participants
332 with EAi (8012, 8014, and 8035) and one with AAi and subsequent rebound (8043) had MVT,
333 affording the opportunity to assess the specificities and kinetics of anAb responses against distinct
334 viral lineages over time (Figure 6). Notably, as the frequency of recombination events was high,
335 determination of a dominant (in general, >50% sequences) and minor (<50% of sequences) clade
336 was imprecise.
337 Sequences from participant 8014 suggest at least two clades (represented by C7.2 and D9)
338 diverging by 92 AA (10.2%) with multiple recombinant lineages. Despite substantial
339 recombination, C7.2 had a larger frequency of related sequences compared with D9. In samples
340 available through 24 weeks of ART, plasma IgG neutralized only the dominant clade. This mirrors
341 the anAb responses that developed after rebound in participant 8043, which neutralized dominant
342 clade but not minor clades or recombinant variants (Figure 6).
343 Participant 8012's virus population was more closely related, with two clades (C8 and M2)
344 diverging by 23 amino acids (2.7%). The M2 clade was nominally more prevalent at ARTi (20/39
345 sequences, 51%). Neutralization of M2, but not C8, was first detected in week 12 plasma IgG and

346 remained the only targeted lineage through 48 weeks. By week 80, however, plasma IgG
347 neutralized both variants with comparable potency. Despite ongoing virus suppression, responses
348 against both lineages generally increased throughout 178 weeks of monitoring (Figure 6).
349 Participant 8035's virus likely represented two lineages with recombination and ongoing
350 diversification, with a dominant lineage represented by sequence G6 (12/15, 80%) and a minor
351 lineage D3 that differed by 28 AA (3.2%) primarily in V4-5. At 12 and 24 weeks of ART, plasma
352 neutralized only D3. More potent responses equally targeting both lineages arose by week 42
353 (Figure 6).
354 Across the participants with MVT who developed anAbs, neutralizing responses on ART
355 increased in potency (rising over sampled timepoints through 24 to 178 weeks) and breadth
356 (targeting the one lineage first, then expanding to recognize the other). As each individual had an
357 initial response against the TF that was present at ARTi (as indicated by escape variants in
358 sequences at ARTi), this indicates that if anAb responses are initiated during viremia prior to ART,
359 they continue to evolve in both potency and breadth over months to years of suppressive ART.
360
361 ***Heterologous neutralizing antibody responses.*** In untreated HIV infection, the earliest
362 anAb responses are strain-specific for the individual's TF virus. Having observed that anAbs
363 develop autologous breadth after early ARTi, we then assessed for heterologous neutralizing
364 activity in the seven EAi participants with detectable anAbs (8012, 8014, 8030, 8035, 8038, 8048,
365 and 8068). Plasma IgG from the timepoint with the highest autologous neutralization potency was
366 used in the TZM.bl assay against a heterologous panel of tier 2 viruses including four viruses from
367 a standardized 'global' panel (TRO.11, X1632, X2278, CE1176) (70), the clade B TF virus WITO

(71), and BG505, an key Env in vaccination strategies. No heterologous neutralization was detected in any participant plasma IgG against any tested virus.

DISCUSSION

Neutralizing antibodies are the primary immune pressure driving virus escape at the HIV Env locus (65) and are increasingly recognized as a potential mechanism of virus control both in unique hosts who naturally suppress viremia (55, 72) and in the context of passive bnAb administration (73-78). Similarly, anAbs suppress reactivation of a subset of reservoir viruses in some individuals with chronic ART initiation (6, 79) and impact rebound virus populations after ART interruption in some individuals with early ART initiation (51). In the modern era of HIV cure, combination immunologic interventions will be necessary to successfully control reactivating reservoir viruses (80); neutralizing antibodies may be an integral part of these strategies. Determining the natural history of the anAb response and the extent to which initial responses mature during ART may inform future efforts to augment these responses. Here, we characterized HIV-specific antibody responses in a well-characterized clinical cohort of PWH initiating immediate ART after diagnosis of acute and early HIV-1.

Our first finding was that participants initiating ART in acute HIV (<60 days; AAi) did not subsequently develop anAb responses during suppressive ART. No AAi participants showed sequence evidence of virus escape from anAbs, nor detectable plasma neutralization, at the time of ARTi (Figures 3, 4, S3-4). This allowed for inference of TF virus(es) and indicated that initial anAb responses had yet to develop. In eleven participants who then maintained virus suppression on ART, no plasma neutralizing activity developed over many months of follow-up (Figure 4A). Tier 1 neutralizing responses were likewise absent at and after ARTi (Figure 2E, G). Binding Ab

responses, in comparison, were driven by viremia over the first 1-3 months, and continued to rise on ART over the next 12 weeks (Fig 2C). Together, results show that antigen exposure following AAi drives limited evolution of humoral immunity, but does not elicit detectable de novo neutralizing responses against trimeric Env even in individuals with lengthy periods of viremia after ARTi.

In contrast to AAi, participants who initiated ART during early HIV (60-128 days; EAi) developed nAb responses prior to ARTi that continued to expand in breadth and potency over time on suppressive ART. At ARTi, plasma antibodies did not neutralize contemporaneous virus, but most did neutralize Tier 1 viruses (Figure 2D, F) and viral sequence diversity reflected selective pressure suggesting escape from the initial anAb response (Figure 3B). Over time on ART, plasma antibodies evolved to neutralize these early escape variants in seven of eight individuals, and further evolved to neutralize divergent variants in two of three participants with MVT (Figure 6). In parallel, anAb responses increased in potency over the duration of the follow up period in four participants with available longitudinal samples, including in one participant followed for several years (8012, 8035, Figure 4C). Tier 2 heterologous neutralization as assessed against a panel of six viruses, however, did not develop in any participant.

Continued maturation of the anAb response on suppressive ART after early, but not acute, ARTi suggests a "threshold" of systemic virus replication prior to ARTi required to sufficiently induce B-cell recognition of trimeric Env epitopes associated with neutralization of TF virus(es). If this threshold is not reached then anAbs do not develop, even with several additional months of detectable viremia on ART. If this threshold is surpassed, anAbs recognizing the TF virus develop and continue to mature on ART, accruing increased breadth and potency over long periods of time without detectable viremia. Thus, substantial systemic virus replication is necessary to initiate the

414 anAb response, but not to mature pre-existing responses. Ongoing HIV replication during
415 suppressive ART has been debated, with evidence suggesting it is likely negligible (81-83). HIV
416 antigen, however, is available to B cells through stochastic expression of infected reservoir cell
417 provirus and within lymphoid tissue via captured virus in FDC endosomal networks. Our results
418 suggest that this continued antigen presentation during ART preferentially engages with memory
419 B cell populations to mature existing responses rather than with naïve B cells to generate de novo
420 responses, though we did not directly assess B cell biology in this study. Further elucidation of the
421 interactions between distinct B cell subsets and different modes of antigen presentation on ART
422 that drive continued anAb responses warrants study.

423 The observation of an apparent "threshold effect," before which anAb responses do not
424 develop and after which they arise and continue to evolve on suppressive ART, was unexpected
425 as the immunologic consequences of earlier ART initiation would seemingly favor anAb
426 development. Initial Env-specific responses skew towards gp41 due to cross-reactive memory B
427 cells targeting gut microbial antigens (57, 58). Replicating virus produces soluble monomeric
428 gp120 and membrane-bound gp120-gp41 monomers, while trimeric Env is sparse, leading to
429 serodominance of 'binding' responses against gp120 that target non-neutralizing epitopes
430 inaccessible on functional trimers (56). These binding antibodies may paradoxically contribute to
431 depletion of uninfected 'bystander' CD4 T cells (84). Naïve B cells recognizing neutralizing
432 epitopes require antigen presentation on FDC networks and T-cell help from T follicular helper
433 (TFH) cells to class-switch and affinity mature (85), which may be greatly impaired by depletion
434 of TFH subsets over longer periods of untreated HIV. Furthermore, the intensely inflammatory
435 milieu resulting from active viral replication interferes with optimal B-cell maturation, driving
436 expansion of short-lived, activated, exhausted memory B-cell populations (37, 86, 87). In the

context of these known and potentially other yet undiscovered mechanisms by which HIV replication impairs effective humoral responses, it is surprising that acute ARTi does not support anAb development, and thus the mechanisms of the 'threshold effect', also seen in other acute ARTi cohorts (17), merits further investigation.

While no AAi participants developed anAb responses on suppressive ART, it is notable that two of three participants with documented rebound viremia produced anAbs at the time or shortly after viremia was detected. In participant 8043, neutralization-resistant variants comprised a larger percentage of the plasma virus population at the timepoint anAbs were detected relative to ARTi, suggesting immune selection imposed by anAb activity. The exact timing of recurrent viremia and nascent anAb emergence were not clear, but the available data suggest rapid induction of anAbs may be possible even many months or years after AAi. Thus, while on-ART viremia of AAi may be insufficient to induce anAb responses, B cells are primed for rapid maturation following a sufficient antigen "boost" such as post-rebound virus replication or potentially, therapeutic vaccination.

To date, therapeutic HIV vaccination strategies have largely aimed to elicit T-cell responses due to the established correlates of CTL activity with spontaneous virus control; only recently has testing of B cell lineage design immunogens been proposed in PWH. In contrast, preventive vaccine efforts have long aimed to elicit broadly reactive nAbs, a formidable challenge. Current approaches to cultivate bnAbs involve targeting rare B cell lineages, then guiding stepwise B cell maturation through a series improbable mutations to train and polish these responses (88). Our findings demonstrate that the period of viremia preceding acute and early ARTi provides a potent and enduring antigen 'prime,' which may be a reasonable target for continued stimulation via vaccination. The EAi MVT participants described here further

460 demonstrate a natural broadening of anAb responses after early ARTi, which may represent
461 development of new B cell lineages or continued evolution of an initial B cell lineage. Thus,
462 therapeutic vaccination with ‘boosting' immunogens represents a distinct and potentially feasible
463 approach to mature and broaden pre-existing anAb responses.

464 Reservoir diversity following chronic ART initiation is vast and the full complement of
465 potential rebound virus populations cannot be reliably predicted by reservoir or pre-ART plasma
466 virus sequencing (89). Acute and early ARTi, however, substantially restricts reservoir size and
467 diversity. Plasma sampling at time of early ARTi allows for more robust prediction of reservoir
468 variants thus assessment of reservoir sensitivity to antibody responses (78, 90). Given the
469 advances in HIV vaccinology, including mRNA-LNP technologies and prefusion-stabilized
470 trimer immunogens that have successfully elicited autologous tier-2 neutralizing antibody
471 responses in HIV-naïve individuals (91), therapeutic vaccination to enhance baseline anAb
472 responses after acute/early ARTi represents an intriguing and potentially feasible approach that
473 merits consideration.

474 This longitudinal cohort-based study has relevant strengths and weaknesses. The study is
475 rooted in validated sequencing methods (SGS)(92, 93) and modeling (star-like phylogeny) (63)
476 that allow inference of the TF virus in AAi and escape of early anAbs in EAi. Limitations include
477 a relatively homogeneous study population of all male, mostly MSM from a single US city, and
478 inter-individual variability in adherence to study visits and ART. However, given the frequency of
479 our study visits (monthly, as well as an additional week 2 visit) and the close relationship with the
480 San Francisco Department of Public Health’s Getting to Zero program (94), aimed at identifying,
481 providing immediate ART, and closely following newly diagnosed individuals with HIV, our well-
482 characterized cohort was able to capture the majority of these data. Further studies in other

populations, including women from diverse regions, are needed to understand the generalizability of findings. Studying virus neutralization also has important caveats. In vitro assays for neutralization potency do not directly represent in vivo activity, but are validated correlates of relative neutralization activity that can be compared across studies and cohorts. Here, we assess neutralization potency on ART with extracted IgG, which is a frequently used, but imperfect correlate of the TZM.bl assays using the standardized plasma dilutions. As the field works to develop other methods, including ART-resistant backbones and distinct viral vectors (17, 51), these assays will become more comparable across studies.

In summary, we found that ARTi in acute HIV prevented subsequent formation of anAbs, unless the participant experienced additional antigen in the form of rebound viremia. ARTi in early HIV, in contrast, enabled development of anAbs that continued to expand in breadth and potency over time on suppressive ART. Given the relative lack of virus diversity in the HIV reservoir after acute and early ARTi, these primed or continuously maturing anAb responses represent an attractive target for therapeutic vaccination with the goal of increasing the breadth and potency of responses to elicit durable virus suppression.

METHODS

Sex as a biological variable. Our study included 23 participants, all of whom reported male sex and male gender. The study population was drawn from clinical cohort that is almost entirely male. This limits the generalizability of the results. Future work in women is needed to confirm findings.

Participants. Individuals with newly diagnosed acute/early HIV infection were consented and enrolled in the UCSF Treat Acute HIV study from December 1, 2015 to November 30, 2020.

Participants were provided immediate ART (tenofovir/emtricitabine + dolutegravir), linked to clinical care, and followed monthly for 24 weeks and then every ~3 months thereafter. For the current study, a total of twenty-three participants were included for whom 1) viremic plasma at ART initiation was available and of adequate quality for SGS, 2) sufficient archived clinical laboratory testing was available to estimate date of detectable HIV infection, and 3) two or more post- ARTi plasma sample timepoints were available. At each visit, detailed interviews included questions regarding current medications, medication adherence, intercurrent illnesses, and hospitalizations were performed. In addition, peripheral blood sampling at each visit was performed to measure plasma HIV RNA, CD4+ T cell count, and clinical labs, as well as blood for storage.

Sample Preparation, Viral Load Measurements and Time to ARTi (EDDI). Whole blood samples were collected in EDTA and ACD tubes and processed within 24 hours. PBMCs and plasma were separated using Ficoll-gradient purification and stored at -80°C. Viral loads were measured by commercially available clinical viral load assays (Abbott Real Time PCR assay, limit of detection < 40 copies/mL). Timing of ARTi was calculated using the Infection Dating Tool (https://tools.incidence-estimation.org/idt/), which included the estimated date of detectable HIV infection (EDDI), along with a "confidence interval" for early probable (EP-EDDI) and late probable (LP-EDDI) date based on each participant's prior clinical HIV test results, as well the baseline study results (52, 53).

Gp120 Binding Antibodies. Gp120 binding antibodies were assessed by indirect qualitative ELISA as previously described (6). In brief, 96 well plates were coated with 2ug/mL recombinant

YU2c gp120 protein (Immune Technology, cat#IT-001-0027p) overnight and incubated with 100uL of heat-inactivated participant plasma in fivefold dilutions (ranging 1/20-1/312,500) as primary antibody, then 100uL HRP-conjugated secondary antibody (Jackson ImmunoResearch, cat#109-035-098) at 1/10,000 dilution. Blocking and dilutions were performed in B3T blocking buffer (150 mM NaCl, 50mM Tris-HCl, 1 mM EDTA, 3.3% fetal bovine serum, 2% bovine albumin, 0.07% Tween 20). After antibody incubations, 100uL KPL Sureblue TMB peroxidase substrate (SeraCare) was added and incubated at room temperature for 10 minutes, after which 100uL 1N sulfuric acid was added and absorbance at 450nm was read (H4 synergy plate reader, Biotek). Two or more replicates were performed for each sample. HIV-negative donor plasma was analyzed in parallel as negative control. Absorbance values were plotted as a function of antibody concentration, and nonlinear curve fit by sigmoidal dose-response (variable slope) model with area under the curve (AUC) measurement was computed in Prism (GraphPad Prism version 10.0.2.). Assay background was calculated as three standard deviations above the mean AUC of HIV-negative donor plasma.

IgG Purification. Plasma was heat-inactivated at 56C for 1 hour. Then, IgG was extracted using the Protein G Gravitrap system (Cytiva) according to manufacturer protocol. Buffer exchange of purified IgGs was then performed by three PBS washes in Amicon Ultra-4 30k cutoff filters (Millipore). IgG was then sterile-filtered in 0.22uM centrifugal filter tubes (Corning Spin-X). IgG concentrations were quantified by absorbance throughput at 280nm on Synergy H4 plate reader (Biotek).

Single Genome Sequencing (SGS). Plasma virus from day of ARTi was characterized by SGS for all participants. Plasma virus at post-ARTi timepoints was additionally sequenced for participants with viremia after ARTi (participants 8028, 8043, and 8016). SGS was performed per previously described methods (73, 89). Briefly, plasma samples were thawed on ice, and viral RNA extraction was performed on a volume of plasma estimated to contain ~20,000 virions per clinical viral load measurement using EZ virus Mini Kit 2.0 (Qiagen) per manufacturer protocol. cDNA template was synthesized by Superscript III system (Life Technologies) using R3B3R antisense primer (5′-ACTACTTGAAGCACTCAAGGCAAGCTTTATTG-3′) (93), and Env region was amplified by nested PCR using high fidelity Platinum Taq DNA polymerase (Life Technologies) in 96 well plates by previously described conditions (73, 89). Input DNA was titrated to yield positive amplicons in <30% of reactions, reducing polymerase-induced errors (92, 93). Reactions containing ~3kb amplicons were sequenced as below.

Sequence Analysis and Identification of TF/ Early Virus. SGS amplicons were sequenced by MiSeq platform (Illumina). Sequence analysis was performed in Geneious Prime Software (v2023.2.1). Raw sequencing reads were aligned to HXB2 (GenBank accession K03455.1) or other clade B consensus sequence. Resultant contigs were inspected for adequate coverage and consensus sequences with large deletions or ambiguous bases at 75% identity were discarded. For participants with few sequences (8011, 8063, 8038), consensus sequences with ambiguous bases at 75% identity were re-analyzed at 50% identity. Sequences containing few (1 or 2) positions with ambiguous bases due to poor sequencing coverage (not ‘double peaks’), ambiguous bases were manually edited to agree with the consensus base at that position for that participant. Consensus sequences of full-length Env gp160 for were aligned by MUSCLE algorithm (95) for each

574 participant. Maximum-likelihood nucleotide trees were compiled by PhyML using Le Gascuel
575 substitution model, 100 bootstrap replicates, and optimized to topology, branch length, and
576 substitution rate.

577 Diversity and multivariant infection were assessed subjectively by visual inspection of
578 nucleotide phylogenetic tree and highlighter plots (Los Alamos National Labs online Highlighter
579 tool) (92), and objectively by fitting alignments to a model of random viral evolution models using
580 the Los Alamos National Labs online Poisson Fitter tool (63). Populations exhibiting SLP or near-
581 SLP by either method were presumed single TF transmission. For participants with plasma virus
582 populations not conforming to SLP, the presence of MVT was determined by inspection of
583 phylogenetic trees and highlighter plots for distinct clades with conserved nucleotide motifs.

584 Amino-acid translations of gp160 sequences were also aligned by MUSCLE algorithm,
585 and phylogenetic trees were assembled by same methods as for nucleotide trees using Geneious
586 Prime software. Sequences with frameshift or nonsense mutations leading to premature stop
587 codons were discarded from amino acid alignments.

588

589 ***TF/Early Virus (Env) Selection and Cloning.*** In participants whose plasma virus populations
590 conformed to SLP, as was the case for AAi participants with single variant transmission, TF virus
591 was inferred as the consensus of the nucleotide sequence alignment (92). In AAi participants with
592 multivariant transmission, we inferred TF viruses as the most recent common ancestors of each
593 individual clade. In EAi participants, all of whom defied SLP, TF could not be inferred and
594 therefore we identified 'Early' virus sequences which were representative of the virus population
595 present at ARTi. In selecting such Early viruses we inspected amino acid highlighters and chose
596 sequences in which the Env regions generally targeted by early anAb responses, namely gp120

variable regions (V1-5) and potential N-linked glycosylation sites, were representative of the individual's broader virus population. For EAi participants with MVT, we selected an 'Early' virus from each distinct clade.

Selected TF and Early viruses were molecularly cloned by amplification of SGS product and insertion into pcDNA 3.1 Zeo(+) expression vector (Invitrogen, Thermo Fisher Scientific) by Gibson assembly. For each participant, bespoke Gibson assembly primers were generated by NEBuilder online tool (New England Biolabs, https://nebuilder.neb.com). Some Env plasmids including Tier 1 viruses MN.3 (HM215430.1) and SF162 (EU123924.1) were synthesized, cloned, and sequenced confirmed by Twist biosciences (synthesized clonal genes were purchased for participants 8010, 8011, 8026, 8061, 8014).

Cloned plasmids were transformed into STBL2 competent cells (Thermofisher) and grown on antibiotic-containing Luria broth (LB) agar plates for 48h at room temperature. After incubation, single colonies were selected and grown overnight in liquid broth at 30C. Plasmid DNA was isolated using the QIAprep Spin Miniprep Columns (Qiagen). Correct insertion of desired Env insert was confirmed by sequencing (Miseq, Illumina).

Pseudovirus Generation and Titration of Infectivity. Pseudovirus was prepared as described previously (89) by co-transfection of env-containing plasmid with SG3ΔEnv plasmid (obtained through the NIH HIV Reagent Program, Division of AIDS, NIAID, NIH: Human Immunodeficiency Virus Type 1 (HIV-1) SG3ΔEnv Non-infectious Molecular Clone, ARP-11051, contributed by Dr. John C. Kappes and Dr. Xiaoyun Wu.) in HEK 293T cells (ATCC) by FuGENE™ 4K transfection system (Promega). Culture supernatants were harvested 48-72 hours after transfection. Cellular debris was removed through 0.4 micron filtration, and resultant viral

stocks were stored at -80C. Multiplicity of infection (MOI) of pseudovirus stocks was determined by TZM-bl beta-galactosidase assay as previously described(89).

Neutralization Assays. Virus neutralization by purified plasma IgG was assessed in TZM-bl assay as described previously (73, 89). In brief, 10,000 TZM-bl cells (obtained through the NIH HIV Reagent Program, Division of AIDS, NIAID, NIH: TZM-bl Cells, ARP-8129, contributed by Dr. John C. Kappes, Dr. Xiaoyun Wu and Tranzyme Inc.) per well in 96 well plates were incubated for 48 hours in presence of: purified participant plasma IgG in fivefold dilutions ranging 1,000-0.0128 ug/mL, 40ug/mL DEAE dextran, and 3,333 infectious units of autologous pseudovirus (MOI of 0.3). Assays were performed in duplicate with challenge of antibody against pseudotyped murine leukemia virus (MLV) in parallel as negative control. After 48-hour incubation, luciferase assay was performed using the Firefly Luciferase assay system (Promega) per manufacturer instructions. Luminescence in relative light units (RLUs) was quantified using H4 Synergy plate reader (BioTek). RLUs were plotted as a function of antibody concentration to generate dose-response curve (GraphPad Prism version 10.0.2). The antibody concentration that neutralized 50% (IC50) of pseudoviral infection was calculated by 4-parameter logistic regression fit. Tier-2 heterologous panel of pseudoviruses and infectious molecular clones was generously provided by the laboratory of George Shaw.

Statistical Analysis. Statistical analyses were performed in Prism (GraphPad Prism version 10.0.2) as outlined in the figure legends, and two-tailed, non-parametric tests were used for all analyses unless otherwise specified.

Study Approval. All participants provided written informed consent, and the institutional review boards of University of California, San Francisco and the University of Pennsylvania approved the research.

Data Availability. Sequence data was submitted to Genbank (Accession#: OR922877-OR923336). See 'supporting data values' file for numerical data underlying figures and means.

AUTHOR CONTRIBUTIONS

SAL, SD, RH, HH, VP, SS, and SGD managed Treat Acute HIV Cohort, collected clinical data, and coordinated sample collection and processing. GDW, JJ, FEM, RK, SM, HC, KP, JC and KJB performed laboratory experiments. GDW, RML, FEM, RK, and KJB analyzed data. GDW and KJB wrote the manuscript; all authors reviewed and edited the manuscript.

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FIGURE LEGENDS

Figure 1: Viral load kinetics

Plasma viral load measurements in copies/mL starting at day of ART initiation (Week 0) and longitudinally on ART for (**A**) Acute ART initiators without rebound (grey, N=12), (**B**) Acute ART initiators with rebound (red, N=3), and (**C**) Early ART initiators (blue, N=8) as measured by commercial clinical assays (limit of detection >40 copies/mL).

Figure 2: Binding and Tier 1 antibody responses

(**A**, **B**, **C**) Plasma gp120 binding antibody responses as measured by qualitative ELISA and presented as area under the curve measurement and (**D**, **E**, **F**, **G**) plasma IgG neutralization of clade B Tier 1 viruses MN.3 and SF162 measured by TZM.bl assay and presented as IC50 in ug/mL for 23 participants. For (**B**, **E**, **G**), AAi without rebound (N=12) are shown in grey, AAi with rebound (N=3) in red, and EAi (N=8) in blue. (**A**) Baseline gp120 binding and responses correlate with time to ART initiation (Spearman correlation), and (**B**) change in binding antibody responses between time of ART initiation (Week 0) and final timepoint (Final TP, range 12-276 weeks) (Wilcoxon matched pairs signed rank test). (**C**) changes in binding antibodies over weeks 0-12 weeks on ART (blue) compared to week 12-24 on ART (orange) for each participant. (**D**, **F**) Baseline Tier 1 responses correlate with time to ART initiation (Spearman correlation), and (**E**, **G**) change in Tier 1 responses on ART (Wilcoxon matched pairs signed rank test).

Figure 3: Viral populations at ART initiation

(**A**) Representative viral populations at ART initiation by SGS of gp160 Env presented as maximum-likelihood nucleotide phylogenetic trees for four participants. **8028** represents AAi

with single-virus transmission, **8043** represents AAi with multivariant transmission (MVT), **8048** represents EAi with single virus transmission, and **8012** represents EAi with MVT. (**B**) Time to ART initiation and 95% confidence interval as estimated by clinical testing (EDDI algorithm, black/grey), and viral population diversity (LANL Poisson-Fitter tool, Orange). For AAi participants with multivariant infection, diversity estimate was also performed within dominant clade only (Green). '*' denotes sequences conforming to star-like phylogeny (SLP).

Figure 4: Autologous neutralizing antibody (anAb) responses

AnAb responses of purified plasma IgG against pseudotyped autologous TF or Early virus measured by TZM.bl assay and presented as IC50 (ug/mL) for twenty-two participants. AAi without rebound (N=11) are shown in grey, AAi with rebound (N=3) in red, and EAi (N=8) in blue. (**A**) AnAb responses at ART initiation (Week 0) and longitudinal timepoints on ART. Statistics represent response rate for relative number of participants with detectable anAbs between groups at each timepoint (Fisher Exact Test. *, $P < 0.05$; **, $P < 0.05$, ***, $P < 0.001$). (**B**, **C**) AnAb responses over time on ART in individual (**B**) Acute participants with rebound and (**C**) Early participants.

Figure 5: Rebound virus populations in three AAi participants

Maximum-likelihood amino acid phylogenetic trees and highlighter plots for the three AAi with rebound participants (**8028**, **8016**, and **8043**). Black nodes represent sequences obtained from plasma at ART initiation, and red nodes represent sequences obtained at rebound timepoint plasma. Numeric values represent final timepoint plasma IgG neutralization IC50 (ug/mL) of selected pre-ART and rebound timepoint Envs by TZM.bl assay.

Figure 6: AnAb responses in EAi participants with multivariant transmission (MVT)

Maximum-likelihood amino acid phylogenetic trees and highlighter plots for the three EAi participants with MVT are presented on left. Pseudotyped dominant clade and minor clade early viruses are denoted in orange and green, respectively. Neutralization IC50 (ug/mL) by longitudinal plasma IgG of each clade is presented on right. Shaded area represents time during which anAbs against only one variant were detected.

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Table 1: Cohort Characteristics

Participant ID	Age	Sex	Race*	Time to ARTi (Days)	Viral Load† (at ARTi)	CD4 count at ARTi	Weeks to VL suppression	Final Timepoint (Weeks)	Viremia post-ARTi: Week post-ARTi	Viremia post-ARTi: Viral Load†
8061	25	M	A	13	400,924	702	8	45	20	43
8028	39	M	W	17	2,026,349	651	10	25	15	6,458
8017	25	M	B	18	2,142,874	246	5	78		
8016	33	M	A	20	247,583	346	8	154	103	23,891
8033	39	M	L	20	>10,000,000	350	8	24		
8031	21	M	L	20	2,235,106	385	4	24		
8010	25	M	L	20	>10,000,000	488	8	25	13	45
8026	44	M	L/NA	25	>10,000,000	366	17	50		
8083	25	M	W/L	27	280,129	687	13	69		
8032	28	M	L	28	65,966	740	3	42		
8011	43	M	L	29	73,272	505	17	274		
8063	35	M	W	33	>10,000,000	568	4	16		
8043	45	M	W	40	>10,000,000	149	50	102	102	3,108
8009	25	M	A	46	4,142	383	2	188		
8036	27	M	W	53	26,354	635	5	54		
8012	45	M	W	66	242,367	267	9	178	17	159
8014	20	M	B	78	9,525	359	2	24		
8022	35	M	B	86	96,222	469	4	24		
8038	45	M	PI	93	12,080	613	4	26		
8048	34	M	L	110	70,145	593	5	24	20	537
8035	29	M	A	118	297,362	271	8	42		
8030	23	M	W/L	121	10,529	276	2	42		
8068	26	M	L	128	66,503	446	4	9		
Median (All)	29			33	247,583	446	5	42		
Median (Acute)	28			25	2,026,349	488	8	50		
Median (Early)	32			101.5	66,503	403	4	25		

* Race Denominations: A = asian, B = black, L = latino, NA = native american, PI = pacific islander W = white; '/' indicates mixed race

† Viral load measurements in copies/mL

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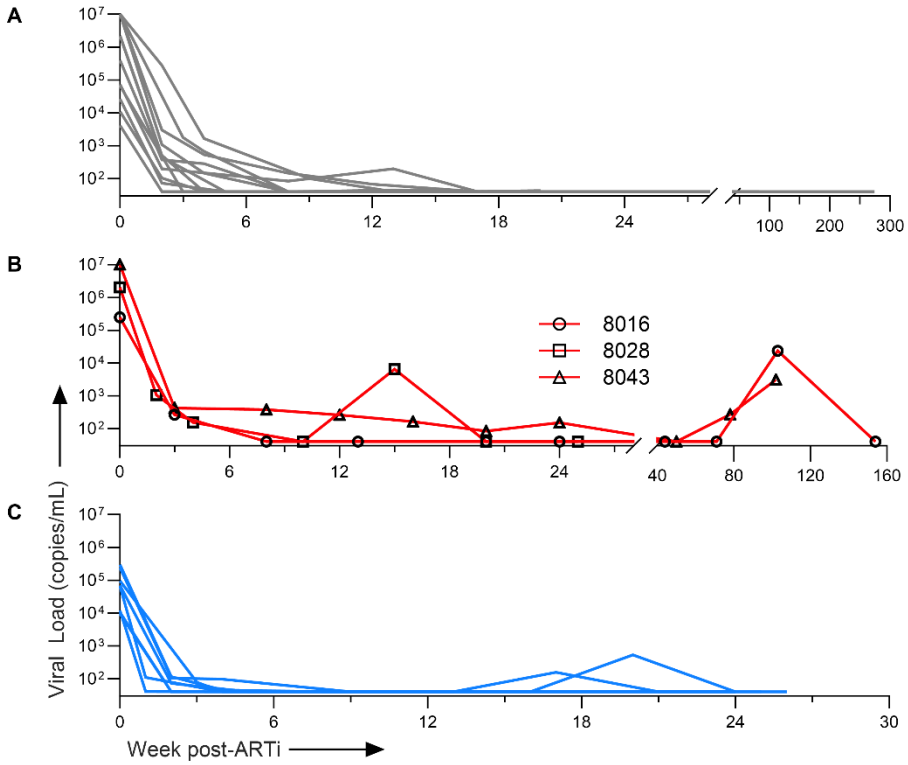
966 **Figure 1**



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968 **Figure 1: Viral load kinetics**
969 Plasma viral load measurements in copies/mL starting at day of ART initiation (Week 0) and
970 longitudinally on ART for (**A**) Acute ART initiators without rebound (grey, N=12), (**B**) Acute
971 ART initiators with rebound (red, N=3), and (**C**) Early ART initiators (blue, N=8) as measured
972 by commercial clinical assays (limit of detection >40 copies/mL).
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Figure 2

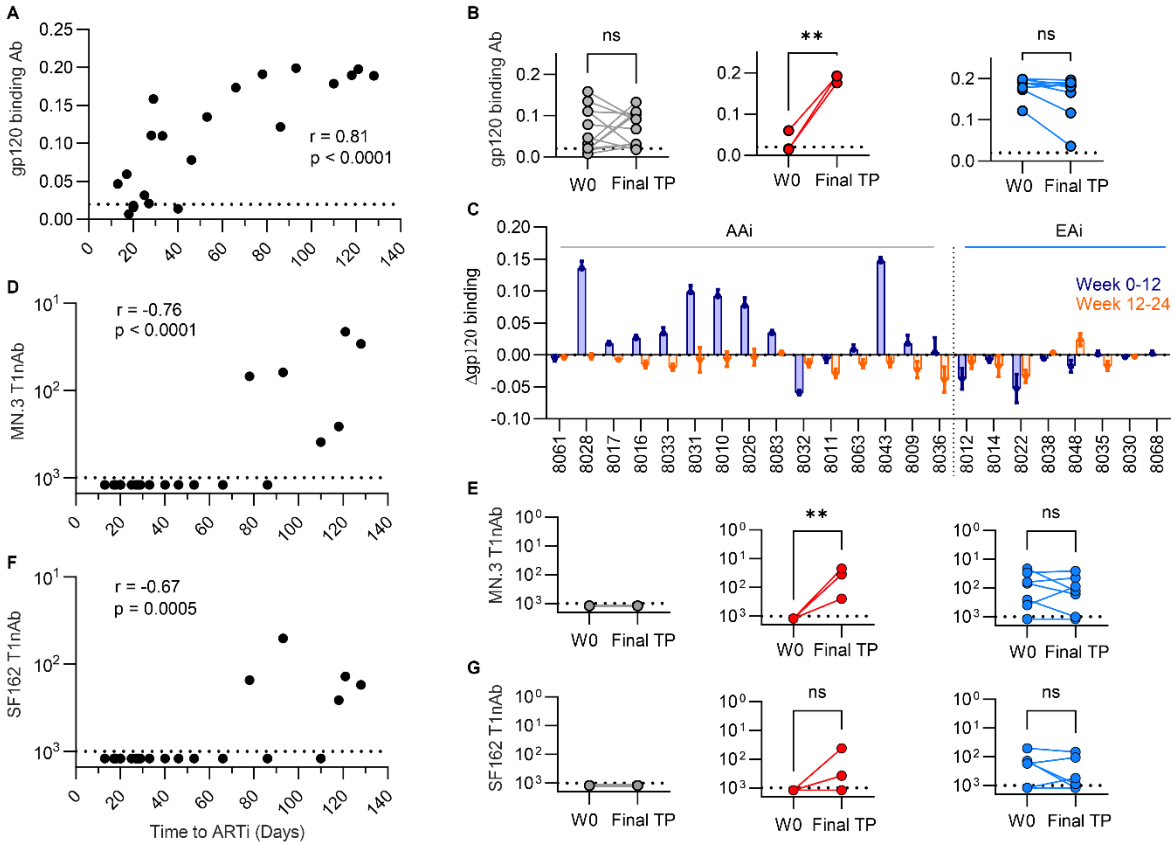

Figure 2: Binding and Tier 1 antibody responses

(**A**, **B**, **C**) Plasma gp120 binding antibody responses as measured by qualitative ELISA and presented as area under the curve measurement and (**D**, **E**, **F**, **G**) plasma IgG neutralization of clade B Tier 1 viruses MN.3 and SF162 measured by TZM.bl assay and presented as IC50 in ug/mL for 23 participants. For (**B**, **E**, **G**), AAi without rebound (N=12) are shown in grey, AAi with rebound (N=3) in red, and EAi (N=8) in blue. (**A**) Baseline gp120 binding and responses correlate with time to ART initiation (Spearman correlation), and (**B**) change in binding antibody responses between time of ART initiation (Week 0) and final timepoint (Final TP, range 12-276 weeks) (Wilcoxon matched pairs signed rank test). (**C**) changes in binding antibodies over weeks 0-12 weeks on ART (blue) compared to week 12-24 on ART (orange) for each participant. (**D**, **F**) Baseline Tier 1 responses correlate with time to ART initiation (Spearman correlation), and (**E**, **G**) change in Tier 1 responses on ART (Wilcoxon matched pairs signed rank test).

Figure 3

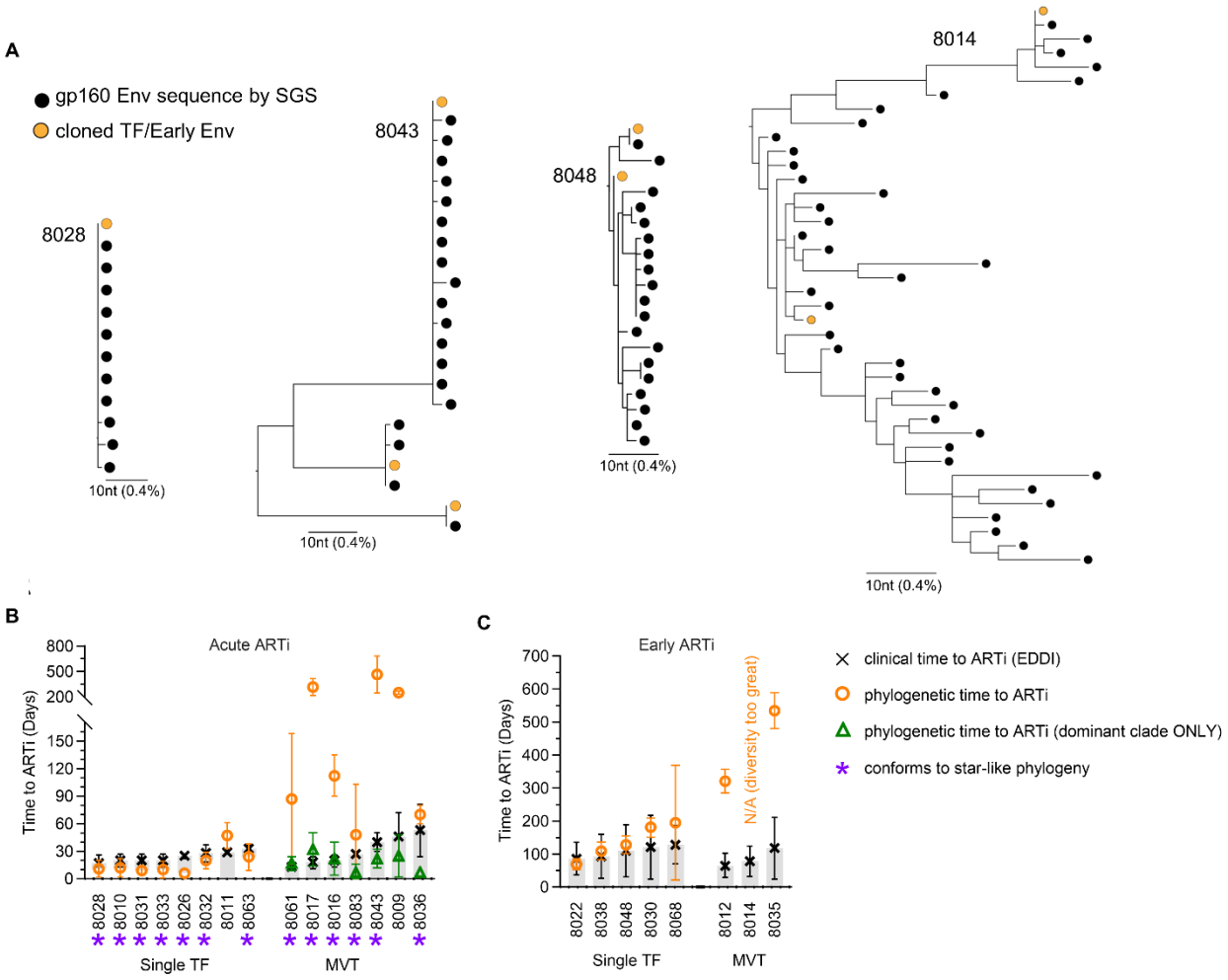

Figure 3: Viral populations at ART initiation

(**A**) Representative viral populations at ART initiation by SGS of gp160 Env presented as maximum-likelihood nucleotide phylogenetic trees for four participants. **8028** represents AAi with single-virus transmission, **8043** represents AAi with multivariant transmission (MVT), **8048** represents EAi with single virus transmission, and **8012** represents EAi with MVT. (**B**) Time to ART initiation and 95% confidence interval as estimated by clinical testing (EDDI algorithm, black/grey), and viral population diversity (LANL Poisson-Fitter tool, Orange). For AAi participants with multivariant infection, diversity estimate was also performed within dominant clade only (Green). '*' denotes participants conforming to star-like-phylogeny (SLP).

Figure 4

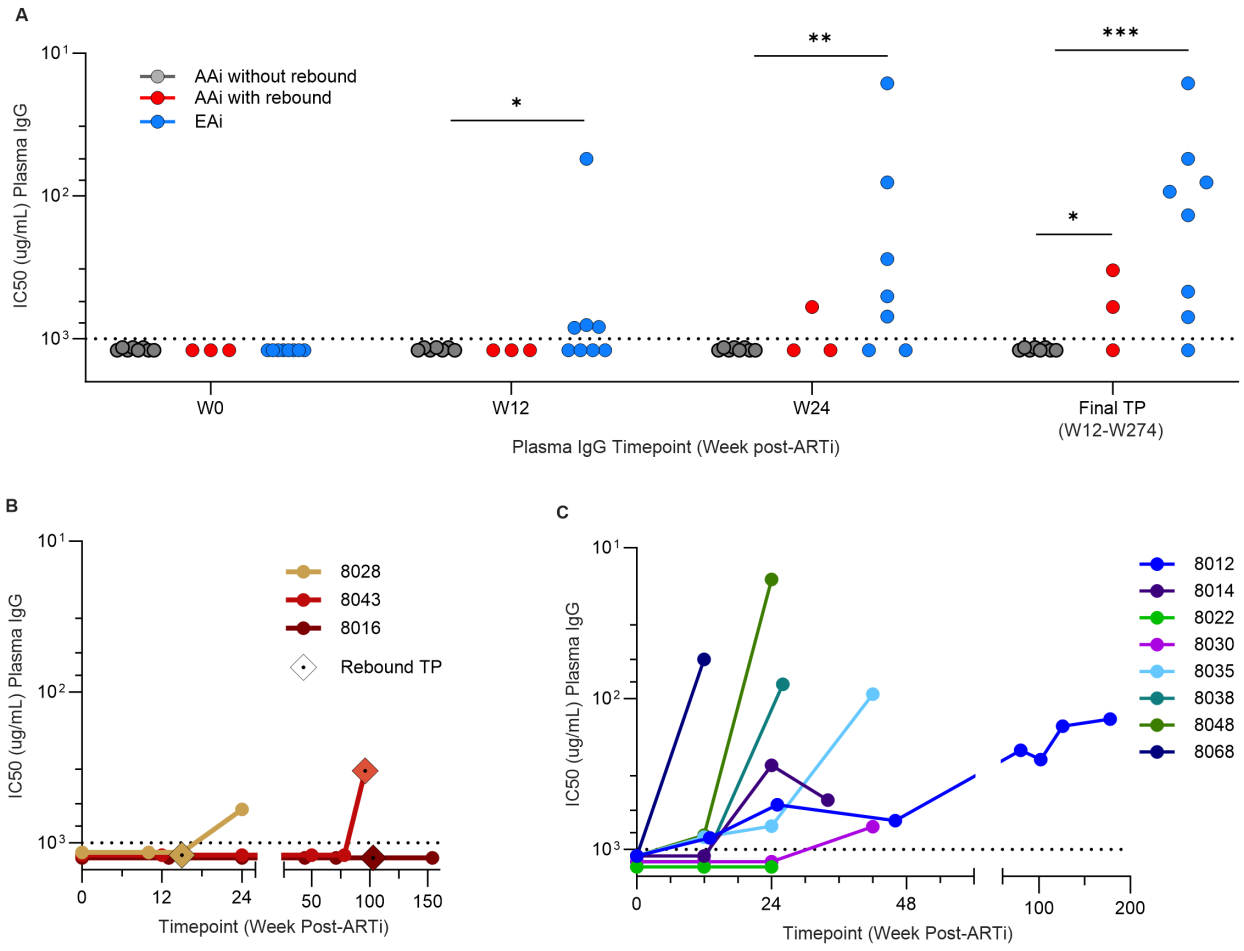

Figure 4: Autologous neutralizing antibody (anAb) responses
AnAb responses of purified plasma IgG against pseudotyped autologous TF or Early virus measured by TZM.bl assay and presented as IC50 (ug/mL) for 22 participants. AAi without rebound (N=11) are shown in grey, AAi with rebound (N=3) in red, and EAi (N=8) in blue. (**A**) AnAb responses at ART initiation (Week 0) and longitudinal timepoints on ART. Statistics represent response rate for relative number of participants with detectable anAbs between groups at each timepoint (Fisher Exact Test. *, $P < 0.05$; **, $P < 0.05$, ***, $P < 0.001$). (**B**, **C**) AnAb responses over time on ART in individual (**B**) Acute participants with rebound and (**C**) Early participants.

Figure 5

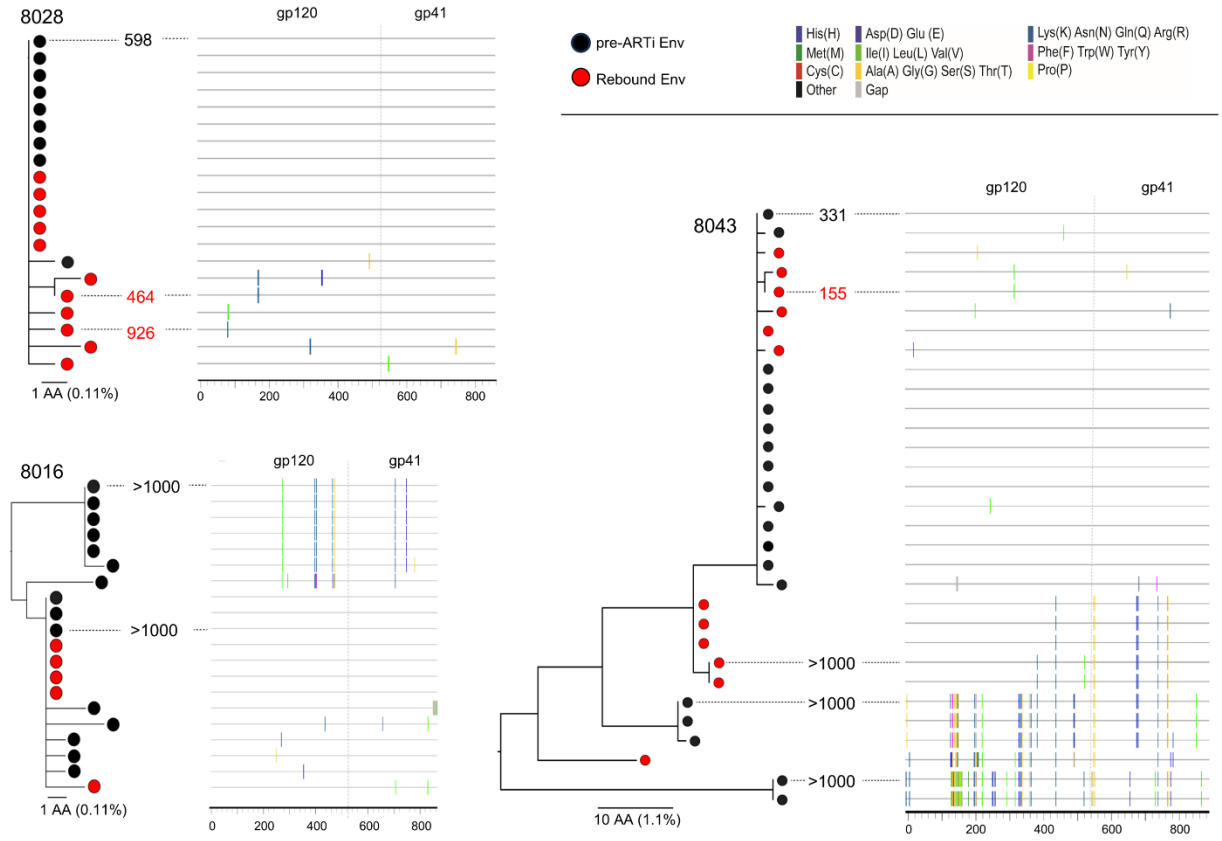

Figure 5: Rebound virus populations in three AAi participants

Maximum-likelihood amino acid phylogenetic trees and highlighter plots for the three AAi with rebound participants (**8028**, **8016**, and **8043**). Black nodes represent sequences obtained from plasma at ART initiation, and red nodes represent sequences obtained at rebound timepoint plasma. Numeric values represent final timepoint plasma IgG neutralization IC50 (ug/mL) of selected pre-ART and rebound timepoint Envs by TZM.bl assay.

Figure 6

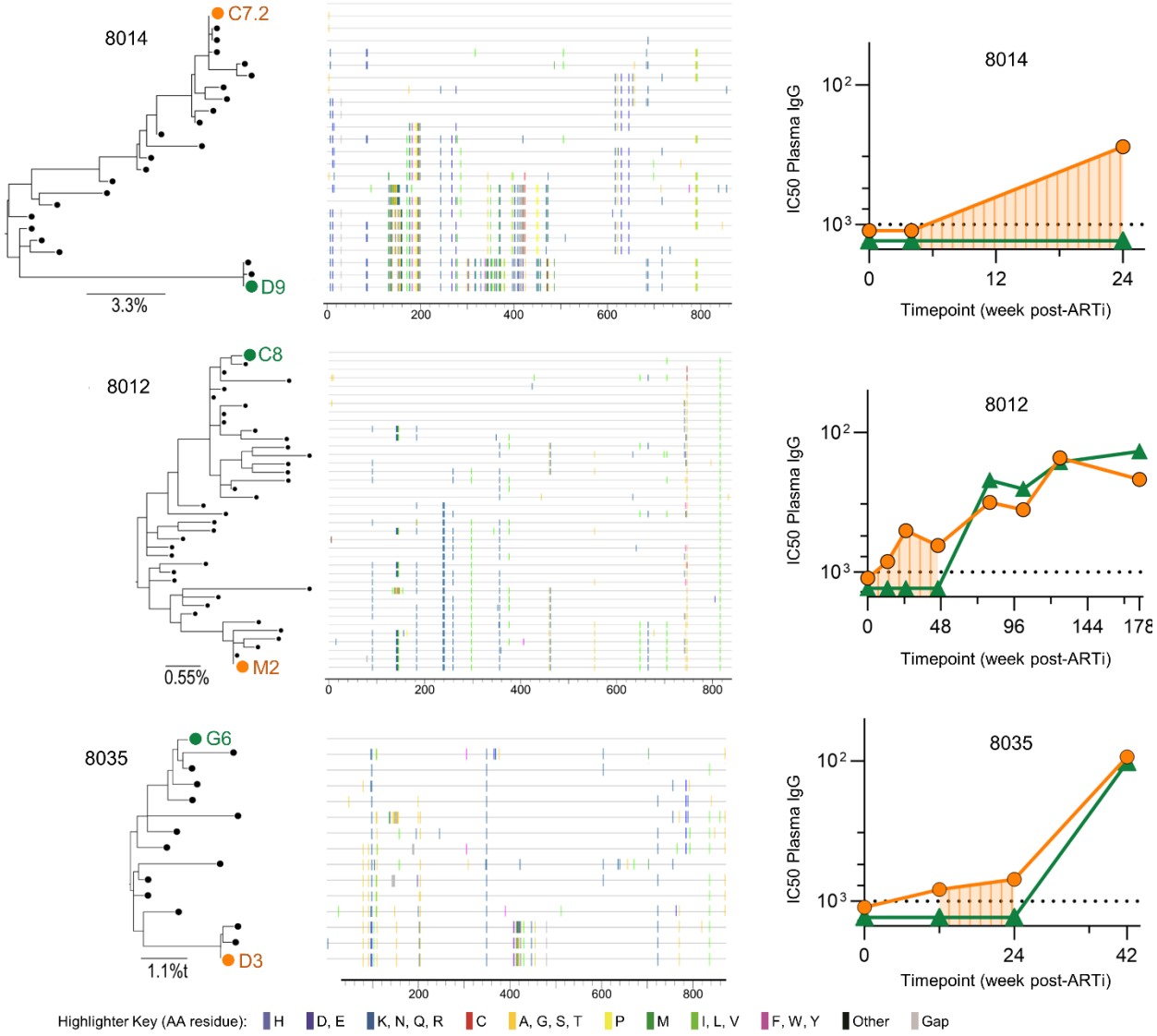

Figure 6: AnAb responses in EAi participants with multivariant transmission (MVT) Maximum-likelihood amino acid phylogenetic trees and highlighter plots for the three EAi participants with MVT are presented on left. Pseudotyped dominant clade and minor clade early viruses are denoted in orange and green, respectively. Neutralization IC50 (ug/mL) by longitudinal plasma IgG of each clade is presented on right. Shaded area represents time during which anAbs against only one variant were detected.