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Abbreviations

Ad5	Adenovirus serotype 5
ADCC	Antibody-dependent cellular cytotoxicity
AHI	Acute HIV infection
AIDS	Acquired immunodeficiency syndrome
allo-HSCT	Allogeneic hematopoietic stem cell transplantation
ARS	Acute retroviral syndrome
ART	Antiretroviral therapy
ATI	Analytical treatment interruption
AZT	Zidovudine
bNAbs	Broadly neutralizing antibodies
CAR-T	Chimeric antigen receptor T cell
cART	Combined antiretroviral therapy
CDC	Center for Disease Control and Prevention
ChAd	Chimpanzee adenovirus
CHAMP	Control of HIV after antiretroviral Medication Pause
CMV	Cytomegalovirus
CROI	Conference on Retroviruses and Opportunistic Infections
CTL	Cytotoxic T lymphocyte
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CXCR5	C-X-C chemokine receptor type 5
dCA	Didehydro-cortistatin A
EC	Elite controller
EEC	Exceptional elite controller
EIAs	Enzyme immunoassays
ELISA	Enzyme-Linked Immunosorbent Assay
Env	Envelope
GALT	Gut-associated lymphoid tissues
HCP	Healthcare providers

HDACi | Histone deacetylase inhibitors
HIV | Human immunodeficiency virus
HLA | Human Leukocyte Antigen
HMT | Histone methyl transferase
HTI | HIVACAT T cell immunogen
HTLV-III | Human T-lymphotropic virus type III
IFN-α | Interferon alpha
IL-6 | Interleukin 6
KS | Kaposi's sarcoma
LAV | Lymphadenopathy-associated virus
LEVI | Long-acting early viral inhibition
LoC | Loss of control
LRAs | Latency reversing agents
mRNA | Messenger RNA
MSM | Men who have sex with men
MVA | Modified vaccinia Ankara
NAT | Nucleic acid amplification test
NK | Natural killer (cells)
NNRTI | Non-nucleoside reverse transcriptase inhibitor
pDCs | Plasmacytoid dendritic cells
PCP | Pneumocystis carinii pneumonia
PD-1 | Programmed cell death protein 1
PIC | Post intervention controller
PIs | Protease inhibitors
PoC | Point-of-care
PrEP | Pre-exposure prophylaxis
PTC | Post treatment controller
PWH | People with HIV
RCT | Randomized clinical trial
RNA | Ribonucleic acid
SIV | Simian immunodeficiency virus

SMC | Safety Monitoring Committees

STING | Stimulator of interferon genes

STIs | Sexually transmitted infections

TasP | Treatment as prevention

TCR | T-cell receptor

TCR-T | T cell receptor-engineered T cell

TIGIT | T cell immunoreceptor with Ig and ITIM domains

TLR | Toll-like receptor

TNF-α | Tumor necrosis factor alpha

VC | Viremic controller

VISCONTI | Viro-Immunological Sustained CONtrol after Treatment Interruption

VL | Viral load

VOA | Viral outgrowth assays

WB | Western blot

WHO | World Health Organization

pVL | Plasma viral load

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Summary

Despite significant advances in antiretroviral therapy (ART), a definitive cure for human immunodeficiency virus (HIV) remains elusive for the 39 million people living with HIV (PWH) worldwide who continue to experience stigma, lifelong medication burdens, and health disparities. The primary obstacle to curing HIV is the persistence of an HIV reservoir, which consists of a pool of cells in which HIV's genome is integrated into the host's DNA. Viral replication can rapidly restart upon ART discontinuation, and the host's antiviral immune response is unable to contain this viral resurgence. Significant efforts have been made over the last 20 years of HIV cure research, aimed at reducing or perturbing the viral reservoir and training the immune system to detect and eliminate HIV-1 infected T cells.

Importantly, cure clinical trials face several challenges. In the absence of validated biomarkers able to predict post-intervention virological control after ART cessation, analytical treatment interruptions (ATI) and inclusion of placebo groups are the only reliable tools to assess the efficacy of a tested strategy. However, ATI harbor several logistical and ethical challenges. Community-driven advocacy has played a pivotal role in shaping the HIV cure research agenda, as well as in reinforcing the necessity of exploring innovative approaches when designing and implementing HIV remission trials. The continued engagement of PWH in clinical trials underscores the collective determination to move beyond ART dependency toward sustainable remission solutions.

In this thesis, I aimed to evaluate the safety, immunogenicity, and efficacy of a novel therapeutic vaccine for HIV, based on the HIVACAT T-cell immunogen (HTI), alone or in combination with the TLR7 agonist Vesatolimod (VES), in PWH treated within the first six months after HIV acquisition in two independent, randomized, double-blinded, placebo-controlled clinical trials.

After the introduction, I provide the description of the **Early-cART** cohort as a research platform from which potential candidates for the discussed HIV cure trials described in Articles 1 and 2 were identified. We also evaluate the impact of the implementation of the Early-cART cohort on the reduction in the time from HIV acquisition to ART initiation in individuals newly diagnosed of HIV in the acute and recent phase of infection and the immunovirological outcomes of early ART initiation. In Article 1 of the compendium, I present the results of the first clinical trial evaluating a combination of HTI-based vaccines in early-treated individuals: **the AELIX-002 trial**. AELIX-002 demonstrated that HTI vaccines were safe and immunogenic, inducing strong, polyfunctional, and broad CD4 and CD8 T-cell responses specific to HTI. Notably, AELIX-002 provided the first clinical signal of efficacy of a therapeutic HIV vaccine, with exploratory analyses suggesting that prolonged ART-free periods in vaccinated individuals lacking protective HLA alleles were associated with vaccine-induced HTI-specific T-cell responses. In Article 2 of the compendium, results from the **AELIX-003 trial** are presented. AELIX-003 study was built on finding from AELIX-002 and evaluated the combination of HTI vaccines with VES also in early treated individuals. Although the combination was safe

and immunogenic, no clear additional benefit over HTI vaccination alone was observed in terms of viral control. However, VES may have contributed to maintaining robust HTI-specific responses through a less complex vaccination regimen.

I then discuss the implications of results from both studies in the HIV cure field, and how can these results can provide clues for the developing future strategies. Finally, in the future perspectives section, I provide an integration of individual participant's data from AELIX-002 and AELIX-003 in a pooled analysis, leveraging the large and homogeneous cohort to validate immunological correlates of ATI outcomes and to explore additional biomarkers predictive of extended ART-free periods and improved viral control during ATI. The results of this combined analysis propose the use of HTI-specific immune responses as a biomarker to guide the design of future HTI-based cure strategies, both for participant selection and for its inclusion as a futility criterion during the intervention phase, in order to avoid unnecessary ATI in participants with a low likelihood of controlling viremia.

Resumen

A pesar de los importantes avances en el tratamiento antirretroviral (ART, por sus siglas en inglés- *Antiretroviral treatment*), una cura definitiva para el virus de la inmunodeficiencia humana (HIV, por sus siglas en inglés, *Human Immunodeficiency Virus*) sigue siendo inalcanzable para los 39 millones de personas que viven con HIV (PWH, por sus siglas en inglés- *People living with HIV*) en todo el mundo, quienes continúan experimentando estigma, la carga de una medicación de por vida y desigualdades en salud. El principal obstáculo para la curación del HIV es la persistencia de los reservorios virales, que consisten en un conjunto de células en las que el genoma del HIV está integrado en el ADN del huésped. La replicación viral es capaz de reanudarse rápidamente tras la interrupción del ART, y la respuesta inmune antiviral del huésped es incapaz de contener este rebote viral. En los últimos 20 años, se han realizado importantes esfuerzos en la investigación hacia la cura del HIV, con el objetivo de reducir o perturbar el reservorio viral y entrenar al sistema inmunitario para detectar y eliminar las células T infectadas por HIV.

No obstante, los ensayos clínicos dirigidos a la cura del HIV enfrentan múltiples desafíos. En ausencia de biomarcadores validados capaces de predecir el control virológico tras la interrupción del ART, las interrupciones analíticas del tratamiento (ATI, por sus siglas en inglés- *Analytical Treatment Interruption*) y la inclusión de grupos placebo se presentan como las únicas herramientas fiables para evaluar la eficacia de las estrategias en investigación. Sin embargo, las ATI conllevan diversos retos, tanto logísticos como éticos. La defensa de los derechos de las PWH, impulsada desde la propia comunidad, ha desempeñado un papel fundamental en el diseño de la agenda de investigación sobre la cura del HIV, así como en la promoción de la necesidad de explorar enfoques innovadores en el diseño e implementación de ensayos de control del HIV en ausencia de ART. La participación continua de las PWH en ensayos clínicos el compromiso de la comunidad por avanzar más allá de la dependencia del ART y hacia soluciones sostenibles de remisión.

En esta tesis, me propuse evaluar la seguridad, inmunogenicidad y eficacia de una nueva vacuna terapéutica contra el VIH, basada en el inmunógeno HTI (HIVACAT T-cell Immunogen), sola o en combinación con el agonista de TLR7 Vesatolimod (VES), en PWH tratadas durante los primeros seis meses tras la adquisición del HIV, en el contexto de dos ensayos clínicos independientes, aleatorizados, doble ciego y controlados con placebo.

Tras la introducción, presento la descripción de la cohorte **Early-cART** como una plataforma de investigación desde la cual se identificaron posibles candidatos para los ensayos de cura del HIV discutidos en los Artículos 1 y 2. También evaluamos el impacto de la implementación de la cohorte Early-cART en la reducción del tiempo desde la adquisición del HIV hasta el inicio del ART en individuos diagnosticados en fase aguda o reciente de la infección, así como los resultados inmunovirológicos del inicio temprano del ART. En el Artículo 1 del compendio, presento los resultados del primer ensayo clínico que evalúa una combinación de vacunas basadas en HTI en individuos tratados precozmente: **el ensayo clínico AELIX-002**. AELIX-002

demostró que las vacunas HTI fueron seguras e inmunogénicas, induciendo respuestas de células T CD4 y CD8 específicas frente a HTI que fueron potentes, polifuncionales y amplias. De forma destacada, AELIX-002 proporcionó la primera señal clínica de eficacia de una vacuna terapéutica contra el HIV, con análisis exploratorios que sugieren que los períodos prolongados sin ART en individuos vacunados que no presentaban alelos HLA protectores se asociaron con respuestas de células T específicas frente a HTI inducidas por la vacuna. En el Artículo 2 del compendio, se presentan los resultados del **ensayo clínico AELIX-003**. AELIX-003 se construyó a partir de los hallazgos del AELIX-002 y evaluó la combinación de las vacunas HTI con VES también en individuos tratados precozmente. Aunque la combinación fue segura e inmunogénica, no se observó un beneficio adicional claro en términos de control virológico en comparación con la vacunación con HTI sola. Sin embargo, VES podría haber contribuido al mantenimiento de respuestas robustas frente a HTI mediante un régimen de vacunación menos complejo.

Posteriormente, discuto las implicaciones de los resultados de ambos estudios en el campo de la cura del VIH y cómo estos hallazgos pueden aportar pistas para el desarrollo de futuras estrategias. Finalmente, en la sección de perspectivas futuras, presento una integración de los datos individuales de los participantes de AELIX-002 y AELIX-003 en un análisis conjunto, aprovechando la amplitud y homogeneidad de la cohorte para validar correlatos inmunológicos de los resultados durante la ATI, y explorar biomarcadores adicionales que predigan períodos prolongados sin ART y un mejor control viral durante la ATI. Los resultados de este análisis combinado proponen el uso de las respuestas inmunes específicas frente a HTI como un biomarcador para guiar el diseño de futuras estrategias de cura basadas en HTI, tanto para la selección de participantes como para su inclusión como criterio de futilidad durante la fase de intervención, con el fin de evitar ATI innecesarias en participantes con baja probabilidad de controlar la viremia.

1. INTRODUCTION

1.1. From community to research

1.1.1. Forty years of HIV epidemic

First cases of acquired immunodeficiency syndrome (AIDS) were described in June 1981 in Los Angeles, California, United States. A brief report of three cases of *Pneumocystis carinii* pneumonia (PCP) published in the *Morbidity and Mortality Weekly Report* of Center for Disease Control and Prevention (CDC) raised alarms about a new illness that had emerged among young gay men in the United States[1]. During the following months, several cases of Kaposi's sarcoma (KS) and opportunistic infections emerged in otherwise healthy men who had sex with other men (MSM) in California and New York[2,3]. This new syndrome, characterized by rapid and fatal progression, occurred in individuals without apparent pre-existing immunosuppressive conditions, raising concerns within the scientific community. A short time later, CDC reported the emergence of cases of PCP and cryptosporidiosis in hemophiliacs [4]. This situation raised the hypothesis that probably AIDS might be a virus-related disease[5] with a probable target in cellular immune system [6]. But unfortunately, given the initial group of affected individuals, young MSM, the cause of the disease was attributed on a specific sexual behavior, and for this reason, AIDS was quickly and strongly stigmatized.

At that time, a long and thorough field of research was initiated, but it was not until 1983, thanks to the collaboration of worldwide scientists, the human immunodeficiency virus (HIV) was identified as the causative agent of AIDS. Initially, a group of European scientists, led by Luc Montagnier and François Barre-Sinoussi, isolated and described a retrovirus, named lymphadenopathy-associated virus (LAV) [7], responsible for this new disease able to devastate the immune system. In 1984, Robert Gallo and colleagues, from the American National Institute of Health, confirmed that this virus was the causative agent of AIDS, termed human T-lymphotropic virus type III (HTLV-III) [8]. In 1986, both French and US scientific groups, renamed the virus responsible for AIDS in the manner we know it today: HIV. The discovery of HIV and its relevance to our history was honored with the Nobel Prize in Medicine in 2008 to Luc Montagnier and François Barre-Sinoussi.

More than four decades after the first description of HIV/AIDS cases, HIV has caused almost 40 million HIV/AIDS related-deaths worldwide, and currently almost 38 million people are living with HIV (PWH) [9].

1.1.2. The natural course of HIV infection. Pathogenesis

The initial uncertainties surrounding HIV were primarily focused on elucidating the virus's mechanism of action, identifying transmission routes, and urgently developing effective therapeutic strategies to halt disease progression and prevent AIDS-related complications. It was soon established that HIV pathogenesis involves the progressive depletion of CD4+ T lymphocytes, causing profound immunosuppression and subsequent development of specific infections and neoplasms, generically named as opportunistic or AIDS-defining diseases, ultimately leading to death, as shown in **Figure 1**.

Following HIV acquisition, approximately 40% of individuals may experience transient symptoms consistent with an acute retroviral syndrome (ARS), which typically persists for no more than four weeks[10]. These nonspecific manifestations—including lymphadenopathy, fever, maculopapular rash, and myalgia—often hinder early diagnosis in the absence of clinical suspicion. After the acute/recent phase, most individuals enter a chronic asymptomatic period that may last years and during which many people remain unaware of their infection, until significant immune decline occurs.

During the chronic phase of HIV infection, conditions like oral thrush, oral hairy leukoplakia, and herpes zoster may arise. While these are not AIDS-defining conditions, they are causally linked to HIV infection, signaling a disturbance in cellular immune response, and their appearance should prompt consideration for a differential diagnosis of HIV infection. In the absence of effective antiretroviral treatment (ART), AIDS-defining illnesses usually appear 8 to 10 years after the initial infection, contributing to AIDS-related mortality[11].

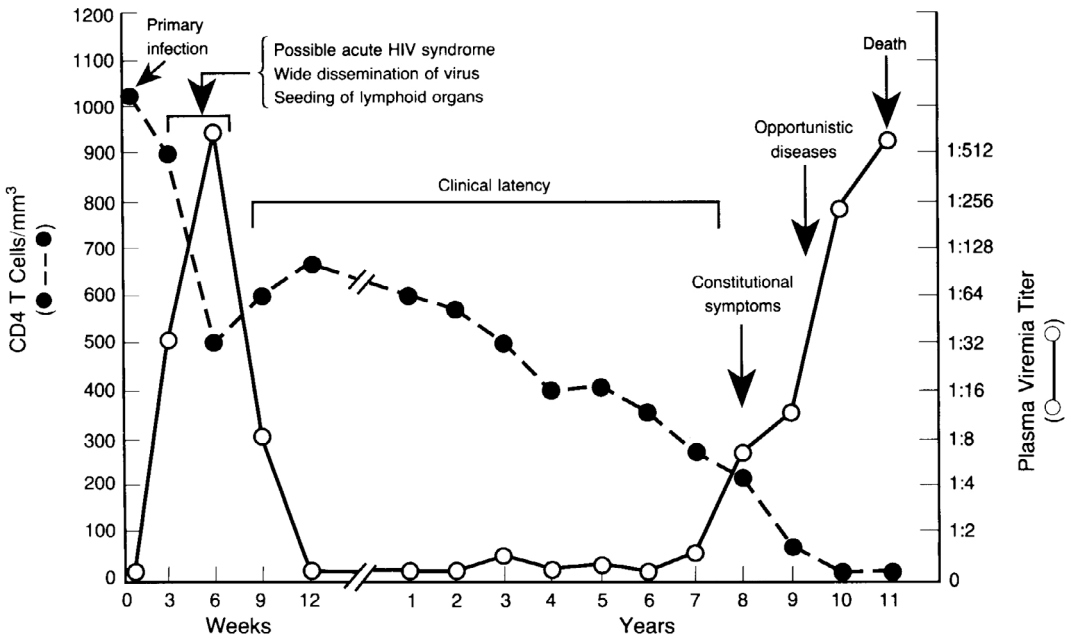


Figure 1. The course of HIV infection without ART, CD4 T-cells and viral load. Adapted from Fauci AS, Lane HC, *N Engl J Med.* 1993.

1.1.2.1. Understanding HIV transmission pathways

Early hypotheses regarding HIV transmission suggested potential associations with cytomegalovirus (CMV) co-infections, intravenous drug use, amyl nitrate (poppers) inhalation, and exposure to sexual fluids such as semen[12]. These speculations initially reinforced misconceptions, particularly attributing the spread of HIV to the MSM community. However, subsequent research elucidated the exact transmission routes of HIV, which include: (1) sexual intercourse with a PWH who has a detectable viral load (VL), (2) sharing of syringes or sharp objects contaminated with blood containing detectable HIV VL, (3) transfusion of HIV-infected blood, and (4) mother-to-child transmission during pregnancy, childbirth, or breastfeeding in the absence of effective ART. At the onset of the epidemic, the lack of awareness regarding needle-sharing risks and blood transfusion-related transmission significantly contributed to the spread of HIV among intravenous drug users and hemophiliacs. Direct contact with bodily fluids—primarily blood, semen, and pre-seminal fluids—containing a detectable HIV VL is necessary for transmission. While mother-to-child transmission remains a concern, particularly in regions such as sub-Saharan Africa, the sexual route continues to be the primary mode of transmission. Several individual risk factors influence acquisition probability, including the type of sexual practice, coexisting sexually transmitted infections (STIs), mucosal integrity, and circumcision status. The highest risk of sexual transmission occurs when engaging with an untreated PWH with a high VL, particularly during acute or recent HIV infection.

1.1.2.2. Focusing on the diagnosis of recent/acute HIV infection

Although both HIV-1 and HIV-2 infect humans, the vast majority of research has focused on HIV-1 due to its wider prevalence. Therefore, 'HIV' will hereafter refer to HIV-1 unless otherwise specified. Studying the early stages of HIV infection in humans is inherently challenging; therefore, a significant part of our current understanding comes from simian immunodeficiency virus (SIV) models in macaques. These studies show that productive infection of CD4+ T lymphocytes can occur as early as two days after viral exposure. The virus initially establishes localized clusters of infection at the mucosal entry site, rapidly spreading to draining lymph nodes, and subsequently to distal lymphoid tissues. In the SIV model, this dissemination unfolds within approximately two weeks. Notably, during acute infection, HIV primarily uses the C-X-C chemokine receptor type 5 (CCR5) to infect CD4+ T lymphocytes, as CCR5 is abundantly expressed on these cells in mucosal tissues. This preferential targeting results in a massive depletion of mucosal CD4+ T cells, which plays a critical role in driving early immunosuppression and disrupting immune homeostasis, thereby increasing susceptibility to opportunistic infections. Individuals carrying the CCR5-Δ32 deletion, which prevents CCR5 expression, are highly resistant to be infected by HIV, underscoring the central role of this correceptor. Over the course of infection, the viral population may evolve in response to selective pressures from the immune system and shifting target cell availability. In the absence of ART, HIV evolution commonly results in a shift from CCR5 to CXCR4 correceptor usage in a significant proportion of individuals [13,14]. The emergence of CXCR4-tropic variants reflects the dynamic nature of HIV pathogenesis, yet the precise mechanisms underlying this tropism switch remain poorly understood.

Typically, new infections are initiated by a single or limited number of genetic variants of HIV[12], reflecting a "transmission bottleneck" that remains incompletely understood. Soon after HIV acquisition, the infection enters an "eclipse phase," during which the infection is established at local tissues and there is no yet detectable systemic dissemination [15]. Upon systemic spread, viral replication accelerates exponentially, with a doubling time of approximately 20 hours[16]. This marks the onset of the viremic ramp-up phase, during which HIV ribonucleic acid (RNA) and p24 antigen become detectable while HIV-specific antibodies are still absent. This seronegative period defines the "window period" of acute HIV infection (AHI). Fiebig et al[16] proposed a classification system that divides acute and early HIV infection into six stages, based on virologic and serologic markers that reflect the progression of viral replication and the evolving antibody response, as shown in **Figure 2**.

- **Fiebig Stage I:** In the initial phase of AHI, during the viremic ramp-up, only HIV RNA is detectable in the blood.
- **Fiebig Stage II:** Approximately seven days later, the p24 antigen test becomes positive. p24, a viral core protein, appears in the blood once HIV RNA levels exceed 10,000 copies/mL but before HIV antibodies are detectable. During this viremic ramp-up, individuals experience a robust innate immune activation, often referred to as a "cytokine storm," [17,18,19] characterized by elevated levels of pro-inflammatory cytokines and chemokines such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), and interferon alpha (IFN-α). This inflammatory environment is driven in part by plasmacytoid dendritic cells (pDCs), which sense viral RNA via Toll-like receptors (TLR) and produce large amounts of type I interferons. Natural killer (NK) cells are also activated, contributing to early antiviral response through cytotoxic activity and cytokine secretion. In parallel, the adaptive immune response begins to develop, marked by the expansion of HIV-specific CD8+ cytotoxic T lymphocytes (CTLs). These T-cells exert selective pressure on the virus, which often leads to the rapid emergence of escape mutants within weeks of infection[19]. This stage is associated with the start of above-mentioned ARS in 40% of individuals, typically emerging two weeks after HIV acquisition, coinciding with peak viremia.
- **Fiebig Stage III:** About five days after p24 antigen detection, first HIV antibodies become detectable via third-generation enzyme immunoassays (EIAs), which primarily include anti-gp41 IgM, sometimes early anti-p24, but the antibody levels are still too low for a Western blot (WB) positivity. Screening EIAs are more sensitive than WB early on because they detect low-level, early antibodies. This stage usually occurs 1–2 weeks after ARS onset.
- **Fiebig Stage IV:** At this stage, an indeterminate WB result emerges, typically three days after positive EIA results. A positive WB typically requires the presence of antibodies to at least two or three proteins from different gene groups, depending on the diagnostic criteria. For instance, CDC rule for positivity requires detection to at least two of the three envelope proteins (gp41-transmembrane, gp120 -surface or gp160-precursor of gp120+gp41), while WHO criteria consider a positive WB if there are detectable

antibody to at least one env protein (gp41, gp120, or gp160) plus antibodies to either gag (p24) or pol (p31/p66) proteins. An indeterminate result is given when there is reactivity to one or a few HIV proteins, but not the full pattern required for WB positivity.

- **Fiebig Stage V:** The transition to a definitively positive WB test (Fiebig stage V) typically occurs 7–30 days after the initial HIV acquisition.
- **Fiebig Stage VI:** A positive p31/32 antibody at 31-35kDA band appears corresponding to the pol-integrase, which is the latest antibody to be detected in the WB test, usually reflecting an infection of more than 90 days. At this point, the plasma viral load stabilizes and reaches the viral setpoint, typically during Fiebig stages V and VI.

Traditionally, WB was the gold standard confirmatory test for HIV diagnosis. WB involves the separation of viral proteins by gel electrophoresis, transfer to a membrane, and detection using serum antibodies. More recently, immunoblot assays like Geenius or INNO-LIA have been introduced as a modern alternative to WB in some settings because they are faster and more standardized, but they still detect similar targets [20]. These assays use recombinant or synthetic HIV proteins fixed directly onto membrane strips, allowing for faster, easier, and more standardized interpretation. Compared to traditional WB, some immunoblots are less sensible in detect certain antibodies, such as p31, may be less useful for the differentiation between Fiebig V and VI stages.

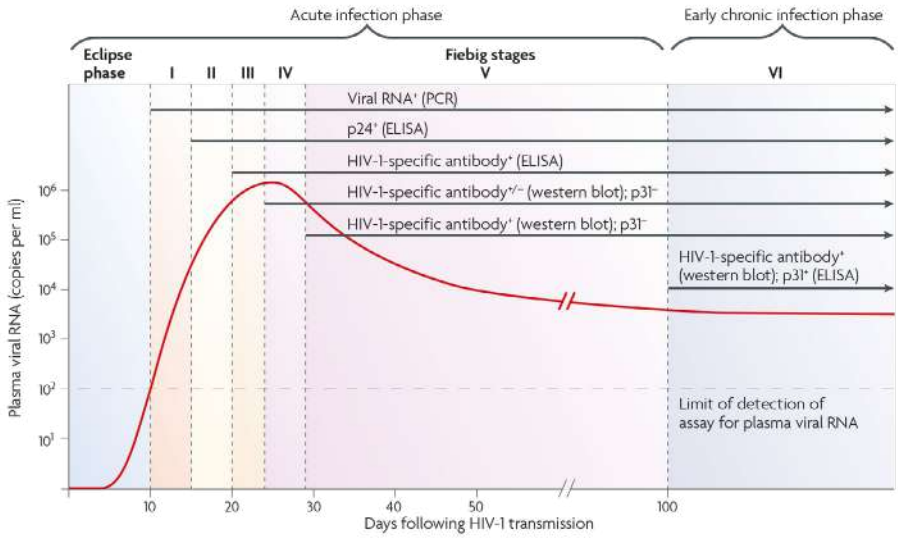


Figure 2. Representation of the stages in the acute HIV infection defined by Fiebig. Image from McMichael *Nat Rev Immunol,* 2010.

Acute vs recent HIV infection

It is important to emphasize that complete seroconversion can take up to six months after HIV exposure, while p24 antigenemia is typically detectable within 3–4 weeks, and viremia can appear as early as 1–2 weeks. While there is no universally accepted definition of **acute HIV infection** (AHI), the most widely accepted criterion is the identification of HIV RNA or p24 antigen in the bloodstream in the absence of antibodies, typically before reaching Fiebig stage III. Thereafter, **recent HIV infection** is defined as the period occurring after seroconversion (positive antibodies) and before six months since transmission, encompassing Fiebig stages III, IV, and V.

Diagnosing acute or recent HIV infection accurately allows for the timely initiation of ART, which can improve clinical outcomes at the individual level and reduce the risk of secondary transmissions. It has long been recognized that the conventional "reactive enzyme-linked immunosorbent assay (ELISA) followed by a full positive WB confirmation" algorithm lacks the sensitivity required to detect early infections, especially when compared to more advanced generations of HIV screening tests. This became particularly evident with the introduction of fourth-generation combined antigen/antibody assays, which include p24 antigen detection (positive in Fiebig II stage as mentioned earlier). While Western blot remains useful for estimating the HIV acquisition date based on the timing of different antibody band patterns, it is no longer considered an appropriate confirmatory test in suspected cases of acute/recent HIV. Therefore, current guidelines recommend that individuals with a reactive screening test (e.g., ELISA) but a negative WB undergo HIV RNA testing to rule out AHI, as outlined in the CDC's 2014 diagnostic algorithm. Of importance, with the advent and increasing use of pre-exposure prophylaxis (PrEP), atypical patterns of HIV seroconversion have been documented. For instance, in breakthrough infections in PrEP users, viral replication may be initially suppressed, leading to delayed or attenuated serologic responses[21]. Also, cases of breakthrough infection have been associated with delayed antibody production and reduced viremia at diagnosis in the context of oral PrEP with tenofovir/emtricitabine, complicating detection of AHI. More recently, the introduction of long-acting injectable PrEP—notably cabotegravir—has been associated with an emerging phenomenon termed *Long-acting early viral inhibition* [22] (LEVI). In these cases, persistent drug levels at the time of HIV acquisition can suppress early viral replication to levels below detection thresholds, resulting in prolonged eclipse phases and atypical or absent seroconversion patterns. These evolving clinical presentations pose additional challenges for early diagnosis and accurate staging of infection. Moreover, they have important implications for clinical management, surveillance strategies, and the design of diagnostic algorithms tailored to populations using PrEP.

1.1.3. The breakthrough of an effective ART

Development of effective ART to control viral replication and prevent progression to AIDS has been a public health priority since the onset of the HIV epidemic. The first breakthrough came in 1987 with the approval of zidovudine (AZT), one the nucleoside-analogue reverse transcriptase inhibitor (NRTI) that, although in monotherapy could only offered partial control over the virus, was the first drug showing an impact on AIDS-related mortality [23,24]. By mid-

1990's, a transformative shift in ART occurred. The advent of non-nucleoside-analogue reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI) allowed the combination of three antiretroviral drugs (combined ART, cART) which, for the first time in history, led to a drastic reduction in new cases of AIDS and mortality associated with HIV infection[25–27]**.** Consequently, between 1994 and 1998, the incidence of AIDS in Europe plummeted from 30.7 to 2.5/100 patient-years. This turning point is clearly reflected in global epidemiological trends, as shown in **Figure 3**, which also includes projections through 2050[28].

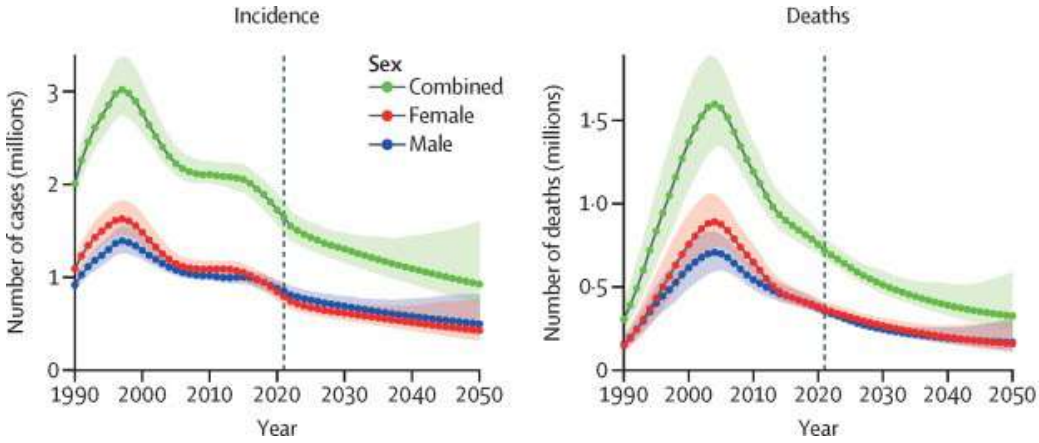


Figure 3. Global trends in AIDS incidence and AIDS-related mortality from 1990 to 2021, with projections to 2050. The introduction of combination antiretroviral therapy (cART) in the mid-1990s coincides with a marked decline in AIDS-related deaths. Figure adapted from the Global Burden of Disease Study 2021, *The Lancet HIV, 2021*.

Despite advances in ART, sustained adherence to old cART regimens was difficult to maintain due to limiting side effects and to pill burden (some of initial cART regiments consisted of more than 20 daily pills taken in 2-3 times). These limitations led to treatment guidelines to recommend deferring ART initiation until CD4+ T cell counts fell below 200 cells/μL[29].

During 2000´s, the development of better-tolerated and easier-to-take drugs, revolutionized cART by minimizing adverse effects and promoting long-term adherence. Additionally, results from studies such as SMART, HTPN052, and START trial[30–32] provided strong evidence that initiating ART regardless of CD4+ T cell count does not only improve individual outcomes but also plays a key role in preventing HIV transmission by lowering community VL—a concept now widely recognized as ***Treatment as Prevention*** (TasP)[33,34]. Therefore, there has been a shift in most international guidelines' recommendations, from reserving ART only to PWH with severe immune suppression to universal ART regardless of CD4+ T cell count. Consequently, **"test and treat," "same-day treatment," and "rapid treatment initiation"** strategies have emerged as preferred approaches to achieving viral suppression as quickly as possible.

Rapid ART initiation in acute and recent HIV infection phase (collectively referred to as Early ART) is particularly relevant, as individuals often present with extremely high VL in blood and semen, significantly increasing the risk of secondary transmissions. Despite its clear benefits, detecting AHI and ensuring immediate linkage to care remain challenging. As mentioned before, recent advances in diagnostic technologies—such as high-sensitivity fourth-generation antigen-antibody assays and nucleic acid amplification tests (NATs)—have substantially shortened the diagnostic window period, thus facilitating the detection of infections at earlier stages. Integrating point-of-care (PoC) technologies with fourth-generation rapid tests and NAT platforms has simplified infrastructure requirements, enabling HIV testing in community settings and maximizing diagnostic opportunities.

Routine screening programs targeting high-risk populations have further facilitated early diagnosis, simultaneously serving as platforms for broader sexual health promotion, including STI management and PrEP counseling. Consequently, by integrating innovative diagnostic strategies with rapid and universal ART initiation, healthcare systems can optimize individual outcomes and substantially improve population-level HIV control, reinforcing early ART as a cornerstone of the global HIV response.

1.1.4. U=U: Undetectable = Untransmittable

The widespread implementation of rapid and universal ART has not only transformed HIV into a manageable chronic condition but has also led to one of the most significant breakthroughs in HIV prevention: ***Undetectable*** = ***Untransmittable*** **(U=U)**. This concept, now endorsed by major health organizations, underscores that PWH who achieve and maintain an undetectable VL through effective ART cannot sexually transmit the virus to their partners.

The scientific foundation for U=U began to take shape in the early 2000s, with studies demonstrating a strong correlation between VL suppression and reduced transmission risk. However, definitive evidence emerged with key clinical trials, including HPTN 052, PARTNER 1 & 2, and Opposites Attract study [31,35,36]. These studies provided robust, real-world data confirming that no cases of HIV transmission occurred between serodiscordant couples when the HIV-positive partner had sustained viral suppression, even in the context of condomless sex. In 2016, the Prevention Access Campaign formally launched the U=U movement, rapidly gaining global recognition. Major public health entities, such as the CDC, WHO, UNAIDS, and ECDC, have since adopted U=U as a cornerstone of HIV prevention, education, and stigma reduction. Beyond its biomedical impact, U=U has played a crucial role in challenging outdated perceptions of HIV, empowering PWH by reinforcing that effective treatment not only preserves health but also eliminates the risk of sexual transmission.

This paradigm shift has profound implications. By integrating U=U messaging into HIV care, prevention strategies, and public health policies, we can strengthen ART adherence, encourage early diagnosis and rapid treatment initiation, and dismantle persistent stigma. Furthermore, U=U complements biomedical prevention approaches like pre- and post-exposure prophylaxis, reinforcing the concept of comprehensive, person-centered HIV prevention. As

the global HIV response moves toward the UNAIDS 95-95-95 goals for 2030, U=U stands as a critical pillar—linking treatment success with prevention, fostering community engagement, and reaffirming that people with HIV, when effectively treated, can lead long, healthy lives free from the fear of transmission.

1.1.5. (Living with) HIV in 2025

The landscape of HIV has changed dramatically since the first years of the epidemic. Advances in ART have transformed HIV from a life-threatening illness into a chronic, manageable condition. Modern ART—primarily single-tablet regimens and long-acting formulations—enable PWH to achieve a life expectancy close to people without HIV, with minimal toxicity, and excellent tolerability. Consequently, global efforts have significantly reduced HIV-related morbidity and mortality. However, as PWH age, the focus is increasingly shifting toward addressing non-AIDS-related morbidity and mortality, including cardiovascular diseases, malignancies, renal dysfunction, and metabolic disorders, collectively recognized as non-HIV comorbidities[34]. Addressing these comorbidities through integrated, multidisciplinary care is now essential to optimize long-term health outcomes and quality of life among aging HIV populations. While addressing non-HIV comorbidities has become increasingly crucial in improving long-term health outcomes for PWH, broader public health challenges remain.

Despite this remarkable progress in testing, treatment, and prevention, HIV remains a major global public health challenge, reflecting persistent inequalities in healthcare access. To date, HIV has claimed approximately 40.4 million lives, and as of 2023, an estimated 39.9 million people were living with HIV worldwide. While sub-Saharan Africa remains the most affected region, notable progress has been made in reducing new infections there. In contrast, HIV incidence has risen in other regions, such as Eastern Europe, emphasizing the need for region-specific interventions. In 2023 alone, 1.3 million individuals acquired HIV, and 630,000 people died from AIDS-related illnesses—figures that underscore the continued urgency of the epidemic[9]. Achieving the goal of ending AIDS as a public health threat by 2030 will require sustained investment in prevention, treatment, and education, along with targeted strategies to address regional disparities.

A central component of the global HIV response is the **UNAIDS 95-95-95 strategy**[37], which aims for 95% of PWH to know their status, 95% of those diagnosed to receive ART, and 95% of those treated to achieve viral suppression. A fourth pillar further emphasizes the importance of enhancing the quality of life for PWH, recognizing that effective HIV care must also address mental health, social support, and overall well-being. Achieving these targets is essential to interrupting transmission, improving health outcomes, and moving closer to ending AIDS as a public health crisis. The 95-95-95 strategy has laid the groundwork for a transformative shift in the epidemic. By promoting early diagnosis and rapid ART initiation, a growing proportion of PWH are achieving undetectable VL, significantly improving their health while also preventing onward transmission (U=U). As community VL decreases, the overall risk of new infections declines, further accelerating progress toward epidemic control. However, these gains remain

fragile in the face of funding cuts and political challenges that threaten the continuity of ART and PrEP programs worldwide[38].

While these achievements mark a turning point in the global HIV response, reaching an undetectable VL in the community does not equate to eradication of HIV. PWH with undetectable VL are no longer at risk of transmitting the virus sexually, yet they continue to live with HIV and remain dependent on lifelong treatment. Persistent challenges—such as treatment burden, costs, HIV and non-HIV-associated comorbidities, and, most significantly, stigma—highlight the need to move beyond viral suppression. In this context, the scientific community is intensifying efforts toward an HIV "cure", which would involve either complete viral eradication or durable remission without ART. Achieving a cure would not only eliminate the need for lifelong ART and reduce the impact of chronic complications but also represent a fundamental breakthrough in dismantling the stigma still faced by PWH today. This next step holds immense promise, as it represents the possibility of not just controlling, but truly ending the burden of HIV for millions around the globe. Realizing this goal would mark one of the greatest milestones in the history of public health and human resilience.

Eradication: *from Latin word radix, meaning "root," eradication refers to the complete elimination of an infectious disease, reducing its global incidence within host populations to zero.*

Human Resilience: *rom Latin resilire "to rebound, recoil", is the ability to cope with challenging situations, and then to recover from them and/or to transform them.*

1.2. Towards an HIV cure

1.2.1. Definition of an HIV cure

The term "HIV cure" remains highly controversial within the HIV community, as it may create expectations that are currently difficult to meet. As a result, in recent years, there has been a growing preference for terms such as "Durable ART-free control of HIV" or simply "Durable control of HIV," which set more realistic expectations. This concept refers to the **sustained suppression of the virus to undetectable or low levels in the absence of ART**.

Durable control of HIV may be achieved by two different approaches: by the elimination of the entire latent reservoir (previously termed "sterilizing cure") or by pursuing functional control of HIV (previously referred to as "functional cure"). **Figure 4** shows a schematic representation illustrating these two approaches. While no definitive strategy has been established, substantial evidence indicates that durable ART-free control of HIV is possible—although not yet in a scalable or widely applicable manner for all PWH.

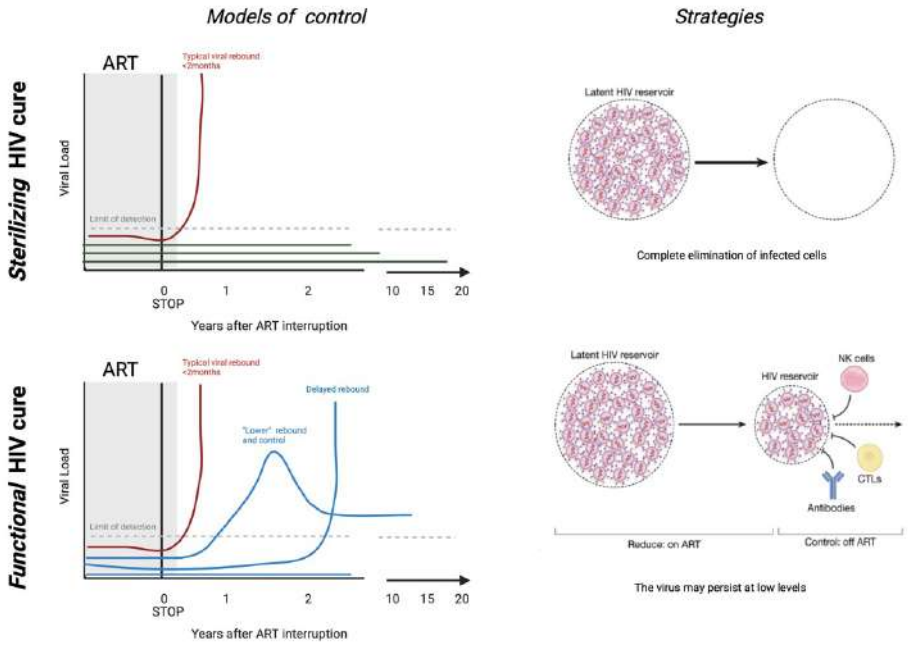


Figure 4. Schematic representation of models of durable control of HIV and potential strategies. Adapted from Landovitz, Nat Rev Microb, 2023 and Ndung´u Nature, 2019.

Several cases of individuals who have achieved permanent control of HIV have been well-documented and extensively studied. Timothy Brown (the "Berlin patient," who remained free of detectable HIV for over ten years) and Adam Castillejo (the "London patient," who has been HIV-free since 2017) were the first recorded cases of complete HIV remission[39, 40]. They represent the pioneering members of the exclusive and expanding group known as the "HIV cure family". However, in these cases, durable ART-free control of HIV was achieved only after undergoing **allogeneic hematopoietic stem cell transplantation (allo-HSCT)** from donors homozygous for the CCR5-Δ32 deletion due to underlying hematologic malignancies. The proposed mechanism for HIV remission in these cases is the complete replacement of recipient hematopoietic cells with donor-derived cells (full donor chimerism), leading to the immune-mediated elimination of latently infected cells through a graft-versus-HIV-reservoir effect. This mechanism refers to donor immune cells recognizing and targeting HIV-infected host cells. In some cases, this effect is further amplified in cases where graft-versus-host disease (GvHD) develops, as the resulting alloreactive immune response may inadvertently enhance the clearance of HIV-infected cells. However, GvHD itself is a distinct, often detrimental, immune directed against host tissues. This treatment involved a procedure that is associated with significant morbidity and mortality. Several of this subset of individuals who have achieved long-term HIV remission have been included in a consortium called IciStem, which conducts in-depth studies on their characteristics and underlying mechanisms[41]. Most IciStem participants demonstrated undetectable HIV reservoirs across multiple compartments, underscoring the profound impact of allo-HSCT on reservoir depletion. Building on the insights gained from HIV remission following allo-HSCT, novel gene-editing and immunotherapy-based strategies are under development to similarly target the latent HIV reservoir. **Clustered regularly interspaced short palindromic repeats- associated protein 9 (CRISPR/Cas9) technology** has been used to engineer HIV-resistant immune cells by disrupting CCR5[42], the key correceptor for viral entry, or by directly excising proviral DNA from infected cells. Furthermore, recent advancements in **chimeric antigen receptor T-cell therapy (CAR-T)** have led to the development of HIV-specific CAR-T cells, currently under phase I clinical testing. Second- and third-generation of CAR-T incorporate broadly neutralizing antibodies (bNAbs) as alternative targeting domains replacing the CD4 domains used in first-generation CAR-T[43]. This modification enhances viral recognition while protecting -CAR-T cells from becoming susceptible to HIV infection themselves. These cutting-edge strategies aim to replicate the immune-mediated clearance observed in transplant recipients, providing promising avenues toward achieving a more scalable and less invasive HIV cure.

Despite these anecdotal cases of HSCT, the complete elimination of the HIV reservoir still remains a distant goal, and achieving a state of 'functional immune control' of the virus may be more realistic. **Functional control** refers to the ability to maintain undetectable or very low levels of viral replication after discontinuing ART, even while proviral HIV DNA may still persist in blood and cells. As described below, this state of control has been observed in various models of natural HIV control, including elite controllers (ECs), viremic controllers (VCs), and post-treatment controllers (PCTs). Understanding these profiles and deepening into the mechanisms behind their control is crucial for developing sustainable strategies for off-ART HIV control.

1.2.2. Models of HIV control

Viremic (VC) and Elite Controllers (ECs) were identified early in the HIV epidemic, when it was observed that some individuals with HIV could control the virus spontaneously, without treatment, and without a decline in their CD4 lymphocyte count. Although ECs (defined as a fraction of VC with sustained VL<50 copies/ml) represent less than 1% of all PWH across multiple cohorts, the mechanisms behind their viral control have been extensively studied. Early investigations proposed that ECs harbored low-replicating viruses; however, subsequent studies refuted this hypothesis [44,45]. Genetic studies revealed protective host genetic factors, such as specific human leukocyte antigen (HLA) class I B alleles (e.g. HLA-B27, HLA-B57) or polymorphisms affecting HLA C expression to be strongly associated with delayed disease progression and with more effective CD8-mediated immune control in ECs. Currently, it is well established that while viral control in ECs is multifactorial, it is primarily mediated through highly efficient HIV-specific CD8+ cytotoxic T lymphocyte (CTL) responses[46]. Several qualitative properties distinguish the CD8+ T cell responses of ECs from typical progressors, including enhanced polyfunctionality, characterized by simultaneous production of multiple cytokines and chemokines, increased degranulation capacity, and higher proliferative and cytotoxic potential as shown in **Figure 5**.

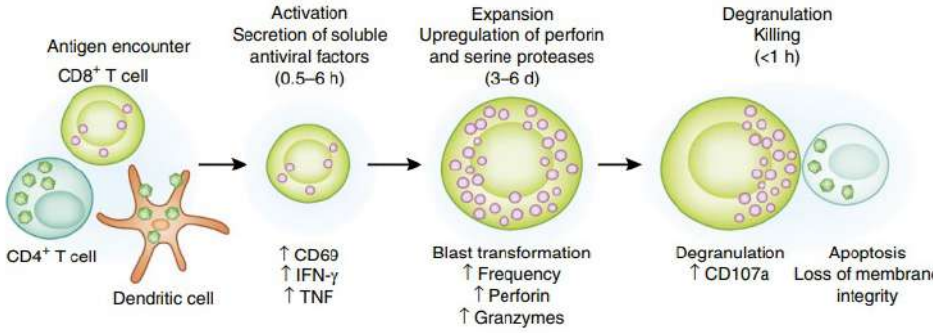


Figure 5. Sequential activation, expansion, and acquisition of cytotoxic functions by memory HIV-specific CD8+ T cells following antigen encounter. Image from Migueles *Nat Immunol,* 2015.

In fact, differences in viral recognition by CD8+ T cells between ECs and progressors are influenced by several factors, including effector-cell characteristics (specificity, breadth, responsiveness to variant epitopes, total frequencies of T cells, clonal composition, and T-cell receptor -TCR- diversity), viral factors (such as deletions or mutations in regulatory genes like *nef* that downregulates MHC), and host major HLA-related factors. The latter include protective polymorphisms and high-affinity epitope presentation. Importantly, the beneficial role of specific HLA alleles in shaping effective CD8+ T cell responses is a hallmark of viral control in ECs, represented in **Figure 6.**

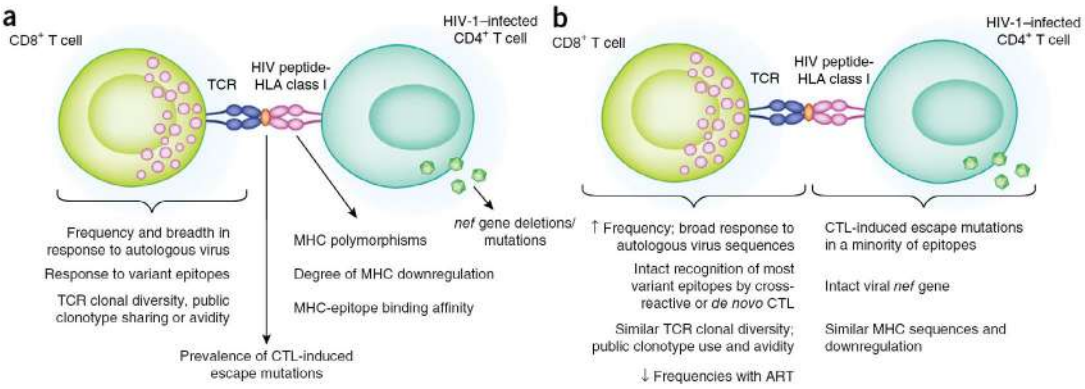


Figure 6. Factors influencing differences in the recognition of HIV-infected CD4+ T cells by CD8+ T cells, contributing to distinct viral control outcomes between ECs in panel a and progressors in panel b. Image from Migueles *Nat Immunol,* 2015.

Furthermore, **Exceptional Elite Controllers (EECs)** are a rare subset of ECs who maintain viral suppression without ART for over 25 years[47]. Interestingly, EECs exhibit weaker HIV-specific immune responses compared to typical ECs, likely due to prolonged viral suppression resulting in minimal antigen exposure and subsequent reduced immune stimulation. Additionally, proviral sequences in EECs show impaired CD4 receptor binding, and contain deletions, hypermutations, or other mutations compromising viral infectivity. Notably, intact proviruses in these individuals are frequently integrated into transcriptionally inactive or genetically silent regions of the genome, limiting their potential for reactivation and contributing to durable viral suppression in the absence of ART. Thus, the exceptional HIV control observed in EECs is likely driven by a combination of initial robust immune responses that effectively reduce viral exposure over time and intrinsic viral attenuation.

Despite these remarkable features, some ECs and EECs may eventually experience loss of control (LoC), characterized by sudden increases in viral replication [48–50]. Studies investigating this phenomenon have identified a reduced capacity of CD8+ T cells to mediate viral suppression in vitro, coupled with increased T-cell activation and exhaustion prior to LoC events[51,52]. Additionally, shifts in viral tropism from CCR5 to CXCR4 have been observed following LoC in some ECs, contributing further to disease progression. A case recently presented at the Conference on Retroviruses and Opportunistic Infections (CROI) described an EEC who experienced viral rebound after maintaining control for over 32 years without ART[53], underscoring the complexity and potential instability of these long-term viral control mechanisms[54–56]. Studying these individuals provides valuable insights for developing strategies aimed at achieving a scalable and durable HIV cure.

In addition to EC and EEC, **post-treatment controllers (PTCs)** constitute a group of PWH with the ability to control HIV in the absence of ART but after a previous course of ART. It is important to note that, unlike ECs, PTCs are not enriched in beneficial HLA-I alleles associated with HIV control, and that their CD8 T cell responses are particularly rich in secreting high magnitudes of interferon-gamma (IFN-γ), suggesting that probably their responses at primary infection are different from those that contribute to control in EC [57,58].

First study of PWH in whom a PTC phenotype was described were retrospectively identified in the French VISCONTI (Viro-Immunological Sustained CONtrol after Treatment Interruption) cohort (*n*=14, etPWH) [59] which included PWH who had initiated ART very early during primary HIV infection and who were able to maintain long-term virological control without ART after interrupting treatment under clinical monitoring. After VISCONTI, several cohorts, including PRIMO, CASCADE, SPARTAC, and OPITPRIM,[60–63] have attempted to estimate the prevalence of PTCs and to better define their clinical and immunological features. These studies highlighted the rarity and heterogeneity of PTCs, as well as the influence of factors such as timing of ART initiation, baseline VL, and host immune responses. Recent findings further suggest that transient viral exposures can drive functionally coordinated humoral immune responses in PTCs, contributing to the diversity of immune profiles observed among these individuals [64].Collectively, these efforts culminated in the CHAMP meta-analysis (Control of HIV after Antiretroviral Medication Pause), which included more than 700 PWH who discontinued ART across 14 trials conducted between 1996 and 2014, providing the most comprehensive estimate of PTC prevalence to date[65] . Considering PTC as those PWH with plasma viral load <400 copies/mL for at least 24 weeks after ART interruption, the CHAMP study estimated that up to 13% of early-treated individuals were PTCs compared with only 5% of PWH that started ART in chronic phases of infection. Further immunological characterization of these individuals, has highlighted key features associated with PTC, including a more stable HIV reservoir, lower $CD4^+$ T cell exhaustion, and notably, enhanced NK cell responses. These findings suggest that functional NK cell activity may play a critical role in maintaining viral suppression after ART cessation[58]. Also, a recent meta-analysis including individual data from 382 participants of 24 prospective ATI trials using more frequent pVL monitoring and employing a stricter PTC definition—pVL <50 copies/mL at day 84 after ART interruption—has provided new estimates on the frequency of PTC[66]. Gunst et al[66] reported a global PTC rate of 4% (6% in early-ART and 1% late-ART), offering a more precise and stringent estimate of the proportion of individuals who maintain viral control after ART interruption. These methodological advancements challenge previous assumptions and provide a more accurate framework for assessing post-treatment HIV control. The understanding of viral dynamics during treatment interruption, along with the expected rates of PTC, is crucial when evaluating the effectiveness of novel strategies in post-intervention control in clinical trials and will inform the design of future randomized clinical trials (RCT). In **Figure 7,** different mechanisms and characteristics between ECs and PTC that have been described across multiple studies are detailed.

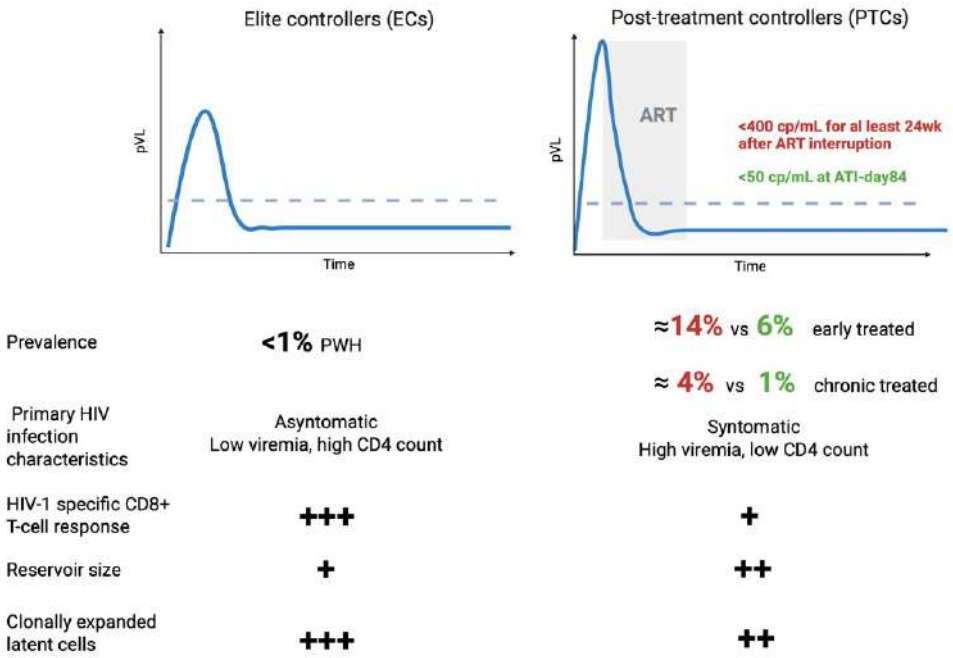


Figure 7. Comparative profiles of ECs and PTCs. In the PTCs group, data is highlighted in red or green according to the different VL criteria for the PTC definition. Created by BioRender based on [58,66,67]

1.2.3. HIV reservoir as a critical barrier of an HIV cure

HIV cure strategies face a major challenge due to the establishment of a latent viral reservoir shortly after HIV acquisition. Within the first 72 hours of infection, viral seeding occurs in lymph nodes and gut-associated lymphoid tissues (GALT), as demonstrated in animal models and studies of individuals with AHI[68, 69]. Despite the implementation of early ART, this process is not fully prevented, as shown in the RV254/SEARCH 010 cohort, where immune activation and the expansion of infected cells occurred even under early ART initiation[70]. The persistence of this reservoir is primarily driven by memory CD4+ T cells harboring integrated HIV DNA, whose low transcriptional activity allows them to evade immune clearance[71, 72]. Moreover, other long-lived immune cells, such as tissue-resident macrophages, have been shown to contribute to viral persistence, particularly during viral rebound after treatment interruption. Importantly, the reservoir is widely distributed across tissues, with only a small fraction detectable in peripheral blood, which adds further complication to quantify and target it effectively.

Following its establishment, reservoir dynamics follow a biphasic decay model upon ART initiation. There is an initial rapid decline during the first months of ART (half-life ≈ 2 weeks), followed by a markedly slower decay phase (half-life ≈ 44 months), and a subsequent

stabilization or setpoint after 2–3 years of sustained treatment. However, recent data suggest that after approximately 7 years of continuous ART, the size of the inducible, replication-competent reservoir no longer declines and may even remain stable due to homeostatic proliferation of infected cells [72, 73] . Moreover, the composition and integration patterns of the reservoir may also evolve over time. Under long-term ART, the reservoir becomes progressively dominated by expanded clones of CD4+ T cells carrying intact proviruses preferentially integrated into transcriptionally repressive regions such as centromeric satellite and microsatellite DNA[74]. This progressive enrichment of proviruses in heterochromatin likely reinforces deep latency by limiting proviral transcription and reducing spontaneous viral reactivation.

Additionally, the latent reservoir is not a uniform entity but rather a heterogeneous collection of infected cells, which can be classified into three main categories according to their capacity to produce the virus and their level of transcriptional activity[75]:

(1) the **defective reservoir**, composed of proviruses carrying mutations, deletions, or hypermutations that prevent them from generating infectious virus;

(2) the **inducible reservoir**, formed by intact proviruses that remain silent under ART but can be reactivated when cells receive appropriate stimuli, potentially leading to viral production;

(3) the **active reservoir**, consisting of cells that, even during ART, express low levels of viral RNA, although this is usually insufficient to produce fully infectious virions.

This conceptual framework is supported by quantitative analyses showing that intact, replication-competent proviruses represent only a small subset of the total HIV-1 DNA found in resting CD4⁺ T cells**. Figure 8** illustrates the relative abundance of different reservoir components—defective, intact but non-induced, and inducible proviruses—as quantified by viral outgrowth assays (VOA) and proviral DNA analyses[71,76].

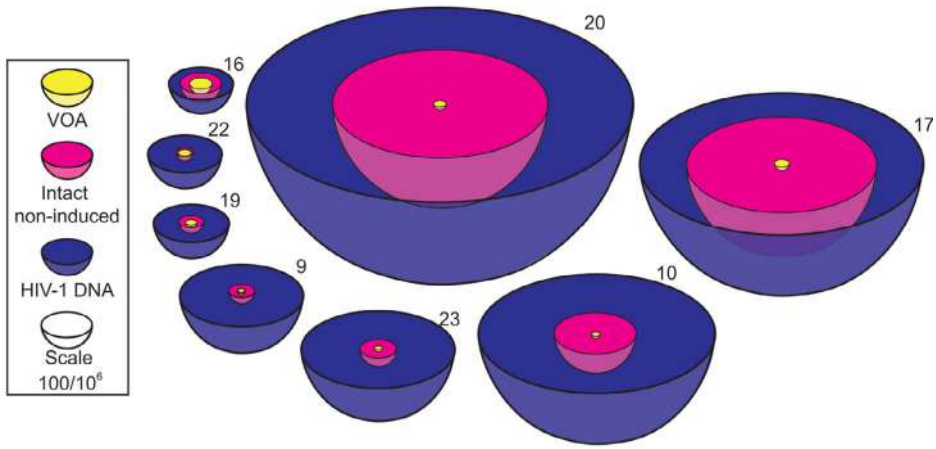


Figure 8. Quantification of the latent HIV-1 reservoir components. Adapted from Ho et al., *Cell*, 2013

Beyond transcriptional activity, this heterogeneity also encompasses cellular identity and anatomical distribution[77], as summarized in **Figure 9.**

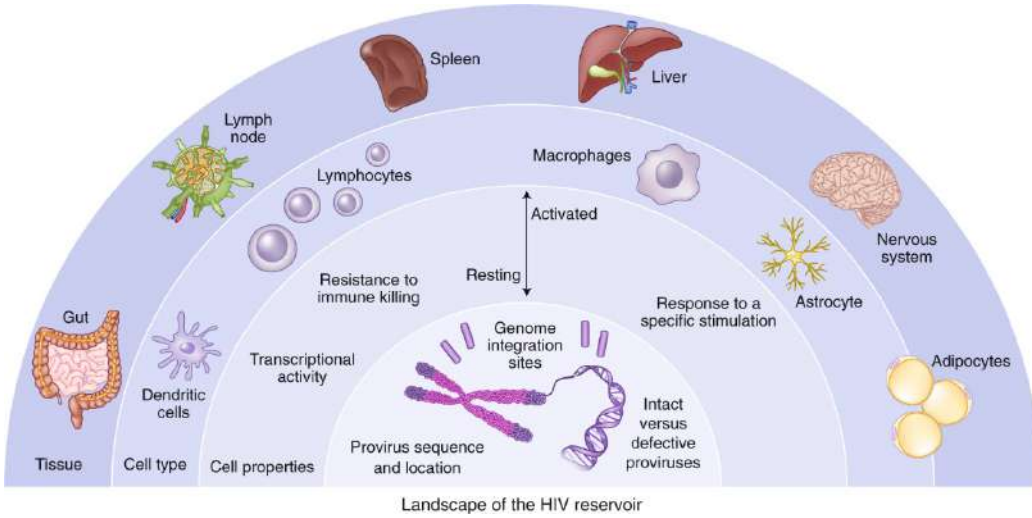


Figure 9. Diagram representing the multifaceted nature of the HIV reservoir, including its cellular and anatomical diversity, distinct proviral transcriptional profiles, and changes over time. Adapted from Deeks et al., *Nature Medicine*, 2021.

Multiple mechanisms contribute to the long-term maintenance of the HIV reservoir. First, **homeostatic proliferation** of memory CD4+ T cells allow the expansion of infected clones, without requiring antigenic stimulation. Second, **antigen-driven proliferation**, results from the chronic stimulation of memory cells by unrelated pathogens or environmental antigens, leading to the preferential expansion of HIV-infected clones[69]. This process has been evidenced by the identification of expanded clones bearing TCRs reactive to non-HIV antigens that nonetheless carry replication-competent proviruses. Third, although ART is highly effective, some evidence suggests that **low-level ongoing viral replication** might persist in anatomical sanctuaries with suboptimal drug penetration, such as lymphoid tissues or the central nervous system[76,78]. Moreover, HIV's ability to undergo **immune escape** via mutations in targeted epitopes, coupled with the gradual **exhaustion and dysfunction of virus-specific T cell responses**, further limits the host's capacity to clear infected cells[72] . These combined mechanisms create a resilient and genetically diverse reservoir that remains refractory to current therapeutic strategies, posing a formidable obstacle to achieving either a sterilizing or a functional cure[75] .

1.2.4. Overview of cure strategies

Building upon the understanding of the establishment, complexity and persistence mechanisms of the latent reservoir, numerous strategies have been developed aiming to overcome these challenges. These approaches generally fall into two broad and complementary categories: interventions directed at reducing, reactivating or permanently silencing the viral reservoir, and immunotherapeutic strategies designed to enhance the ability of the immune system to detect and eliminate infected cells. More recently, combination strategies integrating both components have gained interest, as it has become increasingly clear that neither reservoir reduction nor immune enhancement alone is likely to be sufficient to achieve a functional cure[77,79].

1.2.4.1. Strategies targeting the HIV reservoir

One of the earliest strategies explored to reduce the HIV reservoir focused on intensifying antiretroviral therapy. Following the establishment of triple-combination ART as the standard of care, several studies tested whether adding additional drugs could further suppress residual viremia and limit reservoir size. Trials incorporated agents such as raltegravir or combinations of raltegravir[80] and maraviroc[81] to block eventual low-level viral replication persisting under standard ART. However, these studies consistently failed to demonstrate significant impacts on the reservoir size or on residual viremia, suggesting that intensification alone is insufficient to reduce the established reservoir.

Subsequently, efforts focused on the timing of ART initiation, as early treatment during acute/recent infection was shown to limit reservoir size and diversity and preserve immune function, it was suggested that early ART could lead to PTC. Studies suggest that the reservoir stabilizes within two to three years after infection, highlighting a potential window for intervention. However, even when ART is initiated as early as in Fiebig I stage, as demonstrated in the Thai RV411 study, all participants experienced rapid viral rebound after treatment interruption. These findings indicate that although early ART reduces the reservoir, it is insufficient to achieve durable viral remission by itself. Further insights into reservoir dynamics have shown that most of the replication-competent proviruses found in long-term ART-treated individuals are closely related to the viruses circulating just prior to therapy initiation[68]. This suggests that ART may contribute to the stabilization of the reservoir, highlighting the importance of targeting this specific period to limit reservoir formation.

Given the limited impact of ART intensification and early treatment on achieving sustained remission, subsequent strategies have focused on directly targeting the latent reservoir itself. One of the most explored approaches aims to reverse viral latency to render infected cells visible to the immune system which are then killed by immune-based clearance mechanisms, commonly referred as "**kick and kill strategies**", which will be discussed in detail in later sections. Notably, the "kick" step involves using latency-reversing agents (LRAs) to induce viral gene expression in cells harboring transcriptionally silent proviruses. Several classes of LRAs have been investigated, including histone deacetylase inhibitors (HDACis), protein kinase C agonists, and Toll-like receptor agonists, all seeking to trigger proviral transcription. However,

latency reversal remains an unresolved challenge, as current agents often show limited in vivo potency and may fail to reach all reservoir compartments.

An alternative strategy to latency reversal is the so-called "**block and lock**" approach, which attempts to maintain proviruses in a deeply silent state, reducing the risk of viral rebound upon ART interruption[82]. Mechanistically, this approach involves blocking HIV transcription and locking the viral promoter through epigenetic modifications, effectively rendering the provirus transcriptionally inert. By enhancing the depth and stability of latency, these strategies aim to prevent both spontaneous and stimulus-induced viral reactivation. While still in early stages of development, "block and lock" is regarded as a promising avenue, particularly for achieving long-term viral control without relying on continuous ART or immune-mediated clearance. As illustrated in **Figure 10**, both the "kick and kill" and "block and lock" strategies aim to target the latent reservoir through fundamentally different mechanisms.

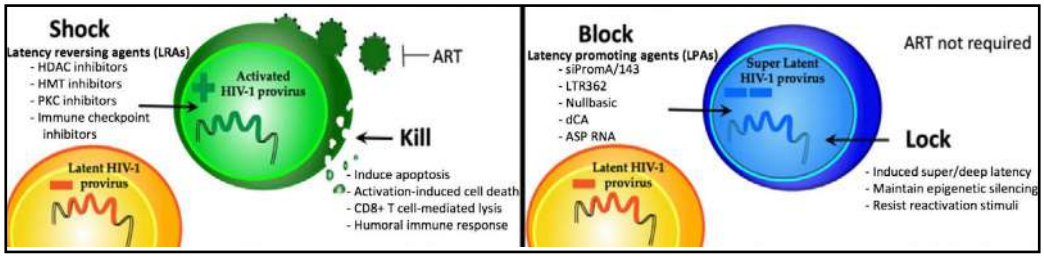


Figure 10. Schematic overview of "kick and kill" and "block and lock" strategies. The figure illustrates two latency-targeting approaches: reactivation and immune clearance in "kick and kill", and transcriptional silencing in "block and lock". *HDAC, Histone deacetylase; HMT, histone methyl transferase; PKC, protein kinase C; dCA, didehydro-cortistatin A; ART, antiretroviral therapy.* Image adapted from Ahlenstiel, C, Front. Cell. Infect. Microbiol, 2020.

1.2.4.2. Strategies targeting the immune response

Given the limitations of reservoir-targeted strategies alone, considerable efforts have been directed toward enhancing the immune system's capacity to detect and eliminate infected cells. A central objective has been to increase the breadth and depth of HIV-specific cytotoxic T lymphocyte (CTL) responses, to better recognize diverse viral variants and reduce the impact of immune escape. Furthermore, improving specificity and overcoming immunodominance patterns are considered essential to broaden the response. Among these, **therapeutic vaccines** are of particular relevance and will constitute a central focus of this thesis, where their underlying rationale, development, and clinical application will be discussed in detail.

In parallel, other strategies aim to enhance the cytotoxic capacity and polyfunctionality of CTLs, boosting their ability to suppress and clear infected cells[83]. Chronic infection, however, drives immune exhaustion, characterized by the sustained expression of inhibitory receptors such as programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte antigen 4 (CTLA-4), or T cell immunoreceptor with Ig and ITIM domains (TIGIT) which has prompted the repurposing of immune checkpoint blockade therapies[83,84]. Another important barrier is the limited migration of effector cells to anatomical sanctuaries such as B cell follicles, a feature being addressed by engineering CXCR5-expressing CTLs[85]. Complementary strategies include the enhancement of innate immunity and the restoration of CD4+ T cell help, both essential for durable and effective reservoir clearance[85]. These combined approaches are now central to the design of next-generation HIV cure strategies (**Table 1** summarizes the major immunological barriers/targets and the therapeutic strategies designed to overcome them).

Table 1. Immunological barriers, immunological targets, and corresponding therapeutic strategies.

Immunological challenge	Objective	Therapeutic strategy
Limited breadth	Increase breadth of HIV-specific CTLs	Therapeutic vaccines expressing full-length proteins ('big antigens').
Rapid viral escape	Broaden epitope recognition (increase depth of HIV-specific CTLs	Therapeutic vaccines expressing *mosaic* antigens
Immunodominance patterns and specificity towards decoy HIV regions	Redirect specificity, shift in immunodominance patterns towards protective HIV regions and optimize TCR repertoire	Therapeutic vaccines expressing conserved non-dominant regions or based on selected beneficial regions (*HTI*)
Reduced cytotoxicity and polyfunctionality	Enhance effector functions of CTLs	Cytokine adjuvants (IL-15 superagonists), functionally enhanced adoptive T cell transfer, CAR-T, soluble TCR, TCR engagers, etc
T cell exhaustion	Restore CTL functionality	Immune checkpoint inhibitors (PD-1, CTLA-4, TIGIT blockade)
Limited migration to anatomical sanctuaries (e.g., B cell follicles)	Promote follicular homing of effector cells	CXCR5-expressing CAR-T cells or vaccines promoting CXCR5+ T cell induction
Impaired innate immunity and CD4+ T cell help	Boost innate immunity and helper T cell function	TLR and STING agonists, vaccines inducing robust CD4+ T cell responses

CTL, cytotoxic T lymphocyte; CAR-T, chimeric antigen receptor T cell; TCR-T, T cell receptor-engineered T cell; PD-1, programmed cell death protein 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; TIGIT, T cell immunoreceptor with Ig and ITIM domains; CXCR5, C-X-C chemokine receptor type 5; TLR, Toll-like receptor; STING, stimulator of interferon genes; HTI, HIVACAT T cell immunogen.

1.2.4.3. Broadly neutralizing antibodies as a dual antiviral&immune strategy

Among the various approaches currently being investigated, broadly neutralizing antibodies (bNAbs) have gained significant attention due to their unique ability to act at the interface between antiviral suppression and immune modulation[86]. bNAbs offer a dual mechanism of action: they directly neutralize circulating virions by targeting conserved HIV epitopes, and they promote immune-mediated clearance of infected cells via Fc-dependent functions such as antibody-dependent cellular cytotoxicity (ADCC). Recent clinical trials have shown that combinations of potent bNAbs targeting non-overlapping epitopes can maintain viral suppression after ART interruption, particularly in individuals without pre-existing resistant variants[87]. However, their therapeutic potential remains influenced by factors such as the sensitivity of archived viruses and the durability of bNAb plasma concentrations. Interestingly, early administration of bNAbs during acute infection has been associated with enhanced endogenous immune responses, suggesting their potential role in reshaping host immunity. In addition, bNAbs are being actively explored during analytical treatment interruptions (ATI), [88, 89] , aiming to control viral rebound while simultaneously boosting immune responses. Moreover, recent clinical studies have also shown that prolonged bNAb therapy can impact the HIV-1 reservoir, leading to measurable changes in the size and composition of the intact proviral pool. These findings highlight the potential of bNAbs not only to control viremia but also to contribute to reservoir reduction strategies[90].

In summary, HIV cure research currently encompasses a broad range of strategies targeting both the viral reservoir and the host immune response. The combination of different approaches will be essential to advance towards a functional cure. This concept is illustrated in **Figure 11**.

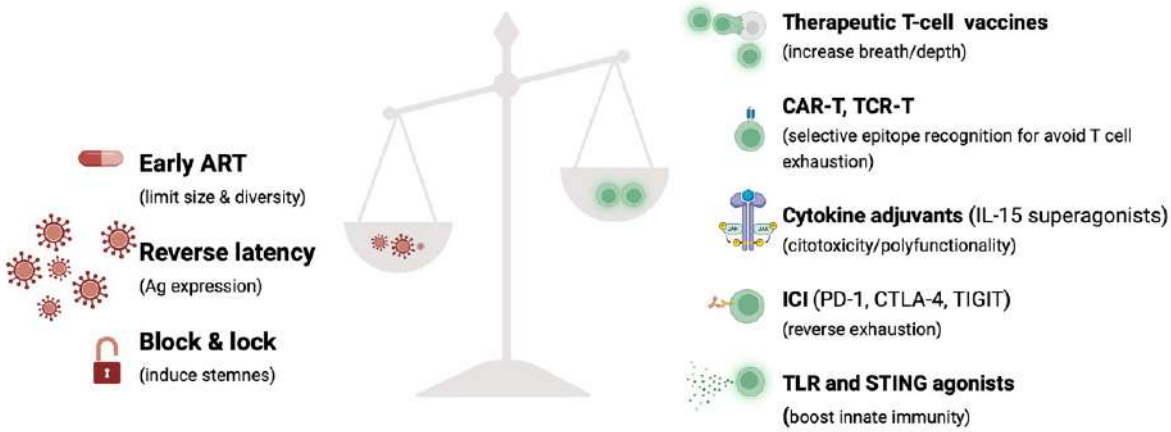


Figure 11. Balance of HIV cure strategies. Overview of reservoir-targeted (in red) and immune-based strategies (in green), highlighting their complementarity in current HIV cure research.

1.2.5. Early ART as starting point toward an improved HIV control

As before mentioned, the timing of ART initiation has emerged as a critical determinant of HIV reservoir formation and immune preservation, and today constitutes one of the pillars of HIV cure research. Initiating treatment during acute or recent infection has consistently been associated with marked reductions in reservoir size, lower viral diversity, and better-preserved immune function. Individuals who start ART early after HIV acquisition harbor smaller reservoirs composed of less immune-escaped viral populations and maintain more effective CTL responses[91], limiting immune exhaustion. This understanding has been shaped by key clinical cohorts. The RV254/SEARCH 010 study provided clear evidence that very early ART, when initiated during the first Fiebig stages, reduces reservoir seeding, immune activation, and systemic inflammation. The IMPAACT P1115 trial[92], focused on perinatally infected infants, demonstrated that early ART not only reduces the reservoir size but, in selected cases, may allow prolonged periods of post-treatment remission. Complementary results from adult cohorts, including EPICAL[70] and FRESH[93], confirmed that early ART consistently preserves immune function and limits reservoir diversification. Moreover, the progressive implementation of test-and-treat strategies and the widespread adoption of INSTI-based ART have likely contributed to these benefits in clinical settings[92], beyond controlled studies. All these factors have contributed to prioritize early-treated individuals in several cure clinical trials.

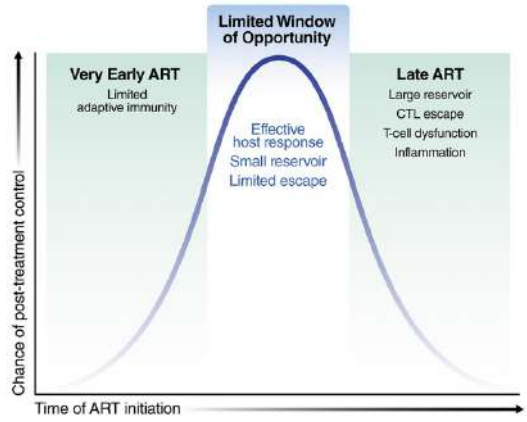


Figure 12. Impact of ART timing on reservoir establishment and immune dynamics. Image adapted form Goulder and Deeks, *PloS Pathogens*, 2018.

However, early ART alone has not consistently translated into durable viral remission. Both pediatric and adult studies have shown that, despite initial post-treatment control, viral rebound occurs in the vast majority of cases. The 2013 "Mississippi Baby"[94] case drew global attention as a promising example of early ART; the infant, treated just 31 hours after birth, achieved undetectable VL and maintained remission for over two years after stopping ART. Yet, viral rebound occurred at 27 months post-interruption, underscoring the complexity of achieving sustained viral control. Additional pediatric cases have consistently demonstrated that, although early ART reduces the viral reservoir, this reduction alone is often insufficient to achieve a cure[95]. Similarly, in the Thai RV411[70] study, adults treated during Fiebig stages I–II also experienced rapid viral rebound after ART interruption, despite reduced reservoir size and preserved immune function. These immunovirological advantages translate into an increased likelihood of achieving prolonged post-treatment control and a greater capacity to respond to therapeutic interventions such as vaccines, immune checkpoint inhibitors, and other immunomodulatory strategies. As a result, most current remission trials strategically

select early-treated individuals, as they represent the population most likely to benefit from combinatorial cure approaches. **Figure 12** summarizes the impact of ART timing on reservoir and immune dynamics, highlighting how early-treated individuals represent a population with a favorable immunovirological profile, providing a critical window of opportunity for HIV remission trials[96].

1.2.6. Therapeutic vaccine as a back-bone of HIV remission strategies

The development of an effective therapeutic vaccine is a cornerstone not only for achieving HIV remission but also for advancing preventive vaccine strategies. Whether the ultimate goal is prevention or cure, both humoral and cellular immune responses will be necessary. Similar to many other vaccines designed to combat infectious diseases, preventive HIV vaccines may not achieve 100% efficacy in blocking infection. Incorporating a cellular immune component into vaccine design could potentially lower the breadth or the threshold of antibody titers required for protection. Moreover, a robust, rapid, cytotoxic, and functional cellular response could substantially complement the immune defense against HIV following acquisition.

Therapeutic vaccines have gained significant attention due to their potential to enhance virus-specific immune responses and promote long-term immune-mediated control of HIV. Among the first platforms explored was **dendritic cell (DC)-based vaccines**, which leverage the potent antigen-presenting capacity of DCs to enhance HIV-specific immunity. These vaccines typically involve the ex vivo manipulation of autologous DCs loaded with HIV antigens, followed by reinfusion into the patient to stimulate a more effective antiviral response. While DC-based vaccines have demonstrated immunogenicity in clinical trials, their impact on sustained viral control remains limited. For instance, autologous dendritic cell vaccination has been associated with only a transient 1-log reduction in VL set points, with the effect waning after treatment discontinuation[97].

In parallel, **T cell-based vaccines** were designed to elicit robust cytotoxic T lymphocyte (CTL) responses, taking advantage of the generated knowledge on immune mechanisms previously described in natural HIV controllers. By enhancing the immune system's ability to recognize and eliminate infected cells, these vaccines have remained at the forefront of HIV cure research. Ongoing advances in vaccine design—including improved immunogen selection, heterologous prime-boost regimens, and combination approaches with immune modulators—offer new possibilities for enhancing their efficacy.

1.2.6.1. Vaccine design

1.2.6.1.1. Immunogen/insert

The **immunogen** is a critical component of a therapeutic vaccine, designed to elicit virus-specific immune responses capable of recognizing and controlling HIV replication. Immunogens consist of viral full-length proteins or selected antigenic regions intended to

stimulate CTLs and helper T cells in PWH, enhancing immune control. One of the main challenges in immunogen design is addressing the extensive genetic diversity of HIV and its ability to escape HLA-mediated immune responses. An optimal immunogen should achieve broad coverage, accommodating the diversity of circulating viral subtypes and the heterogeneity of HLA alleles across different populations. This is particularly relevant in light of studies, such as those by Kawashima et al[98] showing how HIV adapts to distinct HLA profiles in diverse ethnic groups.

Traditional immunogen design strategies have primarily focused on expanding the natural immune response to HIV, a principle underlying **whole protein-based vaccines**[99]. These vaccines typically incorporate entire viral proteins or consensus sequences that represent prevalent viral variants, often targeting highly immunogenic proteins such as Env, Pol, and Gag[100,101]. However, recent advancements have introduced innovative approaches like **mosaic immunogens**[102], which integrate multiple viral variants into a single construct to enhance coverage against HIV's genetic diversity.

Beyond mimicking the natural immune response, alternative strategies seek to reshape immunodominance patterns by directing responses toward more conserved regions shared among different viral variants or by emulating immune responses observed in individuals who exhibit superior viral control (i.e. VC/EC) through potent and highly functional T cell responses. These **antigen-selection-based** approaches focus on conserved elements of the HIV proteome, as exemplified by the HIVconsv design[103], or on critical functional regions essential for viral replication, referred to as "functional targets". Notably, some designs, like the HTI immunogen, have been based on human immunogenicity data, derived from analyses of immune responses in PWH - rather than viral sequence, to identify regions of the proteome that are consistently and effectively targeted by antiviral CD8+ T cells. This strategy aims to prioritize epitope-rich regions that are not only conserved but also immunologically relevant in humans, maximizing their potential to elicit functional responses[104].

A new strategy under preclinical development, is the identification of so-called **networked-informed epitopes**[105], which are defined by their structural connectivity within the three-dimensional viral protein architecture. Targeting these epitopes may help direct immune responses toward regions where mutations are likely to have a high cost in terms of fitness for the virus, limiting its ability to escape. Finally, **personalized immunogen** strategies have been also investigated to elicit immune responses to specifically target viral regions that remain unaffected by the individual's HLA-mediated selective pressure as identified in their own viral reservoir sequences[106]. While this approach holds great promise, its large-scale implementation remains challenging due to the sheer magnitude of the epidemic, which affects over 38 million people worldwide.

The future of immunogen design lies in striking an optimal balance between broad coverage and targeted efficacy, leveraging cutting-edge technologies such as artificial intelligence, epitope prediction tools, and protein engineering to develop more effective and widely accessible vaccines.

1.2.6.1.2. Vectors

Vectors serve as delivery platforms to present immunogens to the immune system efficiently, promoting antigen expression and immune stimulation. For therapeutic vaccines, the main goal is to induce robust and durable T-cell responses, particularly cytotoxic CD8+ T lymphocytes capable of recognizing and eliminating infected cells[107]. Several vector platforms have been evaluated in the context of HIV immunotherapy, including plasmid DNA, viral vectors, and, more recently, messenger RNA (mRNA) technologies[108].

Plasmid DNA vectors represent one of the earliest platforms tested in HIV vaccines due to their safety, stability, and ease of manufacturing[109,110]. DNA vaccines consist of plasmids encoding the immunogen under the control of an eukaryotic promoter. Although DNA vaccines are poorly immunogenic when used alone in humans, they are highly effective as priming agents in heterologous prime-boost regimens, especially when followed by viral vectors to enhance T-cell responses.

Viral vectors have become the most widely used delivery systems in therapeutic HIV vaccines, owing to their strong capacity to elicit potent cellular immune responses [111]. Among these, attenuated non-replicating adenoviral vectors—such as **chimpanzee adenovirus** [112] (ChAd) and human Ad26—have been widely studied. The selected vectors allow evasion of pre-existing anti-adenoviral immunity and efficiently prime T-cell responses in humans. The rationale for their use stems partly from the STEP trial, which used human adenovirus serotype 5 (Ad5) and revealed that pre-existing anti-Ad5 antibodies could interfere with vaccine immunogenicity and even raise concerns about vaccine-enhanced susceptibility to HIV infection[101]. These findings prompted the development of alternative adenoviral vectors with lower seroprevalence in humans, such as Ad26, ChAd, and even gorilla-derived vectors. Recently, rare cases of venous thrombosis with thrombocytopenia syndrome associated with COVID-19 vaccines using ChAd and Ad26 have highlighted the need for careful vector selection and safety monitoring[101,113]. M**odified vaccinia Ankara** (MVA), a highly attenuated poxvirus, has also been commonly employed as a boosting viral vector due to its excellent safety profile and strong immunogenicity. MVA-based vectors are particularly effective at amplifying T-cell responses when used in heterologous prime-boost strategies. Both adenoviral and MVA platforms have demonstrated the capacity to stimulate broad and polyfunctional CD8+ T cell responses in multiple clinical settings [114].

mRNA-based vectors have recently emerged as a versatile and promising platform, particularly following the success of mRNA vaccines to other infectious agents, such as SARS-CoV-2 [115,116]. A major breakthrough in the development of these vaccines was the stabilization of the mRNA molecule through nucleoside modifications and its encapsulation in lipid nanoparticles (LNPs). This innovation protected the mRNA from degradation and enabled efficient intracellular delivery, paving the way for rapid, scalable vaccine production. While LNPs were instrumental in the clinical success of COVID-19 mRNA vaccines, they have also been associated with increased reactogenicity, likely due to innate immune activation triggered by the lipid components[117,118]. These vaccines deliver synthetic mRNA encoding the immunogen,

enabling its expression directly in host cells without the need for a viral carrier. This platform is characterized by its rapid manufacturing capacity, intrinsic adjuvant properties, and ability to induce both humoral and cellular immune responses. While initially mainly explored for prophylactic use, mRNA vectors are increasingly being investigated as part of therapeutic HIV vaccine strategies, alone or in combination with other platforms.

This diversity of platforms and their respective immunological features are schematically summarized in **Figure 13**, which compares the main vaccine technologies under investigation for infectious diseases, including HIV[119] . The safety of these platforms is a critical consideration, and their design ensures that they are replication-deficient and incapable of causing disease while maintaining their immunostimulatory capacity. Furthermore, modern vector development focuses on minimizing anti-vector immunity, which could otherwise impair vaccine efficacy, and on allowing repeated administrations when necessary. Continuous advancements in vector engineering are progressively improving their immunogenicity, safety profile, and scalability, consolidating their role as essential tools in current and future HIV therapeutic vaccine strategies[120].

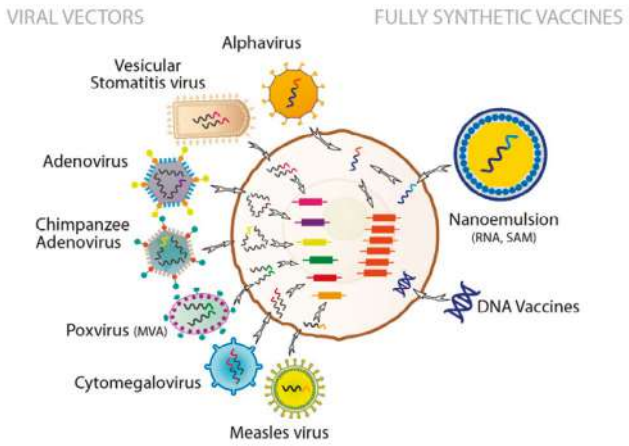


Figure 13. Overview of current vaccine platforms and their immunological properties. Schematic representation of the main characteristics of DNA, mRNA, and viral vector-based vaccines, including their relative capacity to induce innate immunity, cellular and humoral responses, and memory. Figure adapted from Rappuoli et al., *Proc Natl Acad Sci USA*, 2017[119].

1.2.6.1.3. Adjuvants

Adjuvants are critical components in the development of therapeutic vaccines, not only enhancing the magnitude and durability of immune responses but also modulating their qualitative features, which are essential for achieving effective control of viral replication. In therapeutic vaccination settings, adjuvants are particularly relevant for the induction of

potent HIV-specific cellular immune responses, especially CTLs. Among the most commonly employed adjuvants are classical aluminum salts (alum), oil-in-water emulsions (such as MF59 and AS03), Toll-like receptor (TLR) agonists (e.g., CpG ODNs, Poly I:C, or GLA-SE), and cytokine-based adjuvants like IL-12 or GM-CSF[121,122]. These agents can overcome the intrinsic low immunogenicity of protein- or peptide-based platforms frequently used in therapeutic designs[122,123]. Importantly, the advent of systems vaccinology has enabled the dissection of the complex immunological networks triggered by therapeutic vaccination[124], providing valuable insights into how specific adjuvants shape immune responses, influence immunodominance patterns, and modulate the induction of effector and memory populations. In addition to their immune-potentiating role, adjuvants also contribute to improving vaccine scalability by reducing the antigen doses required and can tailor the balance between mucosal and systemic immunity depending on the administration route, a crucial consideration given the compartmentalization of HIV infection.

1.2.6.2. Vaccine Regimen

Various therapeutic vaccination regimens have been tested. In addition to the selection of antigens, vector type, delivery route, dose, adjuvants, deciding on number of immunizations, prime/boost intervals can critically influence the magnitude and quality of the immune response. In general, **heterologous** regimens typically combine different vaccine platforms, often employing prime-boost strategies[125] are used to potentiate immunogenicity. These may include the combination of viral vector-based vaccines with non-viral platforms to deliver HIV antigens, followed by protein or peptide-based boosts to enhance cellular and humoral responses. By engaging different arms of the immune system at distinct stages, heterologous regimens aim to elicit robust and durable responses.

1.2.6.3. HTI: HIVACAT T-cell immunogen

The HIVACAT T-cell immunogen (HTI), was designed at IrsiCaixa and licensed to AELIX Therapeutics for its clinical development. **HTI-immunogen is aimed at redirecting T-cell responses towards the most vulnerable regions of the HIV-1 proteome**. Its design is based on the hypothesis that HIV-specificity matters, that not all HIV-specific T-cell responses are equally effective, and that protective immunity is associated with responses targeting specific regions of the virus.

The design of HTI was based on the systematic analysis of immune data from over 1,000 PWH with multiple viral loads in the absence of ART and combining data from multiple cohorts, including EC and chronic progressors. The analysis mapped the regions within the full HIV-1 proteome that were most frequently targeted by virus-specific CD8+ T cells and associated with lower VL[104] and gave to each overlapping peptide tested, a protective ratio. A total of 26 overlapping peptides were selected by their protective ratio (immunogenicity associated with lower VL), and by their functional relevance and conservation across viral subtypes, minimizing the risk of immune escape. The selection process led to a 529 aminoacid immunogen sequence, including **16 regions**, each ranging from 11 to 78 aminoacids, collectively covering

highly conserved and immunologically relevant segments of the Gag (45%), Pol (44%), Vif (8%), and Nef (3%) proteins, linked by either single, dual or triple alanine residues to induce preferential inducing preferential proteolytic cleavage between segments. Notably, the design intentionally avoided regions frequently targeted by ineffective or immunodominant responses in chronic infection, which are often associated with viral escape mutations. Additionally, the HTI sequence was tailored to maximize HLA class I and II coverage by prioritizing epitopes presented by a wide range of prevalent HLA alleles in the global population. This strategy aimed to ensure broad applicability of the immunogen across diverse populations and to promote the induction of subdominant, yet protective, T-cell responses typically underrepresented in chronic progressors.

Since its conception, HTI has been incorporated into various vaccine platforms, including plasmid DNA, ChAdOx1, MVA, and, in exploratory settings, mRNA. This is schematically represented in **Figure 14**.

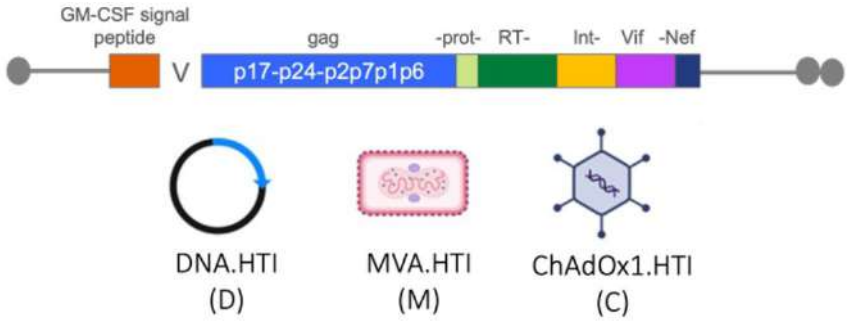


Figure 14. HTI immunogen and its main vector platforms. Schematic representation of the HTI sequence structure and the three vectors used for its delivery in clinical trials: plasmid DNA, modified vaccinia Ankara (MVA) and chimpanzee adenoviral vector (ChAdOx1). Created by BioRender.

To date, HTI has been tested in five independent clinical trials, involving diverse populations and combinations of immunomodulators and vaccine platforms. These studies (summarized in **Table 2**) have substantially contributed to the characterization of HTI's immunogenicity and safety profile.

Note: A detailed analysis of the design, safety, immunogenicity, and virological outcomes of the AELIX-002 and AELIX-003 studies will be presented in article 1 and article 2 of this thesis.

Table 2. Overview of clinical trials evaluating HTI-based therapeutic vaccines. Summary of phase I/II trials conducted with the HTI immunogen in different populations.

	iHIVARNA NCT02413645 & NCT02888756	AELIX-002 NCT03204617	AELIX-003 NCT04364035	BCN03 NCT05208125	HIV-CORE 0051 NCT04563377
Sample size (n)	21 + 70	45	50	30	10
Population	Chronic ART PWH	Early-ART PWH	Early-treated PWH	Chronically-treated PWH	Volunteers without HIV
Intervention	Intranodal mRNA-HTI-TriMix	DDDMM-CCM	CCMM+VES	CM+SOSIPv.7+gp140	CM
Phase	Phase I and IIb	Phase I/II	Phase I/II	Phase I/II	Phase I
Year	2015-2017	2017-2021	2020-2022	2021-2023	2021-2022
RCT / Control	Phase I: dose escalation. Phase IIa: Randomized, double-blinded, placebo-controlled (2:1:1)	Randomized, double-blinded, placebo-controlled (2:1)	Randomized, double-blinded, placebo-controlled (2:1)	Randomized, double-blinded, placebo-controlled (2:1)	Single-arm

1.2.7. Challenges of HIV cure/remission trials

1.2.7.1. Study design

Designing HIV cure/remission trials poses multiple challenges[126]. The selection of the **study population** is one of the first key determinants in trial design. As mentioned previously, several HIV therapeutic vaccine studies focus on individuals who initiated ART during acute or recent infection because they exhibit smaller and less diverse reservoirs, preserved immune responses, and a higher likelihood of PTC, making them ideal homogeneous population for remission-focused strategies at their first clinical testing[127]. However, this approach inherently difficult recruitment, since early-treated individuals represent a small fraction of PWH globally, and limit extrapolation of results to the diversity of the broader HIV population. Another critical aspect when testing immunotherapies such as T-cell vaccines is the distribution of HLA genotypes, which play a central role in shaping HIV-specific immune responses and are strongly associated with differential outcomes both in natural and treatment-induced viral control[128,129]. Certain HLA class I alleles, such as HLA-B57 or HLA-B27, are overrepresented among VC and EC, potentially confounding trial outcomes if they are not adequately balanced across study

arms[129]. To minimize bias and ensure a more accurate evaluation of vaccine efficacy, recent trials as those presented in this thesis have incorporated **HLA stratification** at randomization. Nevertheless, the need to control for HLA distribution, combined with the limited availability of early-treated participants, further complicates recruitment and power calculations. Finally, ensuring **diversity and inclusion** remains an ongoing challenge in HIV cure research, as women, racial minorities, and socioeconomically disadvantaged populations continue to be underrepresented due to structural, logistic, and social barriers, which are further exacerbated by the demands of ATI protocols[130]. Similarly, research efforts must be global in scope and include representation of diverse HIV subtypes, particularly non-B subtypes. Moreover, ATI protocols frequently require the use of pre-exposure prophylaxis (PrEP) by HIV-negative sexual partners of participants and disclosure of HIV status to anonymous or casual partners, which can create barriers to participation [131]. To ensure wide representation and equitable access to HIV cure research, early engagement with community members during trial design is essential. Strategies such as educational programs to reduce participation hesitancy, targeted outreach efforts to underrepresented groups, and revisiting trial criteria to balance safety with inclusivity should be implemented. These measures are crucial for ensuring broader representation and equitable access to HIV cure research.

S**ample size** calculation is also complex, as first-in-human or exploratory studies often lack preliminary data to inform realistic assumptions about intervention efficacy and there are no reliable surrogate markers for predicting long-term durable control. In addition, there is high biological and technical variability both in reservoir and immune parameters' measurements, which also impacts the precision of estimates and increases reliance on the efficacy of the intervention via an ATI. Additionally, inclusion of **placebo arms** remains the gold standard in HIV cure trials, as they are essential to evaluate the safety and distinguish true intervention effects from natural heterogeneity in reservoir, immune responses and remission phenomena. However, their inclusion introduces additional complexity, including trial-specific challenges to ensure study blinding, increased logistical burden, and concerns regarding participants acceptability, as the possibility of receiving placebo may reduce their willingness to be enrolled[132]. A recent meta-analysis has estimated that around 4% of placebo-recipients in controlled ATI studies may achieve spontaneous PTC, with higher rates among early-treated individuals (6%) than in chronically-treated individuals (1%), which has reignited the debate on the necessity of inclusion of placebo arms in future remission studies, at least for the ATI phase [66].

1.2.7.2. Safety

Ensuring participants´ **safety** is paramount. Prior experience with many of the investigational products tested—therapeutic vaccines, LRAs, immune checkpoint inhibitors, etc.—is very limited or they were initially developed for other clinical contexts, mainly other infectious diseases, autoimmune diseases and/or oncology[133], and their short and long-term safety profile in PWH has not yet been well characterized. Some of these interventions may lead to systemic inflammation, immune-related adverse events, cytokine release syndrome, and/or increase the risk of oncogenic transformation or malignancy due to their potential to induce off-target epigenetic modifications[134]. To mitigate these risks, therapeutic vaccine trials incorporate

comprehensive safety frameworks, including the presence of independent Safety Monitoring Committees (SMC), continuous pharmacovigilance, predefined stopping rules, and intensive-care-unit contingency plans for high-risk procedures. In addition, participants usually have access to 24/7 real-time contact with the study team, facilitating early detection and management of adverse events. Given the complexity of these interventions, participants must receive clear and detailed information regarding potential risks, trial objectives, monitoring procedures, and ART resumption criteria. Comprehensive informed consent procedures, supported by educational materials and one-on-one counseling, are crucial to enable autonomous and informed decision-making.

1.2.7.3. Analytical treatment interruption (ATI)

Despite the extensive development of laboratory assays to quantify the HIV reservoir and evaluate the immunogenicity of therapeutic vaccines, there are still no validated biomarkers that reliably predict durable viral remission after ART discontinuation. Consequently, temporary interruption of ART, through ATI, remains the only established method to assess the efficacy of curative interventions.

The design of past studies using ART interruption periods to ameliorate ART-related side effects and pill-fatigue relied primarily on CD4+ thresholds to guide ART resumption[135]. However, the SMART trial showed that uncontrolled viral replication during ART interruption led to increased risk of non-AIDS events and all-cause mortality, and these designs are since thereafter strongly discouraged. Conversely, modern protocols incorporate multiple risk-mitigation strategies, including strict inclusion criteria (excluding individuals with low CD4 nadir, ART resistance, or uncontrolled coinfections/cardiovascular risk factors), frequent virological and immunological monitoring, and well-defined ART resumption criteria[136,137]. Furthermore, current recommendations include active prevention strategies for HIV transmission to sexual partners, such as PrEP provision, STI screening, and behavioral counseling[138].

Actually, ATI designs might differ depending on the specific objectives/endpoints of each clinical trial. Studies aiming **to target the viral reservoir** often rely on short ATIs, using time to viral rebound—typically defined as the first confirmed plasma VL >50 copies/mL—as a primary endpoint, since recrudescence usually occurs within the first 3–5 weeks[139]. In contrast, trials evaluating **immune-based strategies**, such as therapeutic vaccines, generally use longer and more permissive ATIs to assess whether immune interventions can induce sustained post-intervention viral control which can occur after viral recrudescence. Short ATIs, while safer and more controlled, may underestimate the effects of interventions requiring a certain level of immune antigen-driven priming. Conversely, prolonged ATIs offer a more comprehensive assessment of immune-mediated control but carry additional risks, such as immune activation, higher CD4+ T-cell decline, viral evolution, reservoir reseeding, and potential participant attrition. From a scientific perspective, ATI provides invaluable information on the dynamics of viral rebound and the ability of immune interventions to modulate post-ATI viral setpoints[140]. However,

significant inter-individual variability—driven by reservoir size, ART timing, immune function, and host genetics—complicates the interpretation of results[138]. An illustrative evolution of ATI design is shown in **Figure 15.**

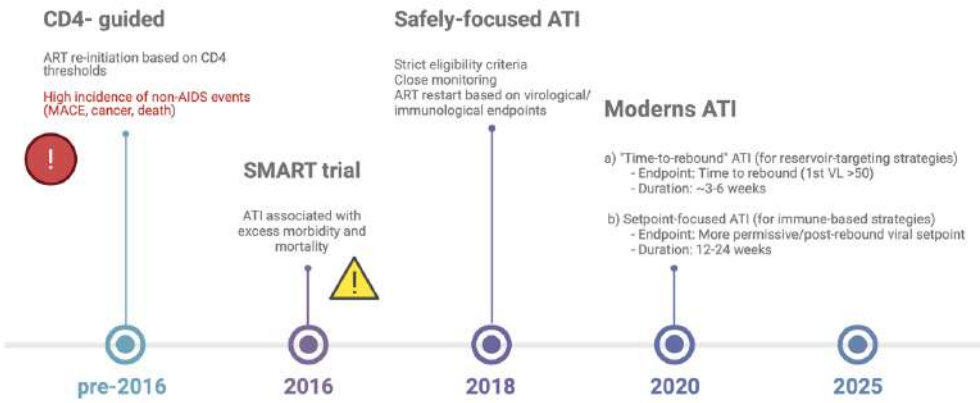


Figure 15. Evolution of treatment interruption designs. Early treatment interruption protocols based solely on CD4 counts were associated with excess morbidity and mortality. Modern designs incorporate safety-focused criteria, tailored durations depending on the intervention type, and structured risk mitigation strategies, although validated predictive biomarkers remain lacking. Created by BioRender, based on[136] .

Beyond HIV and non-HIV related biomedical risks, ATI trials introduce significant psychosocial challenges. Participants often report anxiety, fear of disease progression, and concerns about disclosure and stigma. To address these challenges, therapeutic vaccine trials increasingly integrate community engagement, psychosocial support, and tailored risk-mitigation strategies[141]. These include counseling on partner protection, systematic PrEP provision to HIV-negative partners[131,142], and regular monitoring of sexual health. Importantly, participants are allowed to resume ART earlier if psychological distress or other concerns arise during ATI; however, the implementation of this option remains heterogeneous across investigators and/or trial sites. Ongoing efforts aim to better support participants, reduce participation hesitancy, and ensure the ethical acceptability of ATI protocols. Creating a supportive, non-judgmental environment, where participants feel respected and empowered, is essential to enhance trial retention, improve overall study outcomes, and ensure a sustainable approach to HIV cure research.

Figure 16 summarizes key methodological and ethical challenges in the design of HIV cure trials.

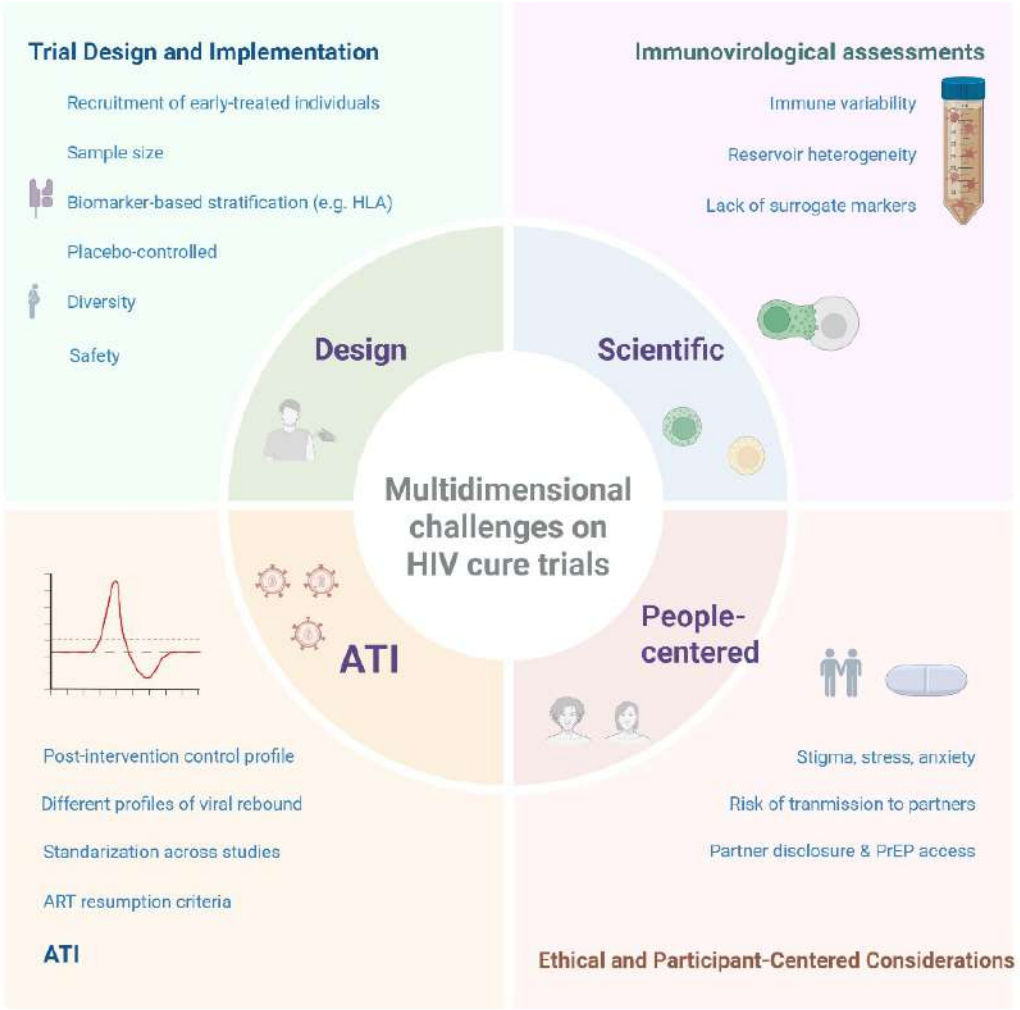


Figure 16. Challenges in HIV cure trial design. HIV cure trials face critical challenges, including defining efficacy endpoints, addressing sample size limitations, and managing the ethical considerations of placebo groups and ATI. Created by Biorender.

1.2.7.4. Interpretation of results

Interpreting the outcomes of therapeutic interventions in HIV cure research remains challenging. Measurements of both HIV reservoir size (e.g., total or intact proviral DNA, quantitative viral outgrowth assays) and/or immunological responses show high biological and technical variability. Moreover, how a reduction in viral reservoir or an increase in magnitude, breadth and/or functionality of HIV-specific response translate into post-intervention control (PIC) is uncertain. **PIC refers to the ability of individuals to maintain some degree of viral suppression after discontinuing ART following an immunotherapeutic intervention**. While the target product profile (TPP) on a successful HIV remission strategy is clearly the ability to induce a sustained virological control <50 copies/ml, other levels of PIC might still be informative to advance HIV cure research[143]. Emerging evidence suggests that PIC is a distinct phenotype[144], its definition varies across studies, making it difficult to compare findings and draw conclusions from different trials. PIC is commonly described by the degree, duration, and pattern of viral control observed during ATI. For instance, different PIC profiles have been described, including delayed viral rebound, non-exponential rebound, oscillatory viral loads, or sustained suppression. These patterns may reflect different underlying mechanisms, including residual antiviral effects, delayed viral reactivation, or enhanced HIV-specific immune responses. In addition, to each of PIC profile, different mechanisms may also be associated and difficult the interpretation of trial results, including early ART initiation, certain HLA class I alleles, and lower pre-ART viral load set points, suggesting overlapping biological pathways[64], represented in **Figure 17**.

Recent work by Sandel, A[144]. et al. underscores the importance of harmonizing the definition of PIC and standardizing reporting criteria across studies. They propose the creation of a centralized database for post-intervention controllers and the development of an infrastructure for biospecimen sharing to enable large-scale analyses. Establishing standardized methodologies would facilitate comparisons across clinical trials and accelerate progress toward long-term ART-free HIV remission.

Given the complexity of PIC, collaboration among research groups will be essential to refining its definition and ensuring consistency in study methodologies. Reaching a consensus on the definition of PIC will be key to optimizing trial design, participant selection, and harmonizing trial endpoints in future HIV cure research.

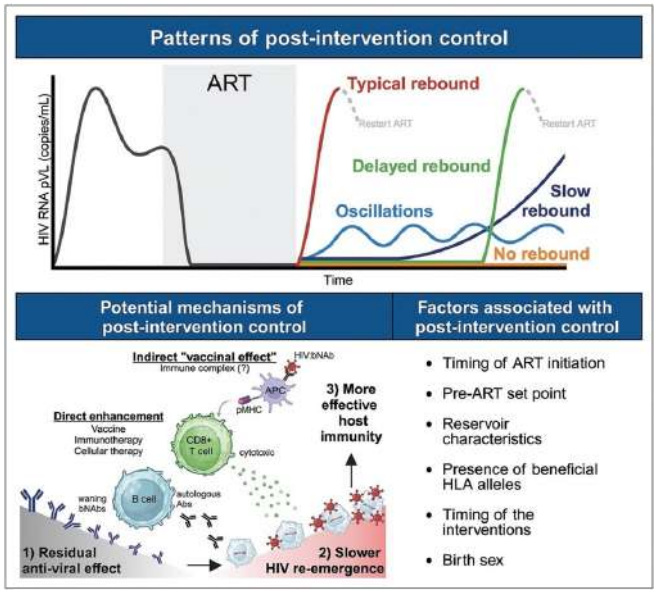


Figure 17. Patterns and potential mechanisms leading to post-intervention control. ART: antiretroviral therapy; HLA: human leukocyte antigen; APC: antigen-presenting cell; bNAbs: broadly neutralizing antibodies; pMHC: peptide-major histocompatibility complex. From Current Opinion in HIV and AIDS20 (1):70-79, January 2025.

In summary, despite the substantial progress achieved in the field of HIV cure research, the path towards achieving long-term ART-free remission remains challenging. Therapeutic vaccines represent a key component within a broader combinatorial strategy aiming to achieve PIC. However, crucial gaps persist, including the limited understanding of the immune mechanisms underlying spontaneous or vaccine-induced viral control, the absence of reliable biomarkers to predict post-intervention outcomes, and the need for strategies that balance scientific rigor with ethical considerations, particularly in the context of ATI-based trials. This thesis aims to contribute to this evolving roadmap towards HIV remission by advancing the clinical development of a novel therapeutic T-cell vaccine strategy and generating new insights into the immunological determinants of viral control. Ultimately, the findings presented here aspire to inform and inspire the design of future remission trials, contributing to the collective effort of the scientific community and PWH to move closer to a functional remission of HIV.

2. HYPOTHESIS

2. Hypothesis

The hypothesis of this thesis is that immunotherapeutic interventions based on therapeutic vaccines expressing the HTI immunogen, administered alone or in combination with other immunomodulators, will be safe, could enhance HTI-specific immune responses and reduce the HIV reservoir, contributing to achieving a durable ART-free remission in virally-suppressed early-treated PWH.

3. OBJECTIVES

3. Objectives

The main objective of this work is to evaluate the safety, immunogenicity and effect on viral rebound of immunotherapeutic strategies based on HTI vaccines, given alone or in combination with a TLR7 agonist- Vesatolimod, in early-treated PWH, recruited from a cohort of individuals diagnosed and treated during acute/recent HIV infection

The **secondary objectives** of this thesis will be:

- **To evaluate the safety and tolerability** of the HTI-based vaccine regimen, either alone or in combination with the TLR7 agonist Vesatolimod, in early-treated individuals with HIV-1 infection.
- **To assess the immunogenicity** of the HTI-based vaccine regimen and the combination regimen, and their **impact on the HIV-1 viral reservoir.**
- **To evaluate the effect of the interventions on viral rebound,** including rebound kinetics and time off ART during analytical treatment interruption (ATI).
- **To investigate the pharmacodynamic effects of Vesatolimod,** particularly regarding cytokine production (e.g., IFN-α, IP-10, ITAC, IL-1RA) and immune-cell activation markers.

4. COMPENDIUM OF PUBLICATIONS

4.1. Description of the Early_cART cohort

4.1.1 Rationale for the creation of the Early_cART cohort

The Early-cART cohort was established in Barcelona in 2014 as part of the collaborative HIVACAT program, a research initiative focused on developing an effective HIV vaccine and strategies for HIV cure. This program aimed to improve the identification of PWH diagnosed during the acute or recent phase of HIV infection and facilitated prompt initiation of ART within 180 days of the estimated date of HIV acquisition.

A cornerstone of this initiative was the partnership between BCN Checkpoint, a community-based sexual health center specializing in point-of-care diagnostics for HIV and other sexually transmitted infections (STIs), and the HIV Unit at Hospital Universitari Germans Trias i Pujol (HUGTiP), along with Fundació Lluita contra les Infeccions and IrsiCaixa. Thanks to this collaborative approach, the Early_cART cohort has also served as a platform to enroll more than 100 participants in clinical trials involving therapeutic vaccines and ART strategies, generating more than 14 scientific publications, and establishing itself as an effective clinical platform for evaluating future HIV cure strategies.

This local initiative was aligned with evolving international consensus, including those from the World Health Organization (WHO), recommending rapid initiation of ART for people newly diagnosed with HIV infection, regardless of their $CD4^+$ T-cell count[32,145,146]. This contributes not only to reduce HIV-related morbidity and mortality, but also to limit onward HIV transmission [31,33,147].

Rapid ART initiation is particularly relevant during the acute/recent phase of HIV infection[148]. The first weeks after HIV acquisition are characterized by high peak viral loads, contributing to a heightened risk of transmission, while critical immunovirological events—such as massive CD4+ T-cell depletion and viral reservoir seeding—set the stage for long-term disease progression70,149. Therefore, rapid initiation of ART during this phase has the potential to limit both secondary HIV transmission as well as reservoir size and diversity[150], reduce immune depletion, preserve CD8+ T-cell functionality [151,152] and improve long-term clinical outcomes.

Diagnosing acute/recent HIV infection remains a significant challenge though because most cases are asymptomatic or present with transient and nonspecific symptoms. Therefore, healthcare providers may overlook early signs. In addition, HIV testing depends on individual risk perception, often hindered by stigma, limited awareness, and access barriers. Regular HIV testing campaigns remain a key strategy to address these challenges, and community-based sexual health centers play an essential role in identifying acute/recent HIV infections, serving as key entry points for diagnosis and linkage to care[153]. These centers often provide anonymous and free testing by on-site and/or home self-sampling, using 4th generation rapid tests which detect both, p24 antigenemia and HIV-specific antibodies, providing positive results as early as 14–20 days after HIV acquisition (Fiebig stages II–III)[154,155]. Additionally, some centers also perform nucleic acid tests (NAT) in those cases with high suspicious of acute infection (i.e., compatible symptoms, recent participation in chemsex, or having a sexual partner diagnosed with HIV within the previous month) with negative or undetermined rapid tests, reducing

the window period to 7–10 days post-acquisition (Fiebig I)[156].Strengthening community-based centers with point-of-care diagnostic tools, enhanced training for community health workers, and streamlined referral to specialized HIV treatment units may significantly improve early identification and management of people with acute/recent HIV infection.

4.1.2. Cohort design

The Early-cART cohort enrolled participants newly diagnosed with HIV infection at a specialized HIV care Unit located at HUGTiP, Badalona, Barcelona, Spain. Individuals aged ≥18 years with a confirmed diagnosis of acute/recent HIV-1 infection and who initiated ART within 180 days of the estimated date of HIV-1 acquisition were included. Early infection was confirmed by at least one of the following criteria: i) positive plasma HIV-1 RNA with negative serology, ii) positive Gag p24 antigen in the absence of HIV-1 antibodies; iii) indeterminate Western blot; iv) absence of the p31 band in a positive Western blot in the context of a known exposure and/or reported acute retroviral syndrome within the previous 180 days, and/or v) positive HIV serology after a documented negative serology or point-of-care test performed within 180 days from ART initiation date.

To promote recognition of acute/recent HIV infections, we conducted specific training for healthcare providers (HCP) in high-HIV-diagnosis sites in the Barcelona metropolitan area consisting of sessions devoted to provide updated knowledge on symptoms associated with PHI, evolution of serologic markers, availability of new point-of-care HIV diagnostic tools. In those session the Early_cART program objectives were presented. HCP included medical doctors, nurses, health promoters/facilitators, and volunteers from community-based centers, as well as hospital and primary care emergency services of the HUGTiP's area of influence. Suspicion of acute/recent HIV infections was promoted in individuals presenting with STI or flu-like symptoms after a recent condom-less sexual encounter, those notified of STI/HIV diagnosis by a recent sexual partner, those displaying a p24 antigen band on a 4th-generation screening test (Alere® Determine HIV-1/2 Ag/Ab), or those testing positive by PoC NAT but negative on the 4th-generation rapid test.

We optimized rapid ART initiation through a streamlined referral system. Briefly, suspected cases were reported to the Early-cART investigators via a cross-platform mobile messaging app (WhatsApp, Meta Platforms Inc.), without sharing personal identifiers. Notifications came from community centers, the hospital emergency department, or primary care clinics. Investigators confirmed appointment availability—ideally within 24–48 hours—which was immediately offered to the patient by the responsible HCP. Upon patient acceptance, the HCP submitted a formal referral to the HIV unit at HUGTiP via the electronic health record system, specifying the pre-arranged Early-cART appointment. Clinical trial assistants (available 8 a.m. to 8 p.m.) monitored referrals and scheduled the patient with the designated HIV specialist accordingly.

At the first visit at the HIV unit, joining the Early-cART cohort, same-day ART initiation was recommended, and individuals were counselled and encouraged for partner notification based

on the estimated date of HIV acquisition. Upon consent, baseline assessments were performed, including confirmatory HIV serology (ELISA and Western blot), plasma viral load (pVL), resistance testing, and biological sample collection (PBMCs and plasma) for storage. Follow-up visits were scheduled at weeks 1 (optional), 4, 12, 24, and 36, and every six months thereafter, or as clinically indicated. The week-1 visit was used when necessary for early clinical monitoring or to reinforce adherence support. **Figure 18A** illustrates the Early-cART cohort recruitment process.

All laboratory tests were performed in the departments of Microbiology, Immunology and Biochemistry of HUGTiP, as per routine care of PWH and following national HIV management guidelines. The processing and storage of biological samples was performed by trained personnel at the sample processing laboratory at IrsiCaixa following standardized procedures.

Acute/recent HIV infection was confirmed after the first visit at the HIV unit, once all laboratory data were available. An in-house developed calculator, based on the Fiebig classification of HIV infection[154,157] and each participant's anamnesis and HIV-1 diagnostic tests, was used to estimate the date of HIV acquisition by taking the average between the estimated dates using each independent confirmation criterion. (**Table 3**). This calculator is not externally validated but was internally standardized and consistently applied across all cases. To increase accuracy of the estimation, if a participant met more than one criterion, a designated expert (PhD candidate) reviewed the estimation, as some criteria might be prioritized over others. Prioritization of criteria was based on clinical judgment and the reliability of available information (e.g., a well-documented acute retroviral syndrome could be prioritized over a Western blot pattern). Estimated date of HIV-1 acquisition was then used to calculate time from `HIV-to-ART' as days from the estimated date of HIV-1 acquisition to ART initiation, and individuals with confirmed time from HIV-to-ART≤180 days were designated as etPWH and regular follow-up visits were scheduled within the cohort as described before. **Link-to-care** was calculated as days between the date of the first positive HIV test and the first appointment at the HIV clinic.

Other clinical data collected from etPWH included: demographics and HIV-related data as HIV transmission route, use of past PEP or PrEP, presence of drug resistance mutations in the genotypic test, and drugs included in the first-line ART regimen. ART regimens were prescribed according to national guidelines, without a study-defined protocol, and changes were allowed based on clinical indications. **Time-to-undetectable pVL** was calculated as days from ART initiation to the first pVL determination <50 copies/mL. **Retention in care** was defined as the proportion of participants who remained actively followed as of December 31st, 2022, which marked the data cut-off for this analysis, although the cohort remains ongoing. **Follow-up time** was defined as the number of days from the first visit to the last documented medical follow-up visit, either in-person or remote, as recorded in the electronic health system.

Table 3 | Calculator of estimated date of HIV-1 acquisition.

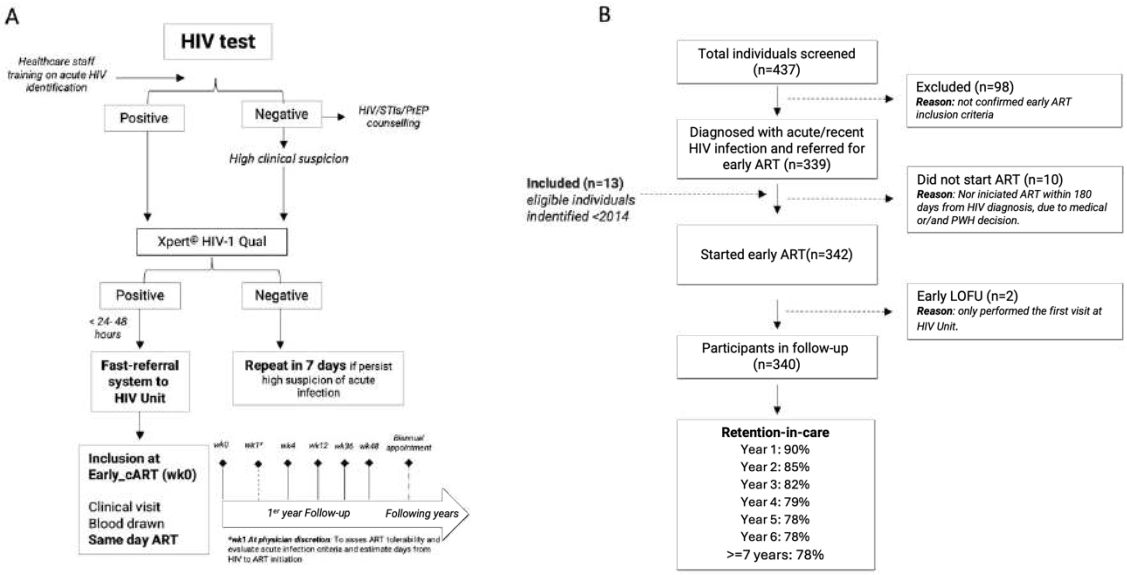
	Fiebig stagea	Estimated range of duration (days)	Date– X days (days)
Known exposure			**dd/mm/yy**$_1$
Documented Symptomatic Primoinfection (date of peak of symptoms)	I	7-21	**dd/mm/yy**$_2$ = dd/mm/yy– 15 days
4th generation test (p24 antigen band only) or p24 antigen positive quantification with negative antibodies	II	18-24	**dd/mm/yy**$_3$ = dd/mm/yy– 21 days
4th generation Determine antibody band positive test (or ELISA) and negative Western blot	III	21-25	**dd/mm/yy**$_4$ = dd/mm/yy– 23 days
Western blotb incomplete (<2 envelope bands)	IV	21-29	**dd/mm/yy**$_5$ = dd/mm/yy– 25 days
Western blot minus p31 (confirms <90 days infection) in the context of a known exposure/reported acute retroviral syndrome	V	30-90	**dd/mm/yy**$_6$ = dd/mm/yy– 60 days
Negative serology – positive serology (<180 days apart).	VI	< 180	**dd/mm/yy**$_7$ = (Last Neg dd/mm/yy–1st positive dd/mm/yy)/ 2
Estimated HIV-1 acquisition			**Average dd/mm/yy** $_{1\text{-}7}$

a Fiebig EW et al. AIDS. 2003;17(13):1871-1879., b Torian LV et al. J Clin Virol. 2011;52 Suppl 1:S41-S44. ELISA, enzyme-linked immunoassay; dd/mm/yy, day/month/year

Since a setpoint of the reservoir size in etPWH is expected to have occurred through natural decay in reservoir levels after 2-3 years from ART initiation157, HIV-1 proviral DNA was measured in PBMCs in a subset of etPWH participants with sustained viral suppression >3 years who had available PBMCs samples and no history of treatment interruption or additional therapeutic intervention beside ART. Lysed extracts from PBMCs were used to measure total cell-associated HIV-1 DNA by droplet digital PCR (ddPCR, Biorad) as previously described92 . Proviral quantification for each individual corresponded to the highest value obtained from the 2 primer sets used (5' long terminal repeats [LTRs] or gag). Primers and probes for the RPP30 cellular gene were used for input normalization.

4.1.3. Recruitment and clinical characteristics of the cohort

Between January 1, 2014, and December 31, 2022, approximately 900 new HIV diagnoses were referred to HUGTiP's HIV unit, 437 of which with suspected acute/recent HIV through the Early-cART cohort. Among them, acute/recent infection was confirmed in 339 cases. Data from thirteen individuals that had been identified <2014 who fulfilled the inclusion/exclusion criteria and had available stored samples, were identified and agreed to be retrospectively included in the study and to be followed thereafter within the Early-cART cohort. Ten individuals (2.3%) with confirmed acute or recent infection opted to defer ART initiation for more than 180 days since the estimated date of HIV acquisition, and 2 individuals attended only the first visit, and were therefore also excluded from the analysis. Consequently, the total number of etPWH with prospective follow-up that were included in the current Early-cART cohort analyses was 340 individuals (**STROBE flowchart, Fig 18B**).



EtPWH accounted for between 37 % and 16% of the annual new referrals of PWH naive to ART to our site from 2014 to 2022. Since 2020, the number of new HIV diagnoses progressively decreased from 137 in 2019 to 90 in 2022, as illustrated in **Figure 19A**. The retention-in-care rate of the Early-cART cohort was approximately 85% at year 2 since inclusion and remained stable over the subsequent calendar years (**Figure 19B**). Overall etPWH had a mean (SD) follow-up period of 5.51 (2.38) years. The majority of Loss to Follow-Up (LOFU) occurred within the first 2 years after inclusion in the Early-cART cohort (**Figure 19C)** and was primarily driven by participant's mobility or convenience regarding proximity to their place of residence.

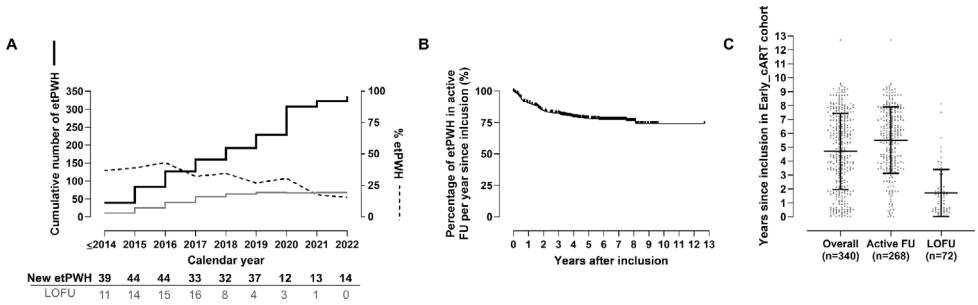


Fig. 19 | Prospective inclusion and follow-up of etPWH. In **A** number of participants included per year (cumulative over time) is shown in bold black line, gray line corresponds to lost-of-follow-up (LOFU) participants (cumulative over time) and dashed line (right-Y axis) corresponds to the proportion of new early-treated PWH (etPWH) included in the cohort over the total of new cART-naïve PWH referred to our site per calendar year. **B** Percentage of participants remaining in active follow-up (FU) across years after inclusion in the Early_cART cohort. **C** Time (in years) on active follow-up for all participants, participants continuing (FU) and dropping out (LOFU) form he cohort (line represents mean and SD).

Table 4 presents main demographic and clinical characteristics of participants in the Early-cART cohort. They were mostly cis-men (97.94%) with a mean (SD) age of 34 (8.77) years at inclusion. Subtype B was identified in 70.8% (221/312) of cases with available determination data. A total of 44.71% of the participants were born abroad Spain, and 245 (72.06%) were referred from a community center for the detection of HIV and other STIs in downtown Barcelona (BCN Checkpoint). Mean (SD) CD4+ counts and $\log_{10}$ pVL at HIV diagnosis were 490 (220) cells/mm3 and 5.10 (1.16) $\log_{10}$ copies/mL, respectively. Mean (SD) time from estimated HIV-to-ART initiation was 60 (34) days, with ART being started at Fiebig stages I-IV in 74 individuals (21.77%). First-line ART consisted of three-drug regimens based on integrase inhibitors (INSTI) in 305 (89.71%) of participants.

Distribution of pre-ART pVL in etPWH per estimated time since HIV acquisition and Fiebig stage is shown in **Figure 20A.** A cube regression model adjusted an estimated pVL setpoint (95% confidence interval) of 4.6 (4.4; 4.8) log10 HIV-1 RNA copies/mL being reached 90 days after HIV acquisition. Notably, mean (SD pVL was significantly higher in individuals who started ART within 30 days of estimated date of HIV acquisition (n=82) (5.90 (1.34) $\log_{10}$ copies/mL) compared with those who started ART ≥30 days after the estimated date of HIV acquisition (4.86 (1.01) $\log_{10}$ copies/mL; t-test, $P < 0.0001$; **Figure 20B**).

Table 4| Study population. Demographic and HIV-related information of participants included in Early cART cohort at study entry (*n*=340).

	etPWH (*n*=340)
Gender, n (%)	
Cis-men	333 (97.94)
Cis-women	5 (1.47)
Trans-women	2 (0.59)
HIV-1 acquisition route, n (%)	
MSM	321 (94.41)
Other	19 (5.58)
Age (years), mean (SD)	34 (8.77)
Country of birth, n (%)	
Spain	188 (55.29)
Other European countries	43 (12.65)
South America	84 (24.71)
Others	25 (7.35)
Referral site, n (%)	
BCN-Checkpoint community center	245 (72.06)
Primary healthcare	22 (6.47)
Emergency room	12 (3.53)
Self initiative	4 (1.18)
*Other*a	57 (16.76)
Subtype B, nb **(%)**	221 (70.83)
CD4+ T cell count (cells/mm3), mean (SD)	490 (220)
CD4/CD8 ratio, mean (SD)	0.65 (0.43)
HIV-1 pVL (copies/mL), mean (SD)	1,404,009 (2,883,061)
Log_{10} HIV-1 pVL (copies/mL), mean (SD)	5.10 (1.16)
Time from HIV-1 acquisition to ART initiation (days), mean (SD)	60 (34)
Fiebig stage at ART initiation, n (%)	
I	11 (3.24)
II	17 (5.00)
III	14 (4.12)
IV	32 (9.41)
V	156 (45.88)
VI	110 (32.35)
ART regimen, n (%)	
Three drugs, INSTI based	305 (89.71)
Two drugs, INSTI based	11 (3.24)
PI based	16 (4.71)
NNRTI based	3 (0.88)
Other	5 (1.47)

Data are presented as mean (Standard Deviation, SD), except when noted.
Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; INSTI, integrase strand transfer inhibitor, MSM, men who have sex with men; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; pVL, plasma viral load.
aOther: refers to additional community-based centers, as well as non-governmental organizations (NGOs) and hospital-based facilities in Barcelona. b Subtype data were available for *n*=312 individuals

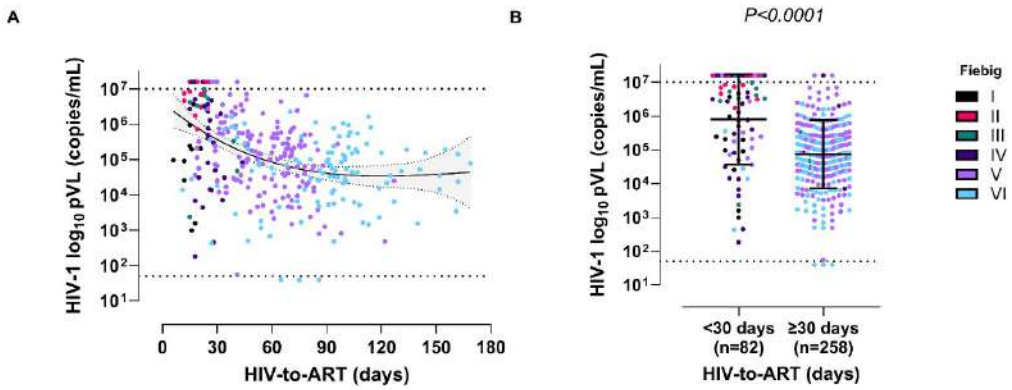


Fig. 20 | Distribution of participant's pre-cART plasma viral load by days from estimated HIV acquisition and Fiebig stage

A Solid line represents cubic regression model adjusted with its corresponding 95% confidence interval (gray shaded area). B T-test comparison of pre-cART HIV-1 $\log_{10}$ pVL (copies/mL) between individuals with <30 days and ≥ 30 days from estimated HIV-to-ART. Mean and SD are shown. Points represent individual values color-coded according to Fiebig stage. Upper dashed line represents upper limit of quantification (10^6 copies/mL). Bottom dashed line represents lower limit of quantification (50 copies/mL).

4.1.4. Immunovirological and clinical outcomes in etPWH

The proportion of participants with pVL <50 copies/mL was 18% at week 4 and 48% and 76% at week 12 and 24 post-ART initiation (**Figure 21A**). Mean (SD) of CD4/CD8 ratio significantly increased from 0.66 (0.43) pre-ART to 1.00 (0.40) and 1.07 (0.47), at 1 and 2 years after early ART initiation (Linear mixed effects model, *P*<0.0001). Importantly, proportion of etPWH with CD4/CD8 ratio <0.5 and >1 after 2 years on ART was 5% and 48%, respectively. (**Figure 21B**).

Total HIV-1 DNA in PBMCs was determined, in 76 participants with >3 years of sustained pVL suppression who had available stored samples. Mean (SD) total HIV-1 DNA levels were 1.62 (0.71) $\log_{10}$ HIV-1 DNA/10^6 PBMCs. Total HIV-1 DNA levels exhibited a segmented linear relationship with the time from HIV-to-ART initiation, defined by two distinct slopes. There was a significant relationship between time HIV-to-ART and the reservoir size in those individuals who started ART within 60 days after the estimated date of HIV aquisition (*P*=0.028), but not therafter (*P*=0.31), underpinnning the relevance of rapid initiation of ART to limit the HIV reservoir size (**Figure 21C**). Aside from time since HIV acquisition, pre-ART pvL (Pearson, r=0.3178, *P*=0.0059) and nadir $CD4^+$ T-cell count (Pearson, r= -0.5438, *P*<0.0001) were negatively and positively correlated, respectively, with the reservoir size.

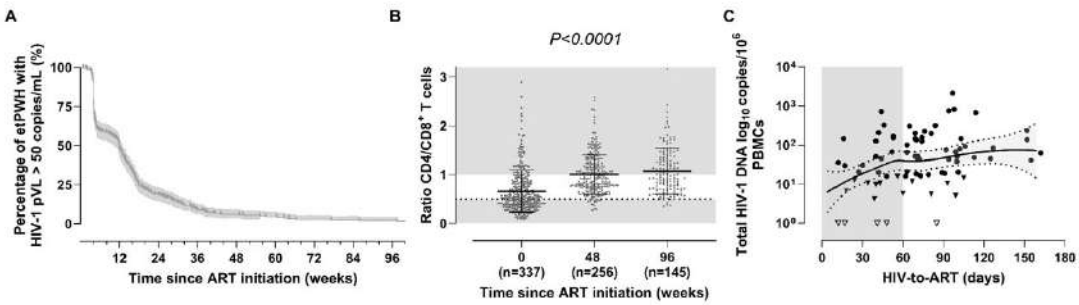


Fig. 21 | Immunovirological response to Early-cART

In **A** proportion of participants with pVL >50 copies/mL during FU is shown by a Kaplan-Meier curve 95% confidence interval is shown in shaded area. **B** Comparison of Ratio CD4/CD8+ T cells pre-ART (week 0), week 48 and week 96 after cART initiation. To assess paired comparisons while accounting for within-subject variability, we used a linear mixed-effects model. Means (in horizontal red lines) and standard deviations are shown. The grey shaded areas shows CD4/CD8+ T cells ratio below 0.5 and above 1. C Locally estimated scatterplot smoothing (LOESS) regression between HIV-to-cART (days) and Total HIV-1 DNA $\log_{10}$ copies/10^6 PBMCs. The black line represents the LOESS fit, with the bands indicating the 95% confidence interval. Each point represent an individual (*n*=76). There are 42/76 (55%) of participants with LoViRet profile (<50 copies HIV-1 DNA copies/10^6 $CD4^+$ T cells) marked in triangles. Open triangles were used for the ones below the limit of detection.

Since the implementation of the cohort, median (min – max) time of link-to-care was significantly reduced, from 11 (0 – 86) days in 2014 to 3 (1 – 25) days in 2022 (Mann-Whitney test, *P*=0.0011). After 2020, 50% of newly confirmed acute/recent HIV cases were visited in a specialized HIV service within 48h and 78% in less than 7 days from HIV diagnosis (first positive test), and were offered rapid ART initiation. In addition, the median (min – max) time from estimated HIV-to-ART initiation was progressively reduced from 73 (11 – 131) days in 2014 to 27 (15 – 141) days in 2022 respectively (Mann-Whitney test, *P*=0.0014), reflecting that etPWH included in the cohort have been progressive identified at earlier stages of HIV infection. **Fig 22**.

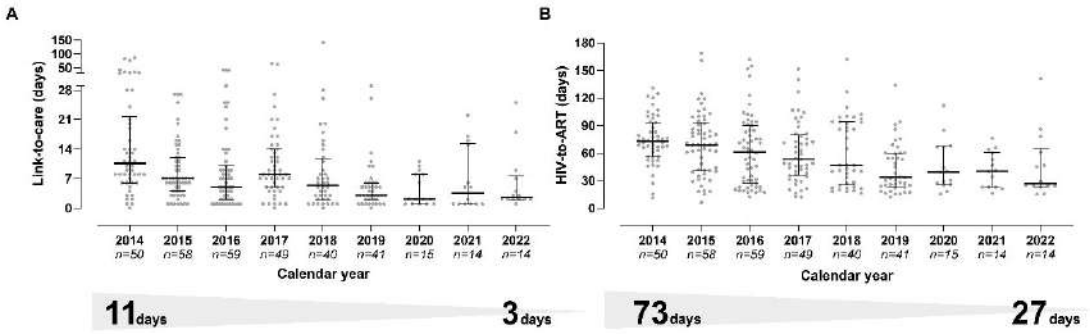


Fig. 22 | Impact of the Early-cART cohort in link-to-care and time from HIV-to-cART

Link-to-care (**A**) and time from HIV-to-ART (**B**) according to calendar year. Horizontal black lines correspond to medians and IQR in each year. On the bottom table the median of 2014 and 2022 are shown.

4.1.5. Discussion of the implementation of the Early-cART program as a research platform for clinical trial recruitment

The Early-cART cohort was established to enable rapid ART initiation in individuals with acute or recent HIV infection through a fast-referral system between community-based centers and a specialized HIV unit. Between 2014 and 2022, the program contributed to a reduction in the median time from HIV acquisition to ART initiation (from 73 to 27 days), despite a decreasing proportion of acute/recent diagnoses among all new HIV cases, likely due to broader outreach and earlier diagnosis efforts.

This structured pathway allowed etPWH to achieve favorable immunovirological outcomes, including rapid viral suppression, robust immune recovery and reduced HIV reservoir size, particularly in those starting ART within 60 days post-acquisition. In a subset of etPWH with >3 years of suppressive ART and no prior ATI, 54% were identified a LoViRet phenotype (<50 copies HIV-1 DNA/10^6 PBMC) [92], reinforcing the long-term benefit of early ART.

The cohort also supported the development of an in-house algorithm to estimate time from HIV acquisition, offering advantages over Fiebig staging for clinical decision-making and partner notification. Community-based NAT PoC testing further enhanced early diagnosis, confirming acute/recent infection in 76% of suspected cases and identifying Fiebig I stage in 3.2%. As PrEP use increases, potentially blunting acute retroviral symptoms and delaying seroconversion, NAT PoC testing will become even more essential[158].

Importantly, the program provided a solid recruitment platform for HIV cure trials. Over 70 etPWH were enrolled in therapeutic vaccine and remission studies , benefitting from early engagement, biobanked pre-ART samples, and high retention (85%). Nonetheless, limitations included underrepresentation of women and non-B subtypes, loss to follow-up, and limited access to confirmatory recency testing[159,160]. Integration of HIV care into community settings may improve long-term retention.

In summary, the Early-cART cohort demonstrates how coordinated, rapid-response systems can improve health outcomes, support translational research, and serve as a model for structured clinical trial recruitment in acute/recent HIV infection.

4.2. Article 1.
The AELIX-002 trial

4.2. Article 1. The AELIX-002 trial

Safety, immunogenicity and effect on viral rebound of HTI vaccines in early treated HIV-1 infection: a randomized, placebo-controlled phase 1 trial

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Safety, immunogenicity and effect on viral rebound of HTI vaccines in early treated HIV-1 infection: a randomized, placebo-controlled phase 1 trial

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HIVACAT T-cell immunogen (HTI) is a novel human immunodeficiency virus (HIV) vaccine immunogen designed to elicit cellular immune responses to HIV targets associated with viral control in humans. The AELIX-002 trial was a randomized, placebo-controlled trial to evaluate as a primary objective the safety of a combination of DNA.HTI (D), MVA.HTI (M) and ChAdOx1.HTI (C) vaccines in 45 early-antiretroviral (ART)-treated individuals (44 men, 1 woman; NCT03204617). Secondary objectives included T-cell immunogenicity, the effect on viral rebound and the safety of an antiretroviral treatment interruption (ATI). Adverse events were mostly mild and transient. No related serious adverse events were observed. We show here that HTI vaccines were able to induce strong, polyfunctional and broad CD4 and CD8 T-cell responses. All participants experienced detectable viral rebound during ATI, and resumed ART when plasma HIV-1 viral load reached either >100,000 copies ml^{-1}, >10,000 copies ml^{-1} for eight consecutive weeks, or after 24 weeks of ATI. In post-hoc analyses, HTI vaccines were associated with a prolonged time off ART in vaccinees without beneficial HLA (human leukocyte antigen) class I alleles. Plasma viral load at the end of ATI and time off ART positively correlated with vaccine-induced HTI-specific T-cell responses at ART cessation. Despite limited efficacy of the vaccines in preventing viral rebound, their ability to elicit robust T-cell responses towards HTI may be beneficial in combination cure strategies, which are currently being tested in clinical trials.

Therapeutic vaccines designed to enhance human immunodeficiency virus (HIV)-specific T-cell immunity have been postulated to be a key component of any HIV cure strategy[1]. Different therapeutic vaccine candidates have been shown to be safe, immunogenic and able to induce broad and functional T- and B-cell immune responses[2–5]. However, no reduction in HIV-1 viral reservoirs, prevention of viral rebound or suppressed viremia off ART have been reported in randomized, placebo-controlled trials of vaccines, given alone or in combination with latency-reversing agents[5–7].

One potential reason for these suboptimal trial outcomes may have been T-cell immunogen designs and the induction of virus-specific T-cell responses with ineffective or insufficient antiviral activity. To overcome this, HTI (HIVACAT T-cell immunogen)-based vaccines were designed to induce functional HIV-1-specific T-cell responses that were

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associated with better viral control in more than 1,000 HIV-1 clade B and C infected individuals within a broad HLA (human leukocyte antigen) class I and class II allele coverage[8] targeting the most vulnerable sites of HIV-1. The HTI immunogen includes 16 HIV-1 regions from Gag, Pol, Nef and Vif that induce T-cell responses of high functional avidity and cross-reactivity and target regions of overall low diversity/entropy, even though these regions were not predicted by stringent conservation algorithms, but were based on human trial data[9,10]. Importantly, in independent cohorts of viremic controllers and individuals with breakthrough infection after being vaccinated with full-length proteins, recognition of viral protein segments covered by HTI were found to be generally subdominant, but, when detected, were associated with better viral control and viral inhibition of clade-matched HIV isolates[11]. The 16 identified HIV-1 regions were assembled in a 529aa immunogen sequence (HTI) and expressed both in a plasmid DNA (DNA.HTI, D)[12] and two viral-vectored vaccines based on a modified Vaccinia virus Ankara (MVA.HTI, M)[13] and a chimpanzee adenovirus (ChAdOx1.HTI, C)[14].

AELIX-002 was a phase I, first-in-human, randomized, double-blind, placebo-controlled study to evaluate the safety, immunogenicity and effect on viral rebound of DNA.HTI, MVA.HTI and ChAdOx1.HTI HIV-1 vaccines administered in a heterologous prime-boost regimen to 45 virally suppressed, early-treated individuals with HIV-1 infection.

Results

A total of 45 participants (44 men and 1 woman), virologically suppressed for at least one year, were recruited from an existing Early-ART cohort[15]. Acute/recent infection at ART initiation was confirmed based on any of the following criteria: (1) positive plasma HIV-1 RNA with negative serology, (2) positive Gag p24 antigen; (3) indeterminate western blot; (4) absence of the p31 band in a positive western blot in the context of a known exposure/reported acute retroviral syndrome; and/or (5) negative HIV antibody test <24 weeks from the first positive test and before starting ART. Participants were randomized 2:1 to receive vaccines or placebo. DNA.HTI or placebo were given at weeks 0, 4 and 8 and MVA.HTI or placebo were given at weeks 12 and 20. All participants completed the first vaccination regimen (DDDMM (n = 30) or placebo (n = 15)). Of them, 42 reconsented to start a second vaccination regimen after a favorable report from the safety monitoring committee (SMC) once the last participant had reached week 32 of the follow-up. The second vaccination regimen started after a minimum of 24 weeks from last MVA.HTI or placebo vaccination. Participants received ChadOx.HTI or placebo at weeks 0, 12 and MVA. HTI or placebo at week 24. Finally, 41 participants (CCM (n = 26) or placebo (n = 15)) entered an analytical treatment interruption (ATI) eight weeks after completing the last series of vaccination (CCM or placebo; Fig. 1).

Demographics

Table 1 presents the baseline characteristics. ART was initiated after a median (range) of 55 (12–125) and 64 (6–140) days after the estimated date of HIV-1 acquisition in placebo and vaccine recipients, respectively. All participants were receiving an integrase strand transfer inhibitor (INSTI)-based ART regimen at inclusion. Median (range) time with undetectable viral load at enrollment was 18 (13–56) and 27 (12–55) months, and median $CD4^+$ T-cell counts (range) were 826 (549–2,156) and 727 (553–1,336) cells per mm^3 in the placebo and in the vaccine group, respectively (not significant for all parameters). Three placebo (20%) and seven (23%) vaccine recipients expressed any HLA class I allele associated with spontaneous control of HIV replication, respectively (that is HLA-B*27:05, -B*57:01, -B*15:17 and/or -B*15:03). In addition, six (40%) placebo recipients and nine (30%) vaccinees expressed HLA class I alleles associated with HIV disease progression (that is, HLA-B*07:02, -B*08:01, -B*35:01/02/03, -B*53:01 and/or -B*54/55/56)[16].

Pre-ART HIV-1 viral sequencing

Full-genome deep sequencing was performed on HIV-1 viral sequences isolated within the first four weeks of ART initiation from 41 participants. Of the 41 participants, 32 (78%) had subtype B viruses. Phylogenetic distance to a reference sequence (HXB2) and the coverage by the HTI immunogen were comparable between placebo and vaccine recipients for any of the HIV-1 proteins included in the HTI immunogen (Extended Data Fig. 1a–c). The median (range) number of pre-ART CTL (cytotoxic T lymphocyte) escape mutants within sequences included in the HTI immunogen was 7 (2–11) and 5 (2–8) in the placebo and vaccine recipients, respectively (Mann–Whitney, P = 0.0364; Extended Data Fig. 1d). The degree of pre-ART CTL escape in HTI-covered regions was not associated with replication fitness of the participants' autologous virus (Extended Data Fig. 1e).

Safety

The severity and intensity of adverse events (AEs) were assessed by the investigator according to the Division of DAIDS table for grading the severity of adult and pediatric adverse events, version 2.1 (March 2017). Overall, vaccines were safe and well-tolerated (Extended Data Table 1). All participants reported solicited AEs related to vaccinations, which were mostly mild (grade 1–2) and transient, except one participant who reported grade 3 asthenia lasting <72 h after the third MVA.HTI vaccination. A total of 440 related AEs were recorded during the entire vaccination phase (111 in placebo and 329 in vaccine recipients), of which 76 and 229 occurred after placebo or DDDMM administrations and 35 and 100 after placebo or CCM (Supplementary Tables 1–4). The most frequent AEs related to vaccinations were pain at the injection site and a flu-like syndrome. There were only two serious adverse events (SAEs) during the study—an episode of acute infectious gastroenteritis due to *Campylobacter jejuni* and an acute appendicitis that required hospitalization, both in vaccine recipients (Extended Data Table 2). No laboratory abnormalities related to vaccinations were reported.

Immunogenicity

Total HIV-1 and HTI-specific T cells were assessed by an ex vivo interferon (IFN)-γ-detecting enzyme-linked immunosorbent spot (ELISpot) assay. Both vaccination regimens (DDDMM and CCM) were immunogenic. The median (range) increase in the total frequencies of HTI-specific T cells from baseline to the peak immunogenicity timepoint after the overall vaccination regimen was 100 (0–498) spot-forming cells (SFCs) per million peripheral blood mononuclear cells (PBMCs) in the placebo group and 1,499 (120–3,150) in the vaccine group (Mann–Whitney t-test, P < 0.0001; Fig. 2a and Extended Data Table 3). This corresponded to an increase in HTI magnitude of more than twofold in ten (67%) and more than threefold in one (7%) of the placebo recipients compared to 29 (97%) and 24 (80%) of vaccine recipients (Fisher's exact test, P = 0.0117 and P < 0.0001, respectively; Extended Data Table 3). To determine the breadth of vaccine-induced T-cell responses, PBMCs obtained at study entry and after DDDMM and CCM or placebo were expanded in vitro and tested against individual 15-mer overlapping peptides (OLPs) covering the HTI immunogen (n = 147). A cumulative breadth over the entire vaccination period of a median (range) of 5 (1–13) IFN-γ-producing responses to individual HTI-covered OLPs was detected in vaccinees without any specific pattern of immunodominance across the HIV subproteins covered by the HTI immunogen, in contrast to 3 (1–8) and predominantly gag-specific responses in placebo recipients (Mann–Whitney t-test, P = 0.0125; Fig. 2b,c). Responses to HTI were already present in 31 participants (20 vaccine and 11 placebo recipients) before ART was initiated. The maximal magnitude of HTI-specific responses achieved during the intervention phase positively correlated with the magnitude of pre-ART HTI-specific T-cell responses (Spearman's ρ = 0.5343, P = 0.0024 and ρ = 0.4632, P = 0.0147 for vaccine recipients at their peak immunogenicity timepoints after DDDMM or CCM, respectively; Extended Data Fig. 2a). Although the HTI magnitude

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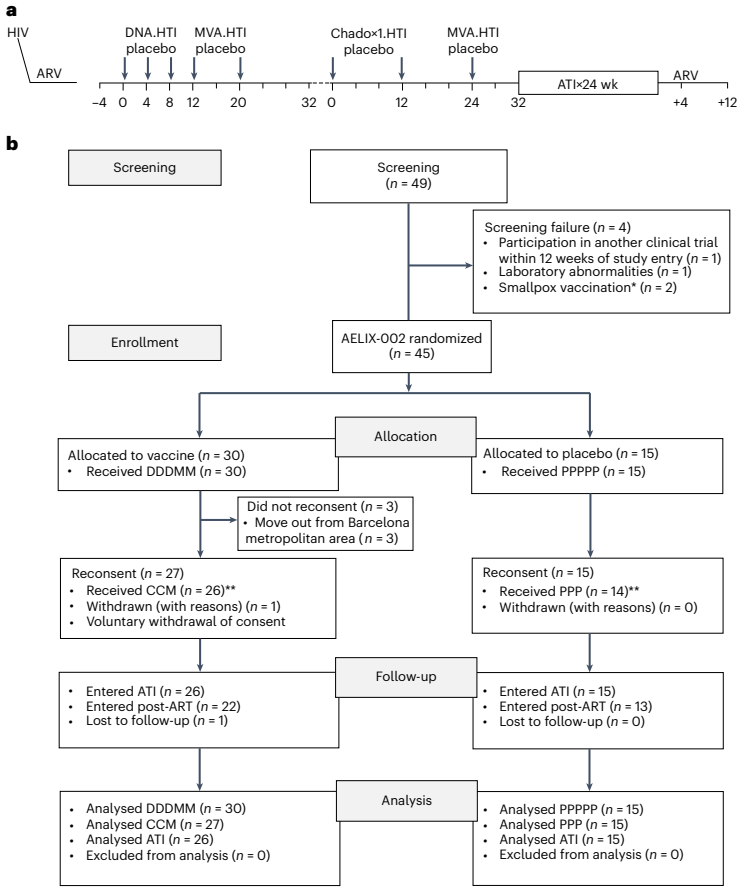

Fig. 1 | Trial design. a, Schematic trial design and study visits. **b**, Consolidated Standards of Reporting Trials (CONSORT) flow diagram for the trial. HIV, human immunodeficiency virus; ARV, antiretroviral therapy; ATI, analytical treatment interruption; D, DNA.HTI; M, MVA.HTI; C, ChAdOx1.HTI; P, placebo.

at the peak immunogenicity timepoint was higher after DDDMM in vaccinees with pre-ART HTI-specific responses compared to those without any HTI-detectable responses before ART initiation (median (range) of 2,203 (460–3,200) versus 808 (60–1,595) SFCs per million PBMCs, Mann–Whitney *t*-test, $P = 0.0380$), these differences were no longer statistically significant at ATI initiation (median (range) of 795 (165–2,705) versus 595 (50–980) SFCs per million PBMCs, Mann–Whitney *t*-test, $P = 0.1012$; Extended Data Fig. 2b). To determine whether HTI vaccination was able to shift the focus of the virus-specific T cells, the percentage of HTI-specific T-cell frequencies divided by the total HIV-1 proteome-specific T-cell frequencies was calculated at each timepoint. At the time of ATI start, the median (range) of 14% (0–50) versus 67% (0–100) of the total anti-HIV-1 T-cell response was HTI-specific in placebo and vaccine recipients, respectively (Mann–Whitney *t*-test $P < 0.001$; Fig. 2d).

To further characterize the vaccine-induced T cells, intracellular cytokine staining for IFN-γ, GranzymeB (GzmB), interleukin-2 (IL-2) and tumor necrosis factor-α (TNF-α) was performed in samples obtained four weeks after the last CCM or placebo vaccination (week 28) with or without in vitro stimulation with four different peptide pools covering the HTI immunogen. T-cell lineage, phenotype, activation and exhaustion surface markers were included in the panel. The results showed that HTI-specific responses, defined as the sum of the HTI-IFN-γ$^+$ populations for each of the four HTI peptide pool stimulations, were both CD4 and CD8 T-cell-mediated (Fig. 2e). Polyfunctionality analyses showed that, compared to placebo recipients, vaccinees had a higher frequency of bi and three-function CD8 T cells expressing IFN-γ/GzmB or IFN-γ/GzmB/TNF-α, whereas CD4 T cells predominantly expressed combinations of IL-2, IFN-γ and TNF-α (Fig. 2f). Importantly, and despite the intense vaccination regimen used in the study (DDDMM-CCM), T-cell exhaustion markers were not increased in HTI-specific T cells in vaccinees compared to placebo recipients after completing the last series of vaccination (Supplementary Table 5).

Finally, we measured the in vitro antiviral capacity of CD8$^+$ T cells by a standard viral inhibition assay (VIA)[17] using autologous CD4$^+$ T cells infected with two laboratory-adapted HIV-1 strains (BaL (R5 tropic virus) and IIIB (X4 tropic virus)) as well as with the autologous HIV virus. Median (interquartile range (IQR)) percentages of inhibition

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Table 1 | Study population

Demographics	Placebo, *n*=15	Vaccine, *n*=30	ITT population, *n*=45
Age, years	34 (20–51)	37 (23–57)	36 (20–57)
Sex at birth, male, *n* (%)	15 (100%)	29 (96.7%)	44 (97.8%)
BMI (kg m^{-2})	22.5 (19.1–31.7)	22.8 (19.1–32.2)	22.8 (19.1–32.2)
Time from estimated HIV transmission to ART initiation (days)	55 (12–125)	64 (6–140)	63 (6–140)
Fiebig stage at ART initiation, *n* (%)[a]			
I	1 (6.7%)	1 (3.3%)	2 (4.4%)
II	0 (0%)	2 (6.7%)	2 (4.4%)
III	2 (13.3%)	0 (0%)	2 (4.4%)
IV	0 (0%)	2 (6.7%)	2 (4.4%)
V	5 (33.3%)	19 (63.3%)	24 (53.3%)
VI	7 (46.7%)	6 (20%)	13 (28.9%)
pVL at ART initiation, log copies ml^{-1}	4.9 (3.7–7)	4.7 (2.9–7)	4.7 (2.9–7)
Current ART, *n* (%)			
DTG/ABC/3TC	7 (46.7%)	9 (30%)	16 (35.6%)
EVG/c/(TAF or TDF)/FTC	4 (26.7%)	13 (43.3%)	17 (37.8%)
RAL+ABC/3TC	1 (6.7%)	2 (6.7%)	3 (6.7%)
RAL+TDF/FTC	3 (20%)	6 (20%)	9 (20%)
Time with undetectable pVL (months)	18 (13–56)	27 (12–55)	24 (11–56)
Absolute CD4 (cells mm^{-3})	826 (549–2,156)	727 (457–1,333)	745 (365–2,156)
Percentage CD4 (%)	39.2 (19–53.9)	35.4 (17.8–63.4)	36.3 (17.8–63.4)
CD4/CD8 ratio	1.1 (0.5–2.66)	1.02 (0.5–3.3)	1 (0.5–3.3)
Beneficial HLA alleles any	3 (20%)	7(23.3%)	10 (22.2%)
B2705	1 (6.7%)	4 (13.3%)	5 (11.1%)
B5701	2 (13.3%)	1 (3.3%)	3 (6.7%)
B1517	0 (0%)	1 (3.3%)	1 (2.2%)
B1503	0 (0%)	1 (3.3%)	1 (2.2%)
Past smallpox vaccination[b]	1 (6.7%)	6 (20%)	7 (15.6%)
CCR5-Δ32 heterozygosity[c]	2 (13.3%)	3 (10%)	5 (11.1%)

Demographic, clinical and treatment characteristics of study participants at study entry (*n*=45). Data presented as median (min–max) except where specified. [a]According to Fiebig[37]. [b]Signs of scarification or history of vaccination reported by the volunteer. [c]CCR5-Δ32 genotype was available for 15 placebo and 26 vaccine recipients (those entering the ATI). Comparisons between study groups by two-sample *t*-test or chi-squared test when corresponding (non-significant for all variables). BMI, body mass index; ART, antiretroviral therapy; pVL, HIV-1 plasma viral load; DTG, dolutegravir; ABC, abacavir; 3TC, lamivudine; EVG/c, elvitegravir/cobicistat; TAF, tenofovir alafenamide fumarate; TDF, tenofovir disoproxil fumarate, ITT, intention-to-treat.

of the BaL-isolate increased in the vaccine group from 46(17; 75)% at baseline to 75(9; 88)% at the end of the intervention (Wilcoxon *t*-test, $P = 0.0805$), but it remained unchanged in the placebo group (34 (17; 60)% at baseline and 37 (14; 63)% at the end of the intervention (Wilcoxon *t*-test, $P = 0.9153$)). When using IIIB viruses and a participant's autologous viruses, significant changes in VIA were detected as well (Wilcoxon *t*-test, $P = 0.0014$ and 0.0176) in vaccinees in contrast to placebo recipients. However, absolute increases in viral inhibition capacity were of minor magnitude, probably due to the high inhibition capacity against the autologous virus already present at study entry, and consistent with early treatment initiation (Fig. 2g).

Effect on viral rebound during an ATI

Of the participants, 41 (15 placebo and 26 vaccine recipients) interrupted ART and were monitored weekly for a maximum of 24 weeks. Criteria for ART resumption included a single HIV-1 plasma viral load (pVL) of >100,000 copies ml^{-1}, eight consecutive determinations of >10,000 copies ml^{-1}, two repeated CD4$^+$ cell counts of <350 cells mm^{-3} and/or development of grade 3 or higher-severity clinical symptoms suggestive of an acute retroviral syndrome (ARS)—whichever appeared first. The ATI period partially overlapped with the first COVID-19 outbreak in Spain, with a State of Alarm declared from 16 March 2020 to 20 June 2020. Risk mitigation strategies were quickly implemented during the pandemic to reduce premature withdrawals while ensuring participants' safety. ATI was tolerated well overall (Supplementary Table 6). The frequency of sexually transmitted infections (STIs) in the study population was similar to those previously reported in MSM (men who have sex with other men)[18], but importantly was relatively lower during the ATI period than during the intervention phase of the study (7 versus 17 cases of STI per 100 persons per year, respectively). Viral suppression to undetectable levels was achieved by the 12th week after ART resumption in all 35 participants assessed at the end-of-study visit.

As shown in Fig. 3a,b, pVL rebound (defined as pVL > 50 copies ml^{-1}) was detected in all 41 participants after ART discontinuation at a median (range) time of 2 (1–6) and 3 (1–9) weeks in placebo and vaccine recipients, respectively (Mann–Whitney *t*-test, $P = 0.1942$). Time to pVL rebound, peak viremia, time to peak viremia, slope of increasing pVL or area under the curve (AUC) pVL during the ATI were comparable between placebo and vaccine recipients (Extended Data Table 4). Of the participants, 25 (61%) resumed ART after one determination of pVL > 100,000 copies ml^{-1}, and one (2%) participant after eight consecutive determinations of >10,000 copies ml^{-1}. Three participants (one in the placebo and two in the vaccine group) showed symptoms compatible with ARS, but they were grade 1–2 and did not proceed to ART resumption. Four (9%) participants resumed ART at weeks 9, 12, 22 and 23 of ATI without reaching any pre-specified ART resumption criteria in the context of the COVID-19 pandemic (details are provided in Supplementary Table 7). Eleven (27%) participants completed 24 weeks of ATI, seven of them with sustained pVL < 2,000 copies ml^{-1}. Five participants resumed ART at week 24, and the remaining six participants (two placebo and four vaccine recipients) opted to remain off ART and entered an ATI-extension protocol with monthly monitoring for up to a total of 72 weeks of ATI (NCT04385875). Four participants (one placebo and three vaccine recipients) completed the ATI extension with sustained pVL < 2,000 copies ml^{-1} after 72 weeks off ART (Extended Data Fig. 3), and then resumed ART. Reasons for starting ART included worries about HIV transmission, previous good tolerability to ART and the burden of additional HIV prevention tools required for viremic individuals. In a post-hoc survival analysis for time off ART during the ATI, participants without any beneficial HLA class I alleles (32 of the 41 participants that entered the ATI period), one (8%) of the placebo and eight (40%) of the vaccine recipients were able to remain off ART for 22 weeks (Δ 32%, 80% confidence interval (CI) (7.6; 55.7) and 95% CI (−1.6; 64.9); log-rank test $P = 0.1834$ for all ATI), with pVL < 2,000 copies ml^{-1} being observed in one placebo and five vaccine recipients, respectively (Fig. 3c)

Exploratory objectives

Reservoir. Amplicon signal issues occurred for six (14%) participants (three placebo and three vaccine recipients) for whom intact proviral DNA assay (IPDA) determinations were not available. Intact HIV-1 DNA represented a median (IQR) of 23% (9; 42) of the total HIV-1 DNA. Total and intact proviral HIV-1 DNA were highly correlated (Spearman's $\rho = 0.6673$, $P < 0.0001$ at study entry and $\rho = 0.8716$, $P < 0.0001$ at ATI

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start). No differences in reservoir decay were found between groups, either measured by total proviral HIV-1 DNA (21% versus 16% decay in the placebo and vaccine groups, respectively, Wilcoxon *t*-test, $P = 0.4291$) or by IPDA (68% versus 66% decay in the placebo and vaccine groups, respectively, Wilcoxon *t*-test, $P = 0.7892$) (Extended Data Fig. 4).

Correlate analyses. Potential immune and viral correlates associated with longer time off ART (that is, less risk to reach the ART resumption criteria of HIV-1 pVL > 100,000 or consecutive HIV-1 pVL > 10,000 for more than eight weeks) were assessed in the subgroup of individuals that did not harbor any HLA class I allele associated with spontaneous HIV control. The magnitude of the HTI-specific T-cell response at ATI start was significantly associated with both prolonged time off ART and with lower pVL at the end of ATI in vaccinees (Spearman's $\rho = 0.6469$, $P = 0.0021$ and $\rho = -0.6837$, $P = 0.0009$, respectively; Fig. 4a,b) but not in placebo recipients. Similarly (albeit not statistically significant), the cumulative breadth of HTI-specific responses at ATI start was associated with longer time off ART (Spearman's $\rho = 0.4235$, $P = 0.0628$; Supplementary Fig. 1). In terms of specificities within HTI, for those vaccinees remaining off ART for longer than 12 weeks ($n = 8$), we did not observe differences in the pattern of responses induced across the different HIV protein segments covered by HTI (Supplementary Fig. 1).

As for T-cell functionality, the frequency of $CD8^+$—and to a lesser extent $CD4^+$—T cells expressing $GzmB^+$ was positively correlated with time off ART and with lower HIV-1 pVL at the end of ATI in vaccine, but not in placebo recipients (Fig. 4c–f). Although vaccinees showed an increase in in vitro viral inhibition capacity, this was not associated with any of the ATI outcomes. As for viral factors, we ruled out the possibility that pre-existing CTL escape in sequences covered by HTI immunogen and/or replication fitness of the participants' autologous virus could have influenced the ability of vaccine-induced responses to control virus replication during ATI. Vaccine recipients that remained off ART for longer periods of time did not show any significant correlation with the number of HLA-adapted footprints in pre-ART sequences (Spearman's $\rho = -0.0160$, $P = 0.9467$; Extended Data Fig. 5a) and were able to control viruses not only with low but also with medium and high replicative capacity (Extended Data Fig. 5b). Levels of total or intact proviral HIV-1 DNA at ATI start were not associated with time to viral rebound or with longer time off ART (Extended Data Fig. 5c,d). However, the majority of participants that remained off ART for >12 weeks were among those with lower reservoir levels.

Finally, as the distribution of time off ART was quite binary rather than continuous (≤12 or >12 weeks), univariate logistic regression models were used to identify factors that could influence length of time to ART resumption. In addition to the pre-ART pVL, most of the immune parameters measured at ATI start increased the odds of time off ART > 12 weeks (for example, HTI magnitude $\widehat{OR}$ (odds ratio) 1.46, 95% CI (1.16; 1.99), $P = 0.0052$; frequency of HTI-specific $CD8^+$ $GzmB^+$ T cells at ATI start $\widehat{OR}$ 1.07, 95% CI (1.01; 1.14), $P = 0.0240$; Fig. 5). Conversely, reservoir levels were not associated with higher chances of remaining off ART in the regression model. Importantly, in a multivariate logistic regression model including most critical demographic covariates, such as pre-ART pVL and CD4/CD8 ratio at AELIX-002 entry, there was an increased probability for being off ART after 12 weeks of ATI for the vaccinees compared to placebo recipients ($\widehat{OR}$ 8.25, 95% CI (1.05; 140.36); Extended Data Table 5).

Discussion

The double-blind, placebo-controlled, randomized AELIX-002 study has demonstrated that HTI vaccines were safe, well-tolerated and able to induce strong, polyfunctional and broad CD4 and CD8 T-cell responses focused on the HTI immunogen sequence. In agreement with preclinical data in NHP (non-human primates)[19] and clinical trials in similar populations using other T-cell vaccines only[5,6], all participants showed detectable viral rebound during the ATI. However, in exploratory analyses we observed a positive efficacy signal on the ability to remain off ART during a 24-week ATI (that is, to avoid reaching an HIV-1 pVL of >100,000 copies ml^{-1} or >10,000 copies ml^{-1} for eight consecutive weeks as per the protocol-defined ART resumption criteria) in vaccinees without beneficial HLA genetics compared to placebo recipients. The AELIX-002 trial is a randomized, placebo-controlled trial testing therapeutic T-cell vaccines in an early ART-treated population that shows a correlation between vaccine-induced immune responses and both lower post-rebound viremia and extended time off ART, providing an opportunity to identify correlates of improved viral control.

The AELIX-002 trial results support the idea that the induction of HIV-specific T cells is a key factor in improving post-rebound viral suppression during an ATI, while validating the design of the HTI immunogen to induce functional T-cell responses to vulnerable sites of the virus. Indeed, the HTI vaccines used in AELIX-002 showed good coverage of the autologous viral sequences, despite some evidence of pre-existing CTL escape[20]. Importantly, HTI vaccination induced strong, long-lasting GzmB-secreting $CD8^+$ T cells along with an improved ability to inhibit replication of CCR5-tropic, CXCR4-tropic and, importantly, autologous HIV virus with a broad range of viral replicative fitness. Additionally, vaccine-induced responses targeted different HTI subunits, confirming that the HTI immunogen design does contain multiple T-cell targets that can mediate effective HIV control ex vivo.

Studies testing a combination of TLR7 agonists and bNAbs in NHP have observed a correlation between lower pre-ART pVL in acute infection and time to viral rebound during an ATI[21]. In contrast, in

Fig. 2 | Vaccine immunogenicity. a, Magnitude (sum of SFCs per 10^6 PBMCs for HTI pools P1–P10) over the AELIX-002 study in placebo (blue) and vaccine (red) recipients over the two vaccination regimens (DDDMM/PPPPP and CCM/PPP) up to the start of the ATI period. **b**, Breadth of vaccine-elicited responses towards individual OLPs spanning the entire HTI sequence in the 15 placebo and 30 vaccine recipients. Boxplots represent the median and IQR, and the *P* values correspond to comparisons between the indicated timepoints using the Wilcoxon signed-rank test. **c**, Distribution of HTI-specific responses within the different HIV-1 subproteins included in the HTI immunogen of cumulative breadth at AELIX-002 study entry (top) and after completion of the last series of vaccinations (bottom) for each placebo (P1 to P15) and vaccine (V1 to V26) recipient. **d**, Average distribution of total HIV-1 T cells according to their specificity at the indicated timepoints. HTI-specific responses are shown for placebo (blue) and vaccine (red) recipients. The other non-HTI HIV-1 specific responses are shown in gray. *P* values correspond to a comparison between the proportion of HTI-specific responses at each timepoint. Fisher's exact test is used for comparisons between groups. **e**, Proportion of HTI-specific $CD4^+$ and $CD8^+$ T cells secreting IFN-γ (left) or both IFN-γ and GzmB (right) after completion of the last series of HTI vaccinations (DDDMM-CCM/PPPP-PPP). Data are presented as median and IQR for the sum of IFN-γ^+ and IFN-γ^+/$GzmB^+$ for each of the four HTI peptide pool stimulations. A Wilcoxon–Mann–Whitney test is used for comparison between placebo ($n = 12$) and vaccine ($n = 20$) groups. **f**, Polyfunctionality of HTI-specific $CD4^+$ and $CD8^+$ T cells analyzed by Boolean gating. Pie charts and boxplots per treatment group (placebo $n = 15$, vaccine $n = 26$) illustrate the relative and absolute proportion of each of the different subsets (cells producing two, three or four cytokines), respectively. On each boxplot, the central line indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to 1.5 times the IQR. *P*-values correspond to the Mann–Whitney test per row, adjusted for multiple comparisons. **g**, Changes in viral inhibition capacity to laboratory-adapted HIV-1 strains (placebo $n = 15$, vaccine $n = 26$) and autologous HIV-1 (placebo $n = 14$, vaccine $n = 23$) at study entry, after DDDMM/PPPPP and CCM/PPP regimens for placebo (blue) and vaccine (red) recipients. Boxplots represent median and IQR, and the *P* values correspond to comparisons between the indicated timepoints using the Wilcoxon signed-rank test. SCR, screening; BSL, baseline; D, DNA.HTI; M, MVA.HTI; C, ChAdOx1.HTI; P, placebo.

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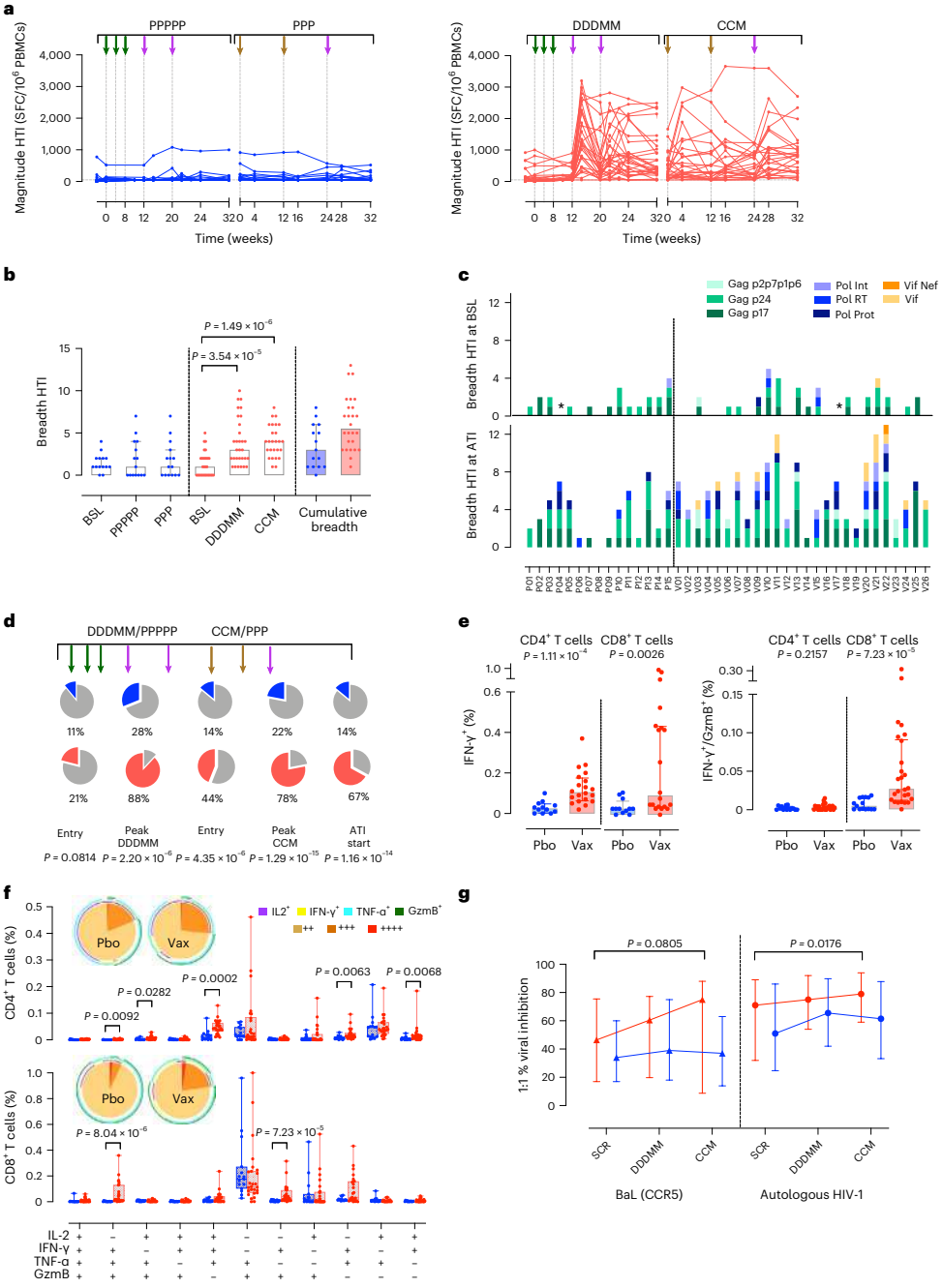

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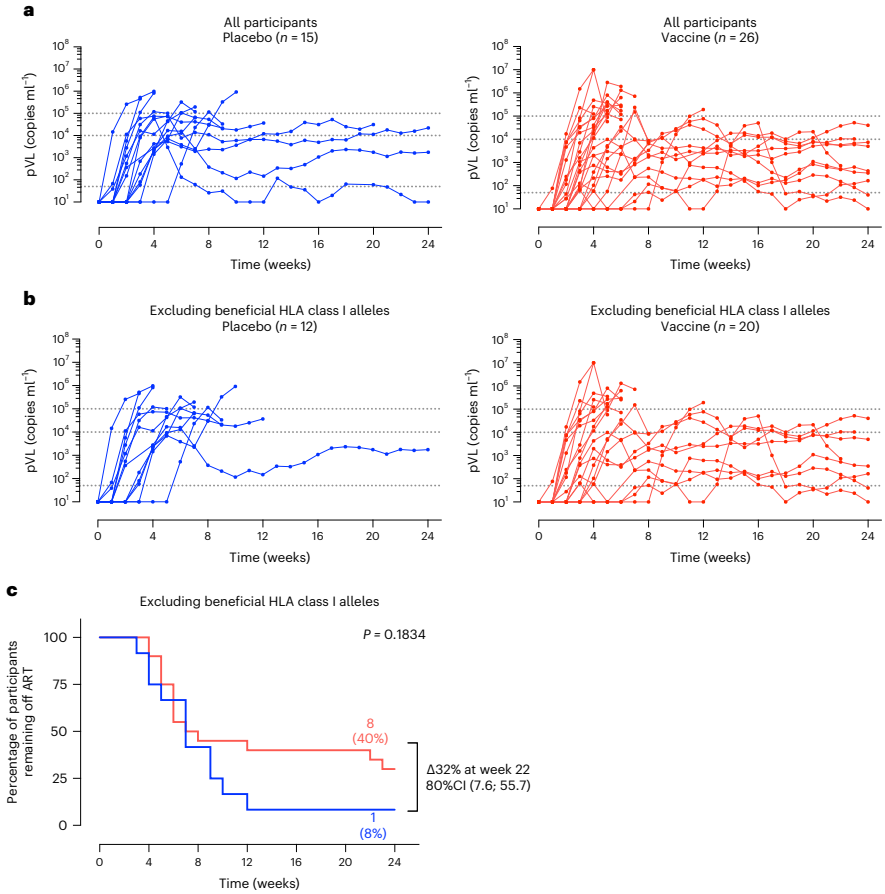

Fig. 3 | ATI period. a,**b**, Individual HIV-1 pVL during the 24 weeks of ATI, shown for all placebo (blue) or vaccine (red) recipients (**a**) and in those without any beneficial HLA associated with spontaneous viral control (**b**). Lines are interrupted at the week of ART resumption. Dotted lines represent the detection limit and the two different virologic thresholds for ART resumption (10,000 and 100,000 HIV-1 RNA copies per ml, respectively). **c**, Proportion of participants without any beneficial HLA allele associated with spontaneous viral control in the placebo and vaccine arms remaining off ART following treatment interruption. The log-rank test is used for comparison between groups over the entire ATI period. The proportion of participants, delta and 80% CI are shown for week 22 of ATI, before the last two vaccine recipients resumed ART for COVID-19-related reasons without fulfilling any per-protocol virological criteria. pVL, plasma viral load; ART, antiretroviral treatment.

AELIX-002, lower pre-ART pVL was not associated with longer time to first detectable pVL during the ATI, but it was positively correlated with time off ART. Importantly, in exploratory multivariate models, the association of vaccination with extended time off ART remained statistically significant, even after accounting for participants' levels of pre-ART viremia and CD4/CD8 ratio.

Different approaches have been developed to establish high-throughput assays to quantify the replication-competent viral reservoir relevant for cure-related trials, including the IPDA assay, which allows measurement of genetically intact proviruses and excludes the majority of defective proviruses[22,23]. In AELIX-002, although the intact proviral HIV-1 DNA declined preferentially over time relative to total proviruses, we did not detect differences in the reservoir decay from baseline to ATI associated with therapeutic vaccination, suggesting that such a reduction reflected natural decay curves due to early treatment[15]. In contrast to others who have reported an association between a delay in viral rebound and lower intact proviral DNA levels after vesatolimod treatment in viremic controllers[24], we did not detect any correlation between levels of intact proviral DNA and time to viral rebound in our early-treated population. Of note, seven (17%) participants that entered the ATI period had no detectable levels of intact HIV-1 proviruses at the time of ART cessation and yet experienced viral rebound during the ATI.

Despite the extended vaccination regimen used in AELIX-002, vaccinations were safe and well-tolerated, and safety profiles were comparable to other HIV vaccines using the same vector platforms both in HIV-negative[25] or HIV-positive individuals[2]. No serious related AEs or laboratory abnormalities were observed after either DDDMM or CCM vaccinations, including any suspected vaccine-induced immune thrombotic

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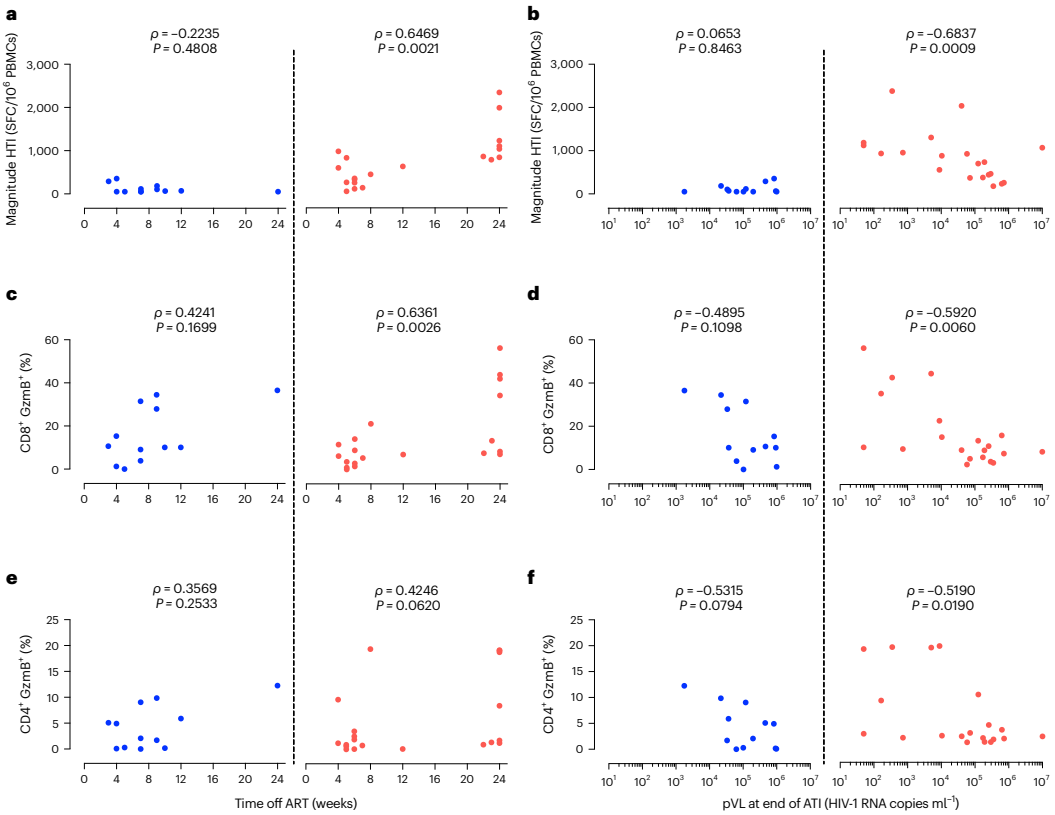

Fig. 4 | Immune correlates with ATI outcomes in participants without any beneficial HLA allele. a–**f**, Correlation between time off ART (left) and HIV-1 pVL at the end of ATI at the ART resumption timepoint (right) for HTI magnitude at ATI start (**a**,**b**) and proportion of $CD8^+$ (**c**,**d**) and $CD4^+$ (**e**,**f**) GzmB-secreting T cells in placebo (blue) and vaccine (red) recipients. Spearman's correlation is used. ART, antiretroviral treatment; pVL, plasma viral load; ATI, analytical treatment interruption.

thrombocytopenia (VITT), as described for ChAdOx1-vectored COVID-19 vaccines[26]; although our sample size was rather limited to detect such rare events. Notewothy, T-cell exhaustion markers were not increased in vaccinees compared to placebo recipients.

Similar to the ATI viral kinetics in the AELIX-002 trial, in which all participants experienced a fast viral rebound, Okoye et al. have recently shown in the NHP model that $CD8^+$ T cells contribute to reduce the viral set point, although they were not able to prevent viral recrudescence[19]. These data suggest that HIV antigenic stimulation might be necessary to trigger an effective immune response during the ATI. This, in turn, has important implications for the design of ATI trials where ART resumption criteria may need to be permissive enough to allow for such a transient viremia[27–29]. Initial peak viremia may, however, also be associated with risks for onward virus transmission, mutational T-cell escape, reseeding of the viral reservoir and/or excessive inflammatory responses giving rise to ARS. Therefore, it is critical to balance research objectives and the well-being of participants while considering, in collaboration with community advisory boards, effective transmission risk-reduction strategies[30]. In AELIX-002, ART resumption criteria during the ATI were well-accepted among participants, as well as all transmission-risk reduction strategies implemented, which included PrEP provision to sexual partners, psychological support and active surveillance for asymptomatic STI. Of note, the AELIX-002 study, and the ATI phase in particular, was ongoing when the first COVID-19 outbreak occurred in Spain. This severely impacted many clinical trial sites, as most non-COVID-related hospital activities, including clinical research, had to be paused. Rapid establishment of a risk-mitigation plan overseen by an external SMC during the emergency outbreak was critical to minimize the impact of the COVID-19 pandemic on the conduct of AELIX-002, as some investigators have recommended recently[31,32].

The main limitations of our trial include the sample size, which did not allow for a powered subgroup analysis in individuals without beneficial HLA genetics, as well as the selected study population, which limited extrapolation of our results to HIV populations other than those treated early during acute/recent HIV infection and in which both cis-gender and transgender women are usually underrepresented. In addition, the regimen used in AELIX-002 consisted of two different vaccination regimens of DDDMM, further boosted by CCM vaccines; overall, this does not represent a clinically feasible vaccination regimen, but it did serve to set up an efficacy proof of concept of the HTI immunogen design. In fact, we acknowledge that the efficacy endpoint of time off ART in our study is a function of the ART resumption criteria

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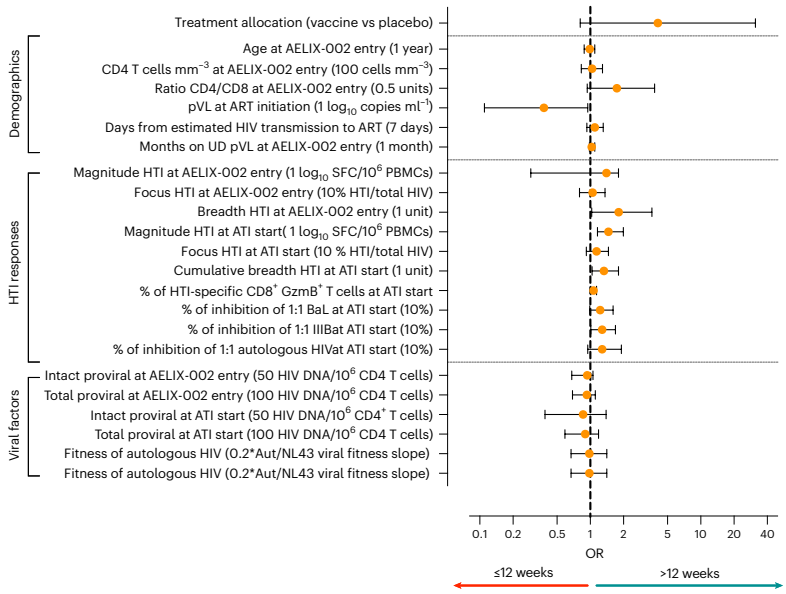

Fig. 5 | Univariate correlate analysis. Odds ratio and its 95% CI of time to ART resumption >12 weeks in univariate logistic regression models (n = 32 participants without beneficial alleles).

used in the protocol and, importantly, is not yet translatable into clinical practice.

Our findings strongly support the further use of HTI vaccines in simpler regimens, given alone or in combination with other immunomodulatory agents to improve their efficacy, to achieve more clinically relevant virological outcomes and to be better aligned with the most current target product profile for an HIV cure indication[33]. For example, to avoid viral rebound, or partially curtail fast and severe viral recrudesence, and to improve the level of virus control, we and others have proposed strategies combining therapeutic vaccines with bNAbs, which at the same time may enhance suppressive capacity of vaccine-induced responses through a vaccinal effect[34–36]. In this sense, the BCN03 and AELIX-003 clinical trials (NCT05208125 and NCT04364035, respectively) are currently exploring the safety and immunogenicity of a ChAdOx1.HTI/MVA.HTI vaccine regimen with a recombinant HIV-1 envelope SOSIP protein (ConM SOSIP.v7 gp140) or with a TLR7 agonist (vesatolimod) including an ATI with the same ART resumption criteria as in AELIX-002.

In conclusion, this first administration of a heterologous prime-boost regimen of HTI vaccines in early ART-treated individuals with HIV infection was safe and immunogenic. In exploratory analyses, AELIX-002 showed a potential signal for improved post-rebound viral control after ART discontinuation in a subset of individuals who did not already possess a beneficial HLA genotype; this requires validation in future studies. These data provide support for the use of HTI vaccines as a T-cell-stimulating backbone for future combination cure strategies, with the addition of immunomodulators, bNAbs or alternative vaccine vectors to boost their efficacy.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-022-02060-2.

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Methods

Study design

AELIX-002 (clinicaltrials.gov NCT03204617) enrolled 45 HIV-positive early-treated individuals at the Infectious Diseases Department of the Hospital Germans Trias i Pujol (HUGTIP), Badalona, Spain. The first and last participants were recruited on 20 July 2017 and 5 June 2018, respectively. The last study visit was conducted on 10 March 2021. AELIX-002 was a phase I, proof-of-concept, first-in-human, randomized, double-blind, placebo-controlled study to evaluate the safety, immunogenicity and effect on viral rebound during an ATI of three novel HIV-1 vaccines (DNA.HTI (D), MVA.HTI (M) and ChAdOx1.HTI (C)) administered in a heterologous prime-boost regimen consisting of DDDMM and CCM versus placebo.

Participants had to be aged 18–65 years and have a history of triple-drug ART initiated within six months after estimated HIV-1 acquisition with an HIV-1 viral load of <50 HIV-1 RNA copies ml^{-1} and $CD4^+$ T cells >400 cells mm^{-3} for at least 12 and 6 months before inclusion, respectively. An in-house algorithm based on the Fiebig classification of HIV infection[15,37] and each participant's available HIV-1 diagnostic tests were used to calculate the estimated date of HIV-1 acquisition for each individual.

Before inclusion, all participants signed an informed consent previously reviewed by a local Community Advisory Board. The study was approved by the institutional ethical review board of HUGTIP (ref. no. AC-15-108-R) and by the Spanish Regulatory Authorities, and was conducted in accordance with the principles of the Helsinki Declaration and local personal data protection law (LOPD 15/1999).

For safety purposes, participants were randomized (2:1) into three sequential recruitment blocks after blinded safety reports were approved by an external SMC. A sentinel group of three participants (two vaccine recipients and one placebo recipient) was first enrolled, and one participant was randomized per day and monitored 24 h after each vaccination (group 1) to allow for the next sentinel participant to be vaccinated. The rest of the participants were part of the non-sentinel groups: group 2 ($n = 12$) and group 3 ($n = 30$). After completion of the first vaccination regimen (DDDMM/placebo), all 45 participants were offered to participate in a second phase of the study, which included a booster vaccination regimen with CCM or placebo (while maintaining the same treatment allocation from the initial regimen) and an ATI period of 24 weeks. Between DDDMM/placebo and CCM/placebo phases of the study, participants were kept on suppressive ART and performed clinical follow-ups every 12 weeks ('roll-over' period).

Criteria to proceed to ATI and resume ART

Eight weeks after the last vaccination (DDDMM-CCM or placebo) participants underwent an ATI of up to 24 weeks of duration if they had (1) received all vaccinations, (2) maintained a pVL of <50 copies ml^{-1} and $CD4^+$ T cells of >400 cells mm^{-3} and (3) there was no evidence of active syphilis, hepatitis B or hepatitis C infections. Before ATI start, HIV-seronegative participants' sexual partners were offered PrEP through a trial-specific PrEP-provision program. During the ATI, weekly visits were performed at HUGTIP, Badalona or at BCN-Checkpoint, Barcelona at participants' convenience. During the COVID-19 pandemic, remote visits and home-based blood draws were carried out. Criteria to resume ART included a single pVL of >100,000 copies ml^{-1}, pVL of >10,000 and ≤100,000 copies ml^{-1} for eight consecutive weeks, $CD4^+$ T cells <350 cells mm^{-3} in two consecutive determinations, development of a ≥grade 3 ARS, at the participant's request or investigator criteria. As part of investigator criteria, active surveillance for STIs was performed during the ATI and, if suggestive of unprotected sex with partners with unknown HIV status and/or HIV-negative partners not taking PrEP, ART was recommended to prevent HIV transmission. All participants off ART after 24 weeks of ATI were offered to resume ART except when pVL < 2,000 copies ml^{-1}. These participants were invited to participate in an ATI-extension protocol (NCT04385875). Criteria for ART resumption during the ATI-extension phase included one determination of pVL > 100,000 copies ml^{-1} or pVL > 2,000 copies ml^{-1} for eight consecutive weeks. Psychological assessments of the impact of the ATI on the emotional and sexual sphere were evaluated using trial-specific questionnaires by clinical psychologists at the HIV unit before entering the ATI, 12 weeks after the ATI, four weeks after ART was resumed and at the participants' request. Participants were followed 4 and 12 weeks after ART was resumed. The protocol and a list of amendments to the protocol are available as Supplementary files 1 and 2.

Study vaccines

The HTI immunogen is a chimeric protein sequence (total length of 529 amino acids (aa)) designed based on human immune reactivity[8] that includes 26 regions in HIV-1 Gag (45%), Pol (44%), Vif (8%) and Nef (3%) proteins identified in these analyses that (1) were preferentially targeted by participants with low viral loads and largely independent of beneficial HLA class I genotypes, (2) turned out to be more conserved than the rest of the proteome and (3) elicited responses of higher functional avidity and broader variant cross-reactivity than responses to other regions[9].

The DNA.HTI vaccine (D) is a circular and double-stranded DNA plasmid vector of 5,676 base pairs derived from the pCMVkan expression vector backbone expressing the codon-optimized HTI gene, preceded by the human granulocyte-macrophage colony-stimulating factor (GM-CSF) signal peptide for better secretion[12]. The DNA.HTI drug substance is manufactured, quality-control-tested and released in accordance with the requirements of good manufacturing practice (cGMP) by the Clinical Biotechnology Centre (CBC), Bristol Institute for Transfusion Sciences, University of Bristol, UK.

The MVA.HTI vaccine (M, modified Vaccinia virus Ankara) is a live, attenuated recombinant vaccinia (pox) virus attenuated by serial passages in cultured chicken-embryo fibroblasts that contains six large deletions from the parental virus genome[13]. The size of MVA.HTI after insertion of a transgene coding for the HTI insert is estimated to be ~179.6 kbp. Production was carried out by the German company IDT Biologika, and preparation, verification of the genetic stability and MSV and WSV storage were carried out at IDT under cGMP conditions and according to EU regulations.

The ChAdOx1.HTI vaccine (C)- is a replication-defective recombinant chimpanzee adenovirus (ChAd) vector based on a chimpanzee adenoviral isolate Y25[14] that encodes the HTI sequence. ChAdOx1.HTI was derived by subcloning the HTI antigen sequence into the generic ChAdOx1 BAC. The plasmid resulting from this subcloning (pC255; 40,483 bp) was linearized and transfected into commercial HEX293A T-REx cells to produce the vectored vaccine ChAdOx1.HTI. The ChAdOx1.HTI batch for non-clinical use was produced at the University of Oxford (UK), and large-scale amplification and purification of ChAdOx1.HTI were performed at ReiThera/Advent (Italy) according to cGMP.

Objectives

The primary objective of the study was to evaluate the safety and tolerability of HIV-1 vaccines DNA.HTI, MVA.HTI and ChAdOx1.HTI, administered intramuscularly as part of heterologous prime-boost regimen (DDDMM-CCM) in early-treated HIV-1-positive individuals. Secondary objectives included (1) evaluating the immunogenicity of DDDMM and CCM, (2) evaluating whether vaccination was able to prevent or delay viral rebound, induce post-rebound viral control and/or prevent or delay the need for resumption of ARV therapy during an ATI and (3) assessing the safety of the ATI period. Further immune (flow cytometry and viral inhibition assay) and viral evaluations (viral reservoir, autologous HIV-1 sequence and replicative fitness) were conducted as exploratory analyses. Post-hoc univariate and multivariate regression models were performed to explore potential correlates of virus control during ATI.

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Safety

Safety was assessed by an analysis of local and systemic reactogenicity and laboratory data. All solicited local and systemic AEs were recorded during seven days after administration of each investigational medicinal product using a 'participant reactogenicity diary card'. Unsolicited AEs and SAEs were recorded at any point during the study. AEs were graded according to the Division of DAIDS table for grading the severity of adult and pediatric adverse events, version 2.1 (March 2017). Throughout the study, AEs were analyzed by period: from screening to ATI start and by DDDMM/CCM or placebo; during ATI and after ART resumption. The primary safety endpoint of the study was the proportion of participants who develop grade ≥3 AEs (including SAE) related to the investigational medicinal product (IMP) administration. AEs were specified as related or unrelated to the IMPs by the investigator. Per the *Manual for Expedited Reporting of Adverse Events to DAIDS* (version 2.0, January 2010), AEs were reported as related if there was reasonable possibility that the AE may be related to the study agent(s), as suggested by a plausible, reasonable time sequence existing in relation to administration of the drug, the observed manifestation coincided with the known adverse reactions profile of the implicated drug, and the event could not be or was unlikely to be explained by a concurrent disease or by other drugs or chemical substances. If there was not a reasonable possibility that the AE was related to the study agent(s), the AE was reported as unrelated.

SMC and risk-mitigation plan during the COVID-19 pandemic

An SMC formed by three external experts in pharmacovigilance and HIV vaccine trials plus four non-voting sponsor representatives reviewed all blinded safety data from the study at pre-specified timepoints (that is, before progressing recruitment groups and every three months thereafter). The SMC also reviewed and approved a risk-mitigation plan established to minimize the impact of the COVID-19 pandemic on the conduct of the trial. This plan included weekly ATI assessments with home-based blood draws by personnel protected with personal protective equipment and remote visits via phone; a taxi service for on-site visits; 24-h/7-d phone availability for reporting any COVID-19 symptoms; SARS-CoV-2 polymerase chain reaction (PCR) testing before any IMP dosing; and provision of ART by courier. The SMC met virtually every week from 16 March 2020 to 28 May 2020 to review all blinded safety and laboratory data, and decisions on whether to continue with the trial were based on the evolving situation of the local epidemic, site capacity and a case-by-case discussion. New ICF versions with emerging information on COVID-19 were also developed and reviewed by the institutional ethical review board of HUGTIP.

High-resolution HLA-A, -B and -C typing

The QIAsymphony DNA kit (Qiagen) was used for genomic DNA extraction. Genomic DNA was genotyped by screening for HLA class I molecules (HLA-A, HLA-B and HLA-C genes) at high resolution at the Histocompatibility and Immunogenetics Laboratory (www.bancsang.net). Briefly, three loci were genotyped simultaneously by an in-house multiplex long-range PCR (LRPCR). The library was prepared (enzymatic fragmentation, adapter ligation and barcoding) from the PCR pools using the NGSgo kit (GenDx) according to the manufacturer's instructions. The final denatured library was sequenced using a NextSeq or MiSeq sequencer (Illumina). HLA class I genotype determination was performed with NGSengine 2.9.1 software (GenDx) using the IMGT database as reference.

CCR5-Δ32 genotyping

DNA was extracted from cryopreserved PBMCs stored from roll-over phase timepoints from participants entering the ATI ($n = 41$). DNA samples were amplified using fluorescent PCR in a 9700 Gene Amp PCR System or 2720 Thermal Cycler (Applied Biosystems) as described in ref. [38]. The forward (TTCATTACACCTGCAGCTCTC) and reverse (FAM-CCTGTTAGAGCTACTGCAATTAT) primers produced a 270-bp product for the CCR5-Δ32 allele and a 302-bp PCR product for the CCR5-WT allele. After amplification, 0.5 µl of PCR products was mixed in a 1:10 dilution with 24 µl of Hi-Di formamide (Applied Biosystems) and 0.7 µl of Gene Scan-500 ROX Size Standard (Applied Biosystems) and denatured at 94 °C for 5 min. The capillary electrophoresis was carried out in a 3130xlGenetic Analyzer (Applied Biosystems) and samples were analyzed with GeneMapper software (Applied Biosystems).

Sequencing

Whole-genome deep sequencing of the HIV-1 genome, including *gag*, *pol*, *vif* and *nef* genes, was performed using the Illumina NexteraXT protocol and a MiSeq platform with 300-bp paired-end sequencing length. Raw sequencing data were analyzed wih PASeq v 1.14 (www.paseq.org[39]). In brief, quality filter and adapter trimming was performed using trimmomatic[40]. High-quality sequences were aligned against the HXB2R reference using Bowtie2[41]. The consensus sequence at 20% frequency threshold was called using samtools[42] and a multiple alignment including all sequences was generated using MAFFT[43]. For each sample-specific consensus nucleotide sequence, subtyping was performed using the COMET online tool[44], and the Tamura–Nei nucleotide and Jones–Taylor–Thornton (JTT) amino-acid distances versus HXB2R and HTI sequences, respectively, were calculated using the R::phangorn package[45]. The number of mismatches (hamming) versus the HTI sequence was also calculated for all segments and aggregated at the protein level. The percentage difference (%AA.mm versus HTI) was calculated over the total length of the segment, correcting for the uncovered position in each sample. Group comparisons were performed using the Mann–Whitney *t*-test.

IFN-γ-ELISpot assay

Total HTI and HIV-1-specific T cells were assessed ex vivo using freshly isolated PBMCs with an IFN-γ-detecting enzyme-linked immunoabsorbent spot assay (ELISpot IFN-γ Mabtech kit) as previously described[2]. 15-mer peptides overlapping by 11 amino acids were combined into ten pools spanning different HIV-1 proteins/subproteins of 7–22 peptides per pool corresponding to the HTI vaccine insert (P1–P10, total $n = 111$ peptides, Thermo Fisher) and eight pools of 62–105 peptides per pool spanning the rest of the HIV-1 viral protein sequences (OUT P1–P8, total $n = 637$ peptides, obtained through the NIH AIDS Reagent Program). All peptide pools used in fresh ELISpots were tested in duplicate with a final concentration of individual peptide of 1.55 µg ml^{-1}. Medium only was used as no-peptide negative control in quadruplicate wells. Positive controls included two peptide pools covering lytic ($n = 16$) and latent ($n = 36$) Epstein–Barr viral proteins (1.55 µg ml^{-1}, Thermo Fisher), phytohaemagglutinin (PHA; 50 µg ml^{-1}, Sigma) and a chicken-embryo-fibroblast peptide pool (2 µg ml^{-1}) consisting of 32 previously defined human CD8$^+$ T-cell epitopes from cytomegalovirus, Epstein–Barr virus and influenza virus (Pantec). Spots were counted using an automated Cellular Technology Limited (C.T.L.) ELISpot reader unit. The threshold for positive responses was set at ≥50 SFCs per 10^6 PBMCs (five spots per well), greater than the mean number of SFCs in negative control wells plus three standard deviations of the negative control wells, or more than three times the mean of negative control wells, whichever was higher.

Mapping of HTI-specific responses

IFN-γ ELISpot assays using 147 individual overlapping peptides covering the entire HTI sequence were performed in in vitro expanded T cells. Participants' cryopreserved PBMCs obtained at baseline (week 0) and after DDDMM (week 24) and CCM or placebo vaccinations (week 28) were expanded using an anti-CD3 mAb (12F6) and kept in culture until sufficient cell numbers were reached for each timepoint[46]. Two consecutive overlapping peptides were considered one individual HTI response, and the highest magnitude of the sequential responses

was taken as the magnitude for each identified response. The results were expressed as the number of positive responses to individual peptides as well as the distribution among the different HIV subprotein regions covered by HTI: Vif-Nef, Pol-Int, Pol-RT, Pol-Prot, Gag-p2p7p1p7, Gag-p24 and Gag p17.

Intracellular cytokine staining assay

Cryopreserved PBMCs from week 28 (four weeks after completion of the last series of vaccinations, DDDMM-CCM) were used for stimulation with four pools of 9–43 peptides per pool spanning p17, p24/p15, Pol and Vif/Nef regions included in the HTI vaccine insert. Peptides were added at a final concentration of 5 μg ml^{-1} of each peptide in the presence of both 1.4 μg ml^{-1} of anti-CD28 (BD Bioscience) and 1.4 μg ml^{-1} anti-CD49d (BD Bioscience). As positive controls for the assay, cells were cultured alone in the presence of (1) anti-CD3/28 Dynabeads (Thermo Fisher Scientific) according to the manufacturer's instructions or (2) 10 ng ml^{-1} phorbol 12-myristate 13-acetate (PMA,Sigma) and 1 μM ionomycin (Sigma). Cells stimulated with only anti-CD28 and anti-CD49d antibodies or with DMSO were used as negative controls. Stimulated cells were incubated for 6 h at 37 °C in 5% CO_2, in the presence of 4 μl of monensin (GolgiStop, BD Bioscience). After 6 h of stimulation, cells were incubated with a Live/Dead fixable Violet Dead cell stain kit (Invitrogen), for exclusion of dead cells, along with the exclusion of monocytes and B cells by including in the dump channel anti-CD14 and anti-CD19 antibodies. Surface markers of T-cell lineage (CD3, CD4 and CD8), follicular T cells (CXCR5 and PD1), T-cell phenotype (CD45RA and CCR7), T-cell activation (CD69 and HLADR) and T-cell exhaustion (TIGIT, PD1) were included as well. Cells were fixed and permeabilized using the Cell Fixation and Cell Permeabilization Kit (Invitrogen) and intracellularly stained for INF-γ, GrazymeB, IL-2 and TNF-α. Details on the used antibodies can be found in the Reporting summary. Cells were resuspended in phosphate buffered saline supplemented with 1% FBS and acquired on an LSR Fortessa flow cytometer (BD, Unidad de Citometria, IGTP) and analyzed using FlowJo. The gating strategy is shown in Supplementary Fig. 2. When needed for variably expressed antigens, fluorescence minus one was included to define the boundaries between positive and negative populations. At least 100,000 total events were recorded. The frequencies of cells that produce all possible combinations of intracellular cytokines were calculated using the Boolean gating function of the FlowJo software. Data were reported after background subtraction (from the unstimulated negative control), and HTI-specific responses were defined as the sum of the specific population for each of the four HTI peptide pool stimulations.

In vitro viral suppressive capacity (VIA assay)

CD8$^+$ T-cell-mediated viral inhibition capacity was measured at 1:1 and 1:10 CD8-effector to CD4-target ratios, as previously described[47,48]. Autologous CD4$^+$ cells were obtained as targets from samples before vaccination where CD8$^+$ cells were depleted by magnetic bead separation (MACS Milteny Biotec). CD8$^+$-depleted cells (CD4$^+$-enriched fraction) were stimulated with PHA for three days and then infected by spinoculation with HIV-1 BAL and IIIB laboratory-adapted strains and autologous HIV-1 viruses at a multiplicity of infection of 0.001. HIV-infected cells were cultured in triplicates in R10 medium with 20 U ml^{-1} of IL-2 in 96-well round-bottomed plates, alone or together with effector CD8$^+$ T cells obtained by positive magnetic bead separation the same day from an additional vial of cryopreserved PBMCs from baseline and after DDDMM (week 24) and CCM or placebo (week 28) vaccinations. Viral replication was measured as the production of HIV-1 antigen p24 in culture supernatants (pg p24 per ml) at day 5 of co-culture using an Innogenetics p24 Elisa kit, and inhibition was expressed as a percentage with respect to the positive control of each virus (that is, infection in the absence of CD8$^+$ T cells).

Total and intact proviral HIV-1 DNA

To distinguish deleted and/or hypermutated proviruses from intact proviruses, total and intact proviral (IPDA) HIV-1 DNA copies in CD4$^+$ T cells were measured at screening and ATI start in extracts of lyzed CD4$^+$ T cells by digital droplet PCR (ddPCR), as previously described[49]. Samples from 41 participants that entered into the ATI period were processed at Accelevir Diagnostics. The DNA shearing index was calculated, and values for intact and defective proviruses were normalized to copies per 10^6 input cells (determined by RPP30, the gene encoding Ribonuclease P protein subunit p30) and adjusted for shearing using the DNA shearing index. Results were expressed as HIV-1 DNA copies (counts) per 10^6 CD4$^+$ T cells.

Viral fitness of participants' autologous HIV-1 viruses

The viral replication capacity of autologous HIV-1 viruses was measured for 38 of the 41 participants that entered into the ATI period. For isolation of autologous HIV-1 viruses, the CD4-enriched fraction of cryopreserved PBMCs stored at HIV-1 diagnosis pre/or within the first weeks of ART initiation were thawed and co-cultured with CD8-depleted PBMCs previously activated from three different healthy donors until HIV-1 was collected from supernatants. To determine the viral replication kinetics, a pool of PBMCs from three healthy donors, previously stimulated with 20 U ml^{-1} of IL-2 and PHA for three days, were infected by spinoculation at a multiplicity of infection of 0.001. HIV-1 antigen p24 was measured in culture supernatants (pg p24 per ml) using a commercial ELISA kit from Innogenetics at days 0, 3, 4, 5, 6 and 7 post-infection, and replication capacity was calculated by fitting a linear model to the log-transformed p24 data during the exponential growth phase. Uninfected cells and those infected with laboratory-adapted CCR5- and CXCR4-tropic viruses (HIV-1_{NL43}, HIV-1_{BaL} and HIV-1_{IIIB} isolates isolates) in the presence and absence of the antiretroviral AZT were used as reference values or controls.

Statistics

There was no power calculation for this study. The sample size was proposed to provide preliminary safety information on the vaccine regimen (primary objective). As a means to characterize the statistical properties of this study for the safety primary endpoint, in terms of the chances of observing an AE, 30 participants in the active group provided a high probability (78.5%) that this study would observe at least one event if the event occurred in the population with a true rate of 5%.

Time to viral load detection was calculated from the ATI start date to the date of first occurrence of pVL of ≥50 copies ml^{-1} and time off ART was calculated from the ATI start date to the date of ART resumption. Participants who prematurely resumed ART for COVID-19-related reasons were not censored for the survival analysis. The time to event was derived using the number of days between the ATI start date and the date of event expressed in weeks (number of days/7). The Kaplan–Meier estimator was used to describe the time to ART resumption, and survival functions were compared using the log-rank test. Differences in medians between groups were compared using the Mann–Whitney test and Fisher test, when corresponding. Spearman's ρ was used for correlations. All tests were two-sided, unadjusted for multiple comparisons, with 5% error rate. Post-hoc univariate logistic regression models (the list of considered covariates is provided in Extended Data Table 5) were considered to select the covariates with $P < 0.25$ to be included in the multivariate models. All selected covariates were analyzed for possible multicollinearity. Considering the final selected covariates, multivariate logistic regression models were adjusted for the binary outcome of time off ART ≥ 12 weeks versus <12 weeks. Analyses were performed using R project 3.6.2 (https://www.r-project.org/) and GraphPad Prism version 9.1.2 for Windows (GraphPad Software, https://www.graphpad.com). Flow cytometry data were preprocessed using FlowJo software version 10.6 and imported into Pestle2/SPICE software v5.35 (Vaccine Research Center, NIAID/NIH) for graphical representation.

Polyfunctional bar plots per treatment group were compared using the Mann–Whitney test per row, with individual ranks computed for each comparison. The two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli was used to control for false discovery rate. All performed analyses matched the pre-specified statistical analysis plan (AELIX002-SAP, version 2, from 10 July 2020).

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this Article.

Data availability

Deep sequencing raw data obtained from sequencing have been deposited in GenBank (accession no. PRJNA751460). Requests for access to the study data can be submitted through the Yale Open Data Access (YODA) Project site at http://yoda.yale.edu.

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Author contributions

C.B., I.M., J.M. and B.M. conceived and designed the study. L.B. and A.L. additionally contributed to the study design in further study amendments. L.B., J.C., C.L., M.L., J.M., B.M., F.P. and A.R. contributed with clinical development of the study. A.L., M.L., B.O.-T., F.P., F.P.E. and D.S. contributed to data management and overall study coordination. T.H. and E.G.W. helped with IMP production. M.C., S.C., T.E., A.L.L., M.P. and M.R.-U. performed the experiments. Y.A.-S., A.L.L., J.M., B.M., M.R.-R. and M.N.-J. undertook the statistical analysis. L.B., C.B., J.M. and B.M. drafted the manuscript. L.B., A.L., I.M., D.S., B.C., C.B., J.M., B.M. and R.P. critically revised the manuscript for important intellectual content. All authors reviewed and approved the final version of the manuscript.

Competing interests

C.B., B.M. and A.L.L. are co-inventors of the HTI immunogen (patent application PCT/EP2013/051596). C.B., B.M. and I.M. are co-inventors of US patent application no. 62/935,519 and US patent application no. 62/851,546, which have relevance to the vaccine regimen used in this study. B.M. reports consultancy personal fees from AELIX Therapeutics SL, as well as speakers' fees from Gilead, Janssen and ViiV Healthcare, outside the submitted work. C.B. is co-founder, CSO and shareholder of AELIX Therapeutics SL and serves as an advisor for Tendel Therapies and OmniScope, outside the submitted work. M.N.-J. is co-founder and shareholder of Nano1Health SL, outside the scope of the submitted work. I.M. is a shareholder of, and acts as a consultant to, AELIX Therapeutics SL. He is also the CMO of Orion Biotechnology, outside the scope of the submitted work. J.M. has received research funding, consultancy fees and lecture sponsorships from, and has served on advisory boards for, various laboratories (MSD, Abbvie, Boehringer Ingelheim, Gilead Sciences, Viiv Healthcare, Janssen-Cilag and Bristol-Myers-Squibb). The remaining authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-022-02060-2.

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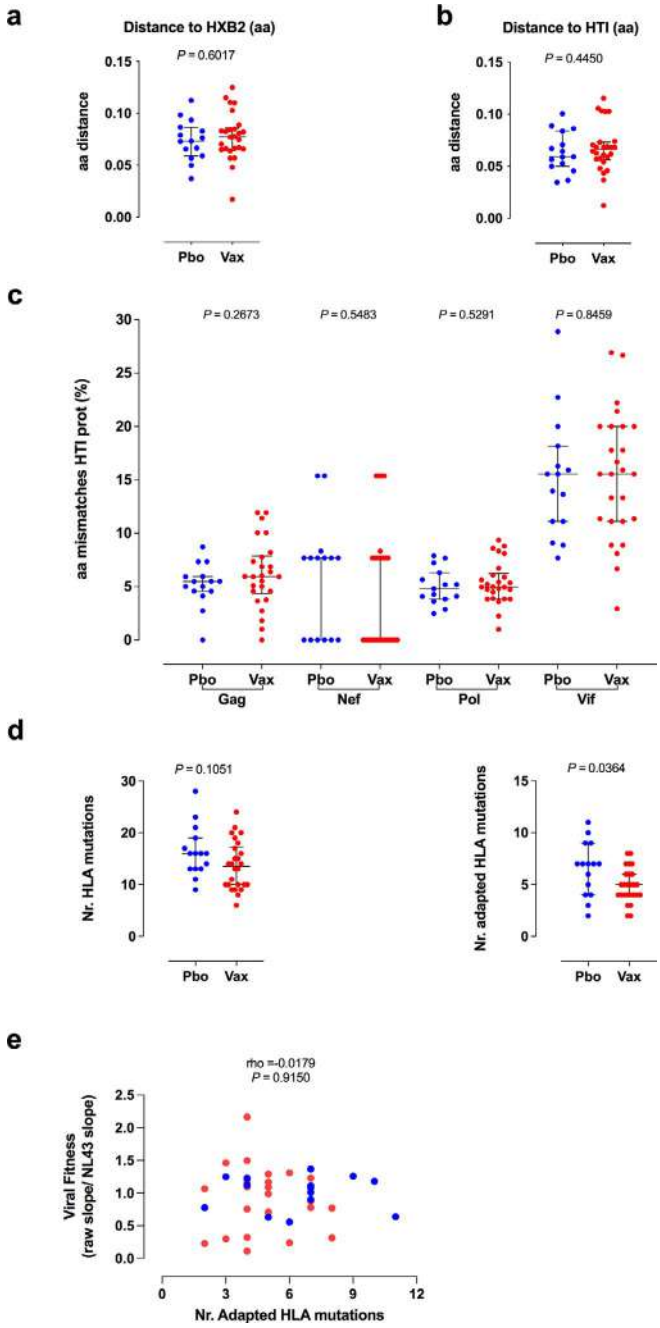

Extended Data Fig. 1 | See next page for caption.

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Extended Data Fig. 1 | Pre-ART HIV-1 sequences. Coverage of the participant's HIV-1 sequences by HTI vaccine. Comparison of distance with HXB2 (Tamura-Nei) in (a) and HTI (JTT) (b) for placebo (n=15, blue) and vaccine (n=26, red) recipients. *Mann-Whitney t-test is shown*. c, Genetic distance between the placebo (blue) and vaccine (red) recipient's pre-ART HIV-1 sequences and different HIV-1 proteins included in the HTI immunogen. *Mann-Whitney t-test is shown* d, Number of total HLA (left) and HLA-adapted (right) polymorphisms on pre-ART HIV-1 sequences from placebo (n=15, blue) and vaccine (n=26, red) recipients. *Mann-Whitney t-test is shown*. For (a)-(d), median with interquartile range is shown. e, Correlation between the number of pre-ART CTL escape footprints and replicative fitness of autologous pre-ART HIV-1 sequences from placebo (n=14, blue) and vaccine (n=24, red) recipients. *Spearman's correlation is used. Pbo:* placebo, *Vax:* vaccine.

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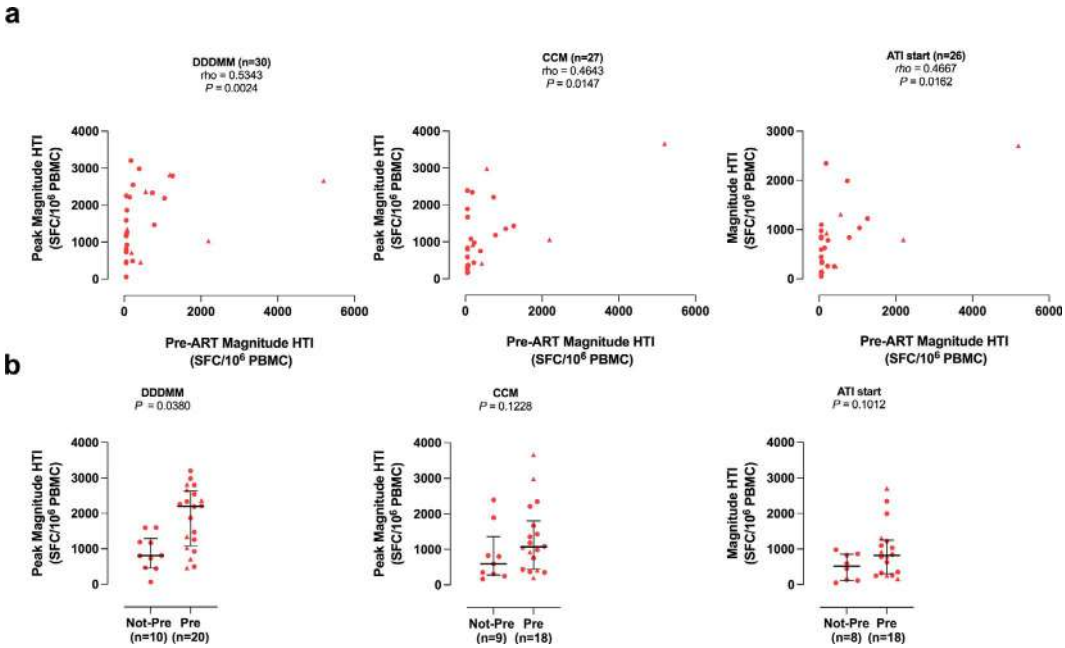
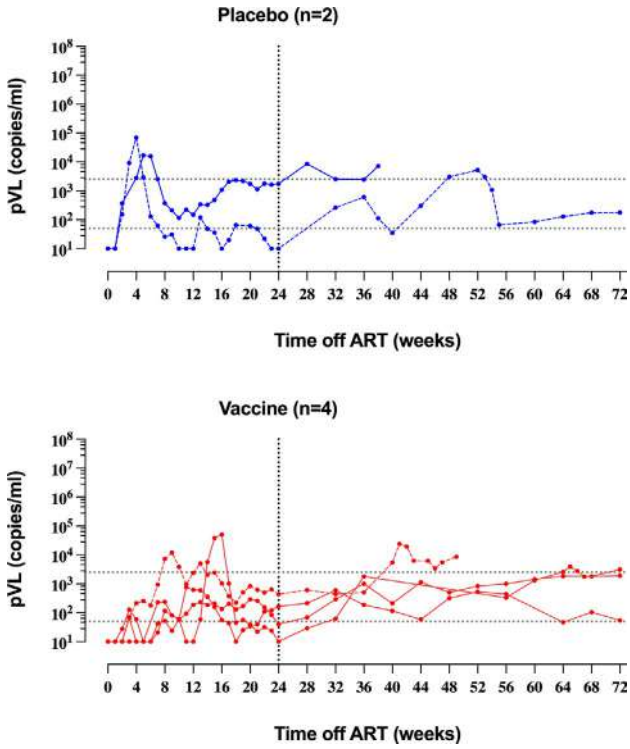


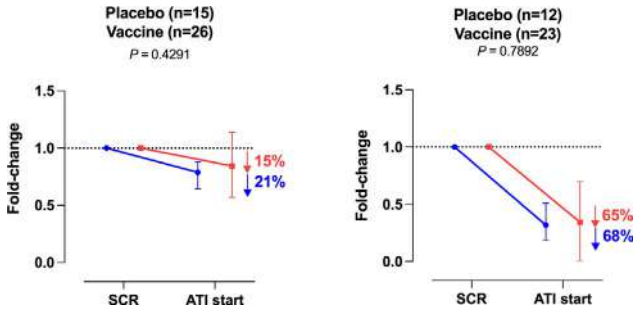
Extended Data Fig. 2 | Role of pre-existing responses to HTI. a, Relation between pre-ART HTI-specific responses and expanded HTI responses at peak immunogenicity timepoints after DDDMM, CCM and ATI start in all vaccine recipients are shown and individuals with any beneficial HLA allele are represented in triangle. *Spearman's correlation is used*. b, Peak HTI- magnitude after DDDMM (n=30), CCM (n=27) and at ATI start (n=26) for all the vaccine recipients having or not having any HTI responses before any ART (*Pre*, defined as HTI magnitude >50 SFC/10⁶ PBMC). Individuals with a beneficial HLA are shown in triangle symbols. Median with interquartile range is shown. Wilcoxon-Mann-Whitney test was used for comparison between treatments. *C:* ChAdOx1.HTI, *D:* DNA.HTI, *M:* MVA.HTI, *P:* placebo, *ART:* antiretroviral treatment, *ATI:* analytical treatment interruption.

Article https://doi.org/10.1038/s41591-022-02060-2



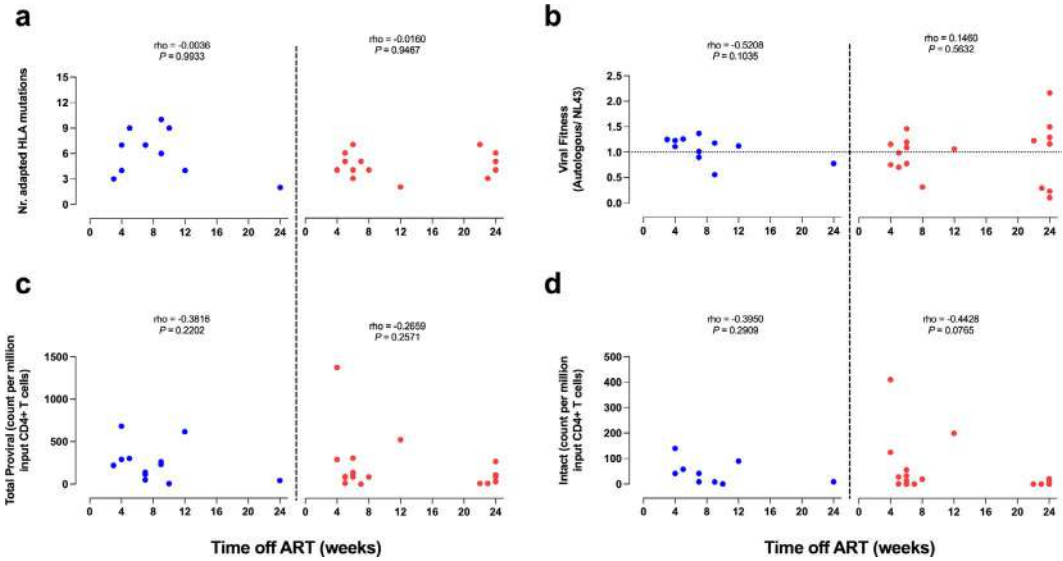
Extended Data Fig. 3 | ATI-extension beyond 24 weeks. Extended individual pVL up to 72 weeks is shown for placebo (blue) or vaccine (red) recipients that entered into the ATI-extension protocol (>24 weeks). Lines are interrupted on day of ART resumption, that is at week 72 or before. Dotted lines represent individuals with beneficial HLA class I alleles. *ATI:* analytical treatment interruption, *pVL:* plasma viral load.

Article https://doi.org/10.1038/s41591-022-02060-2



Extended Data Fig. 4 | HIV-1 reservoir decay throughout the study. Fold-change decay for total (placebo n=15, vaccine n=26) and intact (placebo n=12, vaccine n=23) proviral HIV-1 DNA by treatment group from study entry to ATI start. Median and interquartile range are represented. Mann-Whitney test is used. *SCR*: screening, *ATI*: analytical treatment interruption.

Article https://doi.org/10.1038/s41591-022-02060-2



Extended Data Fig. 5 | Virological correlates with ATI outcomes in participants without any beneficial HLA allele. a, Correlation between number of HLA-adapted polymorphisms in pre-ART HIV-1 sequences and time off ART in placebo (blue) and vaccine (red) recipients. b, Correlation between replication capacity of participant's autologous HIV-1 virus (relative to NL43) replication and time off ART in placebo (blue) and vaccine (red) recipients. Correlation between levels of total (c) and intact (d) proviral HIV-1 DNA at ATI and time off ART in placebo (blue) and vaccine (red) recipients. *Spearman's correlation is used. ART:* antiretroviral treatment, *ATI:* analytical treatment interruption.

Article https://doi.org/10.1038/s41591-022-02060-2

Extended Data Table 1 | Summary overview of all adverse events during the study

	Placebo		Vaccine		Total	
	Episodes n	Participants n (%)	Episodes n	Participants n (%)	Episodes n	Participants (%)
AE information						
Total AEs	238	15 (100)	570	30 (100)	808	45 (100)
Local AEs	12	6 (40)	108	29 (96.7)	120	35 (77.8)
Systemic AEs	226	15 (100)	462	29 (96.7)	688	44 (97.8)
Treatment-related AEs	111	15 (100)	329	30 (100)	440	45 (100)
SAEs related	0	0 (0)	0	0 (0)	0	0 (0)
SAEs	0	0 (0)	2	2 (6.67)	2	2 (4.4)
AEs leading to withdrawal	0	0 (0)	0	0 (0)	0	0 (0)
Deaths	0	0 (0)	0	0 (0)	0	0 (0)
Intensity of AEs						
Grade 1	142	15 (100)	351	30 (100)	493	45 (100)
Grade 2	94	15 (100)	210	29 (96.7)	304	44 (97.8)
Grade 3	2	2 (13.3)	8	6 (20)	10	8 (17.8)
Grade 4	0	0 (0)	1	1 (3.3)	1	1 (2.2)
Study Period						
DDDMM/PPPPP regimen	76	15 (100)	229	29 (96.7)	305	44 (97.8)
CCM/PPP regimen	35	11 (73.3)	100	22 (81.5)	135	33 (78.6)
ATI	22	11 (73.3)	42	18 (69.2)	64	29 (70.7)
Post-ATI	10	4 (30.8)	11	9 (40.9)	21	13 (37.1)

AE, adverse event; SAE, serious adverse event; C, ChAdOx1.MTI; D, DNA.HTI; M, MVA.HTI; P, placebo; ATI, Analytical Treatment Interruption.

Article https://doi.org/10.1038/s41591-022-02060-2

Extended Data Table 2 | Primary endpoint: All Grade 3 or 4 adverse events from baseline to ATI start (sorted by group of treatment)

PT	Group	Grade	Latest vaccination	Outcome	Related	SAE
Epididymitis	Placebo	3	Pbo2	Resolved	No	No
Hypertriglyceridaemia	Placebo	3	-	Resolved	No	No
Appendicitis	Vaccine	4	-	Resolved	No	Yes‡
Depression	Vaccine	3	-	Ongoing	No	No
Vertigo	Vaccine	3	-	Resolved	No	No
Asthenia/Fatigue	Vaccine	3	MVA3	Resolved	Yes	No
Gastroenteritis	Vaccine	3	-	Resolved	No	Yes‡
Gastroenteritis	Vaccine	3	-	Resolved	No	No
Musculoesletetal chest pain	Vaccine	3	-	Resolved	No	No
Myalgia	Vaccine	3	-	Resolved	No	No
Neck pain	Vaccine	3	DNA1	Resolved	No	No

‡ Required inpatient hospitalization.

ATI, Analytical Treatment Interruption; SAE, serious adverse event; Pbo2, 2nd placebo administration; MVA3, 3rd MVA.HTI vaccination; DNA1, 1st DNA.HTI vaccination.

Article https://doi.org/10.1038/s41591-022-02060-2

Extended Data Table 3 | Secondary Endpoint: Increases in HTI magnitude at peak immunogenicity timepoint

Parameter	Placebo (n=15)	Vaccine (n=30)	*p-value*[a]
Immune parameter			
HTI-magnitude	185 (50 to 1,080)	1,593 (170 to 3,660)	*0.0000*
HTI-magnitude change	100 (0 to 498)	1,499 (120 to 3,150)	*0.0000*
Percentage of Responders			
2-fold increase from BSL, n (%)	10 (66.7)	29 (96.7)	*0.0117*
3-fold increase from BSL, n (%)	2 (13.3)	29 (96.7)	*0.0000*
Specific 3-fold increase from BSL, n (%)[b]	1 (7)	24 (80.0)	*0.0000*

[a] *Wilcoxon-Mann-Whitney test or Fisher Exact test for comparison between treatment arms when appropriate.*
[b] *At peak immunogenicity timepoint, increase in HTI magnitude >3-fold from baseline value provided that responses to non-HTI regions are <3-fold from their baseline value*

Article https://doi.org/10.1038/s41591-022-02060-2

Extended Data Table 4 | Secondary Endpoint. Parameters of interest during the ATI

Clinical parameter	Placebo (n=15)	Vaccine (n=26)	p-value
Time to first pVL >50 copies/ml (weeks)	2 (1 -6)	3 (1 – 9)	0.1942
pVL (copies/ml) at first positive determination	553 (59 – 14,632)	214 (52 – 17,180)	0.2314
Time to pVL > 10,000 (weeks)	4 (1 – 19)**	5 (2 – 24$^{\mathrm{T}}$)**	0.1085*
Time to pVL peak (weeks)	5 (3 – 24)	6 (3 – 24)	0.2232
pVL (copies/ml) at peak determination	114,660 (16,868 – 980,171)	212,057 (289 - 10,000,000)	0.9460
Slope (log10(copies)/ml·weeks)	1.07 (0.07 – 1.69)	1.07 (0.04 – 2.18)	0.6847
Last pVL at end of ATI (copies/ml)	63,090 (10– 980,171)	64,866 (10 – 10,000,000)	0.5697
AUC normalized by weeks (log10(copies)/ml)	3.26 (1.80 - 4.30)	3.22 (1.68 – 3.91)	0.4648
Time off ART (weeks)	9 (3 – 24$^{\mathrm{T}}$)**	7.5 (4 - 24$^{\mathrm{T}}$)**	0.5755*
CD4 cell count at ATI start	865 (467 -1,502)	820 (470 – 1,462)	0.5882
CD4 cell count at end of ATI	687 (413 – 1,125)	645 (380 – 1,303)	0.4985

$^{\mathrm{T}}$ Censored at this time point, did not reach the event within 24 weeks of ATI (>24).
** Log-rank test used.*
*** Median survival time, minimum and maximum time presented.*
pVL <20 or <40 cop/ml (UD) is computed as 10 copies/ml & pVL >10,000,000 cop/ml is computed as 10,000,000 copies/ml.
pVL, plasma viral load; ATI, analytical treatment interruption; AUC, area under the curve.

Article https://doi.org/10.1038/s41591-022-02060-2

Extended Data Table 5 | Multivariate logistic regression model for resuming ART >12 weeks considering covariates at AELIX-002 entry (n=32 participants without beneficial alleles)

	$\hat{\beta}$	$s.e.(\hat{\beta})$	$\widehat{OR}$	95% CI ($\widehat{OR}$)
(Intercept)	2.9567	3.2682		
Treatment (Vax)	2.1105	1.1929	8.25	1.05 ; 140.36
pVL at ART initiation (1 log10 copies/mL)	-1.5881	0.7807	0.20	0.03 ; 0.73
Ratio CD4/CD8 at AELIX-002 entry (0.2 units)	0.4070	0.8943	1.50	1.10 ; 65.77

Vax, vaccine; pVL, plasma viral load; ART, antiretroviral treatment.

nature portfolio

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Clinical data was collected thourgh an eCRD designed by Dynasolutions.
Data analysis	Statistical analyses were performed using R version 3.6.2 (2019 The R Foundation for Statistical Computing, https://www.r-project.org/) and GraphPad Prism version 9.1.2 (226) for Windows (https://www.graphpad.com). Preprocessing of flow cytometry data was performed using both FlowJo software version 10.6 and imported into Pestle2/ SPICE software v5.35 (Vaccine Research Center, NIAID/NIH, Bethesda, MD, USA) for graphical representation

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Deep sequencing raw data obtained from sequencing have been deposited in GenBank (accesion PRJNA751460). Requests for access to the study data can be

submitted through the Yale Open Data Access (YODA) Project site at http://yoda.yale.edu. Full answers to the journal´s questions about data access can be found on the Yoda website but briefly, there is a review process to screen applicants who wish to review the study data. The YODA Project has the final decision-making rights regarding these policies and, by extension, the granting or denial of data requests, as per the contractual agreements between the YODA Project and its partners. Participant-level data sets (appropriately de-identified as per current regional standards to protect patient privacy) will be available. Requestors will be asked to sign a Data Use Agreement (DUA). The DUA explicitly states that access to the data will be used to create or materially enhance generalizable scientific knowledge and that findings derived from analysis will initially be publicly disseminated only through the peer-reviewed biomedical literature or scientific meetings.

It is possible to find more information in the following website:
The YODA Project | Policies & Procedures to Guide External Investigator Access to Clinical Trial Data (yale.edu)

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Only sex- based analysis were performed in the study. There were no discrepacies between gender and sex among participants in this study.
Population characteristics	Participants were mostly men (44 men and 1 female), with a median age of 36 years old, median time with undetectable pVL was 24 months and median CD4 count was 745 cell per µL. All participants were in a INSTI-based ART regimen. Median time from estimated HIV trasmission to ART intiation was 63 days and the percentatge of participants who had at least one beneficial HLA alelle was 22.2%.
Recruitment	All study subjects were recruited from an existing cohort of recently/acute infected individuals who had started antiretroviral treatment within 6 months of acquiring HIV infection (IrsiCaixa/FLS- Early_cART cohort). The study was briefly described by phone or during routine visits at the HIV clinics and if interested, a pre-screening visit was scheduled to all candidates who met the inclusion criteria and none of the exclusion criteria per protocol, avoiding selection biases. During the pre-screening visit the study and procedures were discussed in detail, giving time to think about participation. In a second appointment, the screening visit procedures were performed. Participants were compensated with 50€ or 30€ for each study visit performed on-site or remotely, respectively
Ethics oversight	This study was conducted according to Spanish regulations. Protocol was approved by the local Ethics Committee (Hospital Universitari Germans Trias i Pujol and protocol authorization from Spanish Drug Agency (AEMPs). This study is conforming to the standards of "Good Clinical Practice"by ICH E6. Following the "Good participatory practice guidelines" (published by the Joint United Nations Program on HIV/AIDS (UNAIDS), a Community Advisory Committee (CAC) was called with community stakeholders and representatives from the community HIV/STD screening centers, who participated in the review of the protocol design and informed consents.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	There was no power calculation for this study. The sample size was proposed to provide preliminary safety information on the vaccine regimen (primary objective. As a means to characterize the statistical properties of this study for the safety primary endpoint, in terms of the chances of observing an AE, 30 participants in the active group provided a high probability (78.5%) that this study would observe at least 1 event if the event occurred in the population with a true rate of 5%. The study size is in line with Guideline on Requirements for First-in-human clinical trials for potential high-risk medicinal products (EMEA/CHMP/SWP/28367/2007). The inclusion of placebo arm ensure that any potential for bias in the analysis of immune responses is minimised and give greater confidence to assignations of the causality of any adverse reactions observed in this study and will help to maintain blinding of the participants.
Data exclusions	1. A woman who is pregnant or breastfeeding at the screening visit. A men or woman who plans to carry out a conception process during the study. 2. When available, pre-cART genotypic data that demonstrates the presence of clinically significant drug resistance mutations that would prevent the construction of a viable cART regimen post-treatment interruption. 3. Reported periods of suboptimal adherence to cART. 4. History of past antiretroviral treatment interruptions longer than 2 weeks.

5. Participation in another clinical trial within 12 weeks of study entry (at screening visit).
6. Any AIDS-defining disease or progression of HIV-related disease.
7. History of autoimmune disease.
8. History or clinical manifestations of any physical or psychiatric disorder which could impair the subject's ability to complete the study.
9. Receipt of approved vaccines within 2 weeks of study entry and along the duration of the trial. (Efforts were made to ensure all participants included are updated on their Hepatitis A, Hepatitis B and Pneumococcal vaccinations before enrolment. Participants willing to undergo seasonal Flu vaccinations or other licensed vaccinations were excluded if vaccination would be expected to occur throughout the duration of the trial. Administration of approved vaccines were allowed during the Roll-over Phase)
10. History of anaphylaxis or severe adverse reaction to vaccines.
11. Previous immunisation with any experimental immunogens.
12. Receipt of blood products within 6 months of study entry.
13. Treatment for cancer or lymphoproliferative disease within 1 year of study entry.
14. Any other current or prior therapy which, in the opinion of the investigators, would make the individual unsuitable for the study or influence the results of the study.
15. Current or recent use (within last 3 months) of interferon or systemic corticosteroids or other immunosuppressive agents (use on inhaled steroids for asthma or topic steroids for localized skin conditions are permitted).
16. Any laboratory abnormalities including:

Haematology
• Haemoglobin < 10.0 g/dl
• Absolute Neutrophil Count (ANC) ≤ 1,000 /mm3 (≤ 1 x 109 /l)
• Absolute Lymphocyte Count (ALC) ≤ 600 /mm3 (≤ 0.6 x 109 /l)
• Platelets ≤100,000 /mm3, ≥ 550,000 /mm3 (≤ 100 /l, ≥ 550 /l)

Biochemistry
• Creatinine > 1.3 x ULN
• Aspartate aminotransferase (AST) > 2.5 x ULN
• Alanine aminotransferase (ALT) > 2.5 x ULN

Microbiology
• Positive for hepatitis B surface antigen,
• Positive for hepatitis C antibody, unless confirmed clearance of HCV infection (spontaneous or following treatment)
• Positive serology indicating active syphilis requiring treatment (Cases in which positive RPR titres were detected but syphilis has been confirmed to have been properly treated were allowed if treatment has been given in the previous two months).

17. (Phase A participants only) Small-pox vaccination.
• Clinical evidence of vaccinia scarification or self-reported history of vaccinia vaccination.

18. (Phase B participants only). Refusal to an eventual cART interruption within the scope of a future study objective. (A questionnaire related to participation in vaccine/cure trials and cART interruptions were performed during the screening visit to be able to address all participant's expectations and worries in a timely manner to reduce the risk of participants lost to follow-up during the trial.)

Prior to enter to Phase C (CCM regimen and ATI) , all of these exclusion criteria were reviewed and the following were added:
1. Virological failure during Phase A/B, defined as 2 consecutive determinations of pVL > 200 cop/ml.
2. Reported periods of suboptimal adherence to cART during Phase A/B.
3. History of antiretroviral treatment interruptions longer than 2 weeks during Phase A/B.
4. Complete refusal to cART interruption..

Replication

ELISPOT and Viral Inhibition immunogenicity assays were run un duplicates or triplicates, as stated in the methods section. Mean value of QC replicates was used for the analysis. ICS flow cytometry was not tested in duplicates for sample availability.

Randomization

Participants were randomized into 2 arms in a 2:1 (vaccine:placebo) ratio.
Subjects were assigned vaccine or placebo through a randomisation schedule based on the randomisation plan using dedicated computer software.
Allocation to active treatment or placebo was maintained throughout the study (Phase A/B/C).

Blinding

Laboratory staff in charge of processing and performing immunogenicity assays, all clinical investigators (including the Principal Investigator – PI-), study nurses administering the IMP, data entry staff and participants were blinded to treatment allocation. Statistics team with randomization tasks had not direct contact with neither participants nor blinded staff. There were designated clinical research associates (CRAs) in charge of monitoring unblinded procedures (randomization, dispensing, IMP preparation and masking).
Only pharmacy staff and unblinding study nurse team (IMP masking and preparation) had unblinding information.

Unblinding of an individual participant was indicated in the event of a medical emergency where the clinical management of the participant would be altered by knowledge of the IMP assignment. The decision to unblind was immediately communicated to the Sponsor and SMC. No emergency unblinding was needed over the study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

APC-Cy7-labeled anti-human CD3 mAb, Biolegend, Cat#344818, clone SK7, lot# B301842, B285505 and B275133. Dilution 1:333
BV570-labeled anti-human CD4 mAb, Biolegend, Cat# 300534, clone RPA-T4, lot# B319755. Dilution 1:100
BV510-labeled anti-human CD8 mAb, Biolegend, Cat# 301048, clone RPA-T8, lot# B320711, B253532 and B253532. Dilution 1:333
V450-labeled anti-human CD14 mAb, Biolegend, Cat# 325616, clone HCD14, lot# B272019
and B221368. Dilution 1:400
V450-labeled anti-human CD19 mAb, Biolegend, Cat# 302232 , clone HIB19, lot# B275673. Dilution 1:333
BV711-labeled anti-human CCR7 mAb, Biolegend, Cat# 353228, clone GO43H7, lot# B257292
and B303459. Dilution 1:100
BV785-labeled anti-human CD45RA mAb, Biolegend, Cat# 304140, clone HI100, lot# B320193 and B265673. Dilution 1:200
PE-Dazzle-594-labeled anti-human HLADR mAb, Biolegend, Cat# 307654, clone L243, lot# B235176. Dilution 1:250
BV605-labeled anti-human PD1 mAb, Biolegend, Cat# 329924, clone EH12.2H7, lot# B314840. Dilution 1:333
AF700-labeled anti-human IFNg mAb, Invitrogen, Cat# MHCIFG29, clone B27, lot# 2023807 and 2298113. Dilution 1:200
PerCPCy5.5-labeled anti-human IL2 mAb, Biolegend, Cat# 500322, clone MQ1-17H12, lot# B312837. Dilution 1:200
PE -labeled anti-human CD69 mAb, Biolegend, Cat# 310906, clone FN50, lot# B294991. Dilution 1:100
BV650-labeled anti-human CXCR5 (CD185) mAb, BD Biosciences, Cat# 740528, clone RF8B2, lot# 1096639. Dilution 1:2000
PE-Cy7-labeled anti-human TIGIT mAb, ThermoFisher Scientific, Cat# PE-Cy7, clone MBSA43, lot# 2172691. Dilution 1:100
FITC -labeled anti-human Granzyme B mAb, Biolegend, Cat# 515403, clone GB11, lot# B3322790. Dilution 1:35
ALEXA647-labeled anti-human TNF mAb, Biolegend, Cat# 502916, clone MAb11, lot# B301706. Dilution 1:100
Mouse anti-human CD28, BD Bioscience, Cat# 340975, clone L293, lot# 9091671 and 9343750.
Mouse anti-human CD49d, BD BD Bioscience, Cat# 340975, clone L293, lot# 9283590.

Validation

The ICS was performed following SOP multiparametric ICS Flow cytometry for AELIX-002. Manufacturing instructions for each antibody were followed. All antibodies were titrated before use. Whenever the antibody lot was changed, a bridging study was performed.

Validation statements from the manufactures for each antibody:

APC-Cy7-labeled anti-human CD3 mAb, Biolegend, Cat#344818, clone SK7, lot# B301842, B285505 and B275133. Kagoya Y, Nakatsugawa M, Saso K, Guo T, Anczurowski M, Wang CH, Butler MO, Arrowsmith CH, Hirano N. DOT1L inhibition attenuates graft-versus-host disease by allogeneic T cells in adoptive immunotherapy models. Nat Commun. 2018 May 15;9(1):1915.

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BV510-labeled anti-human CD8 mAb, Biolegend, Cat# 301048, clone RPA-T8, lot# B320711, B253532 and B253532. Thakral D, Dobbins J, Devine L, Kavathas PB. Differential expression of the human CD8beta splice variants and regulation of the M-2 isoform by ubiquitination. J Immunol. 2008 Jun 1;180(11):7431-42

V450-labeled anti-human CD14 mAb, Biolegend, Cat# 325616, clone HCD14, lot# B272019
and B221368. John S, Antonia SJ, Rose TA, Seifert RP, Centeno BA, Wagner AS, Creelan BC. Progressive hypoventilation due to mixed CD8+ and CD4+ lymphocytic polymyositis following tremelimumab - durvalumab treatment. J Immunother Cancer. 2017 Jul 18;5(1):54.

V450-labeled anti-human CD19 mAb, Biolegend, Cat# 302232 , clone HIB19, lot# B275673. Joseph A, Zheng JH, Chen K, Dutta M, Chen C, Stiegler G, Kunert R, Follenzi A, Goldstein H. Inhibition of in vivo HIV infection in humanized mice by gene therapy of human hematopoietic stem cells with a lentiviral vector encoding a broadly neutralizing anti-HIV antibody. J Virol. 2010 Jul;84(13):6645-53.

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Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT03204617
Study protocol	Version 7.0, 3rd February 2020 (last version) - submitted as S1. List of study amendments also provided as S2
Data collection	The study was conducted at Hospital Universitari Germans Trias i Pujol, Badalona, Barcelona. The community center Barcelona Checkpoint was a supporting center during ATI visits. Protocol approval/authorization: 05/06/2017 Contract signed: 28/06/2017 Clinical SIV: 30/06/2017 First vaccination: 28/08/2017 Interim report (week 32): 28/06/2018 First participant ATI started: 23/09/2019 Last subject last visit: 10/03/2021
Outcomes	This was an exploratory study with the primary endpoint is to evaluate the safety and tolerability of HIV-1 vaccine DNA.HTI administered IM alone and as part of a heterologous prime-boost regimen with MVA.HTI and ChAdOx1.HTI in early treated HIV-1 positive individuals (DDDMM and CCM). Safety was assessed by an analysis of local and systemic reactogenicity and laboratory data. Participants are monitored for AEs and SAEs during the study. All solicited local and systemic AEs are recorded from the time of administration of each IMP and within 7 days thereafter in a "Participant reactogenicity diary card". AEs assessment (severity/ causality) is based according the Division of DAIDS table for grading the severity of adult and pediatric adverse events, Version 2.1. [March 2017]. Secondary study endpoint included immunogenicity of DDDMM+CCM and effect on viral rebound during an ATI

-Immunogenicity of DDDMM+CCM was assessed by the proportion of participants that developed de-novo T cell responses to HTI-encoded regions measured by IFNg ELISPOT assay. Breadth and magnitude of total vaccine induced HIV-specific responses measured by IFNg ELISPOT too.

-The effect on viral rebound during ATI was evaluated by the following endpoints:
- Percentage of participants with viral remission, defined as plasma viral load (pVL) <50 copies/mL 12 and 24 weeks after start of ATI.
- Percentage of participants with viral control, defined as a pVL <2,000 copies/mL at 12 and 24 weeks after ATI.
- Time to viral detection, defined as the time from ATI start to first occurrence of detectable pVL (>50 copies/mL).
- Time to viral rebound, defined as the time from ATI start to first occurrence of pVL > 10,000 copies/mL.
- Percentage of participants who remain off cART at 12 and 24 weeks after ATI (efficacy endpoint).
- Time off cART, defined as time to cART resumption since ATI start (efficacy endopoint).

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Cryopreserved PBMCs from week 28 (4 weeks after completion of last series of vaccinations, DDDMM-CCM) were used for the stimulation with 4 pools spanning different HIV-1 proteins/sub-proteins of 9-43 peptides per pool corresponding to p17, p24/p15, Pol and Vif/Nef regions included in the HTI vaccine insert. Peptides were added at a final concentration of 5ug/ml of each peptide in the presence of both, 1.4µg/ml of anti-CD28 (BD Bioscience) and 1.4µg/ml anti-CD49d (BD Bioscience). As positive controls for the assay, cells were cultured alone in the presence of 1) anti-CD3/28 Dynabeads (Thermo Fisher Scientific) according to manufacturer´s instructions or 2) 10ng/ml PMA (SIGMA) and 1µM Ionomycin (SIGMA). Cells stimulated with only anti-CD28 and anti-CD49d antibodies or with DMSO were used as the negative controls. Stimulated cells were incubated for 6 h at 37℃ in 5% CO2, in the presence of 4µl of monensin (GolgiStop, BD Bioscience). After 6 hours of stimulation, cells were incubated with Live/Dead fixable Violet Dead cell stain kit (Invitrogen), for exclusion of dead cells, along with the exclusion of monocytes and B cells by including in the dump channel anti-CD14 and anti-CD19 antibodies. Surface markers of T cell linage (CD3, CD4 and CD8), follicular T cells (CXCR5 and PD1), T cell phenotype (CD45RA and CCR7), T cell activation (CD69 and HLADR) and T cell exhaustion (TIGIT, PD1) were included as well. Cells were fix and permeabilized using the Cell Fixation and Cell Permeabilization Kit (Invitrogen) and intracellularly stained for INF-γ, GrazymeB, IL-2 and TNF-α. Cells were resuspended in PBS supplemented with 1%FBS
Instrument	Cells were acquired on a LSR Fortessa flow cytometer (BD)
Software	The frequencies of cells that produce all possible combinations of intracellular cytokines were calculated using Boolean gating function of the FlowJo software. Preprocessing of flow cytometry data was performed using both FlowJo software version 10.6 and imported into Pestle2/ SPICE software v5.35 (Vaccine Research Center, NIAID/NIH, Bethesda, MD, USA) for graphical representation
Cell population abundance	At least 100,000 total events were recorde
Gating strategy	When needed for variably expressed antigens, fluorescence minus one (FMO) was included to define boundaries between positive and negative populations. A forward scatter height (FSC-H) vs forward scatter area (FSC-A) plot was used to exclude doublets. Then an FSC-A vs side scatter area (SSC-A) plot was used to select PBMCs. Live CD3+ cells were gated with the dump channel, which allowed exclusion of dead cells, monocytes (CD14), and B cells (CD19). Within the live CD3+ cell population, expression of CD4 and CD8 was then determined. The evaluation of cytokine production (INF-γ, GzmB, IL-2, and TNFα) (b) in CD4+ and CD8+ T cell populations from unstimulated and anti-CD3/CD28 stimulated conditions in one representative participant is shown in Extended data Fig 7

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Author´s Contribution

Fundació Lluita contra les Infeccions IrsiCaixa

This declaration concerns the following article/manuscript:

Title: Safety, immunogenicity and effect on viral rebound of HTI vaccines in early treated HIV-1 infection: a randomized, placebo-controlled phase 1 trial

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The article/manuscript is: **x Published** / accepted / submitted / In preparation

The phD student has contributed to the elements of this article/manuscript as follows:
A. No or little contribution / B. Has contributed (10-30%) / C. Has contributed considerably (40-60%) / D. Has done most of the work (70-90%) / E. Has essentially done all the work.

Formulation/identification of the scientific problem	A
Planning the experiments and methodology design and development	A
Involvement in the experimental work/data collection	D
Interpretation of results	D
Writing the first draft of the manuscript	E
Finalization of the manuscript and submission	E

The work presented in this article report the results of a randomized, placebo-controlled clinical trial with international relevance, in which the PhD student played a dedicated role in the clinical development phase. Participants were recruited from the Early_cART cohort lead by the PhD student. After recruitment, her responsibilities included active participation in study visits, overseeing a 24/7 line for adverse events throughout the entire trial, performing intense-monitored ATI, managing clinical databases, and collaboration with data analyses, interpretation of results and manuscript writing.

Dr Beatriz Mothe (director) Dr José Moltó (director)

4.3. Article 2.
The AELIX-003 trial

4.3. Article 2. The AELIX-003 trial

Safety, immunogenicity and effect on viral rebound of HTI vaccines combined with a TLR7 agonist in early-treated HIV-1 infection: a randomized, placebo-controlled phase 2a trial

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Safety, immunogenicity and effect on viral rebound of HTI vaccines combined with a TLR7 agonist in early-treated HIV-1 infection: a randomized, placebo-controlled phase 2a trial

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Building on results from the AELIX-002 trial with HIVACAT T-cell immunogen (HTI)-based vaccines, the AELIX-003 (NCT04364035) trial tested the safety of the combination of ChAdOx1.HTI (C) and MVA.HTI (M), with the TLR7 agonist vesatolimod (VES), in a double-blind, placebo-controlled, randomized clinical trial in 50 virally suppressed early-treated men with HIV-1 infection. Secondary objectives included immunogenicity and effects on viral rebound kinetics during a 24-week antiretroviral treatment interruption (ATI). The most common treatment-related adverse events were mild-to-moderate injection-site pain, influenza-like illness, headache, and fatigue. Strong, broad, and HTI-focused T-cell responses were induced by vaccination. All participants experienced viral rebound in ATI; 33.3% and 23.5% ($P = 0.4494$) of CCMM + VES and placebo recipients, respectively, remained off antiretroviral therapy for 24 weeks. Post hoc analysis confirmed a correlation between levels of HTI-specific T cells and prolonged time off antiretroviral therapy. The combination of HTI vaccines and VES was safe and elicited robust T-cell responses.

The lifelong requirement for antiretroviral therapy (ART) in people with HIV (PWH) remains a global challenge, particularly in resource-limited settings[1]. Despite effective ART suppression, ongoing HIV transcription from intact and defective proviruses in the viral reservoir contributes to chronic inflammation and immune activation, contributing to non-AIDS-associated diseases. Achieving an HIV cure or durable ART-free viral remission, thus, remains an unmet clinical need[2].

Therapeutic vaccines expressing the HIVACAT T-cell immunogen (HTI) were designed to elicit T-cell responses associated with spontaneous HIV control in the absence of ART. In the AELIX-002 (NCT03204617) randomized clinical trial[3] of early-treated PWH, a complex combination of HTI vaccines was found to be safe, highly immunogenic, and showed promise in improving virologic control, although the combination did not reduce the viral reservoir, prevent or delay viral rebound upon ART interruption, and/or induce sustained viral suppression to undetectable levels. Results from trials of other therapeutic vaccines given alone [4] or combined with latency-reversing agents such as vorinostat[5] or romidepsin[6,7] have also shown a lack of effect on the viral reservoir, possibly due to insufficient latency reversal and expression of HIV antigens for immune recognition[8], or caused by reservoir-cell compartmentalization in anatomical sites that are poorly accessible to immune effector cells[9], intrinsic resistance to cytotoxic T-lymphocyte-mediated killing[10] and/or insufficient antiviral activity.

Vesatolimod (VES) is an oral toll-like receptor 7 (TLR7) agonist in development as a potential HIV cure regimen component. VES in combination with a therapeutic vaccine and/or broadly neutralizing antibody (bNAb) showed efficacy in delaying viral rebound, decreasing

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viral setpoint, and inducing viral remission after ART cessation in a subset of early-treated SIV-infected rhesus macaques[11–13]. Data from human ex-vivo and in-vivo studies have shown that VES induces potent immune stimulation[14–16]. In a randomized, double-blind, placebo-controlled, phase 1b trial in ART-suppressed viremic HIV controllers, multiple-dose administration of VES up to 8 mg was safe and well-tolerated and promoted a modest delay in time to HIV rebound after cessation of ART[14].

Building on the non-human primate (NHP) studies, the phase 2a, double-blind, randomized, placebo-controlled, multicenter AELIX-003 trial (NCT04364035) was conducted in Spain to assess the safety, immunogenicity, and efficacy of ChAdOx1.HTI and MVA.HTI therapeutic HIV-1 vaccines given in combination with VES in ART-suppressed, early-treated PWH.

Results

Fifty cisgender men with confirmed HIV-1 infection who initiated ART within 6 months of the estimated HIV-1 acquisition date and maintained undetectable viral load for at least 1 year were recruited at nine clinical sites in Madrid and Barcelona, Spain. Participants were randomized 2:1 to receive a heterologous intramuscular vaccine regimen with ChAdOx1.HTI and MVA.HTI (referenced collectively as CCMM) and VES ($n = 33$) or matched placebo ($n = 17$) (Fig. 1a). While on ART, ChAdOx1.HTI or placebo were administered at weeks 0 and 12, followed by MVA.HTI or placebo at weeks 24 and 36. VES or placebo was administered once every other week as a 6-mg oral dose for a total of ten doses, with five doses between week 26 (2 weeks after the first MVA.HTI immunization) to week 34 and the other five doses between week 38 (2 weeks after the second MVA.HTI immunization) to week 46; the last VES dose was administered 2 weeks before entering a 24-week antiretroviral treatment interruption (ATI). The treatment regimen was completed by 30/33 active group (CCMM + VES) and 17/17 placebo group participants. Two (6.1%) participants voluntarily withdrew from the study and one (3.0%) was discontinued by investigator's decision due to transient elevated transaminases present before the first ChAdOx1.HTI dose. The remaining 47 participants received all four planned doses of vaccines or matching placebo, while 21 (63.6%) received all ten doses of VES or matching placebo. All 47 participants who entered the ATI phase received at least eight doses of VES or a matching placebo (Fig. 1b).

Demographics

Characteristics of randomized participants (intent-to-treat population) are shown in Table 1. ART was initiated after a median (range) of 61 (7–170) and 86 (16–167) days after the estimated HIV-1 acquisition date in CCMM + VES and placebo recipients, respectively. All but one participant, who was receiving tenofovir alafenamide/emtricitabine/rilpivirine (TAF/FTC/RPV), were on an integrase strand transfer inhibitor-based ART regimen at study entry. At enrollment, the median (range) time on ART was 41 (16–132) and 43 (17–116) months, and the median CD4 + T-cell counts (range) were 882 (451–1600) and 831 (534–1333) cells/μL in CCMM + VES and placebo recipients, respectively. Six CCMM + VES recipients (18.2%) and four placebo recipients (23.5%) had at least one human leukocyte antigen (HLA) class I allele associated with spontaneous control of HIV replication, including HLA-B27, HLA-B57, HLA-B58:01, HLA-B15:16, and/or HLA-B15:17[17].

Safety

Investigators reported a total of 598 treatment-emergent adverse events (TEAEs) in 32 (97.0%) and 17 (100%) participants in the CCMM + VES and placebo groups, respectively (Supplementary Table 1). Nearly all TEAEs (384/385 events) in CCMM + VES recipients were graded as mild to moderate; one serious event of acute cholangitis in the CCMM + VES group was considered not related to the study treatment (Supplementary Table 1).

Thirty (90.9%) CCMM + VES recipients and 11 (64.7%) placebo recipients had TEAEs related to study drugs (Supplementary Tables 2–5). Most of these treatment-related TEAEs (238/337) were reported in CCMM + VES recipients. The most common treatment-related TEAEs (>30%) in CCMM + VES recipients included injection-site pain (87.9%), influenza-like illness (36.4%), headache (33.3%), and fatigue (33.3%) (Supplementary Table 2). All TEAEs related to study drugs were mild or moderate, and most had an outcome of recovered/resolved or recovering/resolving. There were no serious TEAEs related to study drugs or TEAEs leading to study drug discontinuation (Supplementary Table 1).

In the CCMM + VES and placebo groups, 31 (93.9%) and 13 (76.5%) participants, respectively, recorded a total of 1533 solicited local or systemic reactions in the participants' diaries (Supplementary Table 1). In the CCMM + VES group, but not in the placebo group, five (15.2%) participants reported eight grade 3 or 4 solicited local reactions of pain/tenderness; one event was assessed by the investigator to be related to ChAdOx1.HTI and seven were related to MVA.HTI (Supplementary Table 6). Ten (30.3%) participants in the CCMM + VES group reported 40 grade 3 solicited systemic reactions and three (17.6%) participants in the placebo group reported 27 grade 3 or 4 solicited systemic reactions. In the CCMM + VES group, 27 out of 40 solicited grade 3 or 4 events were assessed by the investigator to be related to the study drugs (20 were related to ChAdOx1.HTI, five were related to MVA.HTI, and three were related to VES) (Supplementary Table 6). The most common solicited systemic symptoms at any dose of the study drug were fatigue/general malaise, muscle aches, and low appetite. All participant-reported solicited local or systemic reactions were investigator-assessed as mild or moderate, with an outcome of resolved (except for one solicited systemic reaction assessed as recovering or resolving).

Vaccine immunogenicity

The magnitude of total HTI-specific T-cell responses was compared between CCMM + VES and placebo participants before and 4 weeks after the first and second ChAdOx1.HTI vaccination (weeks 0, 4, 12, and 16) and the first and second MVA.HTI vaccination (weeks 24, 28, and 36) and at ATI start (week 48). Differences in HTI magnitude at each timepoint relative to baseline (week 0) were calculated. Peak immunogenicity timepoint was defined as the timepoint with the highest HTI magnitude among all timepoints evaluated for each participant.

Persistent, statistically significant differences in absolute magnitude and change in magnitude from baseline of the HTI-specific T-cell responses were observed from week 16 (4 weeks after the second ChAdOx1.HTI) up to ATI start ($P < 0.0001$) between CCMM + VES and placebo participants (Supplementary Table 7). The highest magnitude of HTI-specific T-cell responses was observed at week 36 (12 weeks after the first MVA.HTI vaccination and after five doses of VES), with a difference (95% CI) between CCMM + VES and placebo recipients of 915 (385 and 1645) HTI-specific spot-forming cells (SFCs)/10^6 peripheral blood mononuclear cells (PBMCs) ($P = 0.0002$). HTI responses were well-maintained during the second VES cycle (week 38–46) as well as HTI-specific magnitude at ATI start (week 48); the median (range) was 970 (115–3895) in CCMM + VES participants versus 165 (0–1155) SFCs/10^6 PBMCs in placebo participants ($P < 0.0001$) (Fig. 2a, b).

The focus of the HTI-specific T-cell response, as a percentage of magnitude of the total anti-HIV-1-specific T-cell responses directed against HTI-covered regions, was compared between treatment groups in terms of absolute focus and change from baseline. At ATI start (week 48), the median percentage (95% CI) focus had significantly increased from baseline in CCMM + VES recipients, with a difference of 37.2% (21-53) versus placebo ($P = 0.0002$). Median (range) focus of HTI responses over the total of HIV-1-specific T cells was 47.2% (24–100%) with CCMM + VES and 25.5% (0–82%) with placebo ($P = 0.0024$) (Fig. 2c).

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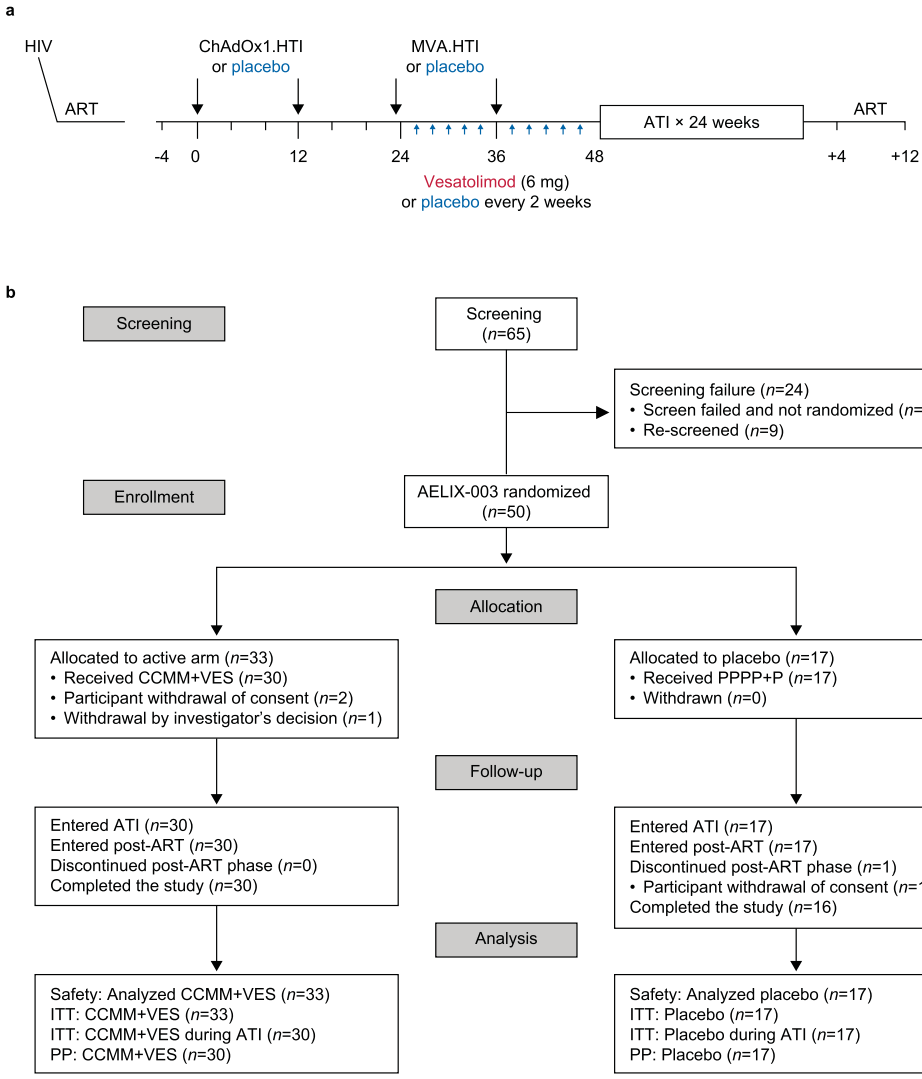

Fig. 1 | Trial design and participant disposition. a Trial schedule and study visits. While on ART, ChAdOx1.HTI or placebo were administered at week 0 and week 12, followed by MVA.HTI or placebo at weeks 24 and 36. VES was administered at a dose of 6 mg orally, once every other week, for a total of ten doses from weeks 26 to 34 and 38 to 46 of the study; the last dose was administered 2 weeks before interrupting ART for a maximum of 24 weeks. **b** Participant disposition (CONSORT flow diagram).

The breadth of the total HTI-specific T-cell response was defined as the number of positive-reactive peptide pools (out of the 10 HTI covering peptide pools used in the ELISpot assay) that exhibited a magnitude above a sample- and timepoint-specific cutoff. The highest breadth of HTI-specific T-cell response was observed in the CCMM + VES group after the first MVA.HTI administration (median of four reactive pools, range 1-8) that was sustained up to ATI start, with statistically significant differences versus placebo recipients, who had a median (range) of one (0–8) HTI-reactive pool at ATI start ($P = 0.0008$) (Fig. 2d). Distribution of T-cell responses within the different HIV subproteins covered by the HTI immunogen sequence showed a broad response toward HTI without revealing any specific pattern of immunodominance in vaccine recipients (Fig. 2e).

VES pharmacokinetics/pharmacodynamics

In the pharmacokinetic substudy population, mean VES concentrations in plasma peaked 2 h after administration and declined in a biphasic manner thereafter (Supplementary Fig. 1a), with a mean

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Table 1 | Study population

Characteristics	CCMM + VES (*n* = 33)	Placebo (*n* = 17)	ITT population (*n* = 50)
Age, years	38 (24–55)	37 (21–59)	38 (21–59)
Sex at birth, male, *n* (%)	33 (100.0%)	17 (100.0%)	50 (100.0%)
BMI (kg m^{-2})	24.1 (16–44)	23.8 (19–33)	24.0 (16–44)
Days from estimated HIV acquisition to ART initiation	61 (7–170)	86 (16–167)	67 (7–170)
Fiebig stage at ART initiation, *n* (%)[a]			
Eclipse	0 (0.0%)	0 (0.0%)	0 (0.0%)
I	2 (6.1%)	0 (0.0%)	2 (4.0%)
II	3 (9.1%)	1 (5.9%)	4 (8.0%)
III	3 (9.1%)	0 (0%)	3 (6.0%)
IV	3 (9.1%)	3 (17.6%)	6 (12.0%)
V	12 (36.4%)	6 (35.3%)	18 (36.0%)
VI	7 (21.2%)	5 (29.4%)	12 (24.0%)
Missing	3 (9.1%)	2 (11.8%)	5 (10.0%)
ART regimen, *n* (%)			
INSTI-based	33 (100%)	16 (94.1%)	49 (98%)
Other, NNRTI-based	0 (0.0%)	1 (5.9%)	1 (2.0%)
Time on ART, months	40.6 (15.7–132.2)	43.46 (17–115.8)	42.03 (15.7–132.2)
Absolute CD4 (cells/mm^3)	882 (451–1600)	831 (534–1333)	872 (451–1600)
CD4 %	39 (24–49)	41 (34–53)	40 (24–53)
CD4/CD8 ratio	1.2 (1–2)	1.2 (1–2)	1.2 (1–2)
Beneficial HLA alleles, any[b]	6 (18.2%)	4 (23.5%)	10 (20.0%)

Baseline clinical and demographic characteristics of the study population (*n* = 50).
Characteristics related to demographics, clinical profiles, and treatment details of randomized study participants at study entry (*n* = 50).
Data presented as median (min–max) except where specified.
BMI body mass index, *INSTI* integrase strand transfer inhibitor, *ITT* intent-to-treat, *NNRTI* non-nucleoside reverse transcriptase inhibitor.
[a]According to Fiebig stage classification.
[b]Includes HLA-B27, HLA-B57, HLA-B58:01, HLA-B15:16, and HLA-B15:17.

half-life ($t_{1/2}$) of 13.3 h (standard deviation [SD] 3.40). Geometric mean (GCV%) VES maximum concentration (C_{max}), concentration at 24 h (C_{24}), and area under the concentration-time curve from time zero to 24 h ($AUC_{0\text{-}24}$) were 3520 (54.1%), 355 (65.4%) pg/ml and 22,400 (53.6%) pg·h/ml, respectively. VES concentrations collected at similar postdose timepoints were consistent across visits (Supplementary Fig. 1b).

VES pharmacodynamics (PD), cytokine levels, and immune-cell activation were evaluated before and 24 h after the first VES/placebo dose (week 26) and before and 24 h after the 10th planned VES/placebo dose (week 46). VES consistently increased plasma levels of interferon-γ (IFN-γ)-inducible protein-10 [IP-10], IFN-inducible T-cell-α chemoattractant [ITAC]), IFN-α, and interleukin-1 receptor antagonist [IL-1RA]) postdose in CCMM + VES recipients (Fig. 3).

Levels of activated (CD25+, HLA-DR+, and/or CD25+/HLA-DR+) CD4 T and CD8 T cells were similar between predose and postdose in both groups (Fig. 4a–f). A significantly higher frequency of activated (CD69+) natural killer (NK) cells was observed at both week 26 and week 46 postdose versus predose ($P = 0.0091$ and $P = 0.0039$, respectively), and versus baseline ($P = 0.0283$ and $P = 0.0034$, respectively) in CCMM + VES recipients but not in placebo recipients (Fig. 4g, h). However, the overall levels of NK activation were similar between groups.

We did not observe an effect of CCMM + VES on plasma viremia during the intervention phase. Six (20.0%) participants in the CCMM + VES group had a single transient increase ("blip") in HIV-1 RNA ≥50 copies/ml (range 52–350) detected during the VES administration period, specifically after doses four and five. Two (11.7%) participants in the placebo group had HIV-1 RNA to ≥50 copies/ml at least once during the intervention period, one of which showed several determinations suggestive of poor ART adherence.

Effects on viral rebound during ATI

Forty-seven participants (30 CCMM + VES recipients and 17 placebo recipients) entered the ATI period for a maximum of 24 weeks. Participants were monitored weekly for clinical symptoms and HIV-1 plasma viral load (pVL), and a safety assessment, including biochemistry and complete blood and CD4 T-cell counts, was conducted every 4 weeks. ART was resumed after a single HIV-1 pVL determination exceeding 100,000 copies/ml, 8 consecutive weeks with >10,000 copies/ml, two consecutive CD4 + T-cell counts below 350 cells/mm^3 and/or a reported grade 3 or higher-severity clinical symptom suggestive of acute retroviral syndrome. Time-to-event endpoints were adjudicated to be the first of at least two consecutive determinations of HIV-1 pVL >50 or >10,000 copies/ml.

HIV-1 rebound (pVL >50 copies/ml) was detected in all participants after ART discontinuation, at a median (95% CI) of 3.1 (2.3–4.0) and 3.0 (2.1–4.0) weeks in CCMM + VES and placebo recipients, respectively (log-rank test, $P = 0.1108$). HIV-1 pVL >10,000 copies/ml was reached in 37 participants (78.7%) after ART discontinuation at a median (95% CI) of 5.1 (4.1–8.0) and 5.0 weeks (3.1–10.9) in CCMM + VES and placebo recipients, respectively (log-rank test, $P = 0.5132$) (Fig. 5a–c). Rebound kinetics parameters, including peak pVL, time to peak pVL, and slope of pVL increase or AUC of viremia from ATI start until ART resumption were comparable between CCMM + VES and placebo recipients (Supplementary Table 8).

Ten of 30 CCMM + VES recipients (33.3%; 95% CI 17.3–52.8) remained off ART for 24 weeks versus four of 17 placebo recipients (23.5%; 95% CI 6.8–49.9) ($P = 0.4494$). Reasons for ART resumption before 24 weeks of ATI (Supplementary Table 9) included reaching an HIV-1 pVL of ≥100,000 copies/ml in 16 (53.3%) and eight (47.1%) participants in the CCMM + VES or placebo groups or having a sustained HIV-1 pVL of ≥10,000 copies/ml on two consecutive weekly tests that did not decrease to <10,000 copies/ml by 8 weeks after the first test in two (6.7%) and five (29.4%) CCMM + VES and placebo recipients, respectively. Two CCMM + VES recipients resumed ART for other reasons; one at ATI week 23 by scheduling mistake (i.e., without meeting ART resumption criteria, confirmed by detection of antiretroviral drugs in plasma at ATI week 24), the other at week 18 due to reactivation of occult hepatitis B co-infection. No participant resumed ART due to CD4 + T-cell decrease or a clinically significant acute retroviral syndrome per protocol criteria. At 12 and 24 weeks of ATI, HIV-1 pVL was <2000 copies/ml in eight (26.7%) and five (16.7%) CCMM + VES recipients versus three (17.6%) and two (11.8%) placebo recipients ($P = 0.3686$ and $P = 0.5872$, respectively).

All participants resumed effective ART at the end of the ATI. One CCMM + VES recipient had HIV-1 pVL >50 copies/ml at the end of the study (12 weeks after ART resumption). This participant resumed ART at ATI week 4 with an HIV-1 pVL >10,000,000 copies and achieved an expected decline in viral load by weeks 4 and 12 after ART resumption (HIV-1 pVL 4460 and 369 copies/ml, respectively).

Viral reservoir

HIV reservoir evaluation was conducted with PBMC samples collected at baseline (week 0) and ATI start (week 48) and included total and intact proviral HIV DNA. Paired determinations of total proviral DNA at baseline and at ATI start were available for 29 (96.6%) and 17 (100%) CCMM + VES and placebo recipients, respectively. Intact proviral DNA data were not available in three (10.0%) and seven (41.2%) participants

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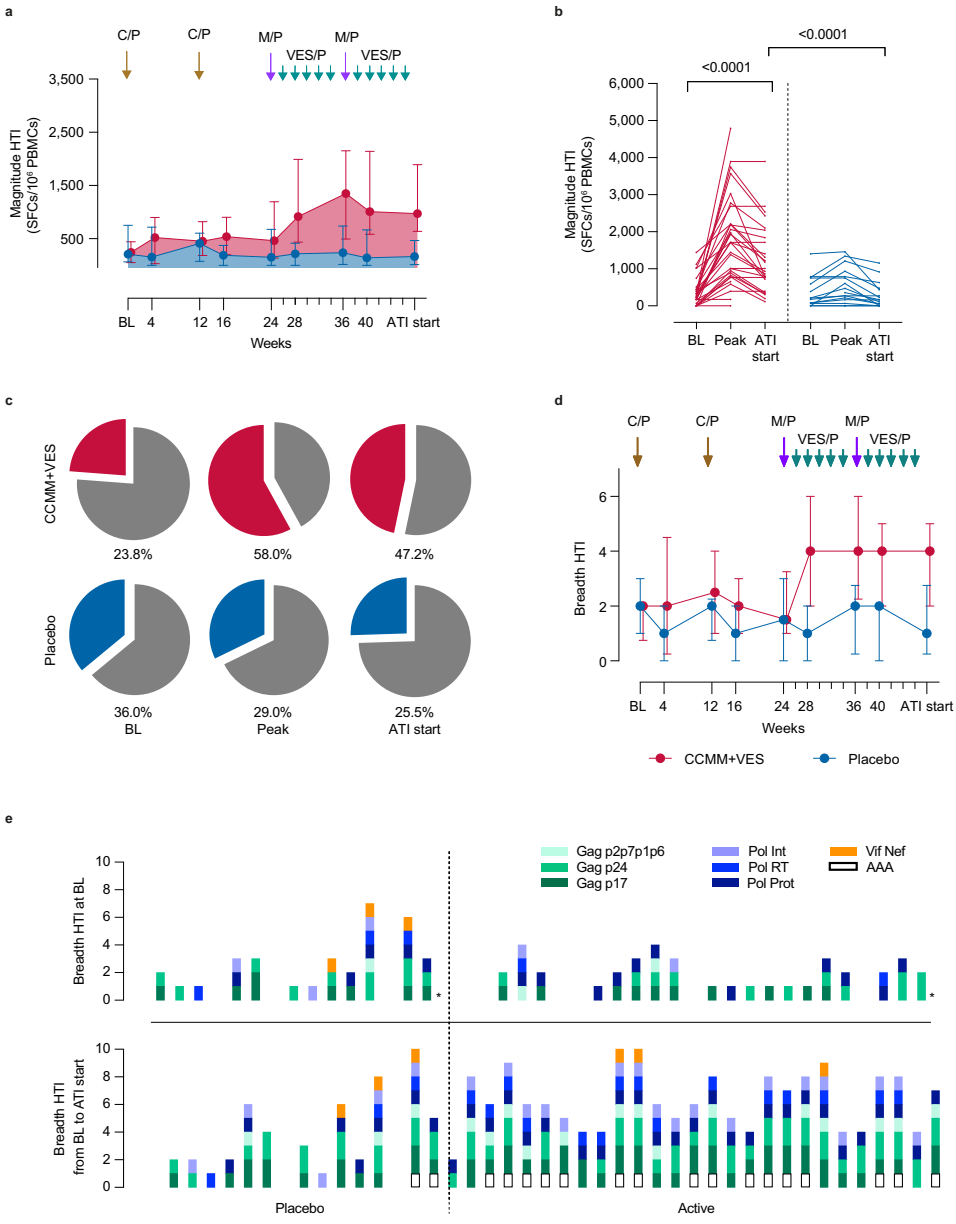

from the CCMM + VES and placebo groups who entered the ATI, respectively, due to Amplicon signal issues. At study entry, reservoir levels were numerically higher in CCMM + VES versus placebo recipients, both for total and intact HIV-1 proviral DNA (median [range], 268 (1.5–2579) total HIV-1 copies/10^6 CD4 + T cells in CCMM + VES recipients and 121 (1.5–842) copies/10^6 CD4 + T cells in placebo recipients). No significant changes from baseline to week 48 (ATI start) were detected in either treatment group ($P = 0.5529$ for total and $P = 0.7902$ for intact HIV-1 proviral DNA), consistent with a population with a median time on ART longer than 3 years at study entry[18]. Similarly, at ATI start, reservoir levels remained numerically higher in CCMM + VES recipients versus placebo recipients, both for total and intact HIV-1 proviral DNA. Median (range) total and intact HIV-1 proviral DNA were 210 (3.2–2432) and 55 (1–797) copies/10^6 CD4 + T cells

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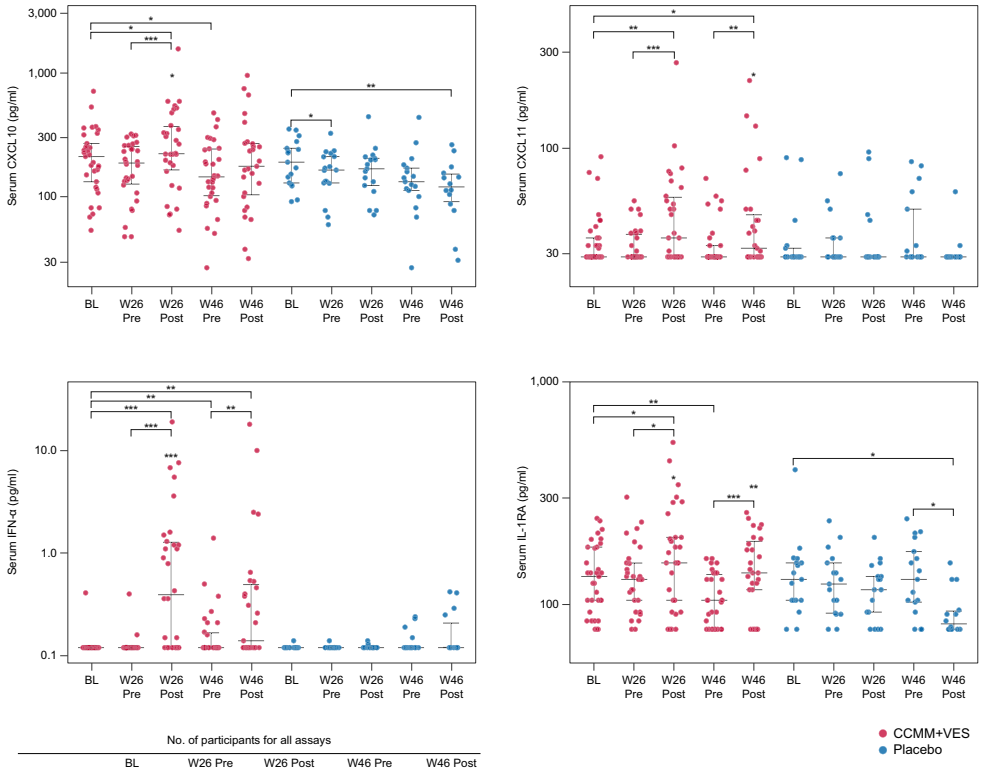
Fig. 2 | Vaccine immunogenicity. a Median and IQR magnitude of total HTI-specific T-cell response (sum of SFCs per 10^6 PBMCs for HTI pools P1–P10) over time in 33 CCMM + VES (shown in red) and 17 placebo (shown in blue) recipients from baseline to week 48. Statistics are derived from data from 24–30 (out of 33) CCMM + VES recipients and 13–16 (out of 17) placebo recipients at each timepoint, depending on sample availability and valid results after assay QC. Arrows indicate vaccination or VES/placebo administration dates. BL baseline, C ChAdOx1.HTI, MVA.HTI, P placebo. **b** Individual magnitudes of HTI-specific response (sum of SFCs per 10^6 PBMCs for HTI pools P1–P10) in CCMM + VES (shown in red) and placebo (shown in blue) recipients, at study entry (BL), the timepoint between study entry and week 48 with the strongest observed total HTI-specific T-cell responses (peak) and at week 48 (ATI start). *P* value tested using a two-sided van Elteren test (*P* value <0.0001; unadjusted for multiple comparisons, with 5% error rate), with a stratification factor for the actual potential for superior viral control (yes/no). **c** Median contribution of HTI-specific T cells to total virus-specific responses, according to specificity. HTI-specific responses are shown in red for CCMM + VES and in blue for placebo recipients; non-HTI HIV-1-specific responses are shown in gray. **d** Median and IQR breadth of total HTI-specific T-cell response (number of reactive HTI pools P1–P10) over time in 33 CCMM + VES (red) and in 17 placebo (blue) recipients from baseline to week 48. Statistics are derived from data of 24–30 (out of 33) CCMM + VES recipients and 13–16 (out of 17) placebo recipients at each timepoint, depending on sample availability and valid results after assay QC. Arrows indicate vaccination or VES/placebo administration dates. **e** Distribution of HTI-specific responses within the different HIV-1 subproteins at study entry (BL) and accumulated up to the start of ATI for each placebo (P01–P17) and CCMM + VES (V01–V30) recipient.



No. of participants for all assays

	BL	W26 Pre	W26 Post	W46 Pre	W46 Post
CCMM+VES	33	30	30	30	27
Placebo	17	17	17	17	14

Fig. 3 | VES PD responses in CCMM + VES and placebo recipients. Serum levels were measured by single-molecule array assay for IFNα and multiplex cytokine assay for IP-10 (CXCL10), ITAC (CXCL11), and IL-1RA at baseline (BL), before and 24 h after the first VES/placebo dose (week 26), and before and 24 h after the 10th (last) VES/placebo dose (week 46). Colored-coded dots represent data collected from individual participants (red color was used to mark participants receiving CCMM + VES and blue for those receiving placebo). Median and IQR are shown with black dots and lines. Wilcoxon test was used to compare data at given timepoints between groups (unpaired) or data from two different timepoints within the same group longitudinally (paired; shown by brackets), reported using nominal *P* values. $^*P \le 0.0500$, $^{**}P \le 0.0100$, $^{***}P \le 0.0010$. CXCL C-X-C motif chemokine ligand.

in CCMM + VES recipients and 86 (4.3–718) and 18 (1.5–391) copies/10^6 CD4 + T cells in placebo recipients, respectively ($P = 0.1238$ and $P = 0.6858$) (Fig. 6a, b). Of interest, one CCMM + VES recipient and two placebo recipients had undetectable intact proviral HIV-1 DNA both at study entry and ATI start. The CCMM + VES recipient remained off ART for the entire ATI period and showed only five detectable determinations of HIV-1 pVL >50 copies/ml, the highest being 104 copies/ml at 21 weeks of ATI. On the contrary, the two placebo recipients with undetectable intact reservoir levels at ATI start showed a fast viral rebound of up to 4 log of HIV-1 RNA copies/ml in both cases and resumed treatment at 16 and 24 weeks of ATI with 19,500 and 4280 copies/ml, respectively.

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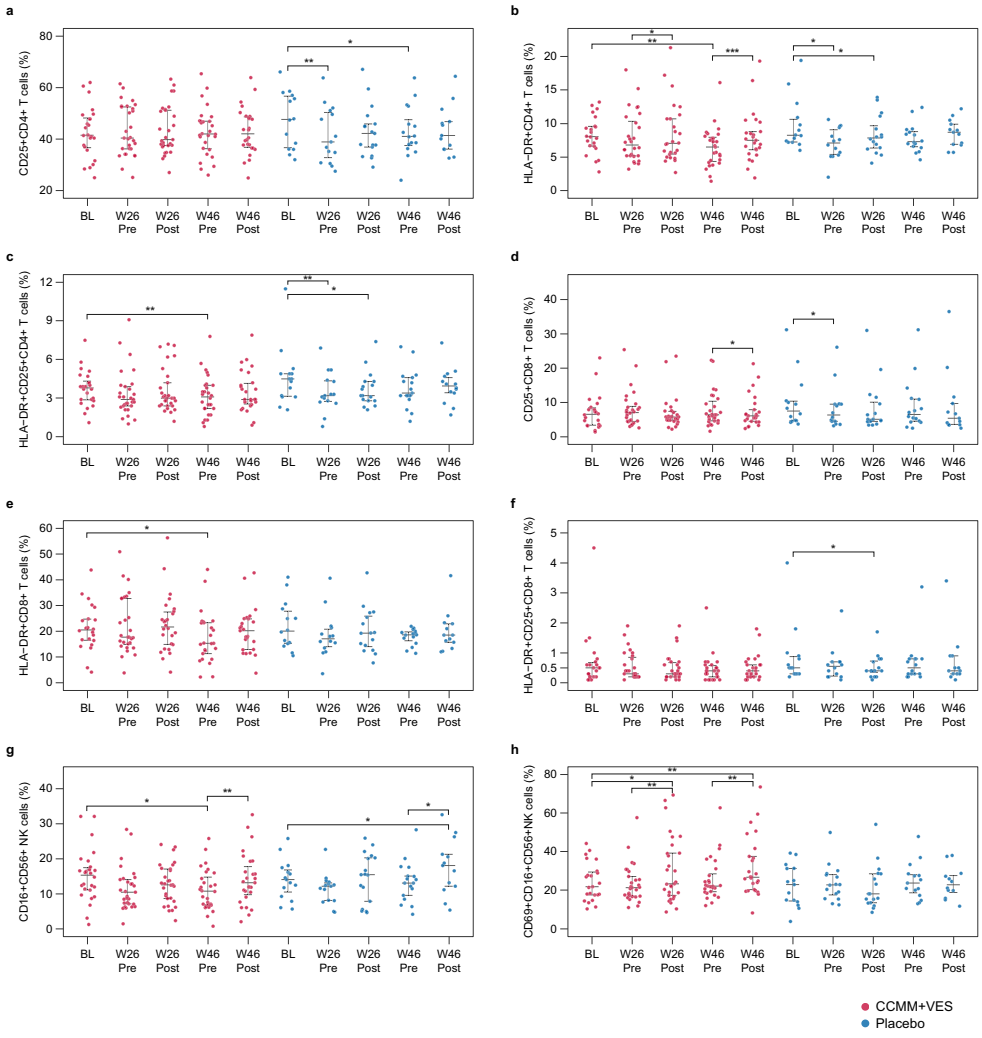

Fig. 4 | Immune-cell activation in CCMM + VES and placebo recipients. Whole blood specimens were collected to evaluate immune-cell activation (T cells, **a**–**f**) and natural killer (NK) cells (**g**, **h**) with flow cytometry at baseline (BL), before and 24 h after the first VES/placebo dose (week 26), and before and 24 h after the 10th planned (last) VES/placebo dose (week 46). Colored-coded dots represent data collected from individual participants (red color was used to mark participants receiving CCMM + VES, blue for those receiving placebo). Median and IQR are shown with black dots and lines. Wilcoxon test was used to compare data at given timepoints between groups (unpaired) or data from two different timepoints within the same group longitudinally (paired; shown by brackets), reported using nominal *P* values. *$P \le 0.0500$, **$P \le 0.0100$, ***$P \le 0.0010$.

Post hoc correlation analysis

We evaluated demographic, reservoir, and immune parameters and other baseline characteristics for potential associations with ATI outcomes, both when considering CCMM + VES ($n = 30$) and placebo ($n = 17$) groups separately and when considering all participants who entered the ATI phase ($n = 47$). Spearman's ρ was used for individual correlations, unadjusted for multiple comparisons, and results are presented in the correlogram in Fig. 7a, b. In CCMM + VES recipients, lower pre-ART viremia and longer time on ART were associated with delayed viral rebound and lower HIV-1 pVL at the end of ATI ($\rho = -0.4399$, $P = 0.0150$ for time to HIV-1 pVL >50; $\rho = -0.3325$, $P = 0.0070$ for time to HIV-1 pVL >10,000 copies/ml; and $\rho = 0.4301$, $P = 0.0177$ for HIV-1 pVL at the end of ATI with pre-ART viremia, respectively; and $\rho = 0.3908$, $P = 0.0327$ for time to HIV-1 pVL >50; $\rho = 0.4648$, $P = 0.0097$ for time to HIV-1 pVL >10,000 copies/ml; and $\rho = -0.3805$, $P = 0.0380$ for HIV-1 pVL at the end of ATI with time on ART, respectively). As for reservoir parameters, lower total and intact levels of HIV-1 proviral DNA at ATI start were associated with longer

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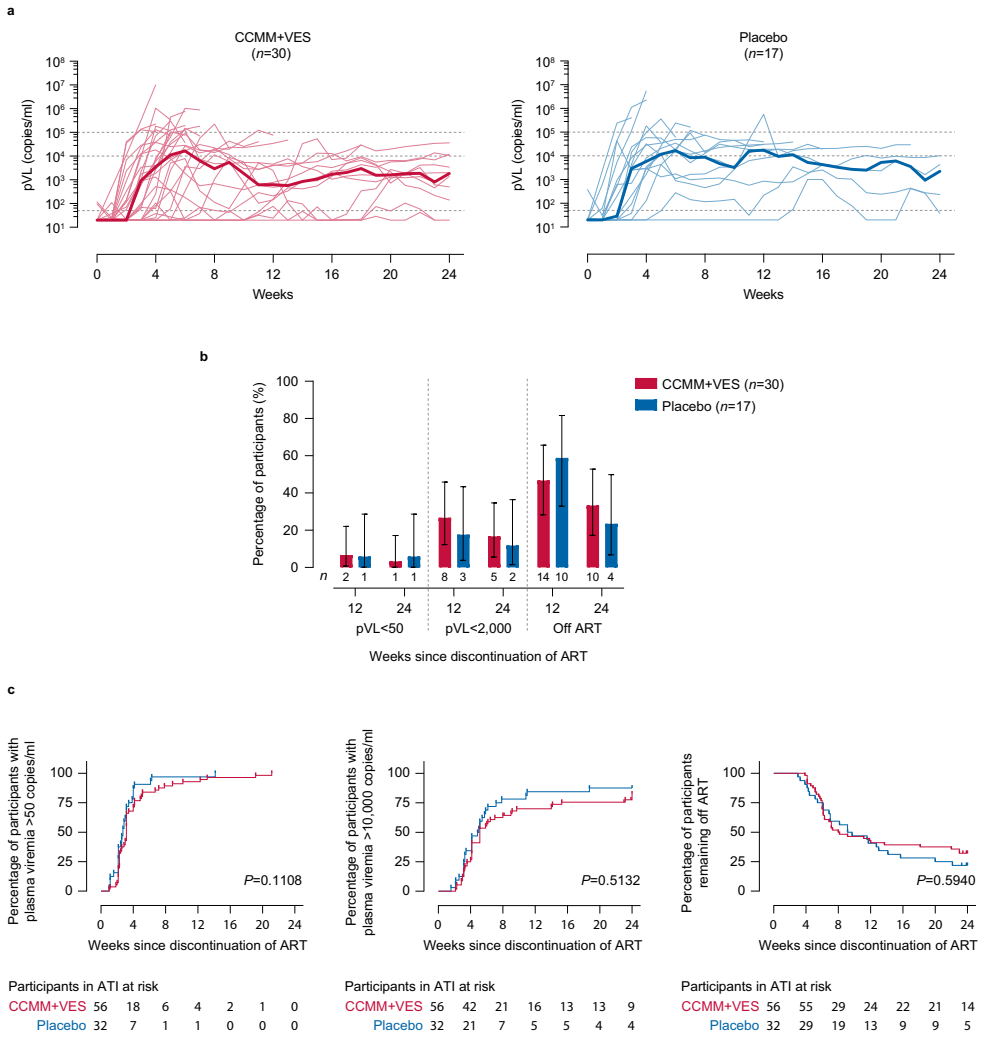

Fig. 5 | HIV-1 RNA pVL during the ATI period. a Individual and mean (thick line) values of HIV-1 plasma viral load (pVL) during the 24 weeks of ATI are represented in CCMM + VES (red) and placebo (blue) recipients. Lines are interrupted at the time of ART resumption. **b** Proportion of participants with HIV-1 pVL <50, <2000 copies/ml and remaining off ART at 12 and 24 weeks of ATI. Data shown for CCMM + VES (red) and placebo (blue) recipients. Error bars represent 95% confidence intervals estimated using the exact (Clopper–Pearson) method. Statistical comparison between treatment groups was performed using a two-sided Cochran–Mantel–Haenszel test, stratified by the potential for superior viral control (*P* values ≤0.05). **c** Time to HIV-1 pVL ≥50, ≥10,000, and percentage of participants remaining off ART during the ATI in CCMM + VES (red) and placebo (blue) recipients. Time to pVL ≥50 copies/ml was adjudicated as the first viral load assessment among the first occurrence of two consecutive visits with pVL ≥50 copies/ml during ATI. Median time to pVL ≥50 and 10,000 copies/ml was estimated in each treatment group using the Brookmeyer and Crowley method and compared between treatment groups using the stratified log-rank test adjusting for stratification factor potential for superior viral control.

time to HIV-1 pVL >10,000 copies/ml after ART interruption ($\rho = -0.3910$, $P = 0.0326$ and $\rho = -0.4374$, $P = 0.0199$, respectively). Of importance, several immune parameters were significantly correlated with ATI outcomes in CCMM + VES recipients. Among these, a higher magnitude of HTI-specific responses, in particular at the start of ATI, was significantly associated with longer time to viral rebound (HIV-1 pVL >50 copies/ml; $\rho = 0.4076$, $P = 0.0388$), slower time to viral rebound (HIV-1 pVL >10,000 copies/ml; $\rho = 0.4823$, $P = 0.0126$) and longer time off ART during ATI ($\rho = 0.4905$, $P = 0.0110$).

We then used univariate Cox proportional-hazard and logistic regression models to identify factors that could influence time to ART resumption, considering all participants (CCMM + VES and placebo)

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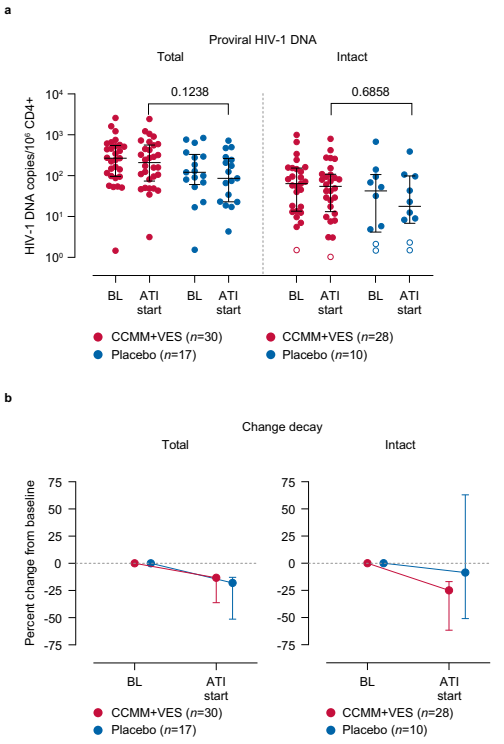

Fig. 6 | HIV reservoir. a Comparison between levels of total and intact proviral HIV-1 DNA at study entry (baseline; BL) and at week 48 (ATI start) in CCMM + VES (red) and placebo (blue) recipients. Participants with undetectable reservoirs are shown in open circles. Median and IQR values are shown. *P* values correspond to the van Elteren test stratifying on the factor for the potential for superior viral control. **b** Median and 95% CI percent change at ATI from baseline is shown for all the participants with a baseline and a post-baseline value at both visits. *P* values correspond to the van Elteren test stratifying on the factor for the potential for superior viral control.

together ($n = 47$). Cox proportional-hazard models treated time to ART resumption as a continuous variable. Logistic regression analyses modeled the proportion of participants with time to ART resumption >12 weeks ($n = 22$) or ≤12 weeks ($n = 25$) as a function of different demographic, reservoir, and immunogenicity parameters. In Cox proportional-hazard models, higher HTI magnitude (HR 0.94, $P = 0.0236$) and lower levels of total HIV-1 proviral DNA (HR 1.12, $P = 0.0047$) at ATI start were significantly associated with longer time to ART resumption (Supplementary Table 10). When using logistic regression models, in addition to the pre-ART HIV-1 pVL, HTI magnitude at study entry and at ATI start significantly increased the odds of remaining off ART >12 weeks during the ATI with odds ratios of 1.34 (95% CI 1.07-1.82; $P = 0.0260$) and 1.09 (95% CI 1.01-1.22; $P = 0.0522$) for each 100 SFC/10^6 PBMC specific for HTI, respectively (Fig. 8).

Discussion

AELIX-003, a phase 2a, double-blind, randomized, placebo-controlled study, confirmed the safety, tolerability, and immunogenicity of HTI-based vaccines (ChAdOx1.HTI plus MVA.HTI; CCMM) when combined with the TLR7 agonist VES in a multi-site trial involving early-treated individuals with HIV. Oral VES 6 mg was generally safe and well-tolerated. In agreement with previous NHP studies combining therapeutic vaccines with VES[13], all participants showed detectable viremia upon ART interruption. However, we did not observe a delay in viral rebound and/or a significant increase in posttreatment control rates, in contrast to previous reports in acutely treated SIV-infected macaques after VES administration in combination with vaccines and/or bNAbs[12]. Post hoc analyses showed that lower pre-ART viremia, smaller reservoir size, and stronger responses to HTI targets correlated with prolonged time to ART resumption. These findings align with and reinforce conclusions from the AELIX-002 trial[3], where an extended HTI vaccine regimen administered alone in a comparable population exhibited similar efficacy. Altogether, our results support further development of HTI vaccines as T-cell targeting backbone components in combination with HIV cure regimens.

Compared with the AELIX-002 trial[3], which tested a more complex and lengthy vaccine regimen comprising a total of eight sequential vaccinations with DNA.HTI, MVA.HTI, and ChAdOx1.HTI (DDDMM followed by CCM), AELIX-003 employed a shorter CCMM regimen. Using this simpler vaccine regimen, AELIX-003 demonstrated sustained strong and broad HTI-specific responses after the first MVA.HTI administration, during all VES dosing periods and upon the second MVA.HTI booster vaccination, and thereafter up to ATI start, potentially supported by the CCMM regimen or the combination with VES. VES PD cytokines were consistently induced over multiple VES dosing, as seen in previous studies in PWH[14,15,19]. However, there was high variability in immune-cell activation in this cohort, and the changes in cellular activation after VES dosing were negligible. This, along with previous data, suggests that a VES dose higher than 6 mg may be required to induce significant T-cell and NK-cell activation in PWH[14,15,19].

Similar to the AELIX-002 trial[3], in which HTI vaccines were administered alone, the combination of HTI vaccines with VES did not affect viral reservoir levels as measured by total or intact HIV-1 proviral DNA. A decrease in HIV-1 intact proviral DNA has been observed in ART-treated HIV controllers treated with VES at doses of 4 to 8 mg[14], but the immune effects of VES on adaptive and innate immune cells were also higher in that setting. Thus, this difference could be attributed to the unique characteristics of the studied HIV controllers, and generalization into wider PWH populations may be limited. Further exploration is warranted into whether the lower dose of VES administered in our study and/or lower resistance to cytotoxic T-lymphocyte-mediated killing in ART-suppressed HIV controllers compared with the AELIX-003 population (which had been ART-treated for a longer period of time), could contribute to this difference. We did not observe consistent increases in HIV-1 RNA measurements that could be related to vaccination and/or VES administration. This is consistent with previous human data, where no viral blips were observed after VES administration[15].

Confirming AELIX-002 findings[3], we observed a positive correlation between the magnitude of HTI T-cell responses and enhanced control over HIV-1 replication after ART interruption. Overall, the total magnitude of HTI-specific responses was associated with a delayed and slower viral rebound, and an extended duration of ART. Interestingly, plasma HIV-1 viremia levels before ART initiation during the acute/recent HIV infection phase were also associated with beneficial outcomes. This observation aligns with findings from NHP studies[11] and AELIX-002[3], and may reflect a lower replicative fitness due to pre-existing, HTI-specific T-cell responses. Also consistent with VES monotherapy studies, lower reservoir levels were correlated with improved ATI outcomes. In AELIX-003, longer periods on suppressive ART, which may contribute to lower reservoir levels, were also correlated with better ATI outcomes, but its role as a potential correlate of control was less clear in univariate Cox

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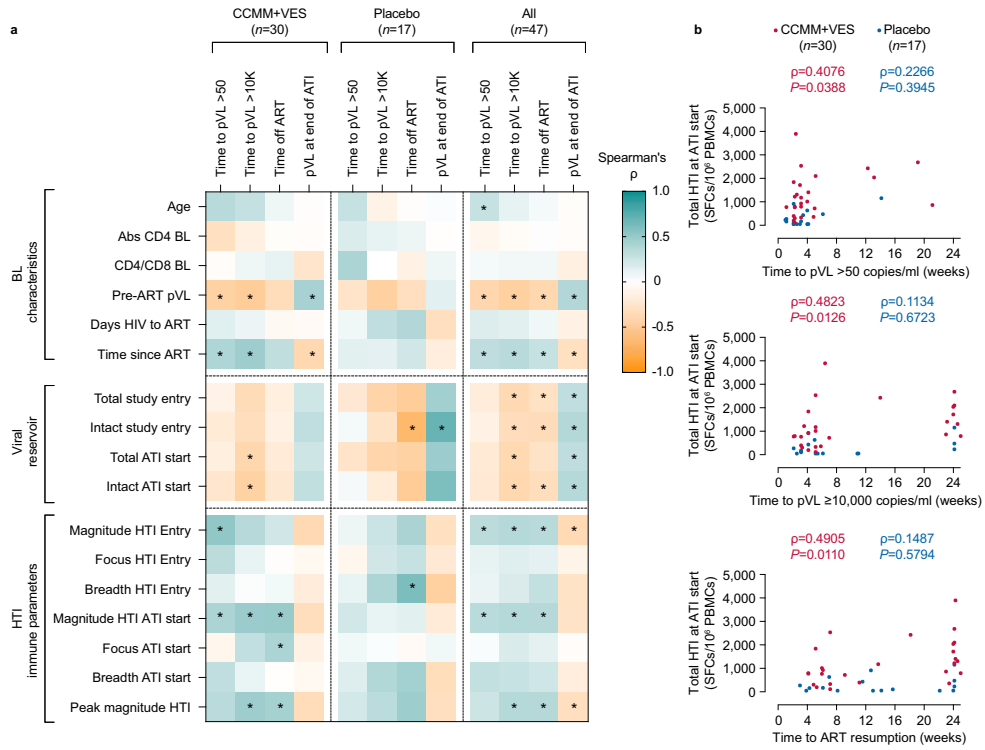

Fig. 7 | Clinical, virological, and immunological correlates of ATI outcomes. **a** are shown in a correlogram for CCMM + VES (left), placebo recipients (middle), and for all (right) participants who entered into the ATI phase ($n = 47$). Spearman's ρ is used for correlations. All tests are two-sided, unadjusted for multiple comparisons, with a 5% error rate. Significant correlations are shown by * when $P < 0.0500$. **b** Individual correlation is shown for HTI magnitude at ATI start and time to either pVL >50, >10,000 copies/ml, or to ART resumption during the ATI in CCMM + VES (red) and placebo (blue) recipients. Spearman's ρ and P values are shown for each treatment group.

proportional-hazard models or when using logistic regression. These findings emphasize the complex interplay of vaccine-induced responses and pre-existing viral characteristics in determining the efficacy of the intervention during ATI.

Limitations of our study include the lack of a single-agent group (receiving only VES or HTI vaccines) to define the VES contribution to CCMM-driven outcomes or to attribute the cytokine changes to VES, vaccination alone, or the combination of both. Based on existing information from NHP studies and AELIX-002[3] with HTI vaccines administered alone in early-treated PWH, the AELIX-003 trial design prioritized including a higher number of participants in the CCMM + VES group over a trial design with multiple single-agent arms to avoid diluting the power to detect meaningful efficacy results between the CCMM + VES and placebo groups. We did not perform additional immune analyses beyond the IFN-γ ELISpot assay in AELIX-003, as the prior AELIX-002 study, using the same HTI immunogen in a comparable trial population, already included a comprehensive characterization of vaccine-induced T cells, including polyfunctionality in both CD4 and CD8 T cells[3]. Notably, in both studies, the magnitude of HTI-specific responses demonstrated similar associations with clinical outcomes, suggesting that any contribution of VES at the tested doses to a distinct functional profile of vaccine-induced responses is likely minimal. Similar to AELIX-002[3] and other contemporaneous HIV cure trials, we were unable to recruit a more heterogeneous and diverse population; thus, extrapolation of our results is limited to those treated early during acute/recent HIV infection, in which both cisgender and transgender women are usually underrepresented. An early-treated population was selected for this translational study because the combination of early ART initiation and length of ART have been found to be beneficial to the preservation of long-lived HIV-specific CD8 + T cells, reducing inflammation, and decreasing HIV reservoir[20,21].

Compared with AELIX-002[3], no clear benefit of CCMM + VES over HTI alone in terms of viral control was observed. However, VES might have contributed to the maintenance of strong HTI responses using a less complex vaccination regimen than was evaluated in AELIX-002[3]. Two ongoing clinical trials (NCT05281510 and NCT06071767) are evaluating VES as an immunomodulator in combination with other modalities such as bNAbs and therapeutic vaccine for HIV remission[22,23]. Our study validates the HTI design and supports the idea that the induction of HIV-specific T cells to vulnerable sites of the virus is a key factor in improving post-rebound viral suppression during an ATI. Avoiding viral rebound, limiting fast viral rebound and/or improving post-rebound control of viremia, and combining therapeutic vaccines with B-cell immunogens or bNAbs (which may also enhance the suppressive capacity of vaccine-induced responses through a vaccinal effect)[24], are of great interest. This is being explored

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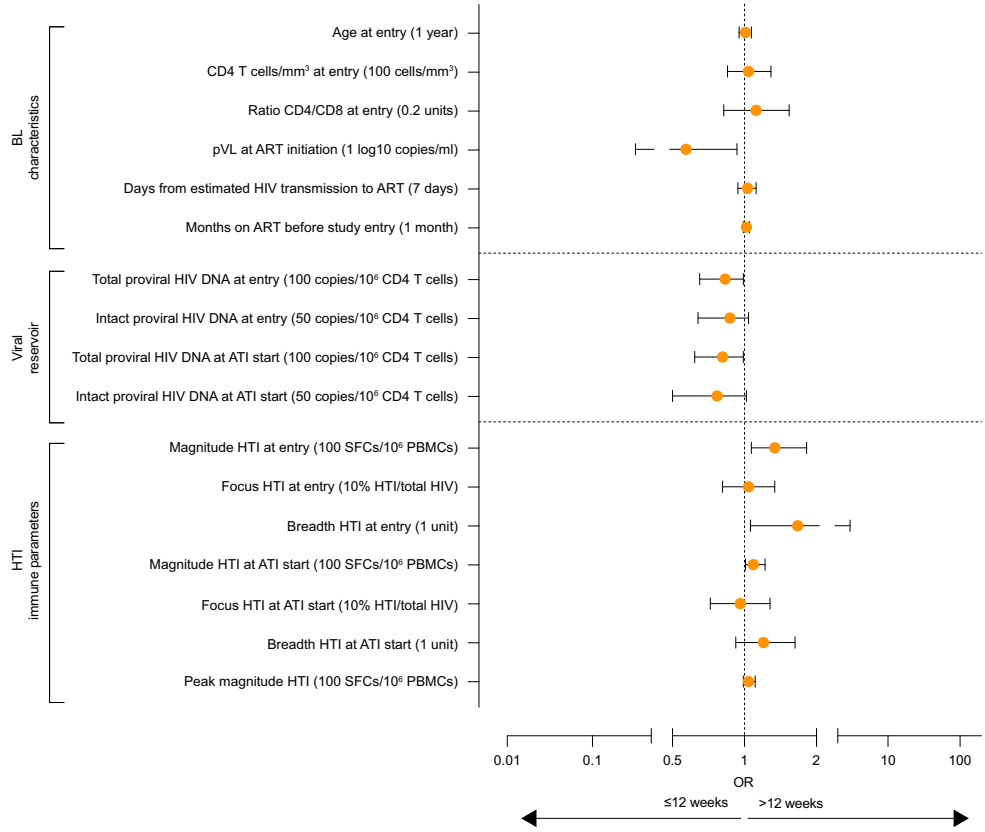

Fig. 8 | Univariate correlate analysis for time off ART. Data were shown for time to ART resumption >12 weeks in univariate logistic regression models in all AELIX-003 participants who entered the ATI phase ($n = 47$). Data were presented as odds ratios (orange-filled circles), and error bars represent 95% confidence intervals. In parentheses, for each variable, the unit of increment is shown for interpretation of the odds ratio. Univariate analyses were not adjusted for multiple comparisons. Abs CD4, absolute CD4 T cells/μl.

in the recently completed BCN03 trial (NCT05208125)[25], which might provide insights into the interaction of humoral responses induced by ConM SOSIP.v7 gp140 vaccines when combined with HTI vaccines.

In conclusion, the combination of ChAdOx1.HTI and MVA.HTI with the TLR7 agonist VES was safe and immunogenic in early ART-treated individuals. This study also validated correlates of improved post-rebound viral control previously identified in AELIX-002[3], which had demonstrated for the first time an enhanced viral control in PWH who mount strong vaccine responses with an extended vaccination regimen. HTI vaccines and VES represent promising components of potential HIV cure regimens, which will need further validation in large clinical trials. Their potential benefits, when combined with other immunomodulators, B-cell vaccines, bNAbs, or alternative vectors to increase HTI vaccine efficacy, warrant continued investigation.

Methods

Study design

AELIX-003 (NCT04364035) was a phase 2a, double-blind, randomized, placebo-controlled, multicenter trial to evaluate the safety, immunogenicity, and efficacy of a heterologous prime-boost HIV-1 therapeutic vaccine regimen (ChAdOx1.HTI and MVA.HTI; CCMM) in combination with a TLR7 agonist (vesatolimod, VES) in 50 early-treated PWH. Participants were enrolled at nine sites in Spain; the first study visit was conducted on May 20, 2019. Study recruitment was interrupted temporarily during the first amendment development and in 2020 due to the disruptions caused by the COVID-19 pandemic. The last participant's last visit was December 16, 2022.

Participants aged 18–61 years old at screening with an HIV-1 confirmed infection treated within 6 months after acquisition (early treatment) with a triple-drug ART regimen were included. Early treatment initiation was confirmed using an in-house-developed algorithm based on Fiebig classification[18,26]. Participants had to be on a suppressive therapy that included ≥3 antiretroviral drugs at study entry, but historical temporary use of a two-drug ART regimen between ART was permitted. Viral suppression (HIV-1 pVL <50 copies/ml) for at least 1 year was required at screening with CD4 T-cell counts ≥450 cells/mm^3 for the past 6 months. Full inclusion/exclusion criteria are available in the protocol as Supplementary Information.

The study was approved by the institutional Ethical Review Committee of Hospital Universitari Germans Trias i Pujol in Badalona, Barcelona, Spain. The study was conducted in compliance with the ethical principles of the Declaration of Helsinki, ICH harmonized

tripartite guideline E6(R2): GCP, and all applicable Spanish regulatory (AEMPS) requirements. Written informed consent (including separate consent for the intensive pharmacokinetic substudy if applicable, including potential updated or new information related to COVID-19, which could impact the study risk-benefit assessment) in compliance with regulatory authority regulations was obtained from each participant before the participant entered the study. All participants received financial compensation for their involvement in the study.

At the screening visit, and after obtaining informed consent, inclusion/exclusion criteria were validated, and blood was drawn for high-resolution HLA typing by next-generation sequencing at Versiti Blood Center of Wisconsin, meeting the American Society for Histocompatibility and Immunogenetics (ASHI) recommendations for resolution of common and well-documented (CWD) alleles. At baseline, participants were randomly assigned in a 2:1 ratio using interactive response technology to receive either CCMM + VES or a placebo. Randomization was stratified by the presence of any HLA class I allele associated with improved natural viral control (any subtype of HLA-B27 and HLA-B57, HLA-B58:01, HLA-B15:16, HLA-B15:17). A subset of participants consented to undergo an intensive sampling for a VES pharmacokinetic substudy. Vaccinations were administered intramuscularly on the non-dominant arm. ChAdOx1.HTI or placebo was given at weeks 0 and 12 weeks followed by MVA.HTI or placebo at 24 and 36 weeks. A total of 10 doses of 6 mg of VES were administered orally, every 2 weeks from weeks 26–34 and from weeks 38–46.

Criteria to enter ATI and resume ART

Two weeks after the last dose of VES or placebo (week 48), participants entered into the ATI period for a maximum of 24 weeks provided that, at week 46, HIV-1 pVL <50 copies/ml and CD4 count >400 cells/mm^3 was confirmed and active infections (hepatitis B, hepatitis C, syphilis, or SARS-CoV-2) were ruled out. Additionally, participants were required to have received at least three doses of the planned CCMM or placebo and at least seven of ten doses of VES or placebo. During ATI visits, symptoms suggestive of acute retroviral syndrome and HIV-1 pVL were monitored weekly, and CD4 T-cell counts on a monthly basis. Criteria for resuming ART included (1) a single HIV-1 pVL ≥100,000 copies/ml, (2) eight consecutive HIV-1 pVL ≥10,000 copies/ml, (3) two consecutive determinations of CD4 count <350 cells/mm^3, and (4) at the investigator's discretion based on clinically significant adverse events, such as grade ≥3 acute retroviral syndromes and/or COVID-19 infection, whichever appeared first. In addition to the abovementioned ART resumption criteria, during the ATI period, intensive efforts were made to monitor for sexually transmitted infections and to implement HIV transmission risk-mitigation strategies. Among those, pre-exposure prophylaxis was provided to HIV-seronegative sexual partners. In cases where the investigator identified activities considered as potentially increasing the risk of HIV transmission, participants were recommended to resume ART per the investigator's criteria. Participants reaching 24 weeks of ATI off ART were then required to resume ART. Time off ART was derived by ART resumption calendar date minus ATI start (week 48) date + 1. Adherence to ART, side effects, viral re-suppression, and CD4 T-cell counts were monitored for 4 and 12 weeks before the study ended.

Study vaccines

The HTI immunogen, comprising a chimeric protein sequence spanning a total length of 529 amino acids covering 26 regions derived from HIV-1 Gag (45%), Pol (44%), Vif (8%), and Nef (3%) proteins, was identified in previous analyses[27] as being (1) preferentially targeted by PWH with low viral loads, (2) having a higher degree of conservation versus other parts of the proteome, and (3) inducing responses characterized by higher functional avidity and broader variant cross-reactivity than responses to other regions[28]. The HTI immunogen is expressed in two viral vectors, ChAdOx1—a replication-defective recombinant chimpanzee adenovirus (ChAd) vector originating from a chimpanzee adenoviral isolate Y25[29]—and the live, attenuated recombinant vaccinia virus, MVA.HTI vaccine, denoted as M (modified vaccinia virus Ankara)[30]. Good manufacturing practice lots for the AELIX-003 trial were produced by ReiThera/Advent/Advaxia (Italy) and IDT Biologika (Dessau, Germany), respectively. ChAdOx1.HTI was dosed at 5×10^{10} viral particles in 0.5 ml and MVA.HTI at 2×10^8 plaque-forming units in 0.5 ml for injection. The matched vaccine placebo included a commercially available 0.9% NaCl solution, delivered as one 0.5-ml intramuscular injection. Syringes were filled and masked by the local unblinded pharmacists and/or study nurses and dispensed to the blinded study personnel for administration. All preparation of the study vaccines/placebos was performed in sterile conditions and following standard procedures.

Vesatolimod

VES was provided by Gilead Sciences, Inc., as unit-dose, 2 mg, round, biconvex, plain-faced, white, film-coated tablets. Placebo tablets matched to the VES tablets were provided as round, plain-faced, white film-coated tablets. Matched placebo tablets for VES were provided as 2 mg tablets of a formulation of lactose anhydrous, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, polyethylene glycol, polyvinyl alcohol, talc, and titanium dioxide. Participants received a total of ten doses of their assigned study treatment (6 mg VES or matched placebo) orally once every 14 days while on ART, with five doses between week 26 and week 34 and the other five doses between week 38 to week 46.

Objectives

The primary objective was to evaluate the safety and tolerability of the heterologous regimen of ChAdOx1.HTI and MVA.HTI administered intramuscularly in combination of the oral TLR7 agonist VES in early-treated PWH. Secondary objectives included (1) evaluating the safety of the ATI period and ART resumption phase; (2) evaluating whether CCMM + VES was able to prevent or delay viral rebound, induce post-rebound viral control, and/or prevent or delay the need for resumption of ART during an ATI period of maximally 24 weeks; and (3) evaluating the immunogenicity of CCMM + VES. Exploratory analyses included VES pharmacokinetic/pharmacodynamic responses and changes in the viral reservoir. Post hoc analyses were performed to explore correlates of ATI outcomes.

Safety

All TEAEs, including serious adverse events, were recorded by the investigators from the time of participant signing the informed consent form until 30 days after the last dose of study treatment. Serious adverse events occurring beyond this period were reported only if considered study drug-related by the investigator. The severity of TEAEs was assessed using the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, corrected version 2.1 (March 2017). The National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE version 5.0) was used to grade cytokine release syndrome. MedDRA v23.0 was used to code all adverse events.

Solicited local or systemic reactions were recorded and graded by the participants for a minimum of 7 days after each treatment administration with the use of diary cards. The severity and relationship to each study drug for these participant-reported solicited events were assessed by the investigator per the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, corrected v2.1, July 2017 (https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf). Investigators recorded any solicited events noted as grade 3 or 4 by the participants or lasting >7 days after study drug administration as an adverse event.

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Risk-mitigation strategies during COVID-19

After the 2020 global declaration of the SARS-CoV-2 pandemic by the World Health Organization, a risk-mitigation plan was implemented to reduce any potential COVID-19-related risks during vaccine or VES administrations. Informed consent was updated continually to provide participants with the latest information on potential COVID-19-related risks.

Individualized assessments were conducted for participants with comorbidities associated with higher risk of severe COVID-19 at study entry. SARS-CoV-2 testing was implemented before interventions and as clinically indicated. Positive test results led to intervention delay or omission, with participants receiving guidance for SARS-CoV-2 clinical care. All COVID-19 events, symptomatic or asymptomatic, were reported as non-related adverse events.

INF-γ ELISpot

HTI-specific and total HIV-1-specific T cells were assessed in cryopreserved and thawed PBMCs with an IFN-γ-detecting enzyme-linked immunoabsorbent spot assay (ELISpot IFN-γ Mabtech kit Cat#3420-2 A) by a central laboratory (Synexa Lab Sciences, London, UK)[3]. Briefly, 15-mer peptides overlapping by 11 amino acids were combined into ten pools spanning different HIV-1 proteins/subproteins of 7–22 peptides per pool corresponding to the HTI vaccine insert (P1–P10; total $n = 111$ peptides, Synpeptide) and eight pools of 62–105 peptides per pool spanning the rest of the HIV-1 viral protein sequences (OUT P1–P8; total $n = 637$ peptides, obtained through the NIH AIDS Reagent Program), tested in duplicate with a final concentration of individual peptide of 1.55 µg ml^{-1}. Medium only was used as no-peptide negative control in quadruplicate wells. Positive controls included two peptide pools covering lytic ($n = 16$) and latent ($n = 36$) Epstein-Barr viral proteins (1.55 µg ml^{-1}, Thermo Fisher), phytohemagglutinin (PHA; 50 µg ml^{-1}, Sigma), and a CEF peptide pool (2 µg ml^{-1}) consisting of 32 human CD8 + T-cell epitopes from cytomegalovirus, Epstein–Barr virus, and influenza virus (Pantec). Spots were counted using an automated Cellular Technology Limited ELISpot reader unit. The threshold for positive responses was set at ≥50 SFCs per 10^6 PBMCs (five spots per well), greater than the mean number of SFCs in negative control wells plus three standard deviations of the negative control wells, or more than three times the mean of negative control wells, whichever was higher.

VES pharmacokinetics

In 30 participants, one pharmacokinetic blood sample was collected within 24 h after vesatolimod dosing at each of weeks 26, 28, 30, 32, 38, 40, 42, and 46. Sixteen participants, with ten and six participants allocated to the CCMM + VES and placebo groups, respectively, were included in a pharmacokinetics substudy with intensive pharmacokinetic blood sampling over 24 h after the first VES dose (week 26). The first sample was collected within 30 min before the first VES dose, and the remaining samples were collected at the following timepoints after vesatolimod dosing: 30 min (±5 min); 1 h (±5 min); 2, 3, 4, 6, 8, and 10 h (all ±30 min); and 24 h (±2 h). The blood pharmacokinetic samples were collected for analysis of plasma VES concentrations using a fully validated high-performance liquid chromatography–tandem mass spectroscopy (LC-MS/MS) bioanalytical method[14]. For the pharmacokinetic substudy population, VES pharmacokinetic parameters were estimated by noncompartmental analysis using WinNonlin.

VES pharmacodynamic evaluation: cytokines and immune-cell phenotyping

The pharmacodynamics of VES were assessed through evaluation of circulating plasma biomarkers reflective of downstream effects of TLR7 stimulation[14], including several cytokines (IP-10, IL-1RA, and IFN-α) and markers of NK/T-cell activation (CD69, CD25, CD38, and HLA-DR) using serum or whole blood collected at baseline (week 0), predose, and 24 h after first (week 26, week 26 + 1 d) and last (tenth) VES dose (week 46, week 46 + 1 d). Serum concentrations of IFN-α were quantified using the ultrasensitive single-molecule array (Simoa), and IP-10, IL-1RA, and ITAC were evaluated with multiplexed immunoassays (Rules Based Medicine, Austin, TX). Whole blood immune-cell phenotyping was performed using a flow cytometry assay (Q^2 Solutions, Durham, NC). Three panels of antibody cocktails were used in the study; all were from BD Biosciences. Panel 1 included anti-CD56 (Cat#562751), anti-CD16 (Cat#563830), Lin3 (Cat#643510), anti-CD38 (Cat#342371), anti-HLA-DR (Cat#339216), anti-CD69 (Cat#340560) and anti-CD45 (Cat#641417); panel 2 included anti-CD4 (Cat#562970), anti-CD8 (Cat#562428), anti-CD3 (Cat#555332), anti-CD25 (Cat#341009), anti-CD45 (Cat#564105), and anti-HLA-DR (Cat#340549); and panel 3 included anti-CD19 (Cat# 562440), anti-CD4 (Cat# 562971), anti-CD3 (Cat# 345764), anti-CD16 (Cat# 332779), anti-CD56 (Cat# 345812), anti-CD45 (Cat# 332784), anti-CD8 (Cat# 345775), and anti-CD14 (Cat# 641394).

Total and intact HIV-1 DNA

Quantification of total and intact proviral HIV-1 DNA copies in CD4 + T cells, aiming to distinguish deleted and/or hypermutated proviruses from intact ones, was performed at screening and at ATI start visits by Accelevir Diagnostics on lysed CD4 + T-cell extracts using digital droplet PCR[31]. The DNA shearing index was computed, and values for intact and defective proviruses were standardized to copies per 10^6 input cells (determined by RPP30, the gene encoding ribonuclease P protein subunit p30) and adjusted for shearing using the DNA shearing index. The results were expressed as counts of HIV-1 DNA copies per 10^6 CD4 + T cells.

ART levels

To ensure ART adherence during the intervention and to rule out ART intake during the ATI, quantification of tenofovir (TFV), emtricitabine (FTC), lamivudine (3TC), and abacavir (ABV) in plasma samples was performed at several study timepoints by a validated method in ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) at Laboratory of Clinical Pharmacology and Pharmacogenetics from CoQua Lab s.r.l., Torino, Italy. Samples from study entry, week 48 (ATI start), and week 72 (end of ATI) were screened for all participants. Additional determination during the ATI period (up to eight determinations per participant) were included depending on the length of the participant's ATI.

Statistics

There were no power calculations for this descriptive study. The sample size was proposed to provide preliminary safety data on the CCMM + VES sequential treatment regimen (primary objective). In terms of the chances of observing an adverse event, 38 participants in the CCMM + VES group provided a high probability (85.8%) that this study would observe at least one event if the event occurred in the population with a true rate of 5%. For each of the primary safety endpoints, the number and percentage of participants were summarized for the safety set by the treatment group. Efficacy endpoints were performed on the intent-to-treat population that entered the ATI period: the number and proportion of the participants remaining off ART, with HIV-1 pVL <50 and <2000 copies/ml at 12 and 24 weeks of ATI were summarized by treatment group, with the associated 95% CI of the proportion using exact (Clopper–Pearson) and compared between groups using the Cochran–Mantel–Haenszel method, stratifying on the factor potential for superior viral control (yes/no) due to the presence of favorable HLA class I genotypes. For the time-to-event endpoints (time to HIV-1 pVL >50, >10,000 copies/ml, and time to ART resumption), the Kaplan–Meier method was used to

estimate the survival function. Median times and the associated 95% CIs estimated by using the Brookmeyer and Crowley method with log-log transformation were reported. Time-to-event endpoints were defined as the time interval (in weeks) between the start date of ATI and the event or censoring date, calculated as (the event or censoring date − the start date of ATI + 1)/7. HTI immunogenicity and reservoir analyses were performed on the intent-to-treat set using only observed values with no imputation in case of missing data. Descriptive statistics were provided for each group at each timepoint for each endpoint: number of participants, standard deviation, median and its 95% CI, min, max, Q1, and Q3. Differences in HTI magnitude at each timepoint, starting at week 4 relative to baseline (week 0), were calculated by Hodges–Lehmann estimates per treatment group, and between-group differences were tested using the van Elteren test with a stratification factor for beneficial HLA genetics. Between-group differences and location shifts are described with an exact CI at the 95% level. Spearman's ρ was used for correlations. All tests were two-sided, unadjusted for multiple comparisons, with a 5% error rate. Post hoc univariate Cox proportional-hazard models, univariate logistic regression models, and Spearman correlations were performed to analyze correlates of ATI outcomes. Non-parametric Wilcoxon tests were used to compare changes in serum/plasma cytokines, gene expression (including IFN-stimulated genes), and immune-cell phenotype/activation at given timepoints between groups (unpaired) or data from two different timepoints within the same group longitudinally (paired). Analyses were performed by PPD Biostatistical team, Gilead study team and AELIX Therapeutic subcontractor Fundació Lluita contra les Infeccions and Marie Pierre Malice of StatAdvice (Brussels) using SAS v9.3 or higher, R project v4.2.1 (https://www.r-project.org/) and GraphPad Prism v10.2.2 for Windows (GraphPad Software, https://www.graphpad.com). All performed analyses matched the pre-specified statistical analysis plan (AELIX-003 SAP, v2, November 2, 2022).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Gilead Sciences shares anonymized individual participant data such as demographics, lab values, etc., as well as related documents such as study protocols and statistical analysis plans, upon request or as required by law or regulation with qualified external researchers based on submitted curriculum vitae and reflecting non-conflict of interest. The request proposal must also include a statistician. Approval of such requests is dependent on the nature of the request, the merit of the research proposed, the availability of the data, and the intended use of the data. The data will be available for 1 year from the signing of Gilead's Data Sharing Agreement contract with an option of 3-month extensions at the deadline if further time is needed for research. Data requests should be sent to datarequest@gilead.com, and requestors can expect a response within 3 business days. For further information regarding Gilead's Data Sharing Policy, please visit GileadClinicalTrials.com.

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Author contributions

B.M., I.M., C.Brander., J.R.A., J.M., and D.S. conceived and designed the study. L.B., I.L., R.G., A.A., and M.G.G. contributed to the study design in further study amendments. L.B., J.M., A.C., J.C., J.C.L.B.Q., I.S., C. Busca, J.A., A.I., S.B., P.S., J.N., J.G.G., L.P.L., J.B., L.J.G.F., G.M.A., J.M.M., S.S., P.D., S.M., J.R.A., and B.M. contributed to clinical development of the study. M.F. and Y.T. conducted the VES pharmacodynamic analysis. I.L., A.A., M.G.G., J.R.A., and B.M. contributed to data management and overall study coordination. Y.A.S. and J.J.W. undertook the statistical analysis. D.L. and S.G. verified the data. L.B., C.Brander, J.M., and B.M. drafted the manuscript. E.V., D.S., J.R.A., J.J.W., and Y.C. revised the manuscript critically for important intellectual content. All authors reviewed and approved the final version of the manuscript.

Competing interests

C.Brander and B.M. are co-inventors of the HTI immunogen (patent application PCT/EP2013/051596). C.Brander, B.M., and I.M. are co-inventors of US patent Application No. 62/935,519 and US Appl. No. 62/851,546, which have relevance to the vaccine regimen used in this study. B.M. reports consultancy, advisory, and/or speaker fees from AELIX Therapeutics, Gilead Sciences, Janssen, ViiV, and MSD. J.M. reports advisory board and speaker fees and grant support from MSD, AbbVie, Boehringer Ingelheim, Gilead Sciences, ViiV Healthcare, Janssen-Cilag, and Bristol Myers Squibb. A.C. reports advisory and speaker fees and grant support from Gilead Sciences, Janssen, MSD, and ViiV Healthcare. P.S. reports advisory and/or speaker fees and/or support for attending meetings from Gilead Sciences, Janssen-Cilag, Merck Sharp & Dohme, Pfizer, and ViiV Healthcare, and has received a research grant from ViiV Healthcare, all outside of the submitted work. J.B. reports speaker fees and grant support from Gilead Sciences and MSD. J.C. reports speaker fees and grant support from Gilead Sciences and MSD. J.A. reports advisory board and speaker fees and grant support from Gilead Sciences, Janssen, MSD, ViiV; data and safety monitoring board membership with Grifols and HIPRA; and is an employee of the European AIDS Clinical Society (guidelines coordinator). A.I. reports advisory and speaker fees and grant support from Gilead Sciences, Janssen, MSD, Theratechnologies, and ViiV. S.M. reports speaking fees and research grants from Gilead Sciences, Janssen-Cilag, MSD, and ViiV. P.D. reports lecture and advisory board fees from ViiV Healthcare, MSD, GSK, Roche, Theratechnologies, Janssen & Cilag, and Gilead. Y.C., Y.T., R.G., D.S., D.L. J.J.W., S.G., and E.V. are employees and stockholders of Gilead Sciences, Inc. M.F. is an employee of ClinData Insight Inc, whose services were funded by Gilead Sciences, Inc. I.M. was an employee of AELIX Therapeutics, is currently an employee of Orion Biotechnology and is a consultant for Synklino. M.G.G. was an employee of AELIX Therapeutics at the time the research was conducted. J.M.M. has received consulting honoraria and/or research grants from Angelini, Contrafect, Cubist, Genentech, Gilead Sciences, Jansen, Lysovant, Medtronic, MSD, Novartis, Pfizer, and ViiV Healthcare, outside the submitted work. J.N. has received honoraria and/or speaking fees and/or financial support for attending conferences from AbbVie, Gilead Sciences, Janssen-Cilag, Merck Sharp & Dome, and ViiV Healthcare outside of the submitted work. C.Brander is the cofounder, shareholder, and CSO of AELIX. J.R.A. reports advisory and speaker fees and grant support from ViiV, Janssen, Gilead Sciences, MSD, and AELIX Therapeutics. The remaining authors declare that the research was conducted in the absence of any conflict of interest.

Additional information

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Author´s Contribution

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Title: Safety, immunogenicity and effect on viral rebound of HTI vaccines combined with a TLR7 agonist in early-treated HIV-1 infection: a randomized, placebo-controlled phase 2a trial

Authors: Bailón L, Moltó J, Curran A, Cadiñanos J, López Bernaldo de Quirós JC, de los Santos I, Ambrosioni J, Imaz A, Benet S, Suanzes P, Navarro J, González-García J, Busca C, Pérez-Latorre L, Berenguer J, García-Fraile LJ, Mejía-Abril G, Miró JM, Scévola S, Moreno S, Domingo P, Tian Y, Frankot M, Lim D, Cai Y, Vendrame E, Guo S, Wallin JJ, Geleziunas R, SenGupta D, Alarcón-Soto Y, Leal I, Aranguen A, Garcia-Garcia M, McGowan I, Brander C, Arribas JR, Mothe B.

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The phD student has contributed to the elements of this article/manuscript as follows:
A. No or little contribution / B. Has contributed (10-30%) / C. Has contributed considerably (40-60%) / D. Has done most of the work (70-90%) / E. Has essentially done all the work.

Formulation/identification of the scientific problem	C
Planning the experiments and methodology design and development	D
Involvement in the experimental work/data collection	D
Interpretation of results	D
Writing the first draft of the manuscript	E
Finalization of the manuscript and submission	E

The work presented in this article report the results of a multicenter, randomized, placebo-controlled clinical trial with international relevance, in which the PhD student played a dedicated role in the clinical development phase. Participants were recruited from the Early_cART cohort lead by the PhD student, being HUGTIP the most-recruiting site within the entire trial. After recruitment, her responsibilities included active participation in study visits, overseeing a 24/7 line for adverse events throughout the entire trial, managing clinical databases, and collaboration with data analyses, interpretation of results and manuscript writing.

Dr Beatriz Mothe (director) Dr José Moltó (director)

5. OVERALL SUMMARY OF RESULTS

5. Overall summary of results

this thesis is based on two randomized clinical trials conducted etPWH, with most participants recruited through the Early-cART cohort—a cohort specifically designed to enable rapid identification and management of individuals initiating ART within six months of HIV acquisition. All participants in the AELIX-002 trial were enrolled from this cohort, while approximately 30% of participants in the AELIX-003 trial were also recruited through it.

The implementation of the Early-cART program significantly reduced the time from HIV diagnosis to ART initiation and was associated with enhanced immunological recovery and a reduced HIV reservoir, particularly among individuals who started treatment within the first 60 days post-HIV acquisition.

HTI-based therapeutic vaccines, administered either alone or in combination with the TLR7 agonist Vesatolimod, demonstrated favorable safety profiles, characterized predominantly by mild and transient adverse events, mainly injection-site pain and flu-like symptoms.

Both regimens elicited strong, broad, and polyfunctional HIV-specific CD4+ and CD8+ T-cell responses, which were sustained over time and not associated with increased T-cell exhaustion. However, **there were no differences in reservoir decay between active and placebo recipients** from baseline to the start of ATI.

Although neither intervention prevented viral rebound during ATI, vaccination was associated with prolonged ART-free period (up to 22-24 weeks), particularly in vaccine recipients lacking beneficial HLA class I alleles associated with spontaneous HIV control.

Higher vaccine-induced HTI-specific T-cell responses at the start of ATI positively correlated with prolonged time off ART during the ATI, particularly in vaccine recipients with smaller viral reservoir sizes and lower pre-ART viremia levels.

At the tested dose, **Vesatolimod induced consistent increases in plasma levels of cytokines commonly linked to innate immune responses** (e.g., IFN-(, IL-1RA and IP- 10 and ITAC), but did not significantly alter levels of T-cell or NK-cell activation.

6. OVERALL SUMMARY OF THE DISCUSSION

6. Overall summary of the discussion

Despite remarkable progress in ART, the development of an effective and scalable HIV cure remains a critical unmet need. The persistence of viral reservoirs and the inability of the immune system to durably control HIV viremia following ART interruption pose major barriers to achieving sustained HIV remission. In this context, testing interventions in individuals treated during acute or recent HIV infection offer a unique opportunity to investigate the mechanisms underlying PTC/PIC and to advance HIV cure research.

6.1. Insights from the implementation of the Early_cART cohort

The widespread adoption of early ART has redefined both clinical and public health paradigms in HIV management, shifting the focus from long-term disease control to potential HIV remission. While "test-and-treat" strategies have proven effective in curbing transmission by reducing community VL—particularly in high-incidence settings—their broader impact lies in how they reshape the immunological landscape of PWH. Early initiation of ART after HIV acquisition not only limits early viral dissemination but also preserves immune function and minimizes systemic inflammation, creating a more favorable environment for cure-directed interventions. Additionally, early ART has also contributed to improved psychological health outcomes by reducing the uncertainty surrounding diagnosis and offering immediate therapeutic engagement. However, these benefits must be weighed against the structural and systemic barriers that continue to limit timely diagnosis and treatment initiation in many settings, particularly among more vulnerable populations such as migrants, women, and other socially marginalized groups, who are at increased risk of initiating ART at more advanced stages of HIV infection. In this context, the implementation of the Early_cART cohort successfully addressed some of the barriers in detecting acute/recent HIV infections and facilitated rapid ART initiation in individuals at higher risk of transmission. This program effectively reduced the time in link-to-care and the time from HIV acquisition to ART initiation since its implementation. This approach also showed significant clinical advantages at an individual level, including excellent immune recovery and lower viral reservoirs, with implications for the testing of HIV remission strategies.

Nevertheless, early ART initiation itself, while necessary, is not sufficient to attain durable ART-free control of HIV. Many studies demonstrated that individuals treated during the earliest stages of infection (Fiebig I/II) experienced viral recrudescence during ATI despite preserved immune profiles[158]. This limitation is equally evident in pediatric populations, where ART is frequently initiated at an extremely early stage—often within hours or days of birth. Despite long-term viral suppression on ART, intact proviral DNA remains detectable in children and adolescents, highlighting the remarkable biological resilience of the reservoir, even under conditions of profound immune preservation[161,162]. Therefore, although early ART confers

significant clinical and immunological advantages, these findings reinforce that timing alone is not sufficient to overcome the fundamental barrier posed by the persistence of the viral reservoir.

The limited rates of durable PTC observed even in individuals treated during acute/recent HIV infection highlights the need to develop novel therapeutic approaches that leverage the virological and immunological benefits conferred by early ART. As such, early ART should be seen not as an endpoint itself, but as an optimal/favorable platform upon which cure strategies can be constructed.

Initial research efforts sought to enhance the benefits of early ART through treatment intensification strategies—adding additional antiretroviral agents to standard 3-drug regimens. However, these approaches showed no measurable effect on reservoir size, reinforcing the notion that the main barrier to remission is not a suboptimal ongoing replication, but the persistence of long-lived, replication-competent viral reservoirs [78]. Yet even in the absence of intensification, early ART alone has not prevented viral rebound.

Beyond their role as platforms for testing HIV cure/remission interventions, acute infection cohorts have been instrumental in deepening our understanding of the fundamental immunovirological dynamics that underlie HIV rebound. The patterns observed during ATI—particularly the kinetics of viral recrudescence, reservoir reseeding and immune responses reinvigoration—closely mirror those seen during primary infection, offering a natural infection model for compare the host-virus interactions that occur when ART is interrupted. Indeed, in this resemblance rely hypotheses such as that of 'autovaccination,' whereby transient antigenic exposure during ATI may enhance subsequent immune control[163]. Nevertheless, these findings reinforce the value of early-treated cohorts in providing a relatively homogeneous setting to test cure-directed strategies and to investigate the immune correlates of PTC/PIC under conditions that most closely approximate the early events in HIV infection. Rather than serving as definitive models of cure, early ART cohorts function as optimal platforms to investigate remission strategies under the most favorable conditions, with the aim of later translating those findings into more complex and diverse clinical contexts. The Early-cART cohort presented in this thesis exemplifies this dual utility: not only improving individual health outcomes but also advancing cure research by serving as a translational platform for the design and implementation of remission-focused trials.

6.2 HTI-based vaccines in the current landscape of HIV cure/remission immunotherapies

Efforts to achieve ART-free HIV remission have long emphasized the need to modulate HIV-specific T cell responses capable of containing or eliminating viral rebound. Therapeutic vaccines have pursued this goal by aiming to induce broad, potent, and durable antiviral T cells. Early trials demonstrated good safety and a certain degree of immunogenicity, but failed to prevent viral rebound during ART interruption[164,165,166]. These early immunogens were typically designed using full-length HIV proteins, which included highly variable regions prone to immune escape. These results underscored the importance of designing vaccine immunogens capable of redirecting HIV-specific immune responses against more vulnerable and conserved regions of the virus—with reduced capacity for escape.

The HTI immunogen-based vaccines evaluated in this thesis were developed to address that need through a focused, rational immunogen design. By directing immune responses towards regions of the HIV-1 proteome associated with spontaneous control or HIV viremia in the absence of ART, HTI aimed to exert high functional antiviral activity on sites with limited potential for immune escape. Across the two clinical trials presented in this thesis, HTI has been delivered using multiple vaccine platforms, including DNA, MVA, and ChAdOx1 viral vectors, in heterologous prime-boost regimens to optimize the magnitude and breadth of T cell responses. Notably, HTI vaccination consistently elicited robust, polyfunctional CD4+ and CD8+ T cell responses, which correlated with antiviral activity ex vivo, particularly in early-treated individuals.

Up to the finalization of this thesis and before acquisition of HTI by Gilead Sciences Inc, HTI vaccines have been evaluated across five independent clinical trials, involving diverse populations and a variety of vaccine platforms and immunomodulatory combinations. These studies have been instrumental in establishing its safety and immunogenic potential. With over 400 doses administered to 135 participants, HTI stands as one of the most extensively studied immunogens in therapeutic HIV vaccine development and the first one capable of showing an impact in virological outcomes upon ART discontinuation. Building on its platform versatility, favorable safety profile and immunogenicity, HTI has the potential to be further tested in future combination strategies.

Three additional trials have been performed with HTI vaccines that have not been discussed in the preceding chapters. First, the iHIVARNAstudies, in which an adjuvanted mRNA-based approach tested the TriMix: CD40L+CD70+caTLR4 RNA expressing the HTI immunogen via intranodal administration[167,168]. Although the vaccine was safe and well tolerated, it failed to induce measurable immune responses in vivo due to an error a second start codon in front of the HTI immunogen coding sequence impacting the expression of the HTI protein from that mRNA vaccine. However, with current advances in mRNA technologies, HTI expression via RNA platform warrant future exploration.

Second, the HIV-CORE0051 study. This was a small pilot study that assessed the safety and immunogenicity of ChAdOx1/MVA.HTI vaccines in HIV seronegative individuals. The rationale behind this study was to test the capacity of HTI vaccines to induce de-novo HTI-specific immune responses, which is somehow challenging in the context of therapeutic trials performed in PWH with already pre-existing responses to HTI.

Third, the recently completed BCN03 trial (NCT05208125) has tested the combination of HTI vaccines with the B-cell immunogen, ConM.SOSIP.v7 gp140, designed to elicit neutralizing antibodies. This combinatorial strategy, tested in chronically treated individuals, aimed to simultaneously stimulate both arms of adaptive immunity. Participants received a heterologous CSSMS regimen (ChAdOx1.HTI, MVA.HTI, and ConM.SOSIP.v7) followed by ATI. While analyses are ongoing, results from this study will be critical to address the challenges of combining T- and B-cell strategies within therapeutic vaccine regimens, and reinforce the importance of population selection and immunogen optimization in achieving meaningful virological impact.

A major limitation observed across therapeutic vaccine studies—including those evaluated in this thesis—is the lack of measurable impact on the size of the latent HIV reservoir. This is likely due, at least in part, to the persistent nature of viral latency, which allows infected cells to evade immune clearance even in the presence of vaccine-induced responses. In light of this, combining therapeutic vaccines with LRAs has emerged as a potential strategy to expose and eliminate reservoir cells—a concept known as the "kick and kill" approach. However, clinical translation of this model has proven difficult. Many LRAs that are potent in vitro trigger broad immune activation, limiting their safety in vivo. Conversely, agents with better tolerability, such as HDAC inhibitors, have shown limited virological impact. For instance, in the RIVER trial, the addition of vorinostat to a vaccine regimen failed to reduce total HIV DNA compared to ART alone[169]. In BCN02-Romi, romidepsin successfully induced viral transcription[170] and was paired with therapeutic vaccination, but sustained viral control during ATI was achieved in only a small subset of participants and could not be associated to vaccine-induced immune responses. Moreover, adverse effects on T cell function—including apoptosis and reduced polyfunctionality—raised concerns about immunological trade-offs. These findings underscore a key challenge: while reactivation of latent virus is feasible, effective elimination of infected cells remains elusive without coordinated immune enhancement.

In response to these limitations, attention has shifted toward immunomodulators with more favorable safety and pharmacodynamic profiles. Toll-like receptor agonists, such as Vesatolimod (VES), emerged as promising candidates. In phase I studies, VES showed consistent dose-dependent induction of interferon-stimulated cytokines and lymphocyte activation, without triggering global T cell activation. Although monotherapy did not impact plasma HIV RNA, these properties provided a rationale for combining TLR7 agonists with therapeutic vaccines to enhance both innate and adaptive immunity in a more physiologically balanced manner, which was also supported in studies in the non-human primate model. Building on these insights, the AELIX-003 trial was designed to evaluate the combination of HTI vaccination

with VES in early-treated individuals, aiming to enhance immune-mediated clearance of the reservoir by pairing a potent T cell immunogen with a targeted innate immune stimulant. While VES consistently induced pharmacodynamic responses, including transient cytokine increases, the levels of CD4/CD8 T-cell or NK activation were minimal at the tested doses, and no additional virological benefit was observed compared to HTI given alone in the AELIX-002 trial. These results suggest that, under the tested conditions, VES did not sufficiently enhance the immunological environment to impact reservoir reduction or induce post-intervention control. However, its safety and immunostimulatory profile contribute to defining the boundaries and future potential of innate immune modulation in therapeutic settings, such as IL-15 superagonists[171,172].

Despite the significant contributions of HTI vaccines to the field of therapeutic HIV immunotherapy—particularly, in terms of safety and immunogenicity—several challenges remain in translating these advances into broadly effective clinical interventions. A major consideration is the translation of the degree of vaccine-induced responses to more diverse populations of PWH different from the early-treated individuals that were included in the AELIX-002 and AELIX-003 trials. Immunogenicity in women and/or in individuals treated at later stages of HIV remains to be fully understood. Also, although HTI was designed to focus responses on conserved regions with broad population coverage and using data from clade B and C cohorts, testing immunogenicity in regions with more non-B subtypes is warranted to ensure universal applicability and/or decide on future immunogen refinements. In addition, while HTI-induced responses have shown antiviral capacity ex vivo, their ability to clear HIV reservoir cells in vivo remains limited. Finally, the efficacy signal observed in AELIX-002 and AELIX-003 is as clear as the need to improve the degree of post-intervention control down to 200 copies/ml or undetectable levels[173].

Further insights from studies in PTC and PIC indicate that virological control after ART interruption may be influenced by a complex interplay of baseline reservoir characteristics, host factors and immune mechanisms that T-cell therapeutic vaccination alone may not be able to replicate[174]. In these individuals, spontaneous post-ATI control has been linked to a unique constellation of features, including smaller intact reservoirs, early ART initiation, robust Gag-specific CD8+ T cell activity, and limited inflammatory responses[175]. For these reason, further studies will be critical to inform which additional immune components—such as antibody-mediated mechanisms, NK cell activity, reversal of anti-apoptotic pathways or tissue-based immunity—may be necessary to achieve durable and clinically meaningful HIV remission. Variables such as timing of vaccination, preexisting immune status, and the nature of combination partners will be critical to its clinical performance. As the field moves forward, success will depend not only on the quality of the immunogen but also on its integration into well-calibrated, multifaceted strategies tailored to the host and virological context.

7. CONCLUSIONS

7. Conclusions

- **The implementation of the Early-cART program effectively reduced key clinical timeframes, including time from HIV acquisition to care linkage (from 11 to 3 days) and to ART initiation (from 73 to 27 days),** thereby facilitating timely ART initiation and potentially reducing secondary transmission. Beyond its clinical impact, the program has established a robust and well-characterized platform that has been instrumental for the design and execution of HIV cure trials such as the ones presented in this thesis.
- **HTI-based therapeutic vaccines were safe and highly immunogenic in etPWH,** eliciting strong, broad, and polyfunctional HIV-specific CD4+ and CD8+ T-cell responses, without increasing T-cell exhaustion. However, vaccination did not significantly reduce the size of the viral reservoir.
- **HTI vaccines given alone or in combination with Vesatolimod did not prevent viral rebound during ATI.** However, higher vaccine-induced immune responses were associated with prolonged ART-free periods and lower viremia at the end of the ATI.
- Despite evidence of **pharmacodynamic effects of Vesatolimod at the tested dose,** it had limited impact on immune cell activation or improving the effect on viral control when combinate with HTI vaccines.

8. FUTURE PERSPECTIVES

8. Future perspectives

8.1. The need for biomarkers to predict control after ART cessation. Pooled analysis of AELIX-002 and AELIX-003 trials

Despite advances in the development of immune strategies aimed at achieving durable HIV-1 control without ART, no validated biomarkers currently exist to reliably predict virological control following treatment interruption. As a result, analytical treatment interruptions (ATI) and the inclusion of placebo groups remain the only dependable approaches for evaluating the efficacy of therapeutic interventions in this context[176,177]. While recently published data may assist in refining sample size calculations and reducing the number of participants required to undergo ATI in future remission trials, identifying robust biomarkers capable of predicting virological outcomes would allow for the avoidance of unnecessary ATI in individuals with a low likelihood of achieving post-treatment control.

The AELIX-002 and AELIX-003 trials—discussed throughout this thesis—demonstrated that HTI-based therapeutic vaccination induces strong, broad, and HTI-focused T-cell responses that are associated with outcomes during ATI. However, due to the limited sample sizes in each individual trial, it was not possible to draw definitive conclusions on efficacy or to define a predictive threshold of HTI-specific T-cell magnitude associated with extended ART-free periods. To overcome this limitation, we are conducting a pooled analysis of individual data from both AELIX-002 and AELIX-003 to identify immunological correlates of virological control following ART discontinuation and to validate the predictive value of HTI-specific immune responses measured at the start of ATI.

This ongoing combined analysis includes data from 88 virologically suppressed individuals who underwent ATI across the AELIX-002 and AELIX-003 trials (vaccine recipients: 55 men, 1 woman; placebo recipients: 32 men) (**Fig. 23a–b**).

During ATI, pVL is monitored weekly, and ART is resumed after a single HIV-1 pVL ≥100,000 copies/ml, eight consecutive pVL ≥10,000 copies/ml, and/or two consecutive CD4 counts <350 cells/mm^3. All participants experienced HIV-1 rebound (pVL >50 copies/ml) after ART discontinuation, with comparable kinetics across studies (**Fig. 23c–d**). Overall, 28% (25/88) of participants remained off ART for 24 weeks.

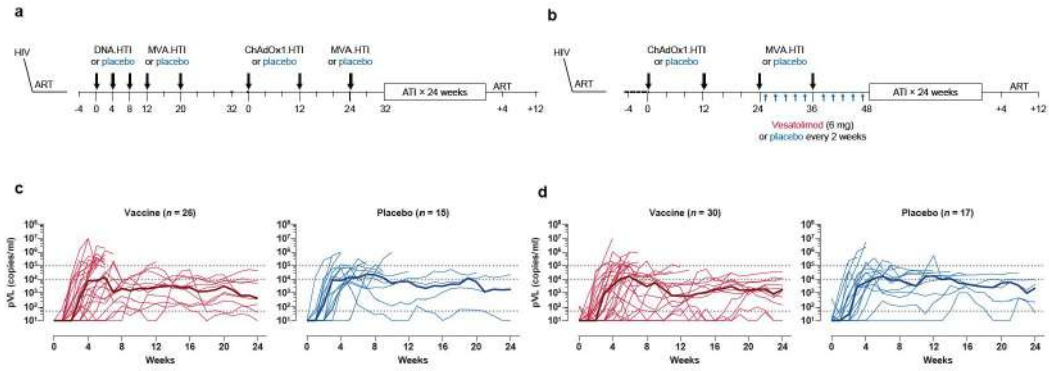

Figure 23 |AELIX-002 and AELIX-003 trial design and HIV-1 RNA pVL during the ATI period. a,b,AELIX-002 and AELIX-003 trial schedule and study visits. c, d, AELIX-002 and AELIX-003 individual HIV-1 pVL during the 24 weeks of ATI, shown for all placebo (blue) or vaccine (red) recipients.

Clinical characteristics, viral reservoir, and immune parameters at study entry and ATI start were assessed for associations with ATI outcomes, both overall and in the active and placebo groups separately (**Fig. 24**).

Preliminary analyses suggest that, in vaccine recipients—but not in placebo recipients—lower pre-ART viremia and longer duration on ART are associated with delayed viral rebound, prolonged time off ART, and lower HIV-1 plasma viral load (pVL) at the end of ATI. Similarly, lower levels of total and intact HIV-1 proviral DNA, both at study entry and at ATI start, appear to correlate with slower viral recrudescence (time to pVL >10,000 copies/ml), extended ART-free periods, and lower pVL at the end of ATI. Notably, higher magnitude and breadth of HTI-specific T-cell responses at ATI start are significantly associated with all ATI outcomes in vaccine recipients, including delayed viral rebound, slower recrudescence, and prolonged time off ART ($P < 0.05$ for all).

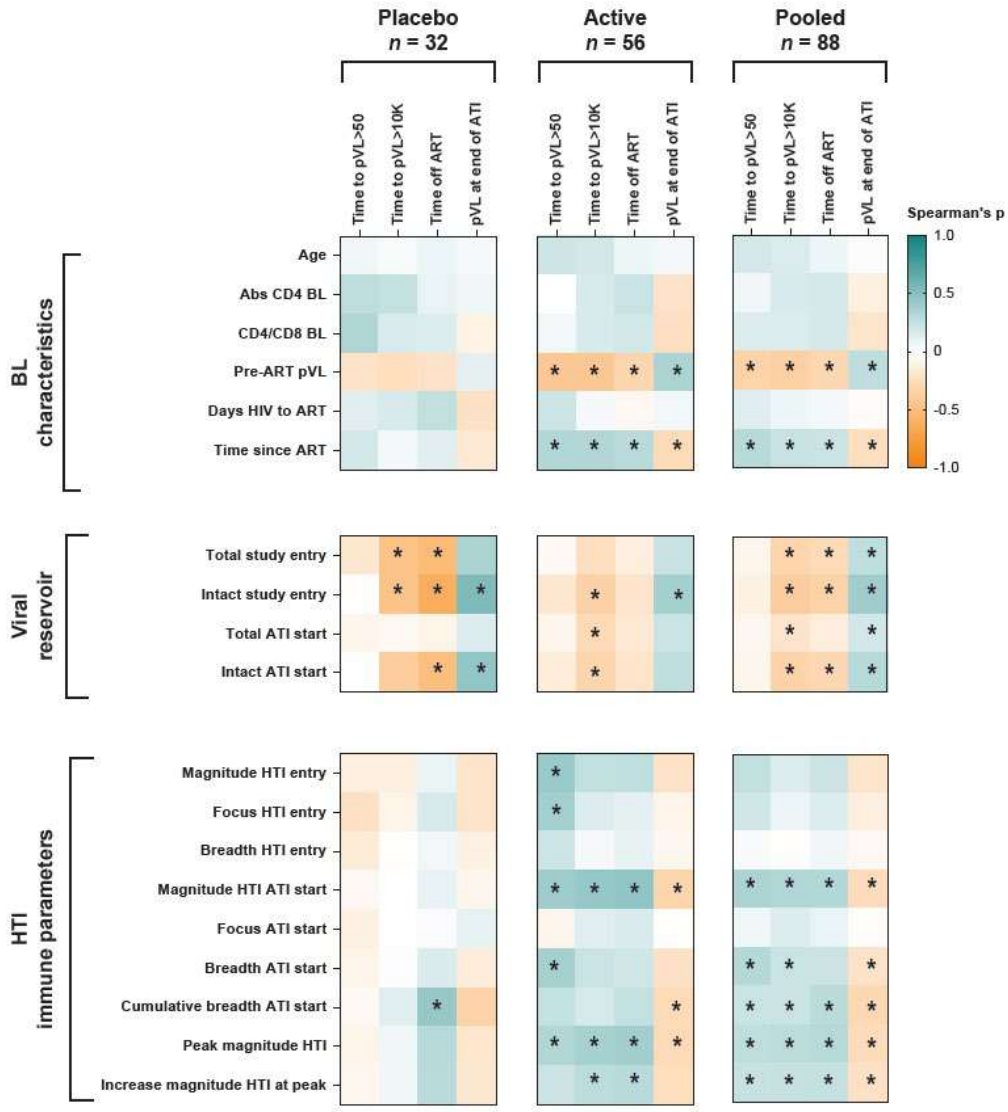

Figure 24 | Clinical, virological and immunological correlates of ATI outcomes. Correlogram for active, placebo and pooled data for all AELIX-002 and AELIX-003 participants. Spearman's ρ is used for correlations. Tests are two-sided, unadjusted for multiple comparisons, with 5% error rate. Asterisks denote significant correlations when $P<0.05$.

Given the binary distribution of time off ART (≤12 or >12 weeks), logistic regression analysis was used to define predictors of remaining off ART for >12 weeks (n=36) versus ≤12 weeks (n=52). After assessing multicollinearity, covariates with P<0.25 in the univariate models were selected for multivariate analysis. Higher magnitude of HTI-specific T cells and lower total proviral HIV-1 DNA at ATI initiation were independently associated with remaining off ART >12 weeks, with odds ratios (95% CI) of 1.13 (1.05-1.23) for each 100 HTI-specific spot-forming cells (SFCs)/106 peripheral blood mononuclear cells (PBMCs) increase, and 0.79 (0.62-0.95) for each 100-copy increase in total HIV-1 DNA/106 CD4 T cells, respectively.

To test whether viral reservoir levels and HTI immunogenicity at ATI start predicted time off ART >12 weeks, we conducted receiver operating characteristic (ROC) curve analyses. Total HIV-1 DNA had poor predictive value (AUC [95% CI] 0.61 [0.49-0.74]) (**Fig.25a**), whereas HTI magnitude ≥835 SFCs/106 PBMCs effectively distinguished participants remaining off ART >12 weeks (AUC [95% CI] 0.68 [0.55-0.81]) (**Fig.25b**). This cutoff value, which corresponded to the median of the observed HTI magnitude across active arms, demonstrated 58% sensitivity, 85% specificity, 75% positive predictive value (PPV) and 73% negative predictive value (NPV) for the entire cohort of participants. Considering vaccinees only, the sensitivity of this threshold increased to 83%, specificity to 76%, and PPV and NPV to 73% and 85%, respectively. Vaccine recipients with HTI-specific magnitude at ATI start ≥835 SFCs/106 PBMCs had significantly delayed and slower viral rebound and longer time off ART versus those below this threshold (**Fig.25c**), whose ATI outcomes were comparable to placebo recipients (Gehan-Breslow-Wilcoxon test, P=0.0077, P=0.0012 and P=0.0014, respectively, **Fig.25d-f**). Median (IQR) time off ART was 23.9 (6.7-24.1) weeks for vaccine recipients with HTI magnitude ≥835 SFCs/10^6 PBMCs at ATI start, versus 6.3 (2.3-9.6) weeks for vaccinees with HTI magnitude < 835 SFCs/106 PBMCs and 9.7 (6.1-22.1) weeks for placebo recipients, only 6% of whom had HTI magnitude ≥835 SFCs/106 PBMCs at ATI start.

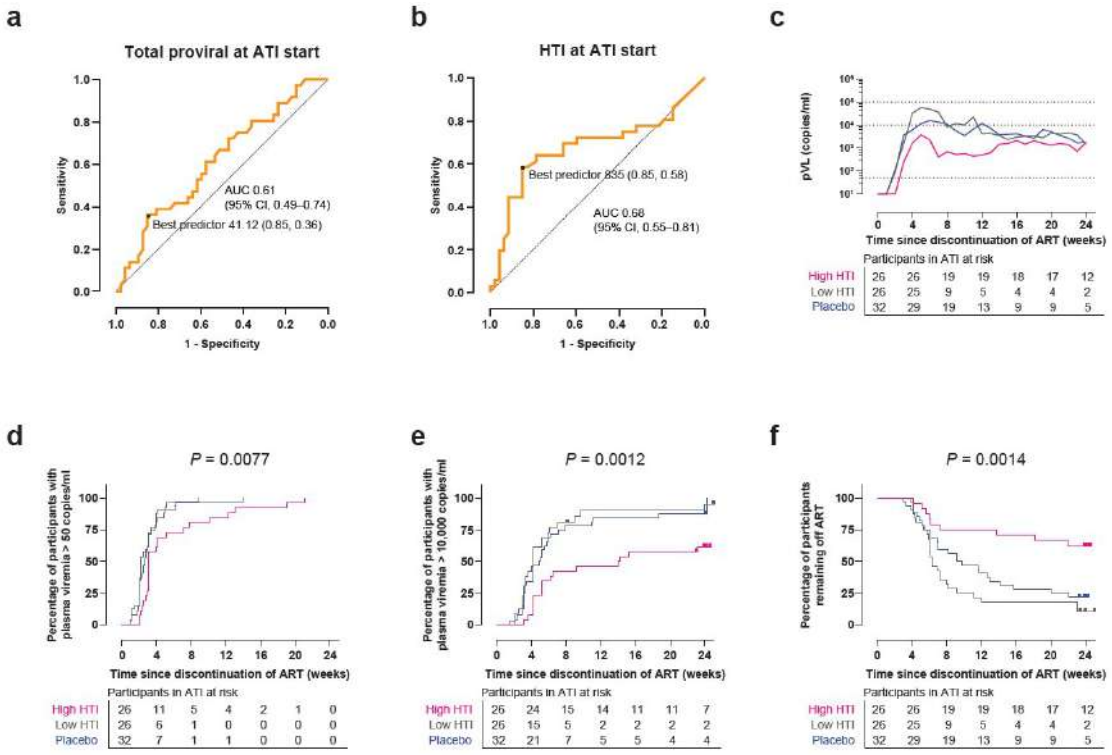

Figure 25 | HIV-1 reservoir and immunogenicity threshold. ROC curves for (**a**) total proviral HIV-1 DNA at ATI start and (**b**) magnitude of HTI-specific T-cell response at ATI start. AUC (95% CI) and best predictor (specificity, sensitivity) are shown. (**c**) Median pVL during ATI, (**d**) time to pVL >50, (**e**) >10,000 copies/ml and (**f**) time off ART in vaccine recipients with HTI magnitude above (high HTI) or below (low HTI) 835 SFCs/10^6 PBMCs at ATI start, and for placebo recipients. Gehan-Breslow-Wilcoxon test is used.

Our findings suggest HTI-specific T-cell magnitude at ATI initiation, measured in cryopreserved PBMCs using an IFN-γ ELISpot assay, is the strongest predictor of ATI outcomes. A threshold of 835 HTI-specific SFCs/10^6 PBMCs predicted extended ART-free time (>12 weeks) with acceptable accuracy, with 75% of individuals above this threshold remaining off ART for >12 weeks versus 27% below. Notably, vaccinees with low HTI responses exhibited similar viral dynamics to placebo recipients, of whom only rare exceptions exceeded the HTI magnitude threshold.

Our findings could have important implications for the design of future combination studies using HTI-based immunotherapies. Incorporating HTI-specific T-cell magnitude as an ATI initiation criterion could reserve ATI for participants more likely to achieve viral control, and their rebound dynamics could be compared with available data from nonintervention cohorts[66]. Moreover, while placebo groups remain valuable for safety and immunogenicity assessments, avoiding unnecessary ATI in placebo recipients and vaccinees unlikely to control

viremia would improve trial acceptability and address several ethical, logistical and economic challenges in current HIV remission research[136]. This approach aligns with a recent report that used machine learning and mathematical modeling to propose decision rules within the first weeks of ATI to identify predictive biomarkers for ATI post-treatment control and recommend earlier ART resumption in those less likely to control[178].

Several considerations should be kept in mind when interpreting these analyses. First, the AELIX-002/003 populations were restricted to etPWH, with limited representation of cis-gender and transgender women, potentially limiting generalizability to individuals starting ART during later stages of HIV. In these cases, HTI-induced responses might differ, and larger and more diverse reservoirs could impact viral control upon ART interruption. Second, our findings are specific to HTI-based vaccines, and other trials testing different immunogens will need to validate their own predictive markers. Lastly, logistic regression models and ROC analysis were used to identify predictors of time off ART >12 weeks. Although this timepoint might appear arbitrary, it reflects the observed distribution of time off ART in our studies, which followed predefined ART resumption criteria, and is supported by recent meta-analytic data on plasma viral load rebound kinetics[15]. That meta-analysis confirmed that sustained viral suppression below 50 copies/ml beyond 12 weeks is rare, and that most individuals establish a post-ATI viral setpoint by this time.

In conclusion, this pooled analysis of AELIX trials identified a cutoff value for the frequency of HTI-specific T cells at ATI initiation that predicts prolonged time off ART. These findings may directly contribute to optimizing the design of future studies evaluating HTI-based combination strategies aimed at achieving durable ART-free HIV control.

8.2 Towards the next generation of HIV remission strategies: scientific innovation and PWH-centered integration

Looking ahead, several areas of research are emerging that could meaningfully shape the next generation of HIV remission strategies. These include improvements in the **timing of intervention**, diversification of **therapeutic combinations**, the **refinement of clinical trial methodologies**, and the monitoring of **long-term outcomes**, all grounded in a **person-centered framework**.

One priority is to better leverage the early stages of HIV infection—not only for epidemiological control, but as a therapeutic window for cure-directed interventions. Acute and recent infection represent a critical period when the reservoir is being seeded, and immune function is largely preserved. Intervening during this phase, using strategies such as therapeutic vaccines, bNAbs, or innate modulators, may interfere with reservoir establishment and foster the likelihood of durable ART-free remission. Clinical trials already underway are exploring interventions initiated within days of HIV infection, marking a shift toward proactively targeting the reservoir at its inception rather than managing it once stabilized.

Advancing toward a functional cure will also depend on increasingly potent and synergistic combination strategies. Two main avenues are emerging: interventions that act directly on the reservoir, and those that enhance immune effector responses. On the reservoir side, next-generation latency reversal agents are being developed with improved specificity and tolerability, including compounds targeting epigenetic modifiers, metabolic pathways, or signaling cascades[179,180]. On the immune side, the field is moving beyond classical vaccination to explore germline-targeting immunogens aimed at inducing bNAbs, soluble T cell receptor (TCR)-based therapies, and adoptive strategies such as CAR-T or TCR-engineered cells[181-183]. HTI-based vaccines remain highly compatible with these innovations, offering a rational backbone for inducing targeted and durable T cell responses. Additional approaches aimed at reversing T cell exhaustion—such as immune checkpoint inhibitors like anti-PD-1—and overcoming resistance to CTL-mediated killing of the infected cells in the reservoir are being investigated to further enhance the effectiveness of these immune-based interventions [184-186]. The convergence of platforms—including mRNA, gene editing, and immune engineering—will likely define the next era of combination cure strategies. As these interventions become more sophisticated, clinical trial design must evolve accordingly. Adaptive trials design that integrate immune biomarkers could improve efficiency and safety by enabling early futility decisions or dynamic cohort selection. Decentralized models—incorporating remote monitoring, home-based sampling, and digital tools—could improve trial access and inclusion, particularly for populations traditionally underrepresented in cure research. A more inclusive and flexible framework is essential to ensure that innovation translates into real-world impact.

Simultaneously, long-term monitoring will be critical to assess both the durability of immunological effects and the safety of novel interventions. While ATI are currently necessary to assess PIC, their potential risks—such as immune activation, reservoir reseeding, and transmission—require ongoing mitigation and oversight. In parallel, the safety and pharmacodynamics of experimental agents such as bNAbs, TLR agonists, and viral vectors must be followed well beyond the intervention window. Longitudinal studies with extended follow-up and tissue-level assessments will be essential to define the true clinical benefit of remission strategies. In this context, recent meta-analyses of ATI in placebo arms provide valuable insights to guide ethical decision-making on whether to stop ART in control participants. Alternative trial designs, where placebo recipients have the option to receive the active intervention during the ATI phase, may not only preserve scientific interest but also improve participant acceptability and trial retention.

An emerging challenge in the cure research landscape is posed by the expanding availability of long-acting antiretroviral formulations. These regimens offer clear clinical benefits—reducing pill burden, improving confidentiality, and potentially enhancing long-term retention in care. However, their extended pharmacologic half-lives impede these individuals undergoing ATI, as residual drug levels may abort viral rebound as well as may promote development of drug resistance mutations, or may confound the interpretation of immunological and virological responses. Looking ahead, the development of ultra-long-acting or implantable ART agents is likely to further magnify these logistical barriers. As cure-directed strategies continue to evolve, parallel efforts will be needed to adapt ATI protocols, develop alternative measures of remission, and integrate robust biomarkers capable of predicting PTC without the need for structured ART interruption.

Throughout all these developments, one constant remains: the centrality of the person living with HIV. Biomedical innovation must be matched by ethical integrity, transparent communication, and sustained community engagement. The success of any future strategy will depend not just on clinical efficacy, but on its relevance, accessibility, and alignment with the needs and preferences of PWH.

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