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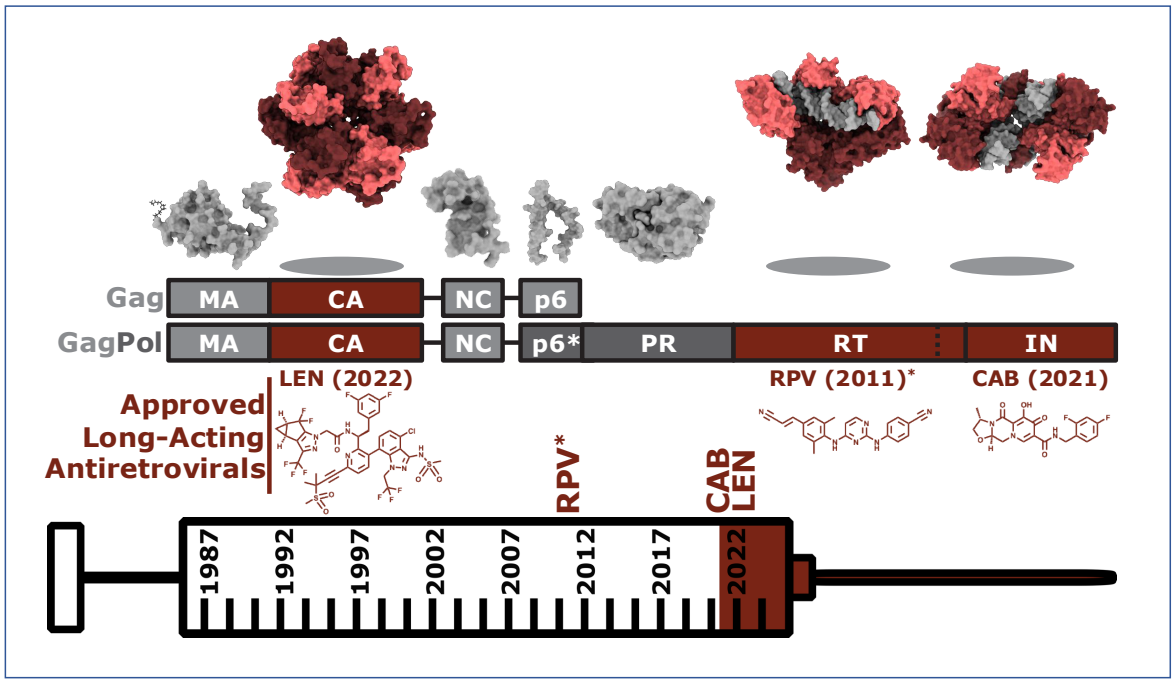

The approved drugs and molecular targets of long-acting antiretroviral therapy (LAART) to treat or prevent human immunodeficiency virus type 1 (HIV–1) infection (Page iv)

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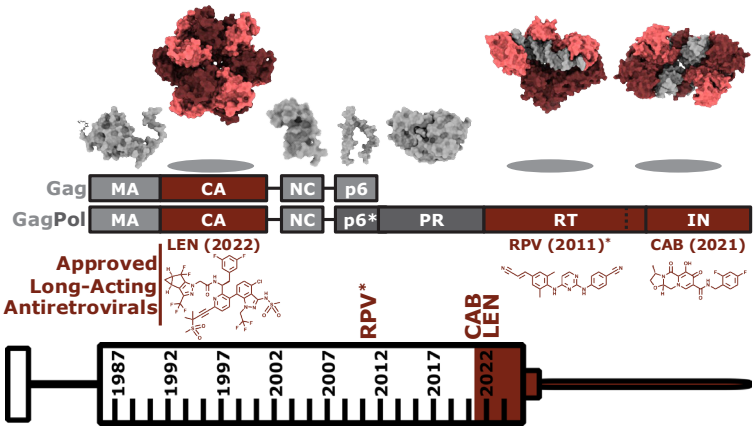
COVER FIGURE



The approved drugs and molecular targets of long-acting antiretroviral therapy (LAART) to treat or prevent human immunodeficiency virus type 1 (HIV–1) infection. The domains of the immature HIV–1 Gag (light grey) and GagPol (dark grey) polyproteins are shown with the corresponding mature protein structure above: matrix (MA; PDB: 2H3Q), capsid (CA; PDB: 4XFX), nucleocapsid (NC; PDB: 1A1T), p6 (PDB: 2C55), protease (PR; PDB: 1A30), reverse transcriptase (RT; PDB: 5J2M), and integrase (IN; PDB: 8W09); structural representations made with UCSF ChimeraX. CA, RT, and IN are highlighted in red as they are targeted by the US FDA-approved compounds lenacapavir (LEN), rilpivirine (RPV), and cabotegravir (CAB), respectively. These drugs are shown below their targets, along with the date of their initial approval. *RPV was first approved in 2011 as an oral formulation and in 2021 RPV was approved in combination with CAB for LAART; LEN was approved in 2022 for highly-treatment experienced individuals. The syringe at the bottom represents a timeline from the first approved anti-HIV–1 drug, zidovudine (AZT), in 1987 to the present with approved LAART injections shaded in dark red.

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EDITORIAL
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Targeting hypoxia-inducible factors in malignancies caused by Kaposi's sarcoma associated herpesvirus

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Abstract: In this editorial, we highlight the potential use of inhibitors of hypoxia-inducible factors (HIFs) for the use in Kaposi's sarcoma associated herpesvirus (KSHV) (also known as human herpesvirus-8) related malignancies. The past 20 years has accumulated detailed knowledge of the role of these factors in ensuring the maintenance of the KSHV in infected cells, in aiding the growth of the virus infected cells and aiding in the spread of virus from infected cells by inducing lytic reactivation. Today, a wide range of inhibitors for HIFs are currently being clinically evaluated for use in treating a variety of cancers. We discuss the current state of this research area as it relates to KSHV malignancies and describe pre-clinical and clinical evidence of drugs that target HIF to back up the idea that these inhibitors could be a novel way to treat KSHV related diseases.

***Keywords*:** hypoxia inducible factor, Kaposi sarcoma associated herpesvirus, human herpesvirus 8, Kaposi's sarcoma

The discovery of hypoxia-inducible factors (HIFs) and their role in orchestrating the response to hypoxia in 1995 was a major advance for which the discoverers were awarded the Nobel Prize (*1*). After their discovery, it soon became apparent that many cancers, especially solid tumors, usurp HIF-induced pathways to promote tumor cell survival, tumor growth, angiogenesis, and even tumor metastasis (*2,3*). Healthy cells generally do not activate the hypoxic pathway except when exposed to low oxygen tension (as in wound healing) or other stresses, and for this reason, HIFs are being studied as potential targets for anti-cancer therapy. There are two principal HIFs, HIF-1α and HIF-2α (also known as endothelial PAS domain-containing protein 1 [EPAS1]). These factors rapidly accumulate in cells exposed to hypoxia, bind to hypoxia response elements (HREs) in the genome, and upregulate specific genes that promote angiogenesis, maintain cell survival and growth etc. The degree of expression of these two forms of HIF varies among different cell types.

It has become apparent that HIFs play a central role in the pathogenesis of Kaposi sarcoma (KS) and other tumors or hyperproliferative diseases caused by Kaposi's sarcoma associated herpesvirus (KSHV) (*4*). KSHV-induced diseases include primary effusion lymphoma (PEL), multicentric Castleman disease (MCD), and KSHV inflammatory cytokine syndrome (KICS). KSHV is a gammaherpesvirus and like other herpesviruses, has both latent and lytic gene expression programs. The promoters of several key KSHV genes were found to have HREs and to be upregulated by HIFs; these include latency associated nuclear antigen (LANA), the lytic switch gene (RTA), and the ORF34-37 lytic gene cluster (*4,5*). Exposure of PEL cells to hypoxia induces lytic replication through the upregulation of RTA by HIF working in concert with LANA. Interestingly, KSHV infection itself leads to an increase in HIFs, so there is a positive feedback loop (*6*). One way HIF inhibitors could help benefit patients with PEL or other KSHV-mediated tumors is to block lytic activation of the infected cells and thereby prevent production of KSHV-encoded cytokines and virus spread. Also, Shrestha *et al*. have shown that if the HIF-1α gene is knocked out in PEL lines, cell growth is severely impaired (*7*). They also showed that the HIF-1 inhibitor, PX-478, which downregulates the mRNA for HIF-1α, severely impairs the growth of PEL cells in normoxia but has no effect on uninfected lymphoma cells (*7*). These studies provide a rationale for exploring the use of HIF inhibitors in the treatment of PEL. In addition, our group and others have demonstrated that KSHV-infected cells harness a significant portion of the hypoxic gene expression signature even under normoxic conditions, suggesting that these specific hypoxic pathways (such as increased glycolysis) could also be targeted in KSHV malignancies (*8,9*). While PEL involves KSHV-infected B-cells, KS primarily involves KSHV-infected endothelial cells, which generally express HIF-2α rather than HIF-1α (*10*). It has been demonstrated that HIF-2α but not HIF-1α is responsible for activating lytic replication in certain KSHV-infected cells by localizing HIF-2α to the endoplasmic reticulum, thus allowing for translation

Table 1. Selected list of drugs shown to inhibit HIF and to have pre-clinical or clinical activity against KSHV-induced tumors

Drugs	Clinical status	Mechanism of HIF inhibition	Effect on KSHV malignancy	*Ref.*
Echinomycin	In clinical use*	Inhibits HIF-1α activity and cMyc	Inhibits cell growth in PEL and KS xenograft mice models	(*14*)
Pomalidomide	Approved for KS	HIF-1α inhibition in endothelial cells	Inhibits PEL cell growth *in vitro*; effective against KS patients	(*7,12*)
Lenalidomide	In clinical use*	HIF-1α inhibition in endothelial cells	Inhibits PEL cell growth *in vitro*	(*13,15*)
Rapamycin	Clinically used in transplant-associated KS	Indirectly inhibits HIF-1α and HIF-2α *via* mTOR pathway inhibition	Inhibits PEL cell growth *in vitro*; effective against KS in renal transplant recipients	(*16,17*)
Rapamycin plus digoxin	Both drugs in clinical use**	Both drugs inhibit HIF-1α and HIF-2α levels and activity	Prevents the growth of KS-like tumors in nude mice	(*18*)
Everolimus	In clinical use*	Indirectly inhibits HIFs *via* mTOR pathway inhibition	Induces apoptosis and inhibits KSHV gene expression and virus production in PEL cell lines	(*19*)
PX-478	Phase I	Inhibits HIF-1α mRNA and protein levels	Selectively inhibits the growth of PEL cell lines *in vitro*	(*7*)

* In clinical use, but not for KS or other KSHV-induced diseases. ** Both drugs in clinical use, but not together against KS or other KSHV-induced diseases. HIF, hypoxia inducible factor; KS, Kaposi sarcoma; PEL, primary effusion lymphoma.

of viral mRNAs (*4*). Therefore, in the case of KS, treating patients with a HIF-2α inhibitor rather than a HIF-1α inhibitor, might be beneficial. Interestingly, a specific HIF-2α inhibitor, belzutifan, which prevents the dimerization of HIF-2α with its partner HIF-1β (needed for its activity) was recently approved by the FDA for the treatment of patients with von Hippel-Lindau disease which leads to overexpression of HIF-2α (*11*).

Several pre-clinical studies have described strategies for targeting HIFs, alone or with other targets, that may be worth exploring in KSHV diseases. In this regard, certain therapies now used to treat KS and other KSHV diseases are known to have indirect or direct effects on HIFs, and this may contribute to their activity. For example, the mTOR pathway is upregulated in KSHV-infected cells by a variety of mechanisms (*4*) and this can increase and stabilize the levels of HIF protein. This may explain in part why rapamycin, which targets mTOR, is useful in the treatment of KS in renal transplant patients. Another FDA-approved treatment for KS is the immunomodulator pomalidomide, an analog of thalidomide (*12*). While it remains unclear why exactly pomalidomide works in KS patients, it has been shown that lenalidomide and pomalidomide decrease angiogenesis and HIF expression in endothelial cells (*13*). Several drugs now in clinical use for other conditions have been shown to inhibit HIF and to have activity in certain models of KS. One is echinomycin, which targets both HIF-1α and cMyc; this has been shown to have activity in pre-clinical models of both PEL and KS (*14*). Table 1 shows selected compounds known to have inhibitory effects on HIF and how they affect KSHV infected cells and/or patients.

In summary, for reasons that are still unclear, KSHV has evolved so that HIFs play an important role in the viral life cycle and in the pathogenesis of KSHV-induced diseases. As such, HIFs may represent a potential target for the treatment of these diseases. Several therapies that modulate HIFs as well as other targets have been shown to be effective in KS, and the effect on HIF may contribute to their activity. It is possible that other HIF-specific approaches may be found to be active in these diseases, and this is an area for future study.

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Conflict of Interest: R. Yarchoan reports receiving research support from Celgene (now Bristol Myers Squibb), CTI BioPharma (a Sobi A.B. Company), PDS Biotech, and Janssen Pharmaceuticals through CRADAs with the NCI. Dr. Yarchoan also reports receiving drugs for clinical trials from Merck, EMD-Serano, and Eli Lilly and preclinical material from Lentigen Technology through CRADAs or MTAs with the NCI. R. Yarchoan and DA. Davis are co-inventors on U.S. Patent 10,001,483 entitled "Methods for the treatment of Kaposi's sarcoma or KSHV-induced lymphoma using immunomodulatory compounds and uses of biomarkers". An immediate family member of R. Yarchoan is a co-inventor on patents or patent applications related to internalization of target receptors, epigenetic analysis, and ephrin tyrosine kinase inhibitors. All rights, title, and interest to these patents have been assigned to the U.S. Department of Health and Human Services; the government conveys a portion of the royalties it receives to its employee inventors under the Federal Technology Transfer Act of 1986 (P.L. 99-502).

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State of the ART (antiretroviral therapy): Long-acting HIV-1 therapeutics

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Abstract: Human immunodeficiency virus (HIV) impacts millions of individuals worldwide, and well over 2/3 of those living with HIV are accessing antiviral therapies that are successfully repressing viral replication. Most often, HIV treatments and prevention are administered in the form of daily pills as combinations of multiple drugs. An emergent and effective strategy for suppressing viral replication is the application of long-acting antiretroviral therapy (LAART), or antivirals that require less-frequent, non-daily doses. Thus far, the repertoire of LAARTs includes the widely used antiviral classes of non-nucleoside reverse transcriptase inhibitors (NNRTIs) and integrase strand transfer inhibitors (INSTIs) and has recently expanded to include a capsid-targeting antiviral. Possible future additions are nucleoside reverse transcriptase inhibitors (NRTIs) and nucleoside reverse transcriptase translocation inhibitors (NRTTIs). Here, we discuss the different strategies of using long-acting compounds to treat or prevent HIV-1 infection by targeting reverse transcriptase, integrase, and capsid.

***Keywords*:** human immunodeficiency virus (HIV), antiretroviral therapy (ART), pre-exposure prophylaxis (PrEP), long-acting formulations, acquired immunodeficiency syndrome (AIDS)

Introduction

In 2023, around 20% of people living with human immunodeficiency virus (HIV) were not treating their infection (*1*). If left untreated, HIV infection progresses to acquired immunodeficiency syndrome (AIDS), which causes over 650,000 deaths annually (*1-4*). While there has been great success in HIV-related healthcare, with 75% of HIV-positive individuals virally suppressed, multiple global regions are underserved in terms of healthcare access and availability (*1,5,6*). Certain regions have a large burden of infections, especially South and West Africa, which comprise 70% of global cases. Other regions have a large proportion of untreated individuals, including the Middle East and Eastern Europe with approximately 50% untreated (*1,4,6-8*) (Figure 1).

An emerging and exciting method of treating HIV infection is the use of a long-acting antiretroviral therapy (LAART) with infrequent doses compared to daily pills (*4,9-13*). LAART can decrease the burden of acquiring and taking a daily medication, and it has received great patient reception for those switching to and initiating LAART to treat or prevent HIV type 1 (HIV-1) infection (*6,9,13-16*). The main utility of LAART is in adding another tool to patient treatment. While it is not expected that all long-acting formulations are favored equally within high-incidence populations, there is a reported bias in LAART uptake toward highly informed individuals and those who practice sex with the use of preventative tools (*6,14,16,17*).

At present, all HIV-1 treatments are administered in combinations of at least two drugs in order to prevent the emergence of antiviral resistance. The coadministration of multiple drugs decreases the chance of antiviral resistance due to the volume of simultaneous mutations needed to escape the drugs' activities (*18-21*). It is possible that LAART helps decrease the occurrence of antiviral resistance by having unwavering continuity of treatment, but this also requires that patients have the opportunity to replenish the LAART at the recommended interval (*10,12,15,22,23*).

Opportunities introduced by LAART include less frequent dosing, avoidance of "pill fatigue", oral dosing being bypassed (with bioavailability near 100%), less adverse events, fewer drug-drug interactions, as well as protection of health privacy, avoidance of HIV-related stigma, and improved consistency of care. Challenges include large limitations due to injection volume restrictions, management of missed doses, pharmacokinetic considerations, possible development of drug resistance, management of drug-drug interactions, management of serious adverse events, and unknown dosing for children and pregnant

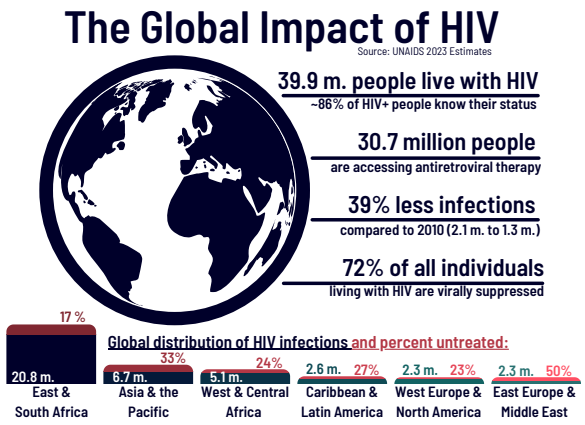

Figure 1. Global statistics of HIV-1 infections, treatment, and access in 2023. Regional-specific data below, showing the total estimated infections (in millions, blue) and percent of untreated individuals (red). Data from the UNAIDS 2023 report, "The urgency of now: AIDS at a crossroads" (*1*).

women. Delivery routes of long-acting antivirals are oral, parenteral, and an implant/device with respective dosing frequencies of more than one week, more than one month, and more than six months (*6,22,24*).

Currently, the approved delivery methods for LAART are intramuscular (Cabenuva) or subcutaneous (Sunlenca) injections (Figure 2). This method of administration has been highly effective; however, there are complications and limitations to receiving injections due to patient anxiety, injection site reactions, the need for a professional healthcare worker for administration/ lack of clinical support, high cost, accessibility, and difficulty in discontinuation once the treatment is injected (*10,14-17,22,25*). Because of these reasons and more, other long-acting treatment options are being developed. These include subdermal implants, intravaginal rings (IVRs), microneedle array patches, long-acting hydrogels, and oral regimens that are dosed

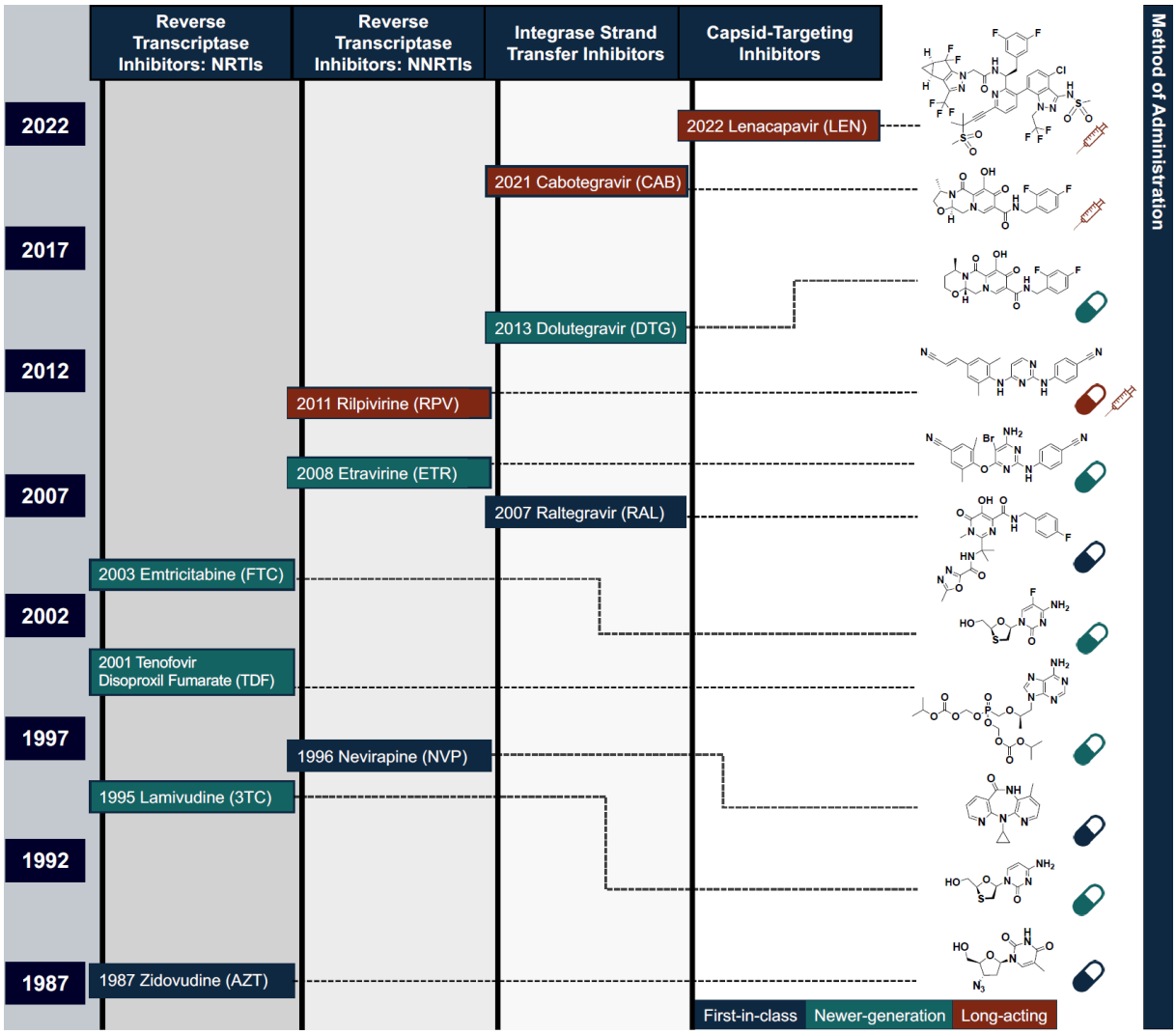

Figure 2. Timeline of some important discoveries, approvals, and advancements leading to long-acting HIV-1 therapeutics. Compounds are categorized as first-in-class (blue), newer-generation (green), and long-acting therapeutics (red) for treating and preventing infection. Chemical structure and delivery method(s) included for each antiviral on the right. Represented inhibitor classes are nucleoside reverse transcriptase inhibitor (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrase strand transfer inhibitors (INSTIs), and capsid-targeting antivirals. Pill and syringe icons designed by Freepik.

less often. Other delivery strategies have been deployed in specific areas, like the ring approved for use in Africa but not by the US FDA (*25*). Of note, adverse reactions can be challenging for LAART, especially for irreversible administration through subcutaneous injections; however, entry periods into the therapy with low and oral lead-in doses, when available, can be useful to test patient tolerance to the new medication before a long-term treatment is in place (*13,23,25,26*).

Thus far, LAART has been successfully employed in clinical trials and as approved medications for HIV-1 treatment and as a preventative measure for pre-exposure prophylaxis (PrEP) by targeting viral proteins required for an HIV-1 infection: reverse transcriptase, integrase, and capsid.

Reverse transcriptase

HIV-1 reverse transcriptase (RT) inhibitors have been used to treat infection since 1987, with the first FDA-approved HIV-1 antiviral zidovudine (AZT). Since the late 1980s, several other RT-targeting antivirals have been developed and approved (*27*) (Figure 2). Currently, there are five non-nucleoside reverse transcriptase inhibitors (NNRTIs) and nine nucleoside reverse transcriptase inhibitors (NRTIs) in the US market, although only five of these NRTIs are recommended (*28*). Another class of RT inhibitors being developed, but not yet FDA-approved, is called nucleoside reverse transcriptase translocation inhibitors (NRTTIs). These inhibitors include islatravir (ISL, EFdA, or MK-8591) and MK-8527.

All of these classes – NNRTIs, NRTIs, and NRTTIs – target HIV-1 RT. This viral enzyme is essential for the HIV-1 replication cycle as it is responsible for converting the positive-sense, single-stranded RNA genome into double-stranded DNA, which is the product that is integrated into the host genome (*29*). NNRTIs, NRTIs, and NRTTIs act through distinct mechanisms of action, though. NNRTIs are allosteric inhibitors that do not target the polymerase active site and instead bind to a region called the NNRTI binding pocket (NNIBP) located at the base of the thumb of RT. When these inhibitors bind, they cause conformational changes to the thumb and surrounding areas of RT, reducing the ability of RT to polymerize (*30,31*). NRTIs are nucleoside analogs and bind at the polymerase active site of RT (*29*). These inhibitors lack a 3'-OH, so once incorporated into the elongating DNA strand, another nucleotide cannot be added. Thus, NRTIs are termed immediate or obligate chain terminators (*32,33*). Differentially, NRTTIs retain the 3'-OH group, allowing another nucleoside to be added (*34*). Thus, NRTTIs can act through multiple mechanisms of action, effectively inhibiting the translocation step of reverse transcription through immediate chain termination, delayed chain termination, or increased misincorporation (*35*).

Non-nucleoside reverse transcriptase inhibitors

Most antiretroviral therapy (ART) treatments, including those based on RT inhibitors, are administered as once-daily oral medications. Rilpivirine (RPV), an NNRTI, was the first RT inhibitor to be approved in long-acting therapies such as Cabenuva. Initially approved in 2011, RPV is considered a next-generation NNRTI due to its capacity to overcome both resistance and safety concerns associated with earlier NNRTIs (*36*). With its extended half-life, RPV allows for monthly dosing using non-oral methods (*37*). Another promising NNRTI, doravirine (DOR), marketed as Pifeltro by Merck & Co., Inc., received FDA approval in 2018 (*38*). While currently available as a once-daily oral medication, efforts are underway to develop its formulation as a long-acting injectable (*39*). VM-1500A, another potent NNRTI, is currently in development by Viriom Inc. Elsulfavirine (ESV), the prodrug of VM-1500A, was approved in Russia in 2017 under the brand name Elpida (ESV 20 mg) as a once-daily oral medication. ESV has a long half-life (*40*) and is being formulated as a once-weekly oral medication and as depulfavirine in a once-monthly nanosphere drug formulation (*41*).

Nucleoside reverse transcriptase inhibitors

Tenofovir, an NRTI, has been used in first-line therapy worldwide for the last twenty years. It has been formulated as tenofovir alafenamide fumarate (TAF), which increases its potency and safety profile. This antiviral is currently being used as a once-daily oral medication but is in preclinical formulation as a long-acting implant for treatment up to six months (*12*). There are currently several implant variations being tested, which include a TAF-filled subcutaneous silicone implant with a polyvinyl alcohol (PVA) coating and orthogonal delivery channels (*42*), a reservoir-style biodegradable implant filled with a TAF/oil formulation (*43*), an implant loaded with TAF pellets sealed in a polyether urethane tube (*44*), and poly(ε-caprolactone) (PCL) reservoir-style implant with a TAF core formulation (*45-47*). Long-acting microspheres are also in development (*48*).

Nucleoside reverse transcriptase translocation inhibitors

ISL is a highly potent NRTTI licensed by Merck & Co. This antiviral has the potential to be long-acting due to its long half-life and favorable selectivity index. It is currently being tested in several clinical trials as a once-daily, weekly, and monthly oral medication partnered with DOR and lenacapavir (LEN) (*49-70*). Preclinical studies of ISL delivered as an injection, implant, and micro patch for long-acting therapies are also underway; ISL has paved the way for other NRTTI development, including MK-8527, a 7-deazadeoxyadenosine analog

that is currently in phase I/II clinical trials with the potential to be used as a once-monthly oral treatment for HIV-1 (*71-74*).

Integrase

HIV-1 integrase protein (IN) is a target of ART formulations that contain integrase strand transfer inhibitors (INSTIs). Currently, there are five FDA-approved INSTIs used in HIV-1 treatments – first generation: raltegravir (RAL), elvitegravir (EVG); second generation: dolutegravir (DTG), bictegravir (BIC), and cabotegravir (CAB) (*75-77*). HIV-1 requires integration of virus-encoding nucleic acid into the host's genome as part of its replication cycle, and IN is responsible for integrating the double-stranded viral DNA (vDNA) resulting from reverse transcription of the viral positive-sense, single-stranded RNA genome (*78,79*). Hence, the integration process has emerged as another ART target, with multiple classes of integration-targeting compounds arising as potential treatments (reviewed in (*80*)). IN was first discovered within an avian retrovirus as a nucleic acid-associating protein that later was found to possess both 3'-processing and strand transfer enzymatic activities (*81-86*). During integration, IN oligomerizes and complexes with vDNA to form the intasome, which contains a conserved integration core (CIC) (*87*). Within the CIC, the IN multimer exposes 3'-OH groups on the vDNA ends, which can then catalyze a strand transfer reaction into the target host DNA (*88,89*). To prevent this from happening, INSTIs contain key structural moieties that block host DNA capture immediately preceding this step. These antiretrovirals have a β-diketo acid-containing a dicyclic or tricyclic pharmacophore to chelate Mg^{2+} ions required to catalyze reactions in the IN active site (*87*). Moreover, INSTIs contain a halogenated benzyl group that performs π-π stacking with the terminal vDNA base, thus preventing its interaction with host DNA (*87*).

RAL became the first FDA-approved INSTI in 2007, followed by EVG in 2012. In building upon these first-generation INSTIs, second generation INSTIs have seen widespread adoption due to their increased tolerability, high barrier to resistance, and low cross-reactivity (*75*). INSTI-containing ARTs typically administer it with two NRTIs, but these combinations can alternatively apply one NRTI and one NNRTI instead. Of these INSTIs, CAB is the only approved long-acting (LA) agent, either in combination with RPV LA as an ART (*90-92*) or on its own for PrEP (*93,94*). CAB is a structural analog of DTG and similarly has a high genetic barrier to resistance, yet it possesses a much longer half-life than DTG does (*95,96*). It also has potent activity at low concentrations, minimal adverse side effects, and little cross-reactivity (*97-99*).

CAB LA, owned by ViiV Healthcare, was approved in early 2021 in combination with Janssen's RPV LA for HIV-1 treatment under the trade name Cabenuva (*100*), becoming the first injectable LAART. This came after the success of the 2020 phase III Antiretroviral Therapy as Long-Acting Suppression (ATLAS) and First Long-Acting Injectable Regimen (FLAIR) studies; these trials confirmed non-inferiority of CAB LA/RPV LA against standard ART in treating and suppressing HIV-1 infection (*90,91*). Following this, the 2020 phase IIIb ATLAS dosed every two months (ATLAS-2M) trial established the non-inferiority of bimonthly CAB LA/RPV LA administration when compared to monthly treatment, leading to the approval of a bimonthly regimen as well (*92,101*). In late 2021, CAB LA itself was FDA-approved as the first LA injectable PrEP and was released under the trade name Apretude (*102*). This announcement resulted from the phase IIb/III HPTN 083 and phase III HPTN 084 studies showing non-inferiority of CAB LA against conventional PrEP treatment (*93,103*).

INSTIs can be given either as oral drugs or as intramuscular injections in the case of CAB LA. INSTI-containing ARTs are orally administered daily, while CAB LA/RPV LA involves a ventrogluteal injection once either monthly or bimonthly (*104*). However, before this treatment starts, individuals may be advised to undergo an oral lead-in period (OLI) to assess their tolerance to CAB and RPV. During OLI, individual tablets of CAB (brand name Vocabria) and RPV (brand name Edurant) are taken daily for at least four weeks. For patients enacting this optional OLI, an immediate switch to CAB and RPV initiation injections (trade names Vocabria and Rekambys, respectively) takes place on the final OLI day; otherwise, the treatment initiation period can directly begin at this step.

Initiation injection schedules for HIV-1 treatment differ between patients undergoing a monthly versus a bimonthly CAB LA + RPV LA dosing timeline. For monthly treatment, initiation injections of CAB LA and RPV LA are each given once in the month prior to the start of their monthly injection schedule at doses higher than during treatment (*104*). For those on a bimonthly schedule, the same monthly initiation injections are administered, though instead for two months before their bimonthly schedule begins (*104*). Upon successful completion of this period, the patient will then begin their prescribed injection schedule (*105*).

Capsid

The HIV-1 virion contains the HIV-1 capsid, the most recent molecular target of an antiviral compound, LEN (previously called GS-6207), approved in 2022 for highly treatment-experienced (HTE) patients (Figure 2). LEN targets the HIV-1 capsid protein (CA) and inhibits viral replication by perturbing the capsid core stability, assembly, and maturation (*19,106-112*); it was developed by Gilead Sciences to disrupt the intricately-

tuned kinetics of capsid assembly by interacting with CA at the phenylalanine-glycine (FG)-binding site (*3,106,113-119*). The mature capsid is essential for the replication of HIV-1 in numerous ways, for example acting as a reaction vessel for RT activity and as a shuttle to carry the viral genome through the cytoplasm and to or through the nuclear pore complex (*114,116,120-127*). The disassembly or "uncoating" of the capsid core needs to be perfectly timed, as early or late uncoating decreases viral fitness (*106,123,124,128-134*). Interfering with post-entry and pre-integration events prevents the establishment of a viral infection, making the mature capsid a great target for preventing or treating HIV-1 (*107,135*). In fact, an interim update of the PURPOSE1 clinical trial (NCT04994509) was recently released, stating that twice-yearly LEN was shown to prevent HIV-1 infection with 100% efficacy as a PrEP regimen in cisgender women in South Africa and Uganda, with 0 reported infections among 2,134 participants who received LEN (*136,137*). The press release also stated that PURPOSE1 is the first phase III HIV-1 prevention trial to report zero infections (*136*).

Interestingly, the first compound reported to target the same site as LEN, the FG-binding site, was PF-3450074 (PF74). Similar to LEN, PF74 contains an amide group and FG scaffold to mimic the FG-containing host factors that bind to the same pocket in CA (*i.e.* Nup153 and CPSF6) (*3,114-119,131,132,134*). Due to these chemical bonds, PF74 has an exceptionally short metabolic half-life and is quickly degraded by cellular enzymes, preventing its further drug development (*3,132,138,139*), although more potent and more stable analogs of PF74 have been published (*139,140*). Intriguingly, this amide bond and general FG structure is still within the LEN molecule, and LEN maintains its exceptionally long-lasting half-life (*3,106,113,141*).

LEN is only approved for use in HTE patients as of June 2024, and not yet as a coformulation (*109*). Specifically, the patients approved for LEN treatment are already taking an ART regimen that is not successfully repressing viral replication. In fact, the CAPELLA trial (NCT04150068) with LEN found that most HTE participants that had established infections with drug-resistant viruses were able to initially suppress viremia (21 of 24), although multiple LEN-associated resistance mutations have been reported shortly after use in CA, including M66I and N74D (*110,142-144*). Multiple clinical trials are ongoing to investigate LEN as a form of treatment or prevention against HIV-1 with either oral formulations or subcutaneous injections in combination with BIC, ISL, emtricitabine/tenofovir disoproxil fumarate (F/TDF), F/TAF, and broadly neutralizing antibodies (bNAbs) (*19,108,111,145*). LEN is typically administered in 927 mg subcutaneous injections in 26-week increments and/or as 300–600 mg tablets as OLI (*111,112,145,146*). Overall, LEN as a first-in-class inhibitor is an exceptionally potent and long-lasting antiretroviral, though its apparent tendency to rapidly select resistance mutations in CA may be a challenge for its future applications.

Conclusions

From many oral doses administered daily and limited drug options to non-daily injections and over 30 FDA-approved antivirals with various delivery strategies, the treatment of HIV-1 infection has evolved and is becoming increasingly accessible to more patients. Many challenges remain to overcome barriers in HIV-1 treatment and prevention. With more and varied treatment options, and numerous clinical and pre-clinical trials underway to use LAARTs, clinicians will be more able to take patient preferences into account and build applicable strategies together to effectively combat HIV-1. Some of the developed LAART compounds expand the existing treatments in antiviral classes like NNRTIs and INSTIs; other novel classes of inhibitors have been reported with long-acting applications, like the NRTTIs and capsid-targeting compounds. As with all antiviral compounds, LAARTs create a selective pressure on the virus and thus, there are known resistance mutations associated with the independent use of a single antiretroviral. Therefore, more and alternative strategies are required to expand the LAART field and optimize combinations of antiviral drugs with applications that meet patient needs.

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Exploration of PrEP/PEP service delivery model in China: A pilot in eastern, central and western region

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Abstract: Since 2017, China has started a pilot exploration of pre-exposure prophylaxis (PrEP)/post-exposure prophylaxis (PEP) service aiming for human immunodeficiency virus (HIV) control. Efforts to summarize the pilot experience and sort out the gaps in service provision must be prioritized. In June-October, 2023, three provincial capital cities with two years of PrEP/PEP pilot experience in eastern, central and western China were chosen. A structural information collective tool was developed, as a framework to identify key links and steps in reviewing service procedures for PrEP/PEP service. Two main service models have been formed, including the independent offline service model led by professional health institutions and Multi-agencies (health institution/Community Based Organizations (CBOs)/Internet platform) online and offline collaborative service model. The pilot experience conceptualizes opportunities to integrate PrEP/PEP into HIV prevention efforts and, illustrates the optimizing path to move forward to reach for a high level HIV prevention and care continuum. Systematic barriers during the process of integration need to be noted and addressed. It is urgent to establish a realistic and feasible online and offline monitoring system to achieve a balance between standardized, safe, simplified and convenient services.

***Keywords*:** human immunodeficiency virus (HIV), pre-exposure prophylaxis (PrEP), post-exposure prophylaxis (PEP), service procedure model, HIV care continuum

Introduction

Since the 1980s, China has gradually formed and developed an independent human immunodeficiency virus/ Acquired Immune Deficiency Syndrome (HIV/AIDS) prevention and control service system (*1*). In 2017, Chinese government released *The 13th five year plan of action for curbing and preventing AIDS in China*, which emphasized the responsibilities and linkage of multi-health care agencies in HIV control (*1,2*). In the process of achieving this goal, China has made a series of basic preparations, such as: China has made remarkable progress in strengthening its primary health-care system (*3,4*). Chinese government has attached significant importance to the participation of Non-government Organizations (NGOs) in HIV prevention in the past 20 years (*5-7*) and in 2015, China established China AIDS Fund for NGOs (CAFNGO) (*8*). Accompanied by Internet development, the in-depth integration of the primary health service system, Community Based Organization (CBOs) and HIV/AIDS service system, have strengthened collaboration among multi-agencies, and have improved the capability of HIV/AIDS prevention and control.

In recent years, the effectiveness of HIV pre-exposure prophylaxis (PrEP)/post-exposure prophylaxis (PEP) has been proven worldwide (*9-12*). As a new biological intervention, PrEP/PEP extends the HIV/AIDS care framework, as well as new challenges and opportunities for the innovation and reconstruction of the current health care system.

After World Health Organization (WHO) proposed PEP guidelines in 2014 (*13*) and PrEP guidelines (*10,13*) in 2015, China started PEP pilot in 2017. In 2019, China issued "Implementation plan for curbing the spread of AIDS (2019-2022)", proposed that PrEP/PEP services should be piloted and provided to target populations including men who have sex with men (MSM) (*14*). In 2020, "Guideline for HIV PEP (on trial)" (*15*) and "Experts consensus for HIV PrEP" (*16,17*) were

issued successively, providing a technical guide for the promotion of PrEP/PEP in China. In 2022, 24 provincial capital cities jointly carried out PrEP Pilot exploration (*18*).

China has accumulated practical experience in the promotion and implementation of PrEP/PEP (*17,19*). PrEP/PEP service procedure model in the pilot area, is potentially valuable to identify opportunities to: *i*) sort out the whole process of PrEP/PEP service initiation and operation; *ii*) modularize and standardize the key PrEP/PEP service process; *iii*) and reexamine the HIV care continuum. In this paper, we summarize the local PrEP/PEP service model, and illustrate the optimizing path to move forward to reach a high level HIV care continuum.

Three pilot capital cities in the eastern, central, and western regions of China

In June-October, 2023, three provincial capital cities in eastern, central and western China, namely Guangzhou, Zhengzhou, Kunming with two years of pilot experience in PrEP/PEP promotion, were chosen.

Guangzhou is the capital city of Guangdong Province, with a total area of 7,434 square kilometers, a resident population of 18,734,100, an urbanization rate of 86.48%, and a per capita GDP of 153,625 RMB in 2022 (approximately USD 22,600). Life expectancy in 2021 reached 83.18 years (*20*). The city uses the Internet platform as the main support, with the joint participation of Center of Disease Control (CDC), medical institutions and CBOs to provide PrEP/PEP services. In 2022, 200 cases of PrEP and 2,395 person-times of PEP visits were completed.

Zhengzhou is the capital city of Henan Province, with a total area of 7,567 square kilometers, a resident population of 13,008,000, an urbanization rate of 80 percent (*21*), GDP per capita 101,500 RMB (approximately USD 14,926), and life expectancy reached 80.48 in 2020 (*22*). The city provides PrEP/PEP services based on VCT outpatient clinics with the participation of CDC, medical institutions, and CBOs. In 2022, 221 cases of PrEP and 357 person-times of PEP visits were completed.

Kunming is the capital city of Yunnan Province, with a total area of 21,012.54 square kilometers, a resident population of 8.6 million, an urbanization rate of 81.1%, GDP per capita 85,146 RMB (approximately USD 12,528), and a life expectancy of 80.37 years in 2022 (*23*). The city's primary health care providers, CBOs, and pharmacies are involved in PrEP/PEP service delivery. In 2022, 369 cases of PrEP and 779 person-times of PEP visits were completed.

We comprehensively reviewed the initiation and implementation procedure of PrEP/PEP service in the project cities; proposed a series of achievable benchmark goals and optimizing paths based on the local implementation status, and analyzed the inspiration from pilot experiences.

Two basic service models were summarized

In three pilot cities, two basic PrEP/PEP service models have been innovated and evolved combining local HIV prevention and control context, which can be summarized as: The independent offline model dominated by CDC or hospital, and Multi-agencies (CDC/Hospital/Community Health Centers (CHCs)/Community Based Organizations (CBOs)/Internet platform) online and offline collaborative operation model.

Independent offline service model dominated by CDC/hospital

During the initiative stage of the project, CDC and HIV/AIDS designated anti-retroviral treatment (ART) hospitals played important roles in the construction, operation and docking of the whole process of PrEP/PEP service. The project cities established a PrEP/PEP service model led by the Voluntary Counselling and Testing (VCT) clinic of CDC/Hospital, and initially constructed a cooperative mechanism in which the staff in VCT clinics initiate counseling and risk assessment, the hospitals assist in testing, prescribing, and the CDC conducts follow-up management. With the gradual improvement of service, online functions have also gradually improved (Figure 1).

For PrEP seekers, after the clinic doctors or VCT counselors conduct pre-medication counseling assessments in the first visit, the seekers are required to give blood samples with informed consent, wait for the laboratory test results and return to the clinic, where a physician will evaluate the results and prescribe PrEP medication for one month, and if the results are abnormal, further diagnosis and treatment will be carried out. After taking PrEP medication, the patient returns to Clinic for follow-up testing and medication refills. Long-term patients with good adherence to PrEP medication may be considered for additional prescriptions of 3 months medication at a time.

For PEP seekers, based on risk assessment, clinicians prescribe a full 28-day course of PEP medication asking the seekers to give blood samples with informed consent. Medical staff will notify the seekers by phone of any abnormalities in the pre-medication test results. If HIV+, further diagnosis and ART will be provided as possible.

Hospitals that can provide laboratory testing results within 1 hour require physicians to issue medication prescriptions referring the results during the first visit. 24-hour PEP service channels at hospitals have also been developed to meet with the demand (Figure 2).

Multi-agencies (CDC/Hospital/CHCs/CBOs/Internet platform) online and offline collaborative model

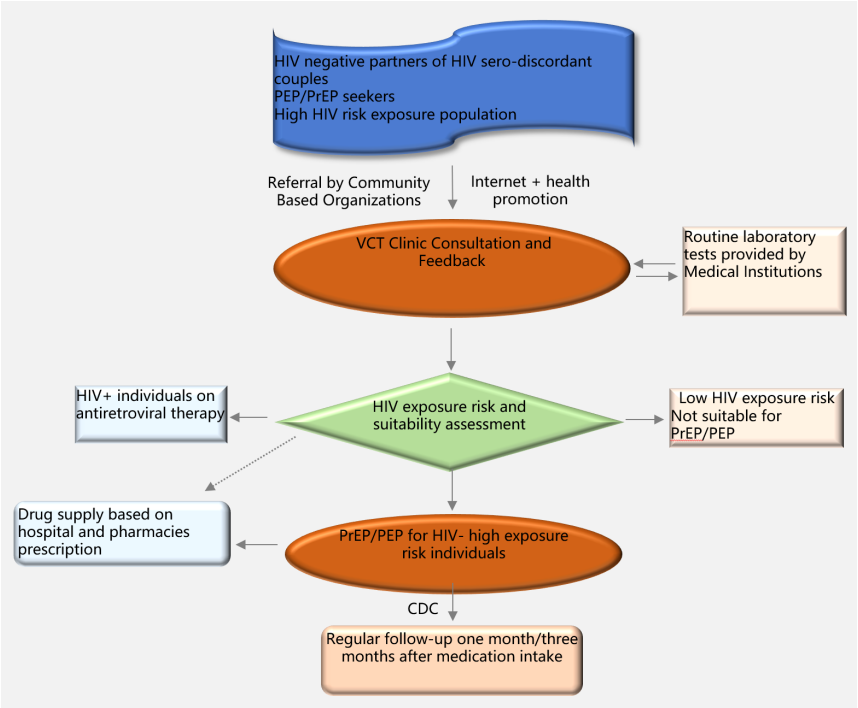

Figure 1. Independent offline service procedure dominated by CDC/Hospital.

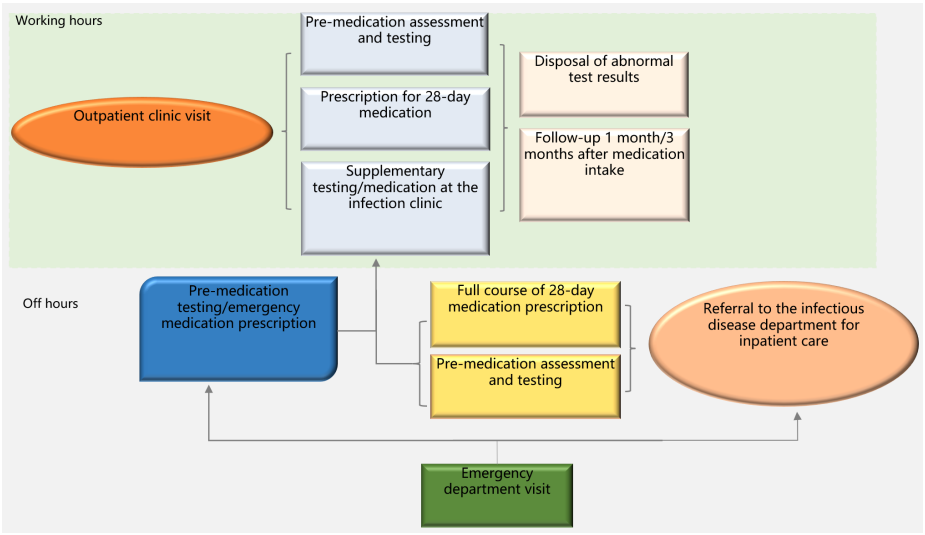

Figure 2. PEP Service procedure working and off-working hours.

Hospital- CDC-CBO -Internet platform collaborative model

In Guangzhou, where the information level is high and CBOs are widely involved in HIV/AIDS prevention, the multi-agencies joint service process was developed as follows (Figure 3):

i) The PrEP seekers initiate an online consultation to make an appointment with the CDC or a designated clinic for risk assessment offline.

ii) After risk assessment offline, the seekers receive rapid HIV/syphilis/HBV/HCV tests, and specimens for liver and kidney function, gonococcus, chlamydia, mycoplasma testing are collected by a third-party testing institution, and all test results are pushed through App to the seekers.

iii) After logging in the platform, the seeker applies for an outcome review with the internet HIV clinician.

iv) The clinician confirms the prescription; the seeker pays for the medicine; and the platform sends the PrEP medicine through logistics. Those with abnormal results are referred back to the designated hospital.

v) The CBOs launch the one-to-one follow-up and reminds them to make return appointments every three months for tests and medication refills.

vi) Those with abnormal HBV/HCV results at any stage, are referred back to the hospitals to take

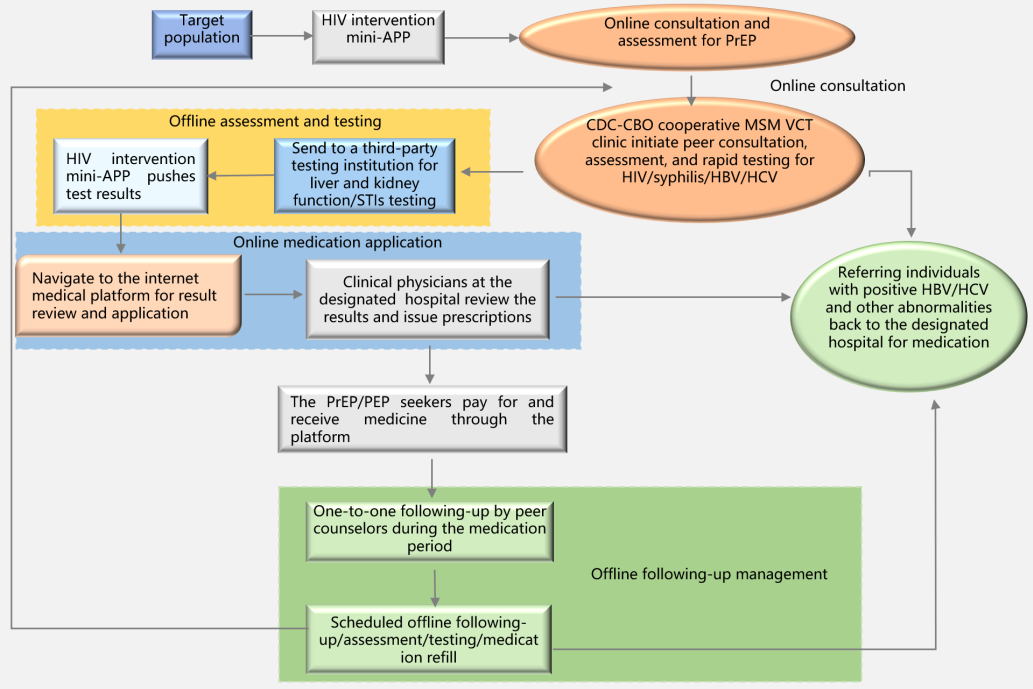

Figure 3. CDC-Hospital-CBOs online and offline collaborative model. *Data source*: Gu Y, Zeng W, Luo Y, *et al.* Exploration and practice of Internet+Pre-exposure prophylaxis model of HIV in Guangzhou. Chinese Journal of AIDS & STD. 2023; 29:1258-1261.

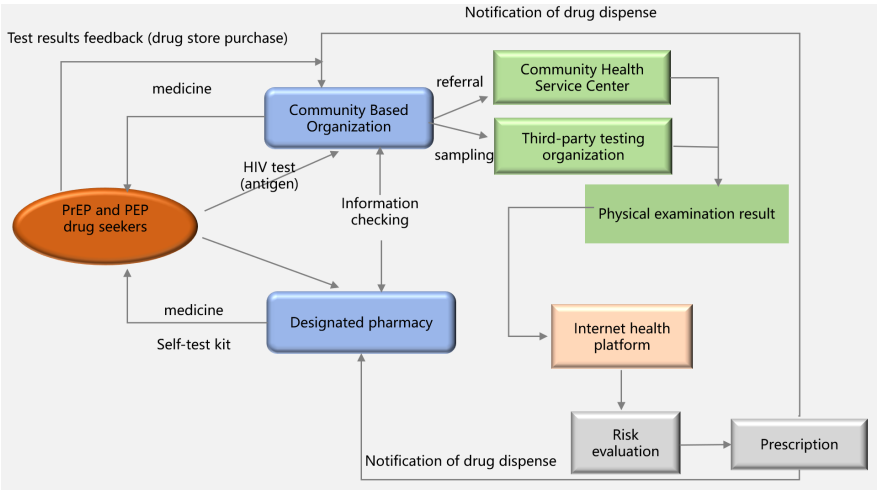

Figure 4. CHCs-CBO-Internet Platform-Pharmacies collaborative model.

medication under the guidance of hepatologists.

vii) Those with HIV+ at any stage, are referred back to the designated hospitals.

CHCs-CBO-Internet platform-Pharmacies collaborative model

In Kunming, HIV/AIDS treatment hospitals, CDC, CHCs and CBO established in-depth cooperation from the beginning of the pilot. Community health providers and pharmacies play important roles in service delivery (Figure 4):

i) CBOs conduct risk assessments for individuals with PrEP/PEP needs, and provide free rapid HIV and syphilis testing with informed consent.

ii) Individuals with PrEP requirements and HIV-test results are referred to cooperative CHCs, and blood samples are sent to third-party testing institutions for pre medication related testing. After receiving feedback on the test results (within 1-3 days), they are uploaded to the online health platform. After evaluating the test results, the online resident doctors issue medication prescriptions, push them to CBOs, and then notify the service recipients to pick up the medication face-to-face or through local express delivery; For seekers who go directly to designated pharmacies to buy medicines through CBOs online information, the staff of pharmacies

will guide them to consult the doctor to conduct online assessment, prescriptions, and free quadruple test reagent kits (HIV, TP, Hepatitis B, and Hepatitis C), checking with CBOs. All personal information is protected.

iii) PEP seekers conduct the HIV risk assessment and rapid HIV tests according to CBOs online guidance. PEP drugs prescriptions are provided through the online health platform for negative HIV testing results. CBOs provide following-up services.

CBOs-Internet platform cooperative model

Multi-agencies collaborative model in Kunming was further simplified. PrEP/PEP service seekers conduct self-risk assessment through the platform, and select to contact an online specialist. After obtaining informed consent online, they are pushed to the online health platform for medication evaluation. After obtaining a prescription and placing an order, the platform notifies CBOs to ship the reserved drugs by local express delivery, along with HIV and syphilis rapid test kits, and encourages service recipients to provide timely feedback on test results to CBOs. CBOs report the abnormal results to CDC, as well as the internet platform (Figure 5).

Achievable benchmarking goals and optimized path

According to the pilot experiences, The PrEP/PEP procedure can be classified into six modules: health education, risk evaluation, laboratory testing, outpatient service, drug provision, and follow-up. The project experts defined the achievable benchmark goals for each module, reflecting the detailed direction for future development, highlighted in increasing the coverage of knowledge dissemination and the number of clinics; improving the accessibility of laboratory testing and drug supply; promoting the feasibility and adherence of follow-up, and strengthening follow-up management. Favorable and practical route were suggested such as: Popularization of relevant knowledge, timely updating of technical guidelines, increasing the number of outpatient clinics, simplifying the risk assessment process, improving the speed of online drug logistics, purchasing follow-up services from CBOs, and lengthening the follow-up interval, *etc.* (Table 1).

Discussion

PrEP/PEP service promotes the cooperation among Multi-agencies in HIV/AIDS prevention and control.

Differentiated care strategies are rapidly becoming the norm for HIV care delivery globally (*24*). In China, HIV/AIDS prevention and control service system owns a relatively independent financing and operating mechanism during the past 30 years (*1*). Experience in PrEP/PEP pilot in project areas, highlights the feasibility of the link between primary health care and HIV care continuum (*25*). It confirmed that tailored, low-barrier approaches (*26,27*) and strategy could be effective for PrEP/PEP service provisions through cooperation of telemedicine (*28-30*), CBO and PHCs.

In 2023, China proposed to comprehensively promote regional health community in county-level, which systematically reshapes the health service system, improves the allocation, integration and efficiency of primary health resources, and prioritizes the fairness and accessibility of services (*31*). In 2022, WHO proposed to simplify the PrEP service process, to medicalize, strengthen the integration of STI/HIV testing consultation and PrEP, and improve the accessibility of PrEP/PEP services through various service supply forms such as health institutions led, community led and online services (*32*). The above factors all contribute to the integration of PrEP/PEP services within the existing service system, and scaled up PrEP/PEP–HIV/STIs testing and treatment for high-risk populations.

In the pilot cities, the advantages and disadvantages of the two kinds of service model (independent offline model dominated by CDC/Hospital, and Multi-agencies online and offline collaborative model) and their impact on HIV/AIDS are still uncertain currently. The potential barriers during the integrative HIV prevention and care continuum need to be noted and effective online and

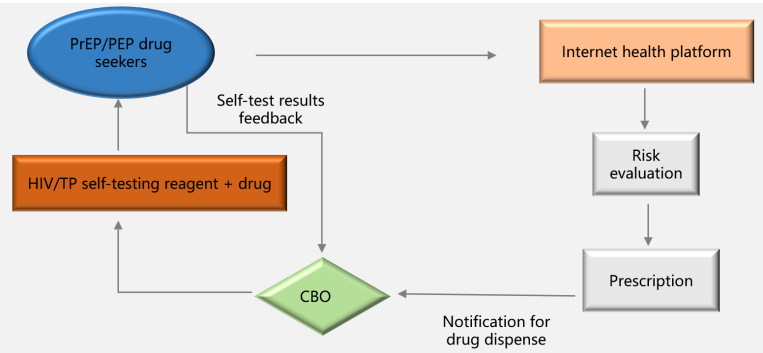

Figure 5. CBO-Internet platform cooperation model.

Table 1. Achievable Benchmark goals for PrEP/PEP service modules

Service module	Benchmark goals	Recommendations
Knowledge dissemination	Health education needs to further integrate information relevant to HIV/STI /PrEP/PEP/ART for high risk population.	Mobilize CBOs to carry out online and offline knowledge dissemination and service promotion for the target population.
Risk assessment	Risk assessment should be strictly conducted in accordance with the technical guidelines.	Strengthen online and offline training for medical professionals.
Laboratory test	1. At least one health institution in urban unit area can provide PrEP/PEP counseling and assessment, laboratory testing, and drug prescription; 2. Simplify the pre-medication testing process with reference to the world authorized PrEP guidelines.	1. Improve the testing capacity of health institutions; 2. Provide autonomous testing services through online platforms for PEP/PrEP seekers covering holidays or off-duty hours; 3. Provide HIV/STI rapid test reagents.
Outpatient services	1. Increase the number of VCT clinics and outpatient clinics for PrEP/PEP services; 2. Emphasize the importance of following-up management to improve medication safety, specification and adherence; 3. Fully acknowledge the history of PrEP/PEP use among newly enrolled patients on ART, in case of suspected breakthrough infections, conducting disease load and drug resistance gene testing; 4. Install a local online APP to provide consultation, appointment, following-up, or testing services.	1. Willingness to launch PrEP/PEP services in multi-agencies; 2. Timely update the technical guidelines in China; 3. Strengthen training and technical guidance for staffs in health institution and CBOs.
Drug supply	1. Equip each clinic with a combination of basic blocking drugs; 2. Establish a seed drug package for each clinic, ensuring that emergency drugs are available, especially for the hospitals in remote areas; 3. Improve online drug logistics and shorten the time to obtain medication.	1. Drug surveillance and management should be integrated into local health administration; 2. Internet platforms should fully upgrade providing timely and convenient logistics delivery.
Following-up management	1. Regular following-up management mechanism needs to be established for the PrEP/PEP drug users; 2. Sentinal monitoring system should be established online and offline.	1. Health education must highlight the importance of following-up visits; 2. Promote CBOs, Internet hospitals to carry out PrEP/PEP following-up visits; 3. PrEP/PEP drug users' following-up management can be integrated into HIV/AIDS care continuum; 4. Constantly updated guidelines and expert consensus on following-up helps to promote the above work; 5. Develop reminding APP to facilitate adherence.

offline monitoring systems need to be developed.

Systemic barriers during the integrative HIV prevention and care continuum need to be noted and addressed

Workers in CBOs and PHCs enabled to improve care access for vulnerable and high risk population at the local level, and enabled an optimization of the care pathway (*7*). However, in the HIV area, the debates about non-specialist PHCs' cultural competence and clinical practice, and the potential for target populations to experience discrimination and homophobia from non-expert health workers need to be noted. In addition, systemic barriers at microsocial (lack of communication about PrEP/PEP from PHCs, lack of familiarity with telehealth, uncertainty around co-navigation workflows), mesosocial (healthcare-service fragmentation, lack of PrEP/PEP-competency, logistical challenges related to insurance and obtaining diagnostic testing, extra work time and resources input), and macrosocial levels (HIV- and sexual-stigma, hesitancy about whether the service model would overcome clients' competing demands or medical mistrust) may constrain and disincentivize engagement with PrEP/PEP (*33,34*).

It is, therefore, important to understand the willingness of target population demand and the feasibility of the service in local areas. With the pilot experience, the project city proposed specific optimization objectives and paths for each service module, provide a reference for the national guidelines, as well as regional adaptable PrEP/PEP strategies (*35*).

It is urgent to establish a realistic and feasible online and offline monitoring system to achieve a balance between standardized, safe, simplified and convenient services.

Multi-agencies cooperation, led to a large amount of information for PrEP/PEP users scattered across different institutions and platforms. It is urgent to establish a systematic and standardized online and offline monitoring system for PrEP/PEP linkage, especially for PrEP care.

The PrEP care continuum sets out the path that potential end-users follow, from: *i*) being aware of, and expressing interest in PrEP, to *ii*) initiating PrEP, *iii*) taking PrEP correctly as long as HIV risk continues, and *iv*) stopping PrEP when no longer at risk for HIV (*36,37*). A monitoring approach is needed to assess whether PrEP programmes accomplish the above mentioned aims, and to identify potential implementation gaps to improve. The development of a PrEP monitoring approach is complicated for several factors, such as no common glossary for abstract terms such as "PrEP uptake", "PrEP coverage" or "PrEP persistence" (*38*). HIV risk is fluid and the diversified medication uptake and use of different formulations accompany the whole process of PrEP drug taking. In light of these challenges, there is a need to advance our understanding of which indicators are valuable for monitoring PrEP, and how these indicators can be operationalized and implemented at a programmatic level (*39*).

WHO suggested that as health services offering PrEP/PEP expand, surveillance, monitoring and reporting systems will need to be implemented alongside services, and their progress evaluated periodically. The indicators including: total PrEP recipient, PrEP coverage, volume of PrEP prescribed as key indicator for PrEP monitoring, and number of PEP recipients, PEP completion, HIV in PEP recipients for PEP monitoring (*40*), were widely recognized. Regional Monitoring and evaluation systems should be simplified and suitable for local context, and contribute to assess the impact of PrEP/PEP on the local HIV epidemic (*41*), and make sure that PrEP/PEP is being delivered safely and effectively, and that services focus on those who would benefit most (*37*).

The limitation of the research is the absence of complete data required to evaluate and compare cost-effectiveness of the two service models. An obstacle to data collection is the lack of a robust and coordinated monitoring system covering PrEP/PEP users online and offline. To address the limitation, as well as to bolster stakeholder interest in the integrative service model, the team will promote a pilot integrating service package, providing Health education- PrEP/PEP -HIV/STIs testing – ART services for target populations, and strengthen the online and offline monitoring systems during 2024-2025.

Conclusion

As China deepens its PrEP/PEP health-care pilot and provision, it has the opportunity to build an integrated, cooperative health-care system for HIV/AIDS control. Systemic barriers during the integrative HIV care continuum need to be noted and addressed. It is urgent to establish a realistic and feasible online and offline monitoring system to guide standardized, safe, simplified and convenient services.

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Advances in HIV management and challenges in Japan: Current situation of pre-exposure prophylaxis in Tokyo

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Abstract: Since the world's first case series of human immunodeficiency virus (HIV) infection were reported, more than 40 decades have passed. The advancement of HIV treatment and prevention has progressed drastically. Especially, the efficacy of pre-exposure prophylaxis (PrEP) for HIV prevention has been proven by a number of trials and the number of new HIV cases has declined over the years due to the large-scale and rapid implementation of PrEP and universal HIV treatment in multiple countries. However, in Japan, PrEP is not approved or officially supported as of June 2024. Despite of the absence of top-down movement, men who have sex with men (MSM)-friendly private clinics initiated prescriptions of generic medicines for oral PrEP with necessary tests in Tokyo, which greatly contributed to improve access to PrEP. It is of note that current situation of bottom-up PrEP implementation using generic medicines in Tokyo is obviously cost-saving, which is needless to evaluate. However, expense of PrEP is fully out-of-pocket, which will hinder those with low or no income from accessing PrEP services despite the low prices of generic medicines. Furthermore, current PrEP implementation based on user-friendly clinics is functioning only in Tokyo. The role of public health authorities is important to solve these financial and geographical disparities in accessing PrEP services, without impairing existing virtues of accessibility and cost-saving in the current system.

***Keywords*:** HIV, antiretroviral therapy, preexposure prophylaxis

Introduction

Since the world's first case series of human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) reported in the United States in 1981 (*1-3*), more than 40 decades have passed. The advancement of HIV treatment and prevention has progressed drastically. Regarding the treatment, combination antiretroviral therapy (cART) became available in 1996 (*4-5*) and has been improving the prognosis of people living with HIV (PLWH) (*6,7*). In addition, the Strategic Timing of Anti-Retroviral Treatment (START) study illuminated that outcomes were better in PLWH who initiated immediate ART (CD4 counts > 500/mm^3) compared to PLWH who initiated deferred ART (CD4 counts < 350/mm^3) (*8*). This promoted early initiation of ART even in asymptomatic PLWH, regardless of CD4 counts, and improved their prognosis. Furthermore, integrase inhibitors (INSTI), especially the third generation INSTI such as bictegravir and dolutegravir, contribute to better quality of life with less adverse effects and less drug-drug interactions compared to other classes of antiretroviral agents (*9-13*). Thus, recent trends of ART are heading to long-acting injection drug and two-drug regimens instead of three-drug for further adherence and theoretically less toxicity, respectively (*14-18*). Along with these accomplishments by ART, current issues in PLWH are mainly related to aging and life style. Causes of death have been changing and the number of non-AIDS defining diseases and mental health related deaths exceeded that of AIDS related (*19*).

The advancement of prevention worldwide has also been significant in this decade. HIV prevention trial network (HPTN) study group conducted the HPTN 052 study, demonstrating that early ART initiation prevented more than 96% of genetically linked infections with the index cases in serodiscordant heterosexual couples (*20*). The extension of this study concluded no linked infections in their partners were observed when HIV viral load was suppressed by ART during more than 5 years of observation (*21*). Another study of serodiscordant gay couples (PARTNER study) also reported similar results, supporting the account that HIV transmission becomes zero when HIV viral load is suppressed (*22*). These findings lead to announce campaigns of "the undetectable equals untransmittable (U=U)". U=U is important in terms of not only public health but also alleviation of stigma of HIV. This concept of treatment as prevention (TasP) has been established through evidence

and contributed to prognosis and public health as well.

However, TasP is not the measure of HIV prevention which people themselves at great risk of HIV acquisition can take voluntarily. In this aspect, pre-exposure prophylaxis (PrEP) has been adopted worldwide as an important preventive strategy. The efficacy of PrEP for HIV prevention has been proven by a number of trials and implementations in many countries (*23-26*). Actually, the number of new HIV cases has declined over the years due to the large-scale and rapid implementation of PrEP and universal HIV treatment in multiple countries (*27-33*). In this review, we discuss current situations and challenges in Japan based upon these worldwide advancements in the field of HIV infection.

Epidemiology of HIV infection in Japan

As of December 2022, the accumulated cases of HIV infection in Japan were 34,421, of which 10, 558 (30.7%) were categorized as AIDS at the HIV diagnosis. The diagnosed cases in each year peaked at 1590 cases in 2012 and showed a decreasing trend to 884 cases in 2022 (*34*). The route of HIV infection is mainly men who have sex with men (MSM) which accounts for 64.5% of the total diagnosis in 2022. This could be an underestimate because of hesitancy in disclosing their sexuality. 12.8% were categorized as unknown for the route of HIV infection, which may reflect the stigma of HIV and being a sexual minority. About 95% of the diagnosed cases were men and 81.8%, 14.6%, 2.0% and 2.3% were Japanese men, foreign men, Japanese women and foreign women, respectively. As for geographical distribution, HIV cases per population in Japan (124,946,789) as of October 2022 (Vital Statistics) was 0.71/100,000 in 2022, which is lower compared to Western countries.

Influence of the COVID-19 pandemic on the decrease in HIV diagnosis since 2020 was concerning based on the decrease in the number of HIV tests provided by public test centers in Japan, which was 142,260 in 2019, 68,998, in 2020, 58,172 in 2021 and 73,104 in 2022 (*34*). However, this decreasing trend, also observed in early 2010s, is likely due to the widespread adoption of the "test and treat" strategy. In this respect, the Joint United Nations Program on HIV/AIDS (UNAIDS)'s 90-90-90 HIV testing and treatment cascade targets by 2020 (90% of people living with HIV know their HIV status, 90% of all people with diagnosed HIV infection will receive sustained antiretroviral therapy, and 90% of all people receiving antiretroviral therapy will have viral suppression), currently 95-95-95 targets by 2025, has been playing an important role worldwide. These cascade targets aimed for 90 % of all PLWH to be diagnosed of their HIV-positive status, 90% of those diagnosed with HIV to receive ART, and 90% of people receiving ART to suppress their HIV viral load. Regarding the 90-90-90 targets in Japan, Iwamoto *et al.*, estimated these targets as follows: 85.6% (22,840/26,670), 82.8% (18,921/22,840) and 99.1% (18,756/18,921), based on the data on universal donation records (2011-2015), national surveillance data, and nationwide questionnaires (*35*).

The first and second 90 target and challenges in HIV testing and early ART initiation

Related to the first 90, the estimated number of undiagnosed PLWH in Japan was between about 4000 and 5000 across studies performed by different methods (*35-37*). As the COVID-19 pandemic undermined HIV test provision systems all over the world (*38-40*), provision of HIV test needs to be resilient and be more accessible in order to achieve the first 95 target. In this sense, self-testing or home specimen collection testing is recommended by the CDC, WHO, and other institutions in the world (*41,42*). Although self-collection testing has been increasing in number (from 26,000 tests in 2005 to 91,000 tests in 2016) in Japan (*43*), these tests are currently not approved and not yet covered by health insurance. Prompt adoption of these modalities will contribute to accomplishing the first 95 target. In addition to self-sampling testing, the number of out-of-pocket HIV testing offered by private STI clinics without health insurance coverage has been increasing especially in metropolitan areas like Tokyo due to its convenience, accessibility and anonymity, complementing the shortage of HIV testing in public health centers. However, these private clinics are located mainly in Tokyo and geographic disparity exists in terms of provision of HIV testing. The capacity of public health centers are limited especially in non-metropolitan areas and public HIV testing programs may be disproportionally affected in these areas by the COVID-19 pandemic.

According to the national surveillance data of HIV by 47 prefectures, the total diagnosed cases of HIV infection between 2018 and 2022 per 100,000 population in descending order by area was Tokyo (2.06), Fukuoka (1.23), Okinawa (1.23), Osaka (1.03) and Kumamoto (0.93) (*44*). The proportion of the number of AIDS cases in the number of total HIV cases in these areas were 18.2%, 38.2%, 41.5%, 23.2% and 48.0%, respectively. The average percentage in Japan was 29%. The high proportion of AIDS cases are thought to be a reflection of insufficient HIV testing provision, given that PrEP is not officially approved in Japan. To close the gap, investment in public health centers and adoption of self-sampling testing need to be promoted.

The second 90 target was also not achieved in Japan, probably due to restrictions in the timing of ART initiation regulated by the disabled person's coverage system. In Japan, ART is quite accessible for most PLWH, being covered by national health insurance plus the disabled person's coverage system. However, there are cases in which the disabled person's coverage is not applicable for PLWH with CD4 counts below 500/mm^3, due to their low HIV viral load. Furthermore, to

obtain the coverage, there is an average wait of two to three months, since two blood tests, 1 month apart, are required to apply for the system (*45*). Although the extent to which these gaps affect the second 90 target is unknown, they should be closed further for PLWH and the community's benefit.

Current situation of PrEP in Japan: The long delay in implementation

The last 90 target is well achieved and there is no gap between Japan and the world. Recent commonly used INSTI powerfully enhanced the achievement of this target in Japan. The biggest gap resides in the field of PrEP. As mentioned above, PrEP is not approved or officially supported as of June 2024. According to previous cohort studies, the incidence of HIV infection among MSM in Tokyo was estimated to be between 3.4 and 3.8 /100 person-years (*46,47*). Prevalence of HIV infection among MSM in Tokyo was reported to be as high as 2.6 to 3.0% in two cross-sectional studies (*43,47*). It is recommended that PrEP be made available for high-risk groups including MSM in Japan. This gap is a serious problem from a perspective of disparity in access to PrEP as well.

Despite of the absence of top-down movement, the number of PrEP users has been gradually increasing in Japan in a grass-roots way, along with an increase in the awareness of PrEP among MSM. A small pilot trial of daily PrEP of Tenofovir disoproxil fumarate (TDF)/ Emtricitabine (FTC) with 124 MSM was initiated in 2018 in Tokyo (*47*), which also contributed to prevalent information on PrEP. Since then, MSM who sought PrEP initiated access by purchasing generic TDF/FTC *via* the internet. According to a large internet-based questionnaire survey for MSM conducted in Japan in 2018, the proportion who knew about PrEP was 36.3% (1,719/4,735) (*48*); 6.8% (116/1,716) had experience in taking PrEP; 64.6% (73/113) obtained PrEP medicine *via* the internet, followed by medical institutions outside of Japan (20.4%, 23/113) and medical institutions in Japan (15%, 17/113). Importantly, only 28.1% (32/114) of PrEP users regularly see a doctor for PrEP follow-up and 43% (49/114) don't see a doctor at all. This "unsupervised use of PrEP" without necessary testing is of great concern, and providing correct information on PrEP use for high-risk groups is essential. In this respect, Kamakura *et al*. reported that 45.4% of physicians have no PrEP knowledge, according to a web-based survey that targeted physicians in specialties of treating STI and/ or HIV in Japan (*49*). People who sought PrEP follow-up tests were likely to be refused at clinics which physicians have no PrEP knowledge. There might also be a gap in knowledge about sexual minorities among medical personnel, which will be another obstacle for HIV prevention in Japan.

To prepare PrEP implementation in Japan, sexual health clinic (SHC) was established in 2017 at National Center for Global Health and Medicine in Shinjuku, Tokyo, where the one of the largest gay towns in Asia was located. The SHC is part of an ongoing prospective cohort study which, as a patient monitoring system, documents HIV and STI incidence while assessing PrEP uptake among MSM who visit the clinic. The inclusion criteria specify MSM aged 16 years or older who have had anal intercourse in their lifetime, are HIV seronegative, and provided written informed consent as described previously (*46,47*). Every three months, eligible participants received free HIV screening and testing for STIs, including early syphilis, chlamydia, and gonorrhea. Participants were given information about PrEP, but PrEP itself was not prescribed at the SHC after completion of the PrEP pilot study abovementioned. They were queried about their sexual behaviors, drug use, and PrEP status during each visit. The accumulated number of PrEP users in the cohort was gradually increased (Figure 1). The breakdown of where the PrEP initiators get PrEP medicines, in descending order, was purchase *via* the internet (62.9%, 631/1,003), clinics in Japan (19.3%, 194/1,003) and clinical trials performed in SHC (13.6%, 136/1,003). It is noteworthy that an MSM-friendly private clinic initiated generic medicine prescriptions for oral PrEP with necessary tests in 2020, which greatly contributed to PrEP access in Tokyo. Many clinics followed and more than ten clinics are providing PrEP in Tokyo at the moment. The Japanese Society for AIDS research announced the guidelines for PrEP use in 2022 in order to inform medical staffs and PrEP users of correct PrEP knowledge in response to rapidly increasing PrEP users and providers (*50*).

Disparities in PrEP implementation in Japan and challenges in the future

Though generic medicine for oral PrEP is remarkably accessible compared to brand-name drugs, expense of PrEP is fully out-of-pocket in Japan. In the SHC cohort,

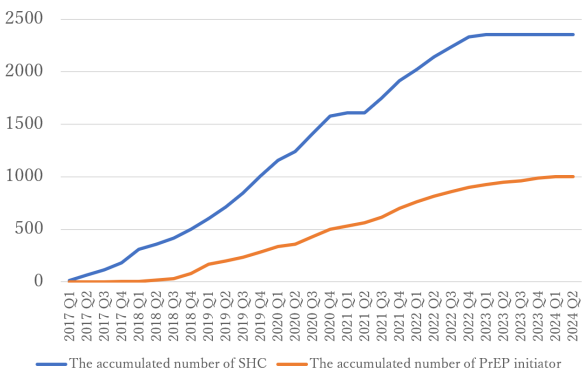

Figure 1. The accumulated number of sexual health cohort participants and PrEP initiators. Although the number of PrEP users is increasing, less than half of the participants in the cohort have not yet started PrEP. PrEP, pre-exposure prophylaxis; SHC, sexual health clinic; Q, quarter.

only half of the study participants are starting PrEP as shown in the Figure 1. The reason is multifaceted but out-of-pocket burden seems to be a main cause. According to the aforementioned survey for MSM, 87.8% (3,956/4,503) can pay less than 10,000 Japanese yen (around 60 to 70 dollars) as a monthly expense for PrEP and 54.0% (2,430/4,503) can pay less than 5,000 yen (*48*). Although some clinics offer PrEP services including HIV, other STIs, and renal function tests at around 10,000 yens at the low end, younger MSM with low or no income at greater risk of acquiring HIV infection may not afford even these reasonable PrEP services. Despite the fact that the PrEP implementation in Japan has progressed in a grass-roots way without top-down regulation and official financial support, relatively feasible access to PrEP service has been achieved. However, certain financial and geographical gaps remain.

The first gap stems from "out-of-pocket" issues which prevent people with low or no income from accessing PrEP services. It is needless to say that this disparity must be solved by official and/or other institutional supports. The second gap is a geographical disparity inside Japan in which MSM living outside of Tokyo have much more limited access to PrEP services compared to MSM living in Tokyo. This geographical matter is also the case with the awareness of PrEP. Under the current situation of PrEP provision in Japan, purchasing generic medicines for PrEP *via* the internet can be an option to mitigate this disparity. However, low awareness of PrEP outside of Tokyo contributes to low numbers of internet-based purchase of PrEP. Furthermore, those who purchase internet-based PrEP must seek out testing on their own, and lower knowledge among physicians outside of Tokyo is a challenge. HIV and syphilis tests regularly provided by public health centers will be a part of the solution, though the capacity of these centers depends on each area. One of the important problems in HIV prevention in Japan is that the testing strategy performed in the public health centers and municipalities does not include treatments and preventive intervention like PrEP, since public health centers are not regulated to prescribe but provide only consultation and testing. As PrEP is not approved at the moment, quite few public health centers are involved in PrEP services. However, recently, public health centers in some areas are entrusting their HIV and other STI testing tasks to MSM friendly clinics, which may not only mitigate shortage of human and financial resources but coordinate tests, treatments, and prevention strategies.

It is essential to improve awareness of PrEP among the involved people including medical staff in Japan. In this respect, approval of PrEP will allow for dissemination of correct information on PrEP. The efficacy and benefit of PrEP are evident in a number of countries. In Japan, high efficacy was reported as well. According to the aforementioned pilot study, HIV incidence rate was significantly higher in the non-PrEP users compared to the PrEP users (3.45/100 *vs.* 0 person-years, $p = 0.01$) (*47*). In terms of cost-effectiveness analysis, two studies demonstrated PrEP as a cost-saving strategy in Japan (*51,52*). It is of note that the current situation of bottom-up PrEP implementation using much cheaper generic medicines in Japan is obviously cost-saving, which is needless to evaluate. In addition to the approval of PrEP, official financial support for PrEP is required; otherwise, authorities in charge will be regarded as free-riders. At the least, the current path of PrEP system that occurred in a grass-roots manner should be paved by authorities without impairing existing virtues of accessibility and cost-saving.

Conclusions and Future directions

Regarding UNAIDS's 95-95-95 HIV testing and treatment cascade, there is room for improvement in the first and second 95, namely, proliferation of HIV testing and early initiation of treatment. However, the largest gap to be filled is the delay in PrEP implementation. Without official support for PrEP, grass-roots PrEP implementation based on user-friendly clinics has been functioning well in some areas like Tokyo. However, there are geographical and financial disparities in access to PrEP services. To establish a more accessible, cost-saving and equitable PrEP system, current existing but isolated entities and strategies related to HIV management needs to be integrated and coordinated with other. In this respect, the role of the authorities is important to close the gaps in PrEP implementation.

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Analysis of tumor infiltrating immune cells in Kaposi sarcoma lesions discovers shifts in macrophage populations

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Abstract: Limited information exists about the types of immune cells present in Kaposi sarcoma (KS) lesions, especially in KS in the gastrointestinal tract. Using previously reported RNA-sequencing results from Kaposi sarcoma lesions in skin and gastrointestinal tract with normal matched tissues from the same patients at the same time, we investigated changes in lymphocytes in these tissues. We employed a computational method that determines changes in cell type distributions using KS lesion transcriptome data compared to a reference set of RNA expression patterns of purified immune cells. Since secreted cytokines and chemokines from KSHV-infected cells may influence the microenvironment of Kaposi sarcoma lesions, we performed cytokine profiling of conditioned media from KSHV-infected primary human dermal lymphatic endothelial cells. We also measured how this conditioned media altered the differentiation of macrophages in cell culture assays. These results suggested that factors in conditioned media from KSHV-infected endothelial cells promoted differentiation of a promonocytic cell line to proinflammatory macrophages.

***Keywords*:** KSHV, cibersort, RNA-sequencing

Introduction

Kaposi sarcoma (KS) is caused by the Kaposi sarcoma herpesvirus (KSHV or HHV8). Besides KS, KSHV also causes primary effusion lymphoma (PEL), multicentric Castleman disease (MCD), and KSHV inflammatory cytokine syndrome (KICS). These conditions can occur alone or together and vary in prognosis (*1*). In the US, KS mainly affects people living with HIV (PWH) and is known as epidemic KS. Despite advances in HIV treatment reducing its incidence, KS remains a common HIV-associated tumor in the US and Southern Africa, where it is a significant health issue. KS commonly occurs in PWH with low CD4+ T cell counts but can also arise from other immune dysregulation. Standard KS treatment for PWH includes antiretroviral therapy (ART). For extensive or ART-resistant disease, systemic therapies like liposomal doxorubicin, paclitaxel, or pomalidomide are used (*1*). KS may recur despite remission and well-controlled HIV, requiring long-term treatment. The reasons for recurrence are unclear.

Little is known about cellular and viral RNA transcripts in KS lesions and their relation to the microenvironment. We previously aimed to understand the changes in human gene expression and viral transcripts in both skin and GI KS. KS tissue was compared to normal tissue using matched patient samples (*2*). KS typically manifests as skin lesions but may also occur in visceral organs including the respiratory and gastrointestinal (GI) tracts in severe cases. Information on their relationships between clinical characteristics, inflammation, immunity and KSHV expression is scarce. We also explored variations in the immune microenvironment based on the lesion location and clinical characteristics. Macrophages play a crucial role in the human immune response to viral infections. As key components of the innate immune system, these versatile cells act as first responders, identifying and engulfing pathogens through phagocytosis. Macrophages then present viral antigens on their surface to prime the adaptive immune response, facilitating the production of specific antibodies and viral-specific T cells. They also release a variety of cytokines and chemokines that recruit and activate other immune cells, such as T cells and natural killer cells, to the site of infection. In solid tumors, tumor associated macrophages (TAMs) form, with aberrant expression of cytokines and chemokines, immunosuppressive tumor microenvironment. KSHV was reported to modulate macrophages directly (*3*) or indirectly (*4*).

To investigate specific cellular genes linked to KS pathogenesis or KSHV infection, we conducted *in vitro* studies using KSHV-infected lymphatic endothelial cells (LECs) to study cytokine production and their effects on

macrophage gene expression.

Materials and Methods

Cell culture, reagents, nucleofection, and KSHV infection

Human dermal lymphatic endothelial cells (HDLECs) were obtained from Promocell and passaged in EGM2 medium (Lonza) for up to 5 passages, with passages 3 to 5 used for experiments. THP-1 cells (American Type Culture Collection TIB-202) were cultured in RPM1640 media (Gibco) supplemented with 10% Fetal Bovine Serum, Penicillin, and Streptomycin (Gibco). HDLEC *de novo* infections were carried out using KSHV-BAC16 (concentrated by ultracentrifugation), diluted in EGM2 medium, at multiplicity of infection of 0.25, 0.5, and 1 infectious units (I.U.). 1.0 I.U. is the amount of virus that is sufficient to infect all cells if virus will be distributed equally, as determined by GFP ectopic marker. Polybrene was added at 8 µg/mL. Uninfected samples were included as negative controls. After 16 hours of incubation, cells were washed and overlaid with fresh media.

Human cytokine analysis

At three days post infection, conditioned media was collected, cleared by centrifugations (500g, 10 minutes, then 2,000g, 20 minutes), and 100 µL for each sample was used for measuring specific cytokines with V-PLEX Proinflammatory Panel 1 Human Kit (Meso Scale Diagnostics). Assays were performed by Clinical Support Laboratory (NCI-Frederick).

THP-1 differentiation assay

2×10^5 THP-1 cells were seeded in 6 well plates in 2 mL complete RPMI media. 10 ng/mL of 12-O-tetradecanoylphorbol-l3-acetate (PMA, Sigma) was added, and cells were incubated for 24 h. 1 mL of media was replaced with 1ml of conditioned media from KHSV-infected HDLEC cells and THP-1 cells were incubated for 24 hours. THP-1 cells were washed with Phosphate-buffered saline (Gibco) and total RNAs were extracted with the Direct-zol RNA miniprep kit (Zymo Research). As a read-out, quantitative reverse transcription-PCR (RT-qPCR) was performed using 500 ng RNA and ReverTra Ace reverse transcription kit (Toyobo), Thunderbird Probe qPCR master mix (Toyobo), and TaqMan probe (*CXCL10* Assay ID: Hs00171042_m1, Applied Biosystems) using the ABI StepOnePlus real-time PCR system (Applied Biosystems). Relative mRNA levels were computed using the threshold cycle (ΔΔCt) method with *RPS13* (Assay ID: Hs01011487_g1, Applied Biosystems) as a reference gene according to manufacturer's protocol.

Bioinformatics analysis

We analyzed our previous RNA-sequencing data (Gene Expression Omnibus accession number GSE241095) of KS lesion specimens (*2*). CIBERSORTx software predictions were based on TPM values using the leukocyte signature matrix (LM22) (*5*). CIBERSORTx was executed using the docker container provided by the authors of CIBERSORTx on their website (*https://cibersortx.stanford.edu*), with default arguments (*i.e.* --absolute FALSE, --rmbatchBmode FALSE and –rmbatchSmode FALSE) results in cell-type proportions relative to the reference cell types in the signature matrix. Cell types with values from at least five patient samples were included for further analysis.

Results

As our previous bulk transcriptome analysis of 22 KS lesion samples showed (*2*) heightened expression in markers associated with activated immune cells, we applied the computational tool, CIBERSORTx (*5*), to interrogate changes in immune cell types in the KS lesions. This method imputes the expression profile of specific marker genes from each cell type and estimates proportions of different cell types in the original cell mixtures. We calculated the relative amounts of cell type specific signatures in KS lesions compared to their matched normal samples (Figure 1, A-B). Heterogeneity in the immune subtype signatures was noted across both skin and GI KS lesions. Follicular helper T cells and regulatory T cells were decreased in many GI samples, but this observation was not noted in the skin KS samples. There were elevated levels of resting memory $CD4^+$ T cells in 5 of 12 GI KS samples as compared to the paired normal tissue and in 1 skin KS sample as compared to the paired normal tissue. Increased signatures of M1 macrophages were observed in the majority (7 of 10 samples, $p = 0.056$) of skin KS samples.

Macrophages play key roles in antigen presentation, inflammation and phagocytosis. M1-classically activated macrophages are pro-inflammatory and promote cell killing while M2-alternatively activated macrophages are anti-inflammatory and promote tissue healing. IFN-γ signaling promotes M0 macrophage polarization to M1 macrophages (*6*). Macrophage polarization is widely exploited by viruses through various mechanisms including manipulating cytokines (*6*). CIBERSORTx analyses predicted differences in M1 and M2 macrophage polarization in the KS tumors compared to normal samples. Within GI KS, RNA expression changes of IFN-γ varied widely as compared to their matched normal samples (*2*), and M1 macrophage signatures appeared variable in both tissues (Figure 1A). Interestingly, there was a strong

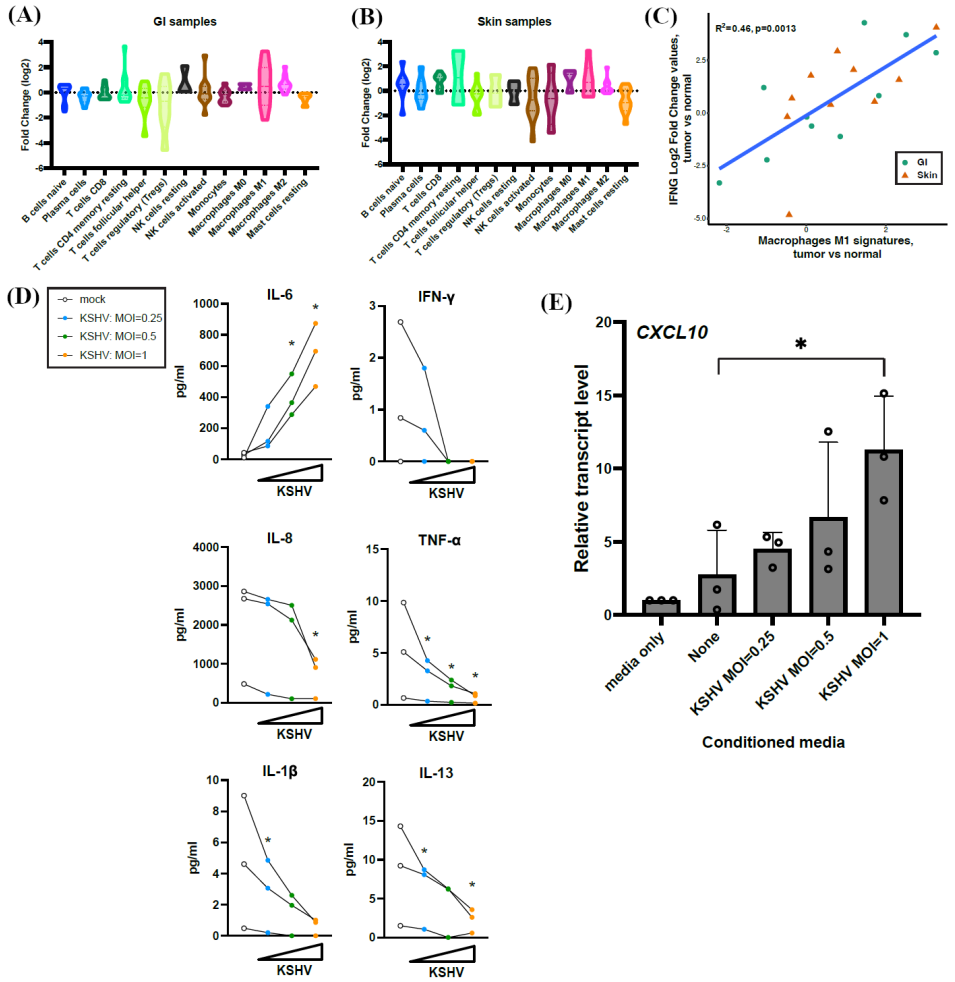

Figure 1. CIBERSORT analysis of KS lesions, cytokine secretion, and macrophage polarization. (A-B) Fold changes of different immune cell types in tumor vs normal samples, were estimated by CIBERSORT. Violin plots show the increased or decreased fold change (log2) of each cell type signature of the KS lesions as compared to normal skin. **(C)** Correlation analysis of changes in *IFNG* RNA expression (tumor *vs.* normal) and changes in M1 macrophage cell signatures (tumor *vs.* normal). **(D)** Proinflammatory cytokine levels in conditioned media of KSHV-infected LECs after 3 days were quantitated with an electrochemiluminescence multiplex immunoassay. Each line represents a separate biological replicate. Only cytokines with concentration > 5 pg/mL in any of samples are shown. **(E)** Macrophage polarization assays with conditioned media and a promonocytic cell line THP-1. THP-1 cells were treated with PMA and then incubated with conditioned media of KSHV-infected LECs (D) for 24 h. Transcript levels of M1 macrophage marker gene *CXCL10* was quantitated with RT-qPCR and normalized to *RPS13*. Multiple paired *t*-test (two sided) was performed. *$p < 0.05$.

positive correlation between IFN-γ expression and M1 macrophage signatures in all tissues ($p = 0.0013$, Figure 1C).

To further characterize the relationship between cytokines, macrophage polarization and the potential role of directing specific cell types in the KS tissue microenvironment, changes in cytokine levels following KSHV infection of endothelial cells were investigated. An initial question was whether IFN-γ expression levels are changed by KSHV infection of LECs or are the changing levels of IFN-γ expression due to other cell types (*e.g.* T and NK cells) in KS lesions. LECs were infected with KSHV in cell culture experiments and the conditioned media from these cells was collected. Changes in cytokine secretion from mock-infected and KSHV-infected LECs were measured at 3- and 5-days post-infection. Conditioned media of KSHV-infected LECs contained higher levels of IL-6 in a multiplicity of infection (MOI)-dependent manner (Figure 1D). This increase in IL-6 levels in KSHV-infected endothelial cells was consistent with previous reports (*7,8*). Other pro-inflammatory cytokines such as IL-8 and IL-1β were reduced in the conditioned media from KSHV-infected cells. Macrophage polarization to the anti-tumor M1 macrophage type is dependent on IFN-γ and TNF-α, and polarization to the pro-tumor M2 macrophage type depends on IL-4 and IL-13. Secretion of all these cytokines was reduced upon infection. IL-4 concentration was less than 5 pg/mL in all samples and was not shown. To assess the effect of conditioned media from KSHV-infected endothelial cells on macrophage polarization, we differentiated a promonocytic cell line THP-1 with conditioned media of infected LECs. THP-1 cells can be differentiated to

M1 or M2 types. Increasing the amount of KSHV in infections polarized THP-1 differentiation more to a M1 profile as measured by an M1 macrophage marker gene, *CXCL10* (Figure 1E). KSHV infection in LECs thus regulates secretion of pro-inflammatory cytokines and may promote M1 macrophage polarization. The M1 polarization observed in KS lesions was unlikely due to changes in IFN-γ secretion from KSHV-infected endothelial cells since IFN-γ secretion levels only decreased with KSHV infection in LECs (Figure 1D). These cell culture experiments (Figure 1, D-E) showing an increase in an M1 macrophage marker was consistent with the increased M1 cell signatures observed in skin KS (Figure 1B), compared to the matched normal skin tissue.

The amount of viral infection varied among the patient samples, as measured by the KSHV transcript reads in the RNA-sequencing results. The KSHV transcript values were summated together into KSHV transcripts per million (KSHV TPM, Figure 2) as a proxy of virus infection levels. These values reflect the total amount of normalized KSHV viral transcript reads per sample. KSHV TPM and CIBERSORT values for changes between tumor and matched normal samples were analyzed using a non-parametric correlation analysis. This method was used to investigate whether predicted changes in cell types were correlative with each other or with the overall level of KSHV infection (KSHV TPM). All samples were used for the correlative analysis in Figure 2A, which found a strong correlation (R^2= 0.85) between the amount of KSHV gene expression (KSHV TPM) and levels of regulatory T cells (Tregs). Since different immune responses may occur between skin and GI locations, additional correlation analyses were conducted separately with only the GI (Figure 2B) and the skin (Figure 2C) samples. In the GI samples, a similar correlation was observed between KSHV infection and levels of Tregs (Figure 2B), which promotes an M2-like TAM formation and immunosuppressive environment. The strongest negative correlation was between Tregs and monocytes in GI samples. In skin samples there was a strong negative correlation between KSHV infection levels and NK resting cells, suggesting that with more KSHV infection, decreased levels of NK resting cells were found in skin KS tumors. A strong positive correlation was also found between changes in M0 macrophages and M2 macrophages, but not with M1, in skin KS. Though CIBERSORTx scores for macrophages are dysregulated both in GI and skin KS compared to normal (Figure 1, A-B), the underlying mechanisms may be distinct.

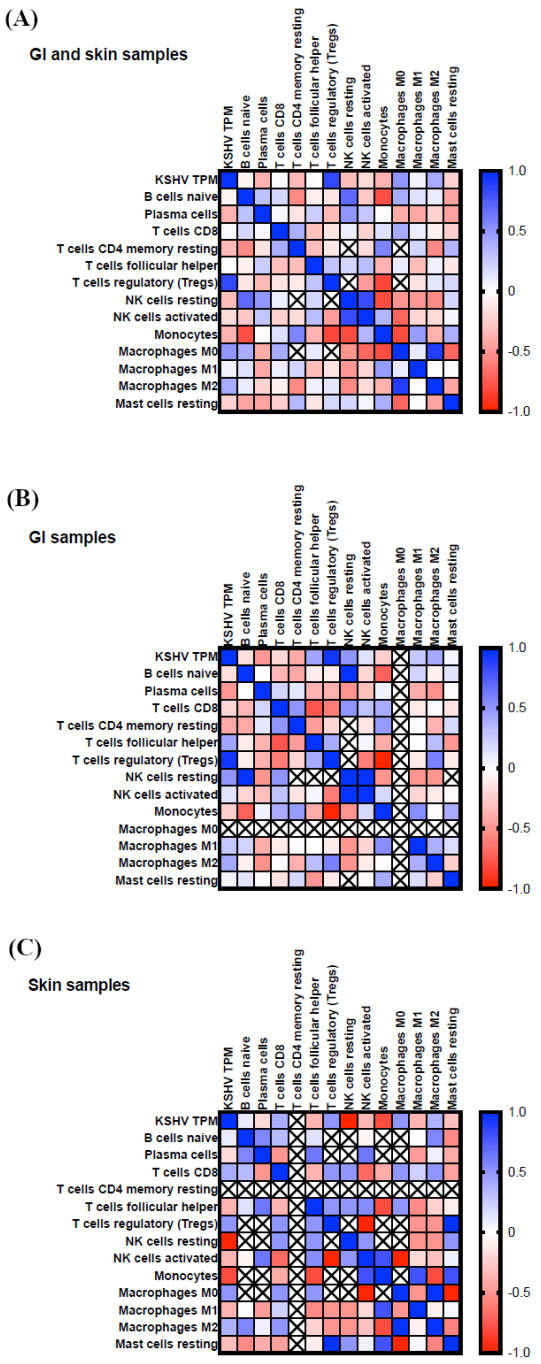

Figure 2. Correlation analysis of CIBERSORT values and KSHV infection. Normalized KSHV transcripts in the RNA-sequencing results were summarized and reported as total KSHV transcripts per million reads (KSHV TPM). Changes in CIBERSORT values were analyzed in combination with KSHV TPM values. Blue colors indicate strong positive correlations and red colors show strong negative correlations in all samples **(A)**, only GI samples **(B)**, and only skin samples **(C)**.

Discussion

Differences in skin and GI lesions gene expression may be associated with immune profiles in respective tumor microenvironments. A recent study using single cell sequencing analysis of KS among participants who were recently diagnosed with HIV and KS who were antiretroviral therapy naïve demonstrated an abundance of T cells and macrophages (*9*). A previous study of skin KS lesions of participants with endemic and epidemic KS from sub-Saharan

Africa demonstrated the distribution of immune cells by infection of KSHV cells. In these analyses, we noted that classically activated M1 macrophages were evenly distributed in KS lesions, whereas alternatively activated macrophages were notable in areas that did not have KSHV infected cells (*10*). M1 macrophages are often amplified by IFN-γ signaling to promote cell killing (*11*). This study also highlighted the abundance of CD8+ T cells in KS lesions compared to normal skin but this was not related to KSHV infected cells.

Limitations of this study are largely dependent on the clinical samples available and the technical shortcomings. The clinical samples that we analyzed had variations in the concurrent diseases occurring at the time of biopsy and some samples were obtained before or after treatment for KS. Additionally, biopsies were obtained from different areas of the skin and gastrointestinal tract and different tissues had various levels of KSHV gene expression. We used a computational method to query different cell types in these samples, based on RNA expression patterns, but complementary approaches (*e.g.* flow cytometry, immunohistochemistry) could strengthen the evidence for these cell type differences between patient samples. These complementary approaches were not feasible in our study, due to the limiting size of the biopsy material.

In our analyses, there were differences noted in the skin and GI KS lesions compared to normal counterparts including dysregulated macrophages and higher resting memory $CD4^+$ T cells particularly in GI KS lesions. One hypothesis was that increased IFN-γ expression in KS lesions might be increasing M1 polarization. However, IFN-γ secretion levels dropped with KSHV infection in our experiments using LECs. In our results using conditioned media from KSHV-infected LECs, we did find an increase in an M1 macrophage marker, *CXCL10*. These results suggest that secreted factors other than IFN-γ in the conditioned media from KSHV-infected cells may be promoting M1 polarization. However, in actual KS lesions, there are infiltrating cells with capability to secrete various cytokines and chemokines beyond KSHV-infected endothelial cells, which may be the overall changes in immune cell population including macrophages. Correlational analysis also demonstrated that such differences may be related to site-specific immune environments between the skin and GI tract rather than broadly associated with KSHV or HIV infection. In GI KS, but not in skin KS, KSHV total TPM positively correlated with regulatory T cells, which promote M2 macrophage differentiation. In contrast, skin KS, only, showed preference to M2 differentiation than M1 in tumor samples compared to matched normal skin. We also note the limitation of this bulk RNA-Seq studies and needs for single cell and spatial analysis to further interrogate the importance of immune cells to regulate tumor microenvironment. Taken together, we showed KSHV's ability to dysregulate immune cells like macrophages *in vitro* and in clinical specimens.

Conclusion

The immune cell composition of KS lesions, particularly in the gastrointestinal tract, remains poorly understood. To address this, we analyzed RNA-sequencing data from KS lesions in both skin and gastrointestinal tissues, using matched normal tissues from the same patients as control samples. We compared the transcriptomes of KS lesions with reference immune cell RNA expression profiles to assess changes in immune cell populations. This analysis found the greatest changes in predicted T cells, monocytes, and macrophages. We conducted cytokine profiling of conditioned media from KSHV-infected human dermal lymphatic endothelial cells, since cytokines and chemokines from KSHV-infected cells may modify the lesion cellular environment. Additionally, we studied how this conditioned media affected the differentiation of macrophages growing in culture. The results showed that factors from the infected cells encouraged the differentiation of promonocytic cells into proinflammatory macrophages (M1). A correlation analysis revealed that as KSHV gene expression increased in tissues, the predicted amount of immune-suppressive T regulatory cells also increased. These studies indicate how KSHV infection and changes in cytokines and chemokines may shape the immune environment of KS lesions.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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Non-acquired immunodeficiency syndrome defining malignancies in people living with haemophilia and human immunodeficiency virus after direct-acting antiviral era

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Abstract: Non-acquired immunodeficiency syndrome-defining malignancies (NADMs) are the crucial cause of mortality in people living with haemophilia and human immunodeficiency virus (PLWHH). We aimed to analyse the types and characters of NADMs in PLWHH after approval of direct-acting antivirals (DAA), considering that most PLWHH are infected with hepatitis C virus (HCV). We conducted a nationwide questionnaire mail survey across 395 HIV core facilities in Japan between May 2022 and February 2023. Eight-year data from 64 respondent hospitals (n = 328 PLWHH; 2015–2022) were collected; 35 NADM cases were identified and analysed. Standardised cancer incidence ratios (SCIRs) were calculated. The median age of PLWHH with NADMs was 51 years (interquartile range: 47–62 years); the SCIR was 2.08 (95% confidence interval [CI]: 1.48–2.90) for all malignancies (including carcinoma *in situ*). Liver cancer accounted for most NADMs (43% [15/35]). The SCIRs of liver cancer (23.09 [95% CI: 13.92–38.30]) and papillary thyroid cancer (9.38 [2.35–37.50]) significantly increased after adjusting for general Japanese male sex and age. Among PLWHH with liver cancers, 73% (11/15) achieved HCV-sustained virological response. Notably, for patients aged ≤ 50 years, 47% (7/15) were affected by liver cancers, and 27% (4/15) succumbed to NADMs. This study presents the largest survey of NADMs in PLWHH after DAA approval. Our findings emphasised the elevated risk of malignancies in PLWHH, underscoring the need for early cancer screening and preventive measures, particularly against liver cancers, even in younger PLWHH.

***Keywords*:** non-AIDS-defining cancers, haemophilia, HIV, HCV, liver cancer, SCIR

Introduction

Improvements in the prognosis of people living with human immunodeficiency virus (PLWHIV) have increased their risk of developing non-acquired immunodeficiency syndrome (AIDS)-defining malignancies (NADMs) (*1-4*). Notably, NADMs have become a major contributor to mortality (23–28%) in

PLWHIV (*2,3*). In Japan, approximately 30% of patients with haemophilia were reportedly infected with human immunodeficiency virus (HIV) through contaminated blood products manufactured in the United States before 1986 (*5*). Subsequently, the number of people living with haemophilia and HIV (PLWHH) was 1,439; currently, this number is approximately 700 in Japan. PLWHH also face an increasing risk of NADMs (*6,7*); however, because of their relatively small global population, a few cases of NADMs have been documented, particularly after the introduction of antiretroviral therapy (ART) (*6,8-11*). No studies have investigated the standardised cancer incidence ratios (SCIRs) for NADMs in PLWHH in only the post-ART era.

In Japan, the introduction of ART in late 1996 greatly enhanced the prognosis of PLWHH. However, almost all Japanese patients with haemophilia during that period were also infected with the hepatitis C virus (HCV), leading to prolonged co-infection since childhood. HCV progression is accelerated in HIV co-infection compared to mono-infection (*12*). Despite the approval of interferon-free direct-acting antivirals (DAAs) for HCV treatment in 2014, Miuma *et al*. showed that liver cirrhosis remained prevalent at 45.5% (Child–Pugh grade A: 31.9%, B: 4.3%, and C: 6.4%) (*13*). Although PLWHH are also recommended to receive HCV treatment, multiple HCV genotypes and/or mental problems of PLWHH have hindered the achievement of sustained virological response (SVR) (*13,14*). HIV co-infection exacerbates liver fibrosis and hepatocellular carcinomas (HCCs) (*15*). Nevertheless, no data exist on NADMs in PLWHH post-DAA initiation.

Recently, breakthroughs in haemophilia treatment have markedly improved patient prognosis and increased the average life expectancy (*16*). The well-being of PLWHH, as well as PLWHIV transmitted through sexual means, is crucial. Therefore, identifying and preventing the risk of NADM development in PLWHH is extremely important. Furthermore, individuals coinfected with HIV and HCV experience an extremely long period of untreated infection (HIV: > 15 years, HCV: > 20 years); therefore, their immunological status is distinctive for cancer development and progression.

In this study, we aimed to elucidate the types of NADMs and the characteristics of PLWHH affected by NADMs; furthermore, we aimed to analyse SCIRs in PLWHH during the post-DAA era. By understanding SCIRs, we conducted a nationwide survey to promote comprehensive cancer care, including diagnosis, treatment, health prevention programs, and education of PLWHH.

Patients and Methods

Study participants

In Japan, most PLWHIV are referred to HIV core hospitals. Physicians and other professionals engaged in HIV care in 395 HIV core facilities and clinics across Japan were contacted for this nationwide mail survey, which was conducted using a questionnaire-based tool between May and June 2022 to gather information on NADMs diagnosed from January 2015 to December 2021 (Supplemental Table S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=89*). A second questionnaire was mailed between January and February 2023 to the facilities that responded to the initial questionnaire (questionnaire 1) for additional details on NADMs diagnosed from January to December 2022 and the numbers of PLWHH attending each facility (Supplemental Table S2, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=89*). Overall, NADM data were collected over 8 years. PLWHH were defined as patients with HIV and haemophilia, or other conditions treated with unheated blood coagulation products (Supplemental Table S3, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=89*). In 2014, the first interferon-free therapy for HCV, daclatasvir/asunaprevir, was launched in Japan. In 2015, sofosbuvir, effective against all HCV genotypes, was approved. Therefore, we decided to collect data on NADMs after 2015. *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=89*) presents the exclusion criteria for NADMs.

Statistical analysis

Statistical analyses were performed using PRISM 8 v8.1.2 (GraphPad Software, San Diego, California, USA). Descriptive statistics comprised medians (interquartile ranges) and proportions. CD4 counts and CD4/CD8 ratio of PLWHH were analysed using the Mann–Whitney test. *P* values less than 0.05 were considered statistically significant. We calculated SCIRs and 95% confidence intervals (CIs) for PLWHH and compared the observed cancer incidence in PLWHH with the expected incidence, standardised based on age-stratified cancer incidence in the general Japanese male population. The sex- and age-specific cancer incidences in the general population were obtained from the National Cancer Centre website (*17*). If the age distribution data for the source population of PLWHH in this study could not be collected, we used the age distribution reported in other national surveillance (*18*). As only one female patient was present despite a significantly larger number of male patients, she was excluded from the SCIR calculation. The CIs for the SCIR were calculated using the formula exp (ln (SCIR) ± 1.96 * (1/cancer incidence$^{1/2}$)) (*19*). If the lower limit of the CI for SCIR exceeded 1, it was considered statistically significant.

Ethics approval and consent to participate

The protocol for this research project was approved by the Ethics Board of the Institute of Medical Science, University of Tokyo (approval no.: 2021-71-1216) and adhered to the principles of the Declaration of Helsinki. The requirement for consent to participate was waived by the Institutional Review Board of the Institute of Medical Science, University of Tokyo, in accordance with national regulations.

Results

Baseline characteristics of people living with human immunodeficiency virus with haemophilia with non-acquired immunodeficiency syndrome-defining malignancies

Sixty-four HIV core hospitals responded to the questionnaire (response rate: 16.2% [64/395]). These hospitals were attended by 328 PLWHH (47.1% of the 697 PLWHH in Japan). Overall, 35 NADM cases were diagnosed for the 328 PLWHH during the 8-year study period; two PLWHH had duplicate cancers, specifically colon and tongue cancers and colon and liver cancers. The characteristics of these 35 NADM cases are presented in Tables 1 and 2. All cases except one were male, with a median age of 51 years (interquartile range: 47–62 years); the youngest PLWHH with NADM was 39 years old and had colon cancer. Furthermore, 74% of these 35 NADMs had haemophilia A, and three PLWHH had > 50 copies/mL of HIV-RNA at diagnosis. Moreover, 97% of the PLWHH with NADMs (33/34, had one missing data) exhibited HCV-antibody positivity; four PLWHH exhibited HCV-RNA positivity. At diagnosis, 6% (2/34) of the PLWHH with NADMs were heavy alcohol drinkers, 18% (6/34) were current smokers, and 29% (10/34) lived alone.

Table 1. Baseline characteristics of PLWHH with NADMs

Baseline characteristics	All patients *n* = 35
Age (years), median (IQR)	51 (47–62)
Haemophilia type	
A	26 (74%)
B	7 (20%)
History of AIDS	5/34 (15%)
Treatment with ART	33 (94%)
Duration of ART (years), median (IQR)	22 (17–25)
HIV viral load < 50 copies/mL	32 (94%)
CD4 cell count (/μL), median (IQR)	405 (281–558)
CD8 cell count (/μL), median (IQR)	470 (304–665)
CD4/CD8 ratio, median (IQR)	0.87 (0.58–1.11)
HBsAg positivity	2/34 (6%)
HCV-Ab positivity	33/34 (97%)
HCV-RNA positivity	4/32 (13%)
Current smoking	6/34 (18%)
Heavy alcohol consumption	2/34 (6%)
Solitary living	10/34 (29%)

Abbreviations: AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; IQR, interquartile range; ART, antiretroviral therapy; HBsAg, hepatitis B surface antigen; HCV-Ab, hepatitis C virus antibody; HCV-RNA, hepatitis C virus RNA; NADM, non-acquired immunodeficiency syndrome-defining malignancy; PLWHH, people living with haemophilia and the human immunodeficiency virus.

Characteristics of non-acquired immunodeficiency syndrome-defining malignancies in people living with human immunodeficiency virus with haemophilia

The observed 8-year cancer incidence was 35, and the corresponding annual incidence was 4.38. The most common NADMs were liver cancer (43% [15/35], one case of liver adenocarcinoma, and remaining of HCCs (Table 2); all PLWHH with liver cancers exhibited HCV-antibody positivity, and 73% (11/15) had achieved SVR. Furthermore, for patients aged ≤ 50 years, 47% (7/15) had liver cancers, and 27% (4/15) died of NADMs.

The next most common NADMs were colon cancer, malignant lymphoma, tongue cancer, and papillary thyroid cancer (in that order). One case of epithelial cancer was observed. Overall, 77% (27/35) were diagnosed during health screenings and examinations performed at regular visits. The remaining 23% (8/35) were diagnosed after symptom appearance. Metastasis was present at diagnosis in 29% (10/34) of the cases. Surgery was required for 60% (21/35); treatment duration exceeded 6 months in 51% (18/35); and complete remission occurred in 68% (23/34) of the cases. However, death from NADM occurred in 18% (6/34) of the cases.

CD4 counts and CD4/CD8 ratios at NADM diagnosis are presented in additional files (Supplemental Figure S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=89*). PLWHH who died exhibited lower CD4 counts (298 [184–429]/μL *vs.* 407 [321–564]/μL) and lower CD4/CD8 ratios (0.49 [0.43–0.89] *vs.* 0.89 [0.66–1.18]) than did those who survived; however, these differences were not significant ($p = 0.156$ [CD4 count] and 0.107 [CD4/CD8 ratio]; Supplemental Figure S1, b and c, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=89*).

Age- and sex-adjusted standardised cancer incidence ratios in people living with human immunodeficiency virus with haemophilia with non-acquired immunodeficiency syndrome-defining malignancies

Figure 1 illustrates SCIRs for all malignancies, including carcinoma *in situ* (2.08 [1.48–2.90]). The SCIR for liver cancer was significantly higher (23.09 [95% CI: 13.92–38.30]) than that for papillary thyroid cancer (9.38 [2.35–37.50]). However, the association was not significant for malignant lymphoma, tongue cancer, or colon cancer. The standardised cancer death ratio was 1.38 (95% CI: 0.62–3.07), which was also not significant.

Table 2. Characteristics and management of NADMs in PLWHH

Characteristics of NADMs	All patients *n* = 35	
Cancer type		
Liver cancer	15 (43%)	
Colon cancer	5 (14%)	
Malignant lymphoma	3 (9%)	
Papillary thyroid cancer	2 (6%)	
Tongue cancer	2 (6%)	
Neuroblastoma	1	
Malignant myeloma	1	
Prostate cancer	1	
Stomach cancer	1	
Renal cell carcinoma	1	
Gall bladder cancer	1	
Buccal mucosa cancer	1	
Pancreatic cancer	1	
Trigger of diagnosis		
Health screening	22 (63%)	
Presentation of symptoms	8 (23%)	malignant lymphomas (*n* = 2), colon cancer (*n* = 2), tongue cancer (*n* = 1), buccal mucosal cancer (*n* = 1), neuroblastoma (*n* = 1), and multiple myeloma (*n* = 1)
Examination during regular visits	sit (14%)	
Metastasis at diagnosis	10/34 (29%)	liver cancer (*n* = 5), malignant lymphomas (*n* = 2), buccal mucosal cancer (*n* = 1), neuroblastoma (*n* = 1), and multiple myeloma (*n* = 1)
Treatment		
Only surgery	15 (43%)	
Chemotherapy + radiation	7 (20%)	
Only radiation	4 (11%)	
Only chemotherapy	3 (9%)	
Surgery + chemotherapy	3 (9%)	
Surgery + chemotherapy + radiation	2 (6%)	
Surgery + radiation	1	
Length of treatment		
< 1 week	11 (31%)	
1 week to 6 months	6 (17%)	
6 months to 1 year	8 (23%)	
1–5 years	8 (23%)	
> 5 years	2 (6%)	
Outcome		
Complete remission	23/34 (68%)	
Partial remission	3/34 (9%)	
Death due to malignancy	6/34 (18%)	liver cancer (n=5), neuroblastoma (*n* = 1)
Death due to other causes	1	
Under treatment	1	

Abbreviations: AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; NADM, non-acquired immunodeficiency syndrome-defining malignancy; PLWHH, people living with haemophilia and the human immunodeficiency virus.

Discussion

In this study, with a SCIR of 2.08 (95% CI: 1.48–2.90) for all malignancies, including carcinoma *in situ*, the risk of cancer was higher in PLWHH than in the general male population in Japan, even after DAA approval. The median age at NADM diagnosis was 51 years, which is relatively young. The most common NADM was liver cancer (43%), with a notably high SCIR of 23.09 (95% CI: 13.92–38.30). In younger patients with NADM aged ≤ 50 years, 47% (7/15) had liver cancers, and 27% (4/15) died of NADMs. The SCIR for papillary thyroid cancer was also relatively high.

Papers were found following a comprehensive search in PubMed using the terms "cancer" or "malignancy" and "haemophilia" and "HIV" including the period from 2000 onwards in the study period, which was the post-ART era. Among the many studies found on cancer deaths in patients with haemophilia without HIV infection, five described cancer incidence in patients with haemophilia and HIV, all of which were small cohorts (*6,8-11*). Three of these reports described cancer types in PLWHH. Biron *et al.* reported seven cases of cancers (leukemia or hematopoietic cancer: 2, HCC: 2, urogenital: 1, respiratory system: 1, and gastrointestinal tract: 1) from France and Belgium during the observation period from 2002 to 2012 (*8*). Similarly, Oka *et al.* reported six cases (thyroid cancer: 3, HCC: 1, pancreas cancer: 1, and neuroendocrine tumor: 1) from Japan during the 2016–2019 observation period (*6*). No SCIRs were reported

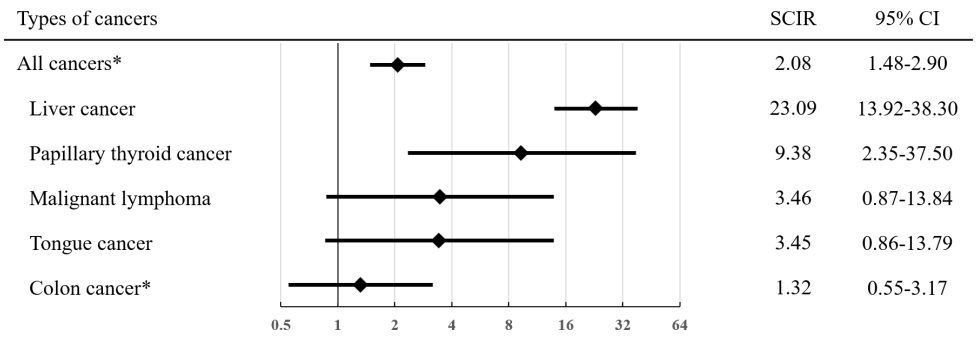

Figure 1. Standardised cancer incidence ratio of various cancers. Comparison of types and standardised cancer incidence ratios of non-AIDS-defining malignancies between PLWHH and the general male population in Japan. *including carcinoma *in situ* AIDS, acquired immunodeficiency syndrome; PLWHH, people living with haemophilia and human immunodeficiency virus; SCIR, standardised cancer incidence ratio; CI, confidence interval.

in either case. Chen *et al.* reported eight cases (oral cavity: 4, non-Hodgkin lymphoma: 3, and leukemia: 1) from Taiwan with respective SCIR (95% CI) of 16.88 (4.54–43.22), 12.66 (2.54–37.00), and 4.22 (0.06–23.48), respectively (*10*). However, no post-DAA era reports of SCIRs for NADMs in PLWHH have been published. This nationwide survey is the largest on NADMs in PLWHH (n = 35) and the first to have determined the SCIRs after DAA approval.

The SCIR results for all malignancies in our study are greatly influenced by the SCIR result for liver cancer. In Japan, the general population affected by liver cancers comprises older adults with complications, such as HCV and hepatitis B virus infections secondary to blood transfusion and mother-to-child transmission. Among the PLWHH with liver cancers in our study, 47% (7/15) were aged ≤ 50 years, and 33% (5/15) died (three died within 1 year of diagnosis). Accordingly, the observed young age of the affected PLWHH in this survey may be a critical finding. Co-infection with HIV and HCV, which increases the risk of HCC, may have contributed to this observation. Factors such as the HCV genotype and immune dysfunction of the patients may have also played roles. PLWHH may be infected with HCVs of multiple genotypes; for instance, HCV genotype 3 is associated with a high incidence of fatty liver, rapid fibrosis development, and HCC (*20-22*). Our findings revealed that 73% of the liver cancer cases in PLWHH had already achieved SVR. Other contributing factors may include rapid disease progression because of immune dysfunction.

In this study, the percentage of heavy alcohol drinkers was low, at 6%. Alcohol consumption is known to promote adipogenesis and fibrosis of the liver, particularly in those who already have liver problems due to HIV and/or HCV infection. HIV infectious disease specialists educate patients about the health risks exacerbated by alcohol consumption. Therefore, to reduce this risk, all PLWHH should avoid alcohol consumption and take steps to prevent fatty liver development for hepatoprotective purposes after achieving SVR. Four cases of HCV-RNA-positive PLWHH were also observed. Following DAA approval, SVR rates have increased; thus, collaboration with hepatologists should be considered to actively promote HCV treatment. The HCC treatment that was administered included various approaches, such as chemotherapy, radiation, and surgery; this reaffirmed the need for continued multidisciplinary treatment of HCCs involving oncologists and liver surgeons (*23*).

Recently, a French study revealed that the incidence of NADMs except for HCCs was higher in PLWHIV with SVR than in PLWHIV without HCV infections, even after excluding cirrhotic cases; this suggested a role of HCV infections in the development of cancers other than HCCs (*24*).

In our study, colon cancer was the second most common NADM; however, the SCIR for colon cancer did not differ significantly. The five most common malignancies in Japanese men are HCCs, prostate cancer, colon cancer, stomach cancer, and lung cancer (*17*). In the future, these malignancies may increase in PLWHH. Therefore, ensuring that PLWHH undergo screening at the same rate as the general population may be necessary.

The next most common malignancy in our study was malignant lymphomas, at 9% (Hodgkin's lymphoma, two cases; unknown, one case). Haung *et al.* reported that in addition to HCCs, leukaemia and lymphomas were common in people living with haemophilia before the introduction of DAA (*9*). The reasons for the higher incidence rates of leukaemia and lymphomas remain unclear; however, they may be linked to frequent blood transfusions in this population. Papillary thyroid cancer was the next most prevalent cancer. Notably, the affected patients were in complete remission after surgical resection. Papillary thyroid cancer generally has a relatively low malignancy, and the reasons for its high incidence in our population remain unclear. Thus, further studies are warranted to elucidate these reasons.

Studies have revealed correlations between NADM incidence and CD4 count and CD4/CD8 ratio in PLWHIV (*25-27*). The mean CD4 count for all PLWHH

has been reported to be 546.9/μL (standard deviation: 246.4/μL) based on publicly available data for Japanese PLWHH (*18*); the median CD4 count for the 35 PLWHH with NADMs in our study appeared lower, at 405/μL. However, the significance of this difference was not determined. Immunological mechanisms may play a role in NADM-associated morbidity in PLWHH. The CD4 count and CD4/CD8 ratio in the six patients who died of NADMs tended to be lower than those in the 28 patients who recovered, although this difference was not significant. Disease progression in PLWHH may also be faster, which should be validated with further research.

The Joint United Nations Programme on HIV/AIDS announced that PLWHIV should have access to integrated health services (*28*) and that PLWHH should be provided with health screening services and cancer treatments. Japanese men have a lifetime cancer risk of 65.5% (*17*). Early detection and treatment are important for PLWHH as they age. In our study, 29% of the PLWHH lived alone during NADM diagnosis, and 51% received cancer treatment for ≥ 6 months. Physical and psychological support are necessary for PLWHH at diagnosis and during treatment. Many PLWHH reportedly experience a limited range of movement secondary to haemophilic arthropathy (*29*); they further tend to avoid malignancy screening tests. Additionally, the strong stigma surrounding these individuals further reduces their likelihood of undergoing malignancy screening. Because PLWHH regularly attend HIV core hospitals, there is a need to establish an efficient system that can detect cancer in its early stages and promote patient education.

Our study had some limitations. First, the small number of PLWHH with NADMs weakened the statistical power of our study. Although data on NADM morbidity were collected, the cancer screening may have been inadequate, potentially leading to undiagnosed NADMs in several cases. It is possible that prostate, colon, stomach, and lung cancers, which are more frequent in our country, might have been reported more if the cancer screening had been conducted adequately. Second, because we did not collect detailed data on liver conditions, we could not directly determine the differences in liver fibrosis between PLWHH with HCCs and those with non-HCC NADMs. Finally, we could not obtain patient blood samples; thus, no immune cell- or serum-based analyses were performed. Further immunological *in vitro* studies are required to elucidate the mechanism of carcinogenesis and progression in PLWHH.

In conclusion, our study investigated the characteristics of PLWHH with NADMs using national surveillance data and focused on morbidities over 8 years (2015–2022); it involved the largest amount of data on NADMs in PLWHH in the post-DAA era. PLWHH were found to be at an increased risk for malignancies. The median age at NADM diagnosis was relatively low, at 51 years, indicating an earlier onset. Among various NADMs, liver cancers were the most prevalent, followed by papillary thyroid cancer. These findings emphasise the importance of cancer screening and preventive measures even in younger PLWHH because early detection is crucial for addressing the elevated risk of NADMs in PLWHH.

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Changes in epidemiological and treatment-related characteristics among newly reported HIV/AIDS cases in an urban area in Shanghai, China from 2001 to 2019: A population-based retrospective study

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Abstract: The HIV/AIDS epidemic has changed significantly over the past 40 years. Changes in AIDS intervention strategies over time and across regions may have influenced epidemiological characteristics and intervention strategies. The aim of the current study was to analyze the changes in multi-year epidemiological characteristics of newly reported HIV/AIDS cases in an urban area (the Fengxian District of Shanghai) from 2001 to 2019 based on the national AIDS comprehensive data information system and Shanghai Statistical Yearbook. In total, the average annual incidence of HIV/AIDS was 1.92 per 100,000 persons. The annual incidence fluctuated and tended to increase from 2001 to 2019 (χ^2 = 128.38, $p < 0.001$). More male patients were reported compared to female patients, accounting for 82.9%. The proportion of patients over 65 years of age increased from 5% in 2009 to 12% in 2019. The majority of cases involved sexual contact (97.7%), early diagnosis (58.8%), full virologic suppression (72.9%), and early antiretroviral therapy (ART) (44.3%). Migrant patients have significantly increased over the years. There were significant differences between local and migrant patients in terms of the age at diagnosis, transmission route, and baseline CD4 count. The disparity in high-risk temporal clusters was also explored to indicate the delay of an epidemic between local patients and migrant patients. HIV remains at a low endemic level. AIDS prevention and control measures have been highly effective, and especially in virologic suppression of ART and early diagnosis. More efforts should be made to enhance early diagnosis and treatment among key vulnerable groups, including the elderly in the local population and young male migrants, and the scale of HIV/AIDS testing should be expanded to the general population to control HIV transmission.

***Keywords*:** HIV epidemic, Shanghai, epidemiology, time clustering, feature analysis

Introduction

Human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) has a negative impact on public health and economic development. According to The United Nations Joint Program on HIV/AIDS (UNAIDS), prior to 2022, approximately 39 million people were estimated to be living with HIV globally, and 29.8 million people living with HIV were receiving antiretroviral therapy (ART) globally (*1*). There have been an estimated 1.3 million new HIV infections, marking a 38% decline in people acquiring HIV since 2010, and 630,000 people died from HIV-related illnesses by 2022 (*1*). Despite extraordinary progress, HIV/AIDS remains a major global public health issue, imposing a serious burden on human life and economic stability (*2*). UNAIDS set the "95-95-95" treatment targets to end the HIV/AIDS epidemic by 2030 (*3*). Higher requirements should be implemented in HIV/AIDS prevention and control. Since the first AIDS case was reported in China in 1985, 60,154 cases and 19,623 deaths were reported in mainland China in 2021 (*4*). At the end of 2022, there were estimated 1.22 million people living with HIV in China (*5*). That said, HIV/AIDS has a low level of incidence and prevalence nationwide. HIV is the leading cause of death from national notifiable infectious diseases (except for COVID-19) in China (*5*).

To prevent the spread of the HIV/AIDS epidemic, China has implemented national policies for HIV/AIDS control for several decades. Through AIDS

intervention strategies, China has achieved substantial progress in combating the HIV/AIDS epidemic. The Chinese Government started the national "Four Frees and One Care" policy in 2003. Moreover, the policy has been adjusted by critically evaluating tailored interventions and iterative assessment and improvement of HIV-related services. The Chinese National Free Antiretroviral Treatment Program (NFATP) for individuals with HIV is part of prevention interventions aimed at reducing HIV transmission. ART is an effective measure to control HIV/AIDS transmission and improve the quality of life of HIV-infected people, prolong their survival, and reduce AIDS-related mortality (*6-8*). Individuals meeting the primary criteria of a CD4 count ≤ 200 cells/mm^3 can utilize the NFATP. While this threshold was revised to ≤ 350 cells/mm^3 in 2008 (*9*). the World Health Organization (WHO) contended that rapid ART imitation is a critical step to reducing HIV transmission in 2015. The eligibility requirement for free ART has been revised and the CD4 count threshold was eliminated in 2016, *i.e.* implementation of the "Treatment as Prevention" strategy. Baseline CD4 cell counts, the time difference between HIV diagnosis and ART initiation, and the viral load (VL) after ART initiation are core treatment-related indicators linked to HIV early detection, early diagnosis, and early treatment. The baseline CD4 count is associated with severe illness. A low CD4 cell count (< 200 cells/mm^3) can indicate late diagnosis. Late diagnosis and ART initiation could lead to an increased risk of negative health outcomes, including opportunistic infections and even death. First VL monitoring is recommended at 6 months after ART initiation under national ART guidelines to evaluate viral suppression. A high VL after ART initiation could reflect failure of virologic suppression and ART treatment. Virologic suppression almost promotes immunologic recovery and reduces the incidence of opportunistic infections and malignancies. Virologic suppression is defined by the WHO as a VL < 1,000 copies/mL. Nevertheless, the threshold could underestimate adverse outcomes. Low-level viremia (LLV) of 50-999 copies/mL could increase the risk of drug resistance. Early detection and ART of HIV infections and viral suppression could avoid negative outcomes and reduce the risk of HIV transmission.

The HIV/AIDS epidemic has changed significantly over the past 40 years, and it has a geographical heterogeneity across China. AIDS intervention strategies have changed over time and across regions. The changes may have affected epidemiological characteristics and intervention strategies. Moreover, an increasing migrant population and aging population in urban areas, and especially in Shanghai, could lead to changes in demographic characteristics and HIV/AIDS transmission patterns. Given the completeness of HIV/AIDS epidemic data and demographic characteristics, the Fengxian District in Shanghai was chosen as a study site in the current study to explore the multi-year epidemiological and treatment-related characteristics of HIV infections and AIDS patients (HIV/AIDS cases) among different sub-populations and to evaluate changes in transmission patterns and the effectiveness of intervention policies.

Patients and Methods

Study site

The Fengxian District, which includes 11 communities, is situated in the southern part of Shanghai at the junction with the Huangpu River to the north. Located southeast of the Yangtze River Delta, it covers an area of 733.38 square kilometers (Figure 1). It is a picturesque coastal urban area with an obvious geographical location. Fengxian is a typical urban area with a high level of industrialization and a large migrant population (*10*). As industrialization has accelerated in the area, the migrant population has grown markedly. According to the Shanghai Statistical Yearbook, the migrant population accounts for half of the total population in Fengxian. Because of the consistency between the demographic characteristics of Fengxian and Shanghai overall, well collected data in Fengxian are considered to be representative of Shanghai, facilitating this study.

This study was approved by the Ethics Administration Committee of the Shanghai Municipal Center for Disease and Prevention (ID: KY-2024-33).

Data source

Individual information on newly reported HIV/AIDS patients with current addresses in the Fengxian District in 2001-2019 were retrieved from the National AIDS Comprehensive Prevention and Control Data Information System, including HIV-infected persons and AIDS patients. Base information on these individuals included sex, age, local address, current address, date of disease onset, date of diagnosis, date of ART initiation, first VL about 6 months after ART initiation, baseline CD4 cell count at diagnosis, and case classification (AIDS or HIV). Yearly aggregated data were calculated based on date of diagnosis. The time range was calculated as the time difference between the date of diagnosis and the date of ART initiation. According to national criteria in different periods, all individuals were divided into 3 groups (2002-2007, 2008-2015, and 2016-2019) based on the year of diagnosis. Population data in the study area were gathered from the Shanghai Statistical Yearbook (*http://tjj.sh.gov.cn/tjnj/index.html*).

In this study, the VL after about 6 months after ART initiation, baseline CD4 cell count, and time

difference from diagnosis to ART can be considered as comprehensive indicators with which to monitor disease procession and the effectiveness of ART therapy. Timely HIV diagnosis and late HIV diagnosis were defined as a baseline CD4 cell count ≥ 200 cells/mm^3 and < 200 cells/mm^3 (*11*). A time from diagnosis to ART within 30 days was considered to be immediate ART, and other times were considered to be delayed ART (*12*). VL is an indicator with which to evaluate the effectiveness of ART therapy and virologic suppression. Full virologic suppression, low-level viremia (LLV), and virologic failure were defined as a VL < 50 copies/mL, a VL < 1,000 copies/mL, and a VL ≥ 1,000 copies/mL (*13*).

Statistical analysis

The incidence of HIV infections and AIDS patients (per 100,000 people) among the overall population, locals, and migrants was calculated from 2001 to 2019 at the study site. The time distribution in the incidence of newly reported HIV infections and AIDS patients was described to indicate potential temporal trends. Descriptive statistics were used to summarize the demographic, behavioral, and treatment-related characteristics of local and migrant HIV/AIDS patients. Significant differences were assessed with Pearson's chi-squared test or Kruskal-Wallis test for unordered categorical data and the Cochran-Armitage test for ordinal categorical data. Demographic, behavioral, and treatment-related variables for local and migrant HIV/AIDS patients over the years were visually depicted to describe the temporal trends in and epidemiological characteristics of HIV/AIDS. The trends in HIV/AIDS cases with respect to different variables during these years was analyzed using the Cochran-Mantel-Haenszel test.

The software SaTScan™ version 10.1 used Kulldorff's retrospective space-time scan statistic based on a discrete Poisson model to explore temporal clusters of HIV infections and AIDS patients during the study period and to identify potential patterns of disease among local and migrant patients (*14*). The maximum temporal size of the clusters was set at 20% for the study period, and the number of Monte Carlo simulations was set at 999. The analysis generates 3 model parameters, including log-likelihood ratios (*LLRs*), *p* values, and risk ratios (*RRs*). A cluster with the maximum *LLR* represents the most likely cluster. The *p* value for the *LLR* parameter can be estimated *via* Monte Carlo simulations to indicate statistically significant clusters if the *p* value < 0.05. The *RR* is the estimated risk within the cluster divided by the risk outside the cluster (*14*).

The study was conducted using the software R version 4.2.3 for data collection and analysis. A *p* value < 0.05 was considered a statistically significant difference.

Results

Demographic characteristics

A total of 388 people with HIV/AIDS were reported in the area from 2001 to 2019, accounting for 41.2% of AIDS patients in Fengxian. The first case was reported in 2001. Figure 1 shows the geographical distribution of the Fengxian District and annual incidence of HIV infections and AIDS patients among the total population, locals, and migrants. The average annual incidence of HIV/AIDS was 1.92 per 100,000 persons (range: 0-4.34 per 100,000 persons). The annual incidence of HIV/AIDS among locals and migrants was 0-5.91 per 100,000 persons and 0-3.64 per 100,000 persons, respectively, with an average annual incidence of 2.75 per 100,000 persons and 0.995 per 100,000 persons. In total, annual incidence fluctuated and tended to increase from 2001 to 2019 ($\chi^2 = 128.38$, $p < 0.001$).

Among the migrant population, the incidence of HIV/AIDS remained at low levels before 2006. The annual incidence of HIV/AIDS increased steadily from 2006 to 2013, and the annual incidence fluctuated and increased from 2014 to 2019 ($\chi^2 = 36{,}67$, $p < 0.001$). The trends in incidence among migrants were similar to those in the total population ($\chi^2 = 120.71$, $p < 0.001$) (Figure 1).

The characteristics of HIV/AIDS cases among locals and migrants are shown in Table 1. Among all reported HIV/AIDS patients, HIV/AIDS cases among migrants increased over the years ($\chi^2 = 11.59$, $p < 0.001$), accounting for 25.5%. Fifty-nine-point-nine percent of local patients and 52.5% of migrant patients were diagnosed in 2008-2015 and 2016-2019, respectively. More male patients were reported compared to female patients, with a male-to-female ratio of 4.88:1. There were no significant differences between or temporal trends in local and migrant patients in terms of sex. In terms of age distribution, the median age (IQR) was 43 (31-54) years. Patients 25-65 years of age comprised 82.7% of cases. There were significant differences and temporal trends in the age distribution of local and migrant patients ($p < 0.001$). The majority of local and migrant patients were 45-65 years and 25-45 years, respectively. The proportion of patients over 65 years of age have increased from 5% in 2009 to 12% in 2019. In addition to the increase in local patients over 65 years of age ($\chi^2 = 6.55$, $p < 0.05$), the proportion of migrant patients ages 25-45 years increased from 17% in 2007 to 24% in 2019 ($\chi^2 = 8.72$, $p < 0.05$). There were no obvious trends in the diagnosis of patients 18-25 years of age (10.1%) (Table 1 and Figure 2).

The level of education of patients was mainly primary school and below, accounting for 56.2% (58.1% for local patients and 50.5% for migrant patients). The proportion of migrant patients whose level of education was primary school or below declined over

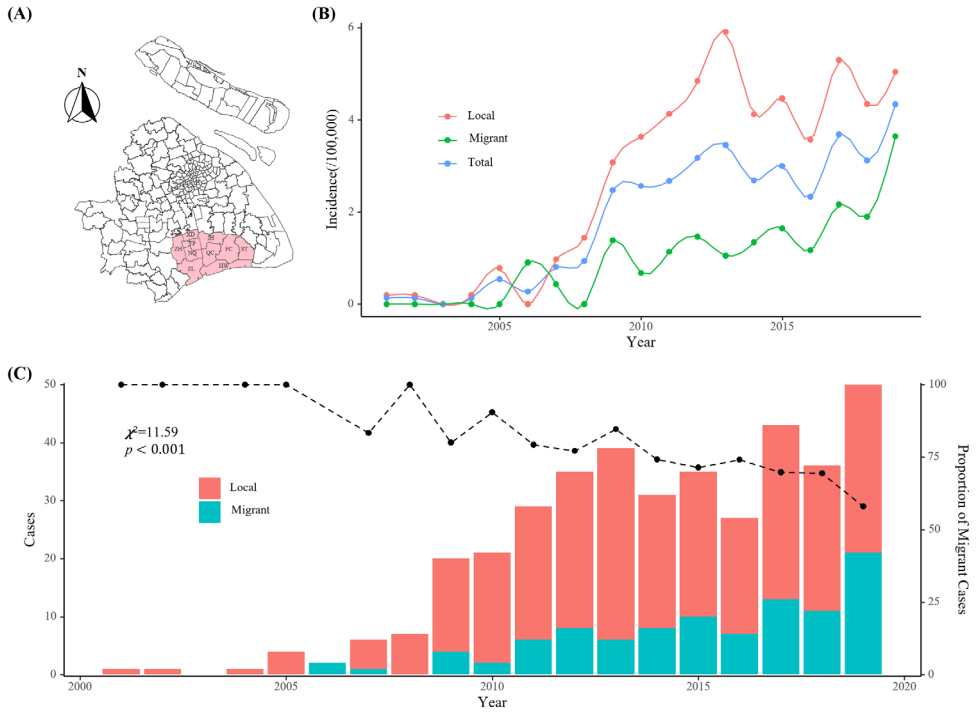

Figure 1. Geographical distribution of Fengxian District in Shanghai and temporal trends in newly reported HIV/AIDS cases among locals and migrants in the area from 2001 to 2019. (A) The geographical distribution of Fengxian District, Shanghai; **(B)** The annual incidence rates per 100,000 persons reported by years among local (green line), migrant (blue line) population and total population (red line) from 2001 to 2019; **(C)** The bar plot of HIV/AIDS local and migrant patients. Line plot shows temporal changes in the proportion of local patients.

the years (χ^2 = 5.02, p < 0.05). Married or cohabiting patients comprised 53.4% of HIV/AIDS patients, and the proportion increased over the years (χ^2 = 16.59, p < 0.001). There were no significant differences in the distribution of marital status among local and migrant patients. The dominant transmission route has been sexual contact over the years (heterosexual contact for local patients and male-to-male sexual contact (MSM) for migrant patients). Fifty-seven-point-five percent of patients were infected *via* heterosexual contact and 40.2% were infected *via* MSM (Figure 2). The proportion of patients infected *via* heterosexual contact (χ^2 = 16.59, p < 0.001) or who were married or cohabiting (χ^2 = 17.21, p < 0.001) tended to increase over the years. The proportion of HIV/AIDS attributed to heterosexual contact as a transmission route increased from 50% in 2007 to 76% in 2019.

Treatment-related characteristics

The median (IQR) baseline CD4 count was 239 (103, 370) cells/mm^3, and 82% of people with HIV had a CD4 count between 200 and 500 cells/mm^3. Forty-one-point-two percent of patients were diagnosed late. There were significant differences in the CD4 cell count of local and migrant patients (p < 0.001). More local patients (44.3%) were diagnosed late than migrant patients (32.3%). Seventy-two-point-nine percent had full virologic suppression, 10.1% had LLV, and 3.1% had a VL ≥ 1000 copies/mL. The majority of those with no VL test were reported from 2013-2015 (57.4%). The time difference between diagnosis and initiation of ART was within 30 days for 44.3%. The median time to ART initiation decreased from 68 months in 2001 to 12 days in 2019. Patients in whom ART was initiated early increased over the years, and especially after 2016. The proportion of patients with LLV (χ^2 = 9.06, p < 0.01), a late diagnosis (χ^2 = 6.42, p < 0.05), and in whom ART was initiated within 30 days (χ^2 = 96.59, p < 0.001) tended to increase over the years. More details are shown in Table 1 and Figure 3.

Temporal cluster analysis

Two significant temporal clusters were found during the study period. The most likely cluster of local patients was from March 2012 to November 2015. RR was 2.27. There were 2.27 times more local patients diagnosed in March 2012-November 2015 than in other years. Moreover, there were 2.78 times more migrant patients diagnosed in August 2016-December 2019 than in other years. More details are shown in Table 2.

Discussion

HIV/AIDS remain a major public health concern in China. With an increasing migrant population and aging population in urban areas, epidemiologic characteristics

Table 1. Characteristics of newly reported HIV/AIDS cases among local and migrants from 2001 to 2019 in the study area

Variables	Patients (percentage, %)			*p* value
	Total	Local	Migrant	
Total	388	289	99	
Sex				0.916
Male	322 (83.0)	239 (82.7)	83 (83.8)	
Female	66 (17.0)	50 (17.3)	16 (16.2)	
Year reported				0.015
2001-2007	15 (3.9)	12 (4.2)	3 (3.0)	
2008-2015	217 (55.9)	173 (59.9)	44 (44.4)	
2016-2019	156 (40.2)	104 (36.0)	52 (52.5)	
Age at diagnosis, years				
Median (IQR)	43 (31, 54)	48 (36, 57)	34 (27, 40)	< 0.001
18-25	39 (10.1)	22 (7.6)	17 (17.2)	< 0.001
25-45	168 (43.3)	99 (34.3)	69 (69.7)	
45-65	153 (39.4)	140 (48.4)	13 (13.1)	
≥ 65	28 (7.2)	28 (9.7)	0	
Education				0.229
Middle school or above	170 (43.8)	121 (41.9)	49 (49.5)	
Primary school or below	218 (56.2)	168 (58.1)	50 (50.5)	
Marital status				0.49
Single, divorced, or widowed	207 (53.4)	151 (52.2)	5 6 (56.6)	
Married or cohabiting	179 (46.1)	137 (47.4)	42 (42.4)	
No data	2 (0.5)	1 (0.3)	1 (1.0)	
Transmission route				0.027
Heterosexual contact	223 (57.5)	176 (60.9)	47 (47.5)	
Male-to-male sexual contact (MSM)	156 (40.2)	105 (36.3)	51 (51.5)	
Other*	8 (2,1)	7 (2.4)	1 (1.0)	
No data	1 (0.3)	1 (0.3)	0	
Viral load				
Median (IQR)	0 (0, 23.78)	0 (0, 25.5)	0 (0, 20)	0.316
Virologic status at first VL after about 6 months on ART, copies/mL				0.296
< 50	283 (72.9)	205 (70.9)	78 (78.8)	
50-1,000	39 (10.1)	30 (10.4)	9 (9.1)	
≥ 1,000	12 (3.1)	11 (3.8)	1 (1.0)	
No test	54 (13.9)	43 (14.9)	11 (11.1)	
Baseline CD4 count, cells/mm^3				
Median (IQR)	239 (103, 370)	226 (83, 360)	268 (148, 389)	0.015
< 200	160 (41.2)	128 (44.3)	32 (32.3)	0.017
200-499	187 (48.2)	137 (47.4)	50 (50.5)	
≥ 500	41 (10.6)	24 (8.3)	17 (17.2)	
Time from diagnosis to ART initiation, days				
Median (IQR)	38 (13, 149)	36 (12, 134)	47 (13, 158)	0.069
< 30	172 (44.3)	131 (45.3)	41 (41.4)	0.733
< 90	94 (24.2)	69 (23.9)	25 (25.3)	
< 180	34 (8.8)	25 (8.7)	9 (9.1)	
< 365	15 (3.9)	9 (3.1)	6 (6.1)	
≥ 365	73 (18.8)	55 (19.0)	18 (18.2)	

*Other transmission routes included injection drug use (IDUs) and blood (plasma) donation.

and intervention strategies for HIV have varied across regions. This study explored changes in the epidemiological patterns of HIV/AIDS and detected the high-risk periods in an urban area of Shanghai. Results indicated that the average annual incidence was 1.92 per 100,000 persons, which is far lower than the incidence in China overall. HIV/AIDS still remains at low endemic levels in Shanghai. The increase in incidence over 20 years might be related to improvements in the HIV testing capacity, standardization of the AIDS prevention and control network and improvement of the national AIDS report system, and expansion of the scale of HIV testing (*15*).

This study found that 83.0% of patients were male. This could be due to the increasing number of MSM. MSM are the highest-risk sub-population for HIV/AIDS infections and are causing an increasing public health burden in China (*16*). Eighty-two-point-seven percent of patients were 25-65 years of age. The proportion of patients 25-45 years of age and over 65 years of age has significantly increased over the years. The young and middle-aged more actively engage in sexual behavior. Neglect of the sexual demand among the elderly population by society and the lack

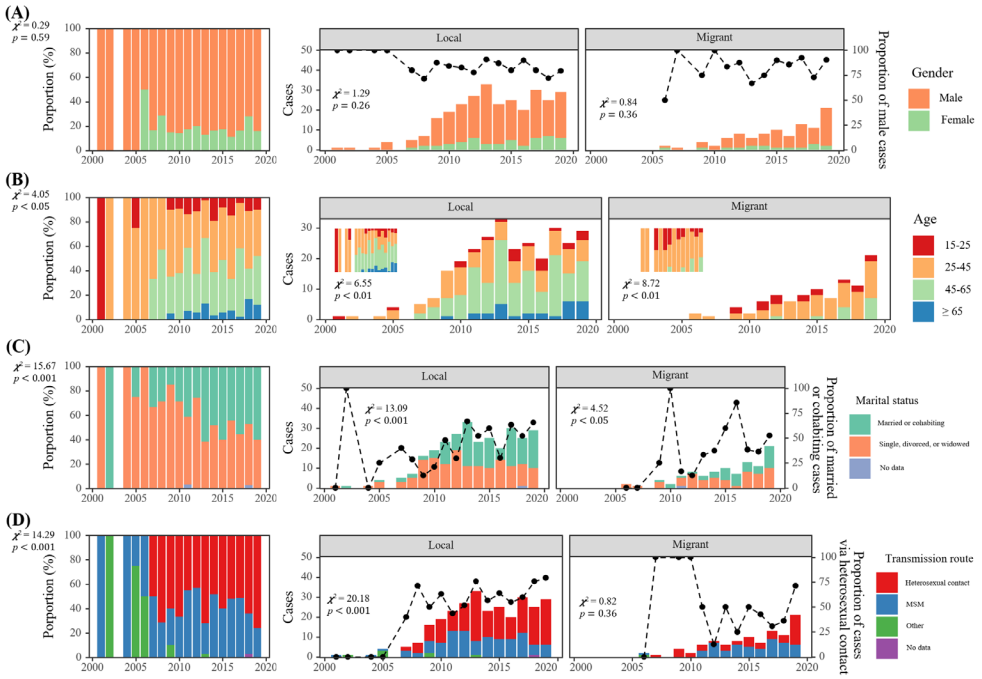

Figure 2. Time distribution of HIV/AIDS cases among local and migrants based on demographic and behavioral variables in 2001-2019. The first column shows the cumulative proportion plot of all HIV/AIDS cases with variables. The second column shows the number of HIV/AIDS cases among local and migrants with variables. **(A)** The annual cases and proportions of HIV infections and AIDS patients by sex. The line plot shows temporal changes in the proportion of male patients; **(B)** The annual cases and proportions of HIV infections and AIDS patients by age group. The bar plot at the top left shows temporal changes in the proportion of cases by age-group; **(C)** The annual cases and proportions of HIV infections and AIDS patients by marital status. The line plot shows temporal changes in the proportion of married or cohabiting patients; **(D)** The annual cases and proportions of HIV infections and AIDS patients by transmission route. The line plot represents the proportion of cases *via* heterosexual contact.

of knowledge of HIV/AIDS can lead to unsafe and unprotected sex, thus increasing the risk of acquiring HIV/AIDS (*17,18*). More efforts should be made to improve active surveillance and HIV prevention education for the accurate and effective prevention and control of HIV among the elderly, and especially those in the local population.

HIV/AIDS patients who have received a high level of education and who are married or cohabiting have increased in recent years. School-based HIV/AIDS education and premarital HIV/AIDS counselling should enhance education on HIV/AIDS intervention to provide information to young people and couples getting married. The dominant transmission route has been sexual contact over the years. Heterosexual contact (57.5%) and MSM (40.2%) were the main transmission routes at the study site over the years. The increase in heterosexual contact among local patients (60.9%) indicates the expansion of the HIV/AIDS epidemic from high-risk groups to the general population (*19*). Based on the high-risk population, intervention strategies for the general population should be enhanced. Moreover, the proportion of MSM may be underestimated due to fear of social discrimination (*20*).

VL, the CD4 cell count, and the time difference between diagnosis and ART initiation in HIV-infected individuals can provide information on the effectiveness and achievement of AIDS prevention, control, and treatment. VL and the CD4 cell count can indicate HIV/AIDS progression and thus the extent of early diagnosis. Early diagnosis and early treatment helps to avoid opportunistic infections and antiretroviral resistance (*21,22*). The rate of full viral suppression among HIV/AIDS patients receiving treatment for at least 6 months was 72.9%, 10.1% had LLV, and 3.1% had a VL ≥ 1,000 copies/mL. Late diagnosis was 41.2% and tended to increase; that proportion was higher than in the rest of mainland China (*19*). Moreover, 67.9% of the elderly (over 65 years of age) were diagnosed late. Late diagnosis is a serious problem. One cohort study found that initiation of ART within 30 days of diagnosis was associated with a 63% reduction in mortality at a 1-year follow-up (*12*). Rapid ART can decrease HIV/AIDS mortality and opportunistic infections. The median time to ART initiation decreased from 68 months in 2001 to 12 days in 2019. These findings could be due to the expansion of intervention and medical care and the subsequent optimization of treatment. Moreover, a lack of knowledge of HIV/AIDS or access to medical services could be related to late diagnosis and treatment (*23*). Furthermore, HIV-related stigma and discrimination surrounding HIV/AIDS is still a severe challenge for achieving the goal of ending the HIV epidemic (*24*). Public health and

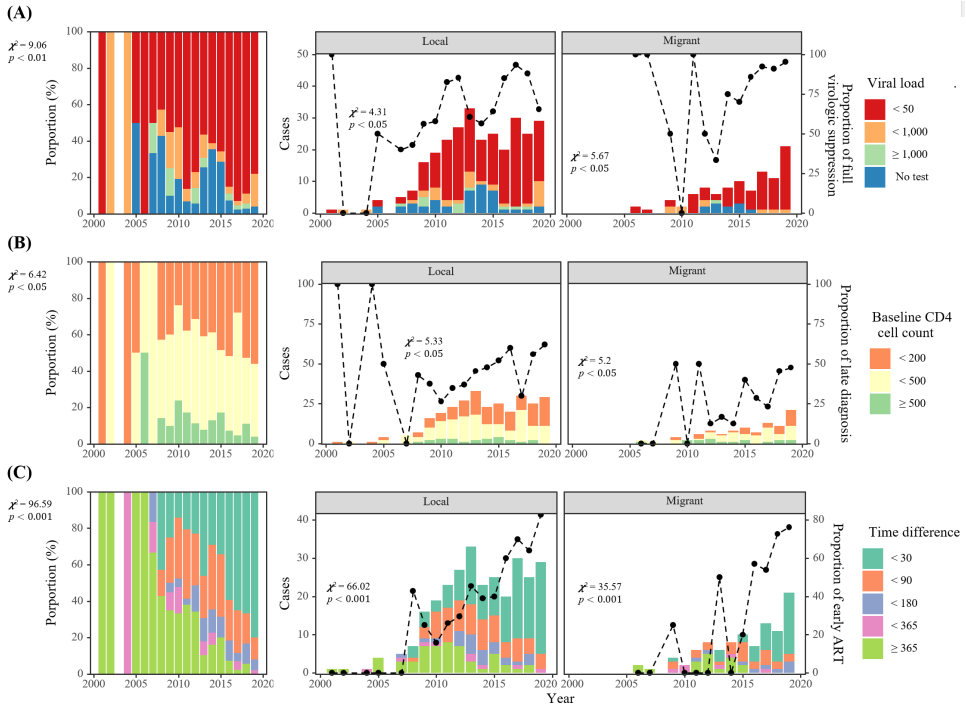

Figure 3. Time distribution of HIV/AIDS cases among local and migrants based on treatment-related variables in 2001-2019. The first column shows the cumulative proportion plot of all HIV/AIDS cases with variables. The second column shows the number of HIV/AIDS cases among local and migrants with variables. **(A)** The annual cases and proportions of HIV infections and AIDS patients by viral load. The line plot shows temporal changes in the proportion of cases of full viral suppression (VL < 50 copies/mL); **(B)** The annual cases and proportions of HIV infections and AIDS patients by baseline CD4 cell count. The line plot shows temporal changes in the proportion of late diagnosis (CD4 count < 200 cells/mm^3); **(C)** The annual cases and proportions of HIV infections and AIDS patients by duration from diagnosis to ART initiation. The line plot shows temporal changes in the proportion of early ART initiation (duration < 30 days).

Table 2. Results of temporal cluster analysis of HIV/AIDS cases among local and migrants from 2001 to 2019

Clusters	Years	Reported cases	Expected cases	*RR*	*LLR*	*p* value
Locals	2012/3-2015/11	107	59.4	2.27	20.69	0.001
Migrants	2016/8-2019/12	50	26.04	2.86	13.12	0.001

medical departments should enhance health education to eliminate all discrimination against HIV-positive patients and facilitate their treatment to promote early diagnosis and treatment (*25-27*). In addition, HIV testing is the key to the early diagnosis of HIV/AIDS (*23,28*).

HIV/AIDS cases among migrants increased over the years (χ^2 = 11.59, p < 0.001), accounting for 25.5%. Moreover, migrant patients were reported to be younger than local patients. The dominate transmission route was MSM. Population migration can hamper the identification and management of cases. Migrants have different a health status than locals (*29-31*). Population migration increases the opportunities for human contact and promotes the spread of HIV as urbanization proceeds. A low level of risk awareness of HIV infection and engaging in high-risk sexual behavior place migrants at higher risk for HIV (*32-34*). The current results revealed that the HIV/AIDS epidemic among migrants may be still be high. Temporal cluster analysis explored the peak in local patients in 2012-2015 and migrant patients in 2016-2019. The disparity in temporal clusters of local and migrant patients indicates the delay of the HIV/AIDS epidemic in migrants. These findings suggest that more interventions and education should be provided to young male migrants.

This study had several limitations. First, it was a county-level cross-sectional study on newly reported HIV/AIDS data. In this study, the Fengxian District was considered to be representative of Shanghai, but the reported number of cases was relatively few. Considering the low prevalence in Shanghai and similar demographic characteristics, the epidemiological patterns in the Fengxian District may reflect those in Shanghai. The study only qualitatively analyzed the potential epidemiological patterns of HIV/AIDS. Influencing factors could be explored in a further study. Moreover, age-specific populations could not be identified, leading to lack of age-adjusted incidence.

Finally, this study found that the dominant transmission route has been heterosexual contact. HIV transmission routes rely on self-reports and could be misclassified as a result of stigma and discrimination. To effectively and efficiently identify high-risk groups, molecular epidemiological methods could be used to explore transmission routes.

In conclusion, HIV/AIDS prevention and control measures have achieved substantial progress at the study site. Nevertheless, there are still several emerging challenges. To tackle future challenges of the AIDS epidemic and further advance progress towards achieving the goal of curbing AIDS, the next stage of HIV/AIDS prevention and control should be focused on these key high-risk populations. HIV screening services and the surveillance network should be enhanced for these high-risk populations (migrants and elderly in the local population) to promote early diagnosis, interventional policies and strategies should be tailored, and prevention and control programs should be implemented for sub-populations, and especially the elderly population and MSM population, as well as the general population. Health surveillance and management of the migrant population should be enhanced.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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BRIEF REPORT
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HIV disease progression among heterosexually-infected individuals before the introduction of universal ART in China: A linear mixed-effects model

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Abstract: In 2016, China introduced universal antiretroviral therapy (ART) for all HIV-infected individuals regardless of CD4 cell count. However, the natural history and rate of CD4 count decline among heterosexually-infected individuals remain uncharacterized. Analyzing national surveillance data can address this gap and shed light on the pathogenesis of HIV in this population. We used a linear mixed-effects model to assess CD4 trajectory over time before ART initiation and estimated the median time from HIV seroconversion to reaching CD4 thresholds of < 500, < 350, and < 200 cell/mm^3. From the Chinese HIV/AIDS Comprehensive Response Information Management System, 59,085 eligible individuals were identified, with 113 having data to estimate the date of HIV seroconversion. The linear mixed-effects models estimated an intercept of 23.64 (95% confidence interval [CI]: 22.41 to 24.87) and a slope of -1.32 (95% CI: -1.34 to -1.30) for males, and an intercept of 22.70 (95% CI: 21.00 to 24.40) and a slope of -1.29 (95% CI: -1.31 to -1.27) for females. The estimated median times from HIV seroconversion to reaching CD4 count thresholds of < 500, < 350, < 200 cells/mm^3 were 0.97, 3.74, and 7.20 years for males, and 0.26, 3.09, and 6.48 years for females, respectively. Males consistently took longer to reach these CD4 count thresholds compared to females of the same age group. Older individuals (≥ 40 years) reached CD4 thresholds faster than younger individuals (15-29 years), indicating more rapid disease progression in older people living with HIV.

***Keywords*:** HIV/AIDS, disease progression, CD4$^+$ T cell count, linear mixed-effects model

Introduction

HIV remains a major public health issue globally and in China. By the end of 2019, approximately 37.9 million people were living with HIV (PLHIV) worldwide (*1*). In China, an estimated 1.25 million people were living with HIV in 2018, with 73.1% infected through heterosexual contact (*2,3*). Effective clinical and public health management rely on accurate estimates of new HIV infections and their distribution by factors such as age, sex, and transmission mode. Understanding the natural history of HIV/AIDS and its disease progression is essential for this purpose (*4*).

The most renowned study on the natural history of HIV, conducted by Fauci and colleagues in 1996, showed that the rate of CD4 level decline decreases over time before antiretroviral therapy (ART) initiation based on clinical assessments and laboratory measurements of CD4 counts and virus loads (*5*). Similar studies (*6-8*) have been conducted in low and middle-income regions, as well as in Europe and the United States, involving cohorts of individuals with HIV not on ART (*9-11*). However, no such studies have been conducted in China (*12*).

In 2015, World Health Organization (WHO) recommended ART for all PLHIV regardless of CD4 level. China adopted this policy in the national treatment guideline in June 2016 (*13*). Understanding the natural history of HIV infection and disease progression in China has become challenging since the implementation of the "treat-all" policy.

CD4 cell count is a crucial biomarker for studying HIV disease progression and prognosis (*14,15*). The rate of CD4 decline since infection can be used in statistical models to estimate HIV incidence (*5*). Two back-calculation methods have been developed for estimating the rate of decline of CD4 count: a multi-state modelling method and a CD4 depletion modelling method. The multi-state model categorizes HIV infections by CD4 level (*16,17*), while the CD4 depletion model uses longitudinal data of individually repeated measurements of CD4 count before ART

initiation in a linear mixed-effects model (*4,7,18*).

Advantages of the CD4 depletion modelling method include: *i*) handling correlation between CD4 measurements without assuming independence; *ii*) not requiring complete historic data on HIV diagnoses, which is useful when there are fewer CD4 counts available in the early years of a study (*19*); and (3) back-calculating seroconversion time for each individual, describing the distribution of new infections over a calendar year. These advantages enable the estimation of new HIV infections in China and the time from seroconversion to various CD4 cell count thresholds.

To our knowledge, and based on a systematic literature search in PubMed, Embase, Web of Science, and Chinese database, no published studies have addressed the natural history and disease progression indicated by the rate of decline in CD4 cell count among heterosexually infected HIV patients in China. Using data from the Chinese HIV/AIDS Comprehensive Response Information Management System (CRIMS), we conducted an analysis of heterosexually-infected patients and applied a linear mixed-effects model (adapted from the CD4 depletion modelling method) to estimate the rate of CD4 decline before ART initiation and the time from HIV seroconversion to CD4 cell count thresholds of < 200, < 350, < 500 cell/mm^3.

Patients and Methods

Data source and study population

HIV has been a notifiable infectious disease in China since 1985. Established in 2005, CRIMS is an integrated internet-based system for real-time collection and reporting of newly diagnosed HIV/AIDS cases in China. Local physicians and health professionals report newly diagnosed HIV/AIDS cases to CRIMS using a uniform case reporting form (CRF) that includes personal information, such as date of birth, sex, residence, occupation, date of diagnosis, and testing results. All cases are validated by local clinicians and uploaded to China CDC for analysis and monitoring.

Eligible subjects included all HIV cases reported to CRIMS who met the following criteria: *i*) diagnosed with HIV between January 2006 and May 2019 (as the reporting quality in CRIMS improved starting in January 2006; *ii*) aged 15 years or older at the time of diagnosis; *iii*) self-reported infection through heterosexual contact; and *iv*) had at least two CD4 measurements before ART initiation. Exclusion criteria included incomplete records and non-citizens of China.

Statistical Methods

CD4 counts are periodically measured in PLHIV in China. We used these periodic assessments to construct an analytic dataset with CD4 counts over time for each subject. A linear mixed-effects model with random slope and/or random intercept was used to fit variations within and between individuals' CD4 measurements (*20*).

The study included three key variables: *i*) seroconversion date, estimated as the midpoint between last negative HIV test date and the first positive test date within a three-year interval; *ii*) t_1, the interval between the HIV seroconversion date and each CD4 test date, used as the independent variable in the initial regression sqrt(CD4) = $a + b \bullet t_1$; and *iii*) t_2, the intervals between the first CD4 test date and subsequent CD4 test dates, used as the independent variable in the second regression sqrt(CD4) = $a' + b' \bullet t'_2$.

The model fit the square root of CD4 over time: $\sqrt{CD_4(t)} = a_i + (b_i \times t)$ (*5,19*), where the variable t denotes time from date of infection to CD4 test date. We first estimated the intercept (a_i), the average square root of CD4 count at the date of seroconversion, and the slope (b_i), the rate of change in the square root of CD4 count over time. In the initial regression, sqrt(CD4) = $a + b \bullet t_1$, a was treated as a fixed intercept (assuming all individuals had the same CD4 count at time of seroconversion), and b as either fixed or random. Fixed b values were expected to be close to the mean of random b and fixed b'. The fixed b' values were estimated using data from all individuals in the second regression, sqrt(CD4) = $a' + b' \bullet t'_2$. In this regression, b' was treated as a fixed slope (assuming all individuals had the same depletion rate), and a' as a random intercept (each person had a different value of CD4 count on the first CD4 test date). Assuming linearity in the CD4 depletion model during the study period, b and b' should be statistically equivalent. However, a and a' are different (with $a > a'$).

We used the model to estimate the time from seroconversion to reaching CD4 cell count thresholds of < 200, < 350, < 500 cell/mm^3 to assess disease progression among men who have sex with women (MSW) and women who have sex with men (WSM).

Analyses were performed using SAS 9.4 (Statistical Analysis Software 9.4, SAS Institute Inc, Cary, North Carolina, USA). All tests were two-tailed with p values of 0.05 or less considered statistically significant.

Ethical review

The study was approved by the Ethics Committee of the National Centers for STD/AIDS Prevention and Control, China CDC (Ethical approval number: X190311565).

Results and Discussion

Our analytic dataset included 59,085 eligible cases. Of these, 113 cases had data to estimate the date

of seroconversion, defined by a negative HIV test followed by a positive HIV test within three years. Table 1 summarizes the characteristics of the eligible cases used for fitting the initial and second regression models. The median of first CD4 counts was 489 cells/mm^3 (interquartile range [IQR]: 372-620 cell/mm^3), with 48% of cases having an initial CD4 count ≥ 500 cells/mm^3.

For CD4 cell count declines without ART, Table 2 presents the intercept and slope estimates from the linear mixed-effects CD4 depletion model by age and gender. For males, the rate of decline in the square root of CD4 count (slope) ranged from -1.21 (95% CI: -1.24 to -1.18) in the youngest group (15-29 years) to -1.48 (95% CI: -1.51 to -1.44) in the oldest group (≥ 40 years). For females, the rate of decline ranged from -1.22 (95% CI: -1.25 to -1.19) in the youngest group to -1.46 (95% CI: -1.50 to -1.41) in the oldest group. The intercepts, representing the square root of the model-estimated CD4 cell count at the time of seroconversion, varied slightly, with the lowest intercept observed in the 30-39 age group for both males and females. Intercepts for females were consistently lower than those for males. The intercept for males in the 15-29 and ≥ 40 age groups were 24.42 (95% CI: 22.64 to 26.20) and 24.04 (95% CI: 21.28 to 26.80), respectively. For females, the intercepts were 23.80 (95% CI: 21.49 to 26.12) and 22.62 (95% CI: 20.36 to 24.87), respectively. Overall, the average intercept and slope were 23.64 (22.41 to 24.87) and -1.32 (-1.34 to -1.30) for males, and 22.70 (21.00 to 24.40) and -1.29 (-1.31 to -1.27) for females.

A monotonic decline in CD4 count over time since seroconversion was observed, with steeper declines in the earlier years post-infection for both males and females across all age groups (Figure 1). Among males, the CD4 count trajectories of the 30-39 and ≥ 40 age groups crossed after four years of infection, indicating greater CD4 depletion in later years among older males (≥ 40 years). The 15-29 year group consistently exhibited the highest CD4 cell count over time since seroconversion for both males and females.

To our knowledge, this study represents the first national-level exploration of HIV disease progression among heterosexually infected individuals in China. We estimated the date of HIV seroconversion using national surveillance data and applied a mixed-effects CD4 depletion model to estimate the time from seroconversion to reaching three CD4 count thresholds.

Compared to Lodi *et al.* (*9*), our estimated times from seroconversion to reaching CD4 thresholds of < 500, < 350, and < 200 cells/mm^3 were shorter by approximately 1, 4, and 8 years, respectively. Although the reasons for these differences are unclear, they may be attributable to variations in race/ethnicity, HIV subtype, baseline disease condition, and non-ART treatment (*11,15*).

The linear mixed-effects model in this study effectively handles random effects between multilevel variables and is widely used in longitudinal data analyses involving repeated CD4 measurements (*19,21*). Our model demonstrated a negative relationship between CD4 count and time since infection, with a greater rate of decline among older adults. These findings align with results from studies conducted in the United States (*9*), Brazil (*10*), and Europe (*15*).

Table 1. Characteristics of 59 085 HIV/AIDS cases included in this study

Variable	*n*	(%)
Gender		
Male	34 337	58.11
Female	24 748	41.89
Age group*		
15-29	19 984	33.83
30-39	16 499	27.93
≥ 40	22 594	38.25
Education		
Primary/illiterate	19 245	32.57
Junior high	25 560	43.26
Senior high and above	14 280	24.17
Ethnic group		
Han	43 506	73.63
Others	15 579	26.37
First CD4 cell count (cells/mm^3)		
< 200	4 261	7.21
200-349	7 749	13.12
350-499	18 778	31.78
≥ 500	28 297	47.89
Total	59 085	100.00

Note: *There were 8 cases with missing information on age.

Table 2. Intercept and slope estimates from liner mixed-effects CD4 depletion model, by age and gender

Gender	Age group	Cases for intercept estimates	Intercept	95% CI	*p* value	Cases for slope estimates	Slope	95% CI	*p* value
Male	15-29y	22	24.42	22.64 - 26.20	< 0.001	9656	-1.21	-1.24 - -1.18	< 0.001
	30-39y	28	23.17	21.25 - 25.08	< 0.001	10005	-1.27	-1.30 - -1.24	< 0.001
	≥ 40y	26	24.04	21.28 - 26.80	< 0.001	14675	-1.48	-1.51 - -1.44	< 0.001
	subtotal	76	23.64	22.41 - 24.87	< 0.001	34336	-1.3	-1.34 - -1.30	< 0.001
Female	15-29y	10	23.80	21.49 - 26.12	< 0.001	10328	-1.22	-1.25 - -1.19	< 0.001
	30-39y	9	22.55	17.73 - 27.37	< 0.001	6494	-1.27	-1.31 - -1.23	< 0.001
	≥ 40y	18	22.62	20.36 - 24.87	< 0.001	7919	-1.46	-1.50 - -1.41	< 0.001
	subtotal	37	22.70	21.00 - 24.40	< 0.001	24741	-1.29	-1.31 - -1.27	< 0.001

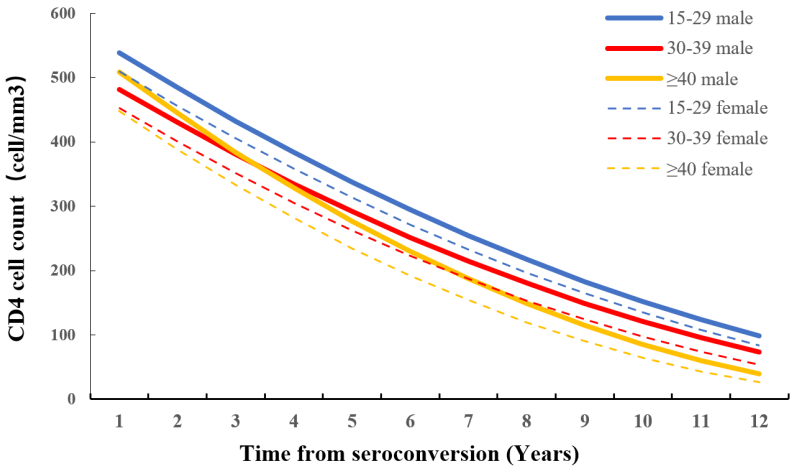

Figure 1. CD4 cell count decline over time since HIV seroconversion by age and gender based on the linear mixed-effects CD4 depletion model.

Table 3. Median estimated time from seroconversion to CD4 cell count < 500, < 350, < 200 cell/mm^3 by age

Variable	CD4 < 500		CD4 < 350		CD4 < 200	
	Male	Female	Male	Female	Male	Female
15-29y						
Median (95% CI)	1.70 (0.23-3.25)	1.18 (0.00-3.16)	4.72 (3.17-6.35)	4.17 (2.23-4.23)	8.49 (6.85-10.22)	7.92 (5.88-10.07)
30-39y						
Median (95% CI)	0.64 (0.00-2.19)	0.15 (0.00-4.07)	3.51 (1.96-5.14)	3.02 (0.00-7.04)	7.11 (5.47-8.82)	6.62 (2.74-10.75)
≥ 40y						
Median (95% CI)	1.13 (0.00-3.08)	0.18 (0.00-1.78)	3.60 (1.70-5.62)	2.68 (1.10-4.37)	6.69 (4.73-8.79)	5.81 (4.15-7.61)
Total						
Median (95% CI)	0.97 (0.04-1.93)	0.26 (0.00-1.61)	3.74 (2.76-4.74)	3.09 (1.75-4.48)	7.20 (6.17-8.25)	6.48 (5.12-7.89)

Table 3 shows estimated time intervals from HIV seroconversion to reaching CD4 cell count thresholds of < 500, <350 and < 200 cells/mm^3 by age (Year) and gender. Notably, females tend to reach these thresholds sooner than males, while the youngest age group (15-29 years) reached thresholds the slowest, highlighting faster disease progression in females and slowest progression in the youngest age group. Specifically, males took 6-10 months longer to reach the CD4 thresholds compared to females of the same age. Median times from HIV infection to reaching CD4 thresholds of < 500, < 350, and < 200 cells/mm^3 were 0.97, 3.74, and 7.20 years for males, and0.26, 3.09, and 6.48 years for females. These findings contrast with a previous study showing longer times for women to reaching CD4 cell counts of < 500, < 350, and < 200 cells/mm^3 than men, despite a similar pace of CD4 decline (*11*). CD4 stands as a pivotal biomarker for monitoring HIV disease progression, and researchers generally refer to median time after HIV seroconversion as an indicator of disease progression (*22*). Our results reveal shorter median times from seroconversion to the three CD4 cell count thresholds for women than for women. The disparity might potentially be attributed to varying initial CD4 levels between genders. Lodi and colleagues proposed that men experience a steeper CD4 decline than women after seroconversion. However, our analysis found little difference in CD4 decline rate by gender, which was not influenced by regional or ethnic stratification. A Singapore cohort study emphasized a significant impact of the infecting subtype, especially subtype CRF01_AE, on the rate of CD4 decline (*14*). Therefore, further exploration is needed to understand the relationship between HIV subtype and the rate of CD4 decline by gender.

Our analysis revealed that the rate of CD4 decline was faster among older individuals and that females experienced faster disease progression than males. Among heterosexually infected individuals, females had faster disease progression, with an estimated time from seroconversion to reaching CD4 thresholds six to ten months sooner than males. The initial CD4 cell counts at HIV seroconversion were highest in the 15-29 age group, with the rate of CD4 count decline increasing with age.

Since China implemented the "treat-all" policy in 2016, studying the natural history and disease progression of PLHIV has become more challenging. Using the national surveillance database and a linear mixed-effects model based on CD4 depletion modelling

methods, we were able to explore HIV disease progression at the national level. The estimated rate of CD4 decline can inform back-calculation of the distribution of years since infection, aiding estimation of new infections in China.

Our study has several limitations. First, the estimation for seroconversion date as the midpoint between the last negative HIV test date and first positive HIV test date within a three-year interval may compromise accuracy and precision. Additionally, the small number of cases with a negative HIV test within three years prior to a positive test could impact the precision and generalizability of the intercept estimates. However, the slope estimates, based on all 59 085 eligible patients, mitigate these concerns. The lack of information on HIV subtype and other disease prognostic factors also limits our ability to address patient heterogeneity.

In conclusion, the rate of CD4 decline was faster among older ages and females in China, with progression to CD4 thresholds occurring faster in women and older age groups (≥ 40 years) than men and younger age group (15-29 years). Further studies should utilize the modeled CD4 decline rate to estimate new infections who acquired HIV through heterosexual contact, which can help decision-makers evaluate the effectiveness of HIV prevention and control measures in China.

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BRIEF REPORT
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The association between HIV infection and perimenopausal syndrome: A matched cross-sectional study of women living with HIV/AIDS and their uninfected counterparts in rural areas of Anhui, China

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Abstract: The study compared the level of perimenopausal syndrome (PS) among women age 40 or older living with HIV/AIDS (WLWH) and their HIV-negative counterparts in rural areas of Anhui, China and it analyzed the association between HIV infection and PS. From March 2018 to February 2019, WLWH ≥ the age of 40 and their aged-matched HIV-negative female neighbors in 8 townships in the cities of Fuyang and Bozhou in Anhui Province, China were selected by cluster random sampling to respond to a questionnaire survey. Multivariable logistic regression analysis was performed. Responses from a total of 464 participants were analyzed, including 220 HIV-positive women and 244 HIV-negative female neighbors. The average score for PS was 18.02 and the prevalence of PS was 85.0% in the HIV-positive group, both of which were higher than those in the control group ($p < 0.05$). The most common PS symptoms among WLWH were irritability (83.2%), followed by fatigue (79.5%) and arthralgia myalgia (68.2%). The risk of developing moderate to severe PS in HIV-uninfected women was 0.605 times that in WLWH. Other significant risk factors included being older, a history of chronic diseases, poor sleep quality, and poor appetite. In the future, more attention should be paid to the prevention of PS in WLWH while actively providing antiretroviral therapy and follow-up.

***Keywords*:** HIV/AIDS, women, perimenopausal syndrome, prevalence

Introduction

Perimenopause is the transitional phase in women from reproductive vigor to decline, and its average duration is 3 to 4 years. Perimenopausal syndrome (PS) refers to a series of symptoms in women started by changes in menses and/or development of hypoestrogenic symptoms around the time of the transition to menopause, including vasomotor symptoms (*e.g*. hot flashes and sweating), sleep disturbance, cognitive changes, urogenital symptoms, and sexual dysfunction (*1,2*). Individuals with a previous major depressive disorder have an increased risk of major depressive disorder over the transition to menopause (*3*).

In China, the female population ages 40–60 number about 229 million in 2021 and had a life expectancy of 80.88 years (*4*). Previous studies in China indicated that the incidence of PS is 68.1% (*5*), and insomnia, fatigue, and mood swings might be the three most prevalent menopausal symptoms in middle-aged Chinese women (*6*). From 2007–2018, a total of 272,611 female patients with HIV/AIDS were reported in China (*7*). As the life span of people living with HIV/AIDS increases, the issue of aging among women living with HIV (WLWH) is becoming increasingly serious, as more are entering perimenopause (*8*). A previous study suggested that WLWH undergo menopause at a younger age than women not living with HIV (*9*). The WLWH face the severe impacts of HIV as well as challenges associated with the perimenopausal period upon reaching that age (*10*). WLWH may experience earlier menopause and a higher symptom burden than women without HIV (*11*). However, little attention has been paid to PS among WLWH in China, and data from other countries are also limited.

The aims of the current study were to investigate the prevalence of PS among WLWH age 40 or older and their HIV-negative counterparts in rural areas of Anhui,

China and to examine the association between HIV infection and PS.

Patients and Methods

Study design

This study used a matched cross-sectional design. From March 2018 to February 2019, cluster sampling was used to select eight townships in the cities of Fuyang and Bozhou in Anhui Province, China. Ethical approval for this study was obtained from the Anhui Provincial Center for Disease Control and Prevention. This study was conducted in accordance with the Declaration of Helsinki.

Participant recruitment

WLWH and their neighbors who did not have HIV were included in the study. Inclusion criteria for the patient group were as follows: women ≥ the age of 40, confirmed to be HIV-positive, have intact ovaries and uterus, and no history of chemotherapy or radiotherapy. The inclusion criteria for the control group were as follows: women with an age difference of within 2 years compared to the patients, HIV-negative, have intact ovaries and uterus, and no history of chemotherapy or radiotherapy. The exclusion criteria were cognitive impairments or difficulty communicating, artificial menopause, and unwillingness to participate in the survey.

Data collection

Potential participants provided written informed consent prior to study enrollment. A structured questionnaire was administered by a well-trained research staff member to each participant at a local public health facility. Each interview took approximately 25-30 min to complete. The participation rates of the HIV-positive and HIV-negative groups were 86% (220/255) and 91% (244/267), respectively. The information was dually entered by personnel specifically designated by research team, and checks for logical consistency and duplicate data were conducted.

Measures

An on-site questionnaire survey was conducted, which included demographic information, medical history, health status, and PS status. The PS assessment used the Modified Kupperman Index (MKI), consisting of 13 items, to evaluate the severity of various symptoms such as sweating and hot flashes, insomnia, paresthesia, depression, irritability, fatigue, vertigo, arthralgia myalgia, palpitations, headache, a tingling sensation on the skin, sexual problems, and urinary system infections. Each item was rated on a scale from 0 to 3, with higher scores indicating more severe symptoms. The criteria for classification were as follows: a total score of ≤ 6 indicated normal health, 7 to 15 indicated mild symptoms, 16 to 30 indicated moderate symptoms, and a score ≥ 30 indicated severe symptoms (*12*).

Statistical analysis

A database was created using the software EpiData 3.1. Descriptive analyses were performed for all study variables. Categorical variables were expressed as frequencies and proportions, and continuous variables were expressed as means and standard deviations (SD). Univariate logistic analyses were then performed to explore the relationships between independent variables and PS. Multivariable logistic regression was finally used to examine the associations between independent variables and PS. A p-value < 0.05 was considered to indicate a significant difference. All analyses were performed using the software SPSS 23.0.

Results and Discussion

Participant characteristics

A total of 464 individuals were surveyed, including 220 WLWH and 244 controls. The average age of the participants in the WLWH and control group was 52.4 ± 6.0 and 53.4 ± 7.3 years, respectively, with no significant difference in the age distribution between the two groups (t = -1.602, p = 0.110); 41.8% had completed primary school or higher, 86.6% were married, and 62.4%% had a chronic disease. The proportions of participants with good sleep were 51.4% of WLWH and 66.4% of controls. In the WLWH group, 63.2% had a good appetite while 80.7% in the control group had a good appetite (Table 1).

Perimenopausal symptoms in total and by strata

In the WLWH group, the average PS score was 18.02 ± 10.60 points, with a total prevalence of 85.0%, and a higher proportion of moderate to severe PS (44.1% and 13.6%, respectively). In the control group, the average PS score was 14.3 ± 9.18 points, with a total prevalence of 78.7%, and a higher proportion of mild to moderate PS (37.3% and 35.2%, respectively) (Table 2). PS scores differed significantly between the two groups (t = 3.981, $p < 0.001$).

The PS score for and the prevalence of PS in WLWH in this survey were higher than those in studies on the general Chinese population. A study by Du *et al.* (*13*) with a large sample that included 3,147 women ages 40-60 from 16 communities in Shanghai, for example, found that the total prevalence of PS was 73.80%. In 2022, a survey of women ages 40-60 at a hospital health

Table 1. Distribution of participant characteristics

Characteristics	*n* (column%) / mean ± SD			Statistical values*	*p*
	Patient group	Control group	Total		
Age (years)				6.578	0.087
40-44	23 (10.5)	37 (15.2)	60 (12.9)		
45-49	55 (25.0)	43 (17.6)	98 (21.1)		
50-54	59 (26.8)	57 (23.4)	116 (25.0)		
≥ 55	83 (37.7)	107 (43.9)	190 (40.9)		
Mean ± SD	52.4 ± 6.0	53.4 ± 7.3	52.9 ± 6.7	-1.602	0.110
Education				11.489	0.001
no formal education	146 (66.4)	124 (50.8)	270 (58.2)		
primary school or higher	74 (33.6)	120 (49.2)	158 (41.8)		
Marriage				10.072	0.002
married	178 (81.3)	222 (91.4)	400 (86.6)		
divorced/widowed, *etc*.	41 (18.7)	21 (8.6)	62 (13.4)		
Chronic disease				0.003	0.954
no	82 (37.4)	92(37.7)	174 (37.6)		
yes	137 (62.6)	152 (62.3)	289 (62.4)		
Sleep quality				10.825	0.001
good	113 (51.4)	162 (66.4)	275 (59.3)		
poor	107 (48.6)	82 (33.6)	189 (40.7)		
Appetite				17.850	< 0.001
good	139 (63.2)	197 (80.7)	336 (72.4)		
poor	81 (36.8)	47 (19.3)	128 (27.6)		

*A Chi-square test was used for categorical variables, and the Chi-square value and p-value are reported. The Mann-Whitney *U*-test was used for continuous variables that were normally distributed, and the Z score and *p*-value are reported.

Table 2. Perimenopausal syndrome scores for and categorization of survey respondents [Number/(Proportion/%)]

PS level	HIV/AIDS patient group	Control group	χ^2	*p*
Normal	33 (15.0)	52 (21.3)	15.071	0.002
Mild	60 (27.3)	91 (37.3)		
Moderate	97 (44.1)	86 (35.2)		
Severe	30 (13.6)	15 (6.1)		

management center in Sichuan Province indicated that 46.44% had mild PS and 24.07% had moderate or severe PS (*3*). This suggests that the prevalence of PS in female HIV/AIDS patients in Anhui Province is concerning, with a higher proportion of moderate and relatively severe disease. In addition to antiretroviral therapy and follow-up, attention should also be paid to the prevention of PS in WLWH.

Types of perimenopausal symptoms reported

Of various perimenopausal symptoms, irritability had the highest prevalence in the WLWH group (83.2%), followed by fatigue (79.5%) and arthralgia myalgia (68.2%); symptoms with a lower prevalence included urinary system infections (23.2%) and a tingling sensation on the skin (26.4%). In the control group, irritability also had the highest prevalence (69.3%), followed by arthralgia myalgia (61.5%) and vertigo (60.2%); symptoms with a lower prevalence were urinary system infections (15.2%) and a tingling sensation on the skin (16.8%). Fatigue, palpitations, irritability, and 8 other symptoms differed significantly between the two groups ($p < 0.05$) (Table 3).

"Irritability" had the highest prevalence among both the WLWH group and the control group, and it was significantly higher in the former compared to the latter. Eight other PS symptoms were more prevalent in Chinese WLWH. However, a survey of Spanish women revealed that 75.1% experienced symptoms, with hot flashes being the most common among those in the perimenopausal group (*14*). This suggests that mood swings are most typical among HIV-positive women with PS in rural China. Health interventions for these populations should focus on education regarding emotional management, and raising awareness of preventing PS among WLWH in particular. In addition, the survey found that "fatigue" had a higher prevalence among WLWH compared to the normal control group, which is likely related to the HIV infection itself. Given the significant effectiveness of current antiretroviral therapy, AIDS has become a long-term chronic infectious disease, with a prolonged course potentially lasting decades. The unavoidable adverse effects of long-term medication may contribute to increased fatigue among HIV-infected individuals. This indicates that female HIV/AIDS patients are prone to fatigue, which is influenced by multiple factors. They should seek to rest and avoid excessive physical labor in their everyday lives.

Risk factor analysis of perimenopausal symptoms

Participants were divided into two groups based on their

PS scores: a normal to mild PS group (score ≤ 15) and a moderate to severe PS group (score > 15). Six factors — HIV infection status, age, marital status, a history of chronic diseases, sleep quality, and appetite — differed significantly between the two groups ($p < 0.05$) (Table 4). Using the forced entry method with PS score as the dependent variable (0 = "score ≤ 15", 1 = "score > 15"), and including criteria while excluding HIV infection status, age, marital status, a history of chronic diseases, sleep quality, and appetite as independent variables, multivariate analysis indicated that the risk of developing moderate to severe PS in women not infected with HIV was 0.605 times that in WLWH, while being older, a history of chronic diseases, poor sleep quality, and a poor appetite were identified as risk factors for developing moderate to severe PS (Table 5).

The adverse effects of HIV infection on an individual include both physiological functions and psychological states, which may increase the severity of PS, but the exact mechanisms are still unclear (*15,16*). Similar domestic studies have also reported that age was a potential influencing factor. Some studies have suggested that the incidence of PS is higher with age among women ages 40-60 in rural areas (*5,17*). Therefore, attention should be paid to PS in postmenopausal women as well as in perimenopausal women.

A previous study (*18*) has found that chronic diseases are positively correlated with various syndromes of perimenopause. Individuals with physical illnesses are more likely to experience sexual problems, depression,

Table 3. Prevalence of symptoms related to perimenopausal syndrome among survey respondents [number/(proportion/%)]

Syndrome	Patient group		Control group		χ^2	p
	Yes	No	Yes	No		
Sweating and hot flashes	89 (40.5)	131 (59.5)	91 (37.3)	153 (62.7)	0.486	0.486
Paresthesia	97 (44.1)	123 (55.9)	76 (31.1)	168 (68.9)	8.289	0.004
Insomnia	147 (66.8)	73 (33.2)	132 (54.1)	112 (45.9)	7.808	0.005
Palpitations	148 (67.3)	72 (32.7)	122 (50.0)	122 (50.0)	14.187	< 0.001
Irritability	183 (83.2)	37 (16.8)	169 (69.3)	75 (30.7)	12.241	< 0.001
Depression	111 (50.5)	109 (49.5)	92 (37.7)	152 (62.3)	7.642	0.006
Arthralgia myalgia	150 (68.2)	70 (31.8)	150 (61.5)	94 (38.5)	2.277	0.131
Vertigo	144 (65.5)	76 (34.5)	147 (60.2)	97 (39.8)	1.342	0.247
Fatigue	175 (79.5)	45 (20.5)	141 (57.8)	103 (42.2)	25.214	< 0.001
Headache	140 (63.6)	80 (36.4)	144 (59.0)	100 (41.0)	1.040	0.308
Tingling sensation on the skin	58 (26.4)	162 (73.6)	41 (16.8)	203 (83.2)	6.300	0.012
Urinary system infections	51 (23.2)	169 (76.8)	37 (15.2)	207 (84.8)	4.839	0.028
Sexual problems	149 (67.7)	71 (32.3)	145 (59.4)	99 (40.6)	3.434	0.064

Table 4. Univariate analysis of perimenopausal syndrome

Variables	Normal to mild perimenopausal syndrome group		Moderate to severe perimenopausal syndrome group		χ^2	p
	n	%	*n*	%		
HIV infection status					12.350	< 0.001
yes	93	39.4	127	55.7		
no	143	60.6	101	44.3		
Age					31.493	< 0.001
40-49	109	46.2	49	21.5		
≥ 50	127	53.8	179	78.5		
Education					1.006	0.316
no formal education	132	55.9	138	60.5		
primary school or higher	104	44.1	90	39.5		
Marriage					8.065	0.005
married	213	91.0	187	82.0		
divorced/widowed, *etc.*	21	9.0	41	18.0		
History of chronic diseases					22.446	< 0.001
no	113	48.1	61	26.8		
yes	122	51.9	167	73.2		
Sleep quality					46.626	< 0.001
good	176	74.6	99	43.4		
poor	60	25.4	129	56.6		
Appetite					47.305	< 0.001
good	204	86.4	132	57.9		
poor	32	13.6	96	42.1		

Table 5. Multivariable logistic regression analysis of perimenopausal symptoms

Factors	β	S.E.	*p*	OR	95% CI
HIV infection status					
yes				1	
no	-0.503	0.219	0.022	0.605	(0.394-0.929)
Age					
40-49				1	
≥ 50	0.959	0.232	< 0.001	2.609	(1.656-4.110)
Marriage					
married				1	
divorced/widowed, *etc*.	0.313	0.323	0.333	1.367	(0.726-2.576)
Chronic disease					
no					
yes	0.685	0.222	0.002	1.984	(1.284-3.063)
Sleep quality					
good				1	
poor	0.937	0.220	< 0.001	2.553	(1.659-3.928)
Appetite					
good				1	
poor	1.061	0.256	< 0.001	2.889	(1.750-4.770)

anxiety, and vasomotor symptoms than those without physical illnesses. Sleep problems, including insomnia, not only reduce physical immunity but may also affect the psychological state of menopausal women. This can exacerbate endocrine disorders and potentially lead to a nervous breakdown and an increased risk of cardiovascular diseases (*19*). Psychological guidance should be enhanced for WLWH to improve their sleep quality or treat sleep problems through medical intervention (*20,21*). For WLWH, maintaining a good appetite can promote normal eating, help them obtain sufficient nutrition, and provide a sense of satisfaction and pleasure during meals, thereby alleviating mood swings. To prevent and treat PS in WLWH, special attention should be paid to women who are older, have a history of chronic diseases, poor sleep quality, and a poor appetite. Targeted health education and behavioral interventions should be provided for these individuals (*22*).

In conclusion, this study found that the prevalence and severity of PS in WLWH were higher than those in the control group, and mood swings were most evident in PS. While actively providing antiviral therapy and following up with WLWH, attention must be paid to female reproductive health, and relevant education, emotional management, and health education must be provided.

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BRIEF REPORT
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Identification of new circulating recombinant form of HIV-1 CRF139_02B in Japan, and search for the origin

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Abstract: Many circulating recombinant forms (CRFs) of HIV-1 have been reported, resulting in complex molecular epidemiology of HIV-1 infection. In this study, we newly identified CRF139_02B in Japan from 4 cases of antiretroviral therapy naïve people living with HIV. Near full-length genome sequences of CRF139_02B were determined using Illumina MiSeq. Basic Local Alignment Search Tool (BLAST) revealed that there were several sequences having the same breakpoints as CRF139_02B in the UK and Nepal, though its full-length genome sequences were not available. Maximum clade credibility tree analysis using the region of protease and reverse transcriptase of HIV-1 estimated that the time to the most recent common ancestor of CRF139_02B variants found in Japan was 2017.6 (95% highest posterior density interval: 2015.9-2019.3), and that among the UK, Nepal, and Japan was 2010.4 (2007.8-2012.5). These results suggested that CRF139_02B circulated in Japan recently and domestically. Furthermore, the origin of CRF139_02B could be in the UK. Because there is a possibility that further international circulation of CRF139_02B may be observed in the near future, continuous monitoring of HIV-1 molecular epidemiology will be needed.

***Keywords*:** HIV, Japan, circulating recombinant form, phylogenetic tree analysis, time to the most recent common ancestor

Introduction

Development of antiretroviral therapy (ART) averted approximately 20.8 million AIDS-related deaths in the past three decades (*1*). However, the AIDS epidemic is still a public health threat and should be ended (*2*). In 2022, people living with HIV (PLWH) were 39 million, people acquiring HIV were 1.3 million, and people dying from HIV-related causes were 630,000 (*3*). HIV-1 group M is the majority of the global epidemic of HIV infections (*4*) and has diverged into 10 subtypes (A-D, F-H, J-L) (*5*). Furthermore, many inter-subtype recombinants of HIV-1 have been identified (*4*). Typically, recombinants found in 3 or more epidemiologically unrelated PLWH were distinguished as circulating recombinant forms (CRFs) and were numbered sequentially. In May 2024, over 150 CRFs were reported to the HIV Sequence Database of Los Alamos National Laboratory (*https://www.hiv.lanl.gov/content/index*). Recombination of HIV-1 makes viruses diversified (*6,7*) and molecular epidemiology complicated (*8,9*).

In Japan, newly diagnosed cases of HIV-1 infection were 884 in 2022, which was the lowest number in the past 20 years (*10*). On the other hand, the percentage of people who were diagnosed with HIV infection per PLWH was estimated at 85% (*11,12*) and was below the 95% targeted by WHO (*13*). The dominant strain of HIV-1 was subtype B (82.8%), followed by CRF 01_AE (7.2%), among people newly diagnosed with HIV in Japan from 2003 to 2019 (*14*). Two CRFs have been identified in Japan and were composed of subtype B and CRF01_AE (*15,16*). Recently, we found a candidate for a new CRF in PLWH visiting our outpatient clinic. The objective of this study was to determine whether these HIV-1 strains were new CRFs and to explore their molecular epidemiology.

Materials and Methods

Participants and sample collection

In AIDS Clinical Center, National Center for Global Health and Medicine (NCGM), pre-treatment HIV-1 drug resistance testing was performed before the introduction of ART by determining protease and reverse transcriptase sequences in 2,482 ART-naïve PLWH from January 2003 to June 2022. During the analysis of these sequences, we noticed that 4 cases had a similar unique recombination pattern in protease and reverse transcriptase of HIV-1

composed of subtype B and CRF02_AG. Their stocked plasma or serum samples and clinical information at (or nearby) the first visit were used for analysis anonymously. The study was approved by the Human Ethics Committee of NCGM (#NCGM-A-000172-01), and each participant also signed informed consent in accordance with the Declaration of Helsinki.

Near full-length genome sequencing of HIV-

Near full-length genome (NFLG) sequencing of HIV-1 (HXB2 position: 706-9531) was performed using the previously published method (*17,18*) with little modification. Briefly, viral RNA was extracted from plasma or serum samples using QIAamp Viral RNA Mini Kit (Qiagen., Venro, Nederland). Reverse-transcription followed by polymerase chain reaction (PCR) and nested PCR was performed to amplify two (or four) segments of NFLG of HIV-1 using PrimeScript II High Fidelity One Step RT-PCR Kit and PrimeSTAR GXL DNA Polymerase (Takara Bio Inc., Shiga, Japan) (*19,20*). Nextera XT DNA Library Preparation Kit and MiSeq (Illumina, San Diego, CA, USA) were used for library preparation and next generation sequencing (NGS). Vicuna was used for the de novo assembly of short read NGS data (*21*). Short read NGS data were mapped into a tentative NFLG sequence made by Vicuna using BWA-MEM (*22*). Mapped NFLG sequence data was visualized using IGV (*23*) and polished to get final consensus sequence. Anti-HIV drug resistant mutation was checked by HIV DRUG RESISTANCE DATABASE of Stanford University (*https://hivdb.stanford.edu*). Viral tropism of coreceptor usage was checked with geno2pheno [coreceptor] (*https://coreceptor.geno2pheno.org*).

Analysis of breakpoints and phylogenetic tree

Breakpoints of recombination were estimated using jpHMM (*24*) and RIP (*25*). Reference sequences for phylogenetic tree analysis were obtained from the Nucleotide Basic Local Alignment Search Tool (BLAST) (*https://blast.ncbi.nlm.nih.gov/Blast.cgi*), and from the HIV Sequence Database of Los Alamos National Laboratory. Phylogenetic tree analysis was conducted by MEGA7 using the neighbor-joining method with Kimura two-parameter model and 1,000 bootstrap replications (*26*). Time to the most recent common ancestor (tMRCA) was estimated by maximum clade credibility (MCC) tree analysis using the Bayesian Markov chain Monte Carlo method. The analysis was conducted by BEAST v2.4.6 with 100,000,000 states using GTR +G +I, relaxed clock log normal, and Coalescent Bayesian Skyline model (*27*).

Results and Discussion

The clinical information of the 4 participants is shown in Table 1. They were all men who have sex with men. Two of them were Japanese, and the remaining 2 cases were non-Japanese. A certain epidemiological link was not observed among them. We succeeded in NFLG sequencing of HIV-1 in all participants. No sign of dual infection was found in the result of the de novo assembly made by Vicuna and in the mapping images depicted by IGV. We registered 4 NFLG sequences of HIV-1 to DDBJ. Accession numbers are as follows: ACCRF1, LC762505; ACCRF2, LC762506; ACCRF3, LC762507; and ACCRF4, LC762508. Next, we analyzed the recombination pattern of these sequences. First, jpHMM analysis suggested that these 4 sequences had the same recombination pattern composed of subtype A, B, G, and CRF01_AE (Figure 1A). Second, RIP analysis suggested that these 4 sequences were more likely composed of subtype B and CRF02_AG, rather than subtype A, G, and CRF01_AE (Figure 1B). To confirm the recombination pattern suggested by RIP, we conducted phylogenetic tree analysis in 3 regions; region I (HXB2 position:706-3141), region II (3142-4855), and region III (4856-9531) (Figure 1C). It revealed that these 4 sequences made significant clusters (bootstrap score 99) and belonged to CRF02_AG in region I (Figure 1D), to subtype B in region II (Figure 1E), and to CRF02_AG in region III (Figure 1F).

Nucleotide BLAST with our NFLG sequences revealed that even the most similar sequence to ACCRF1 had only 91.11% identity, suggesting that no sequence was close to ACCRF1. We also checked known CRFs composed of subtype B and CRF02_AG in the HIV DATABASE of Los Alamos National Laboratory. Three CRFs (CRF56_cpx, CRF94_cpx, and CRF95_02B) were found, though their recombination patterns were different from our 4 sequences. Thus, we contacted HIV DATABASE to resister our new CRF. After that, HIV DATABASE assigned the following code to the new CRF: CRF139_02B. This was the third CRF of HIV-

Table 1. Information of study participants at (or nearby) first visit

ID	Sex	Transmission route	Age	Nationality	Last HIV negative	Sampling	CD4 (/μL)	Viral load (/mL)
ACCRF1	Male	MSM	20s	China	2020/6	2020/9	326	789,000
ACCRF2	Male	MSM	30s	Japan	NA	2020/10	322	22,800
ACCRF3	Male	MSM	30s	Mexico	2019	2021/10	263	140,000
ACCRF4	Male	MSM	20s	Japan	2021/8	2022/1	430	405,000

MSM: men who have sex with men; NA: not available.

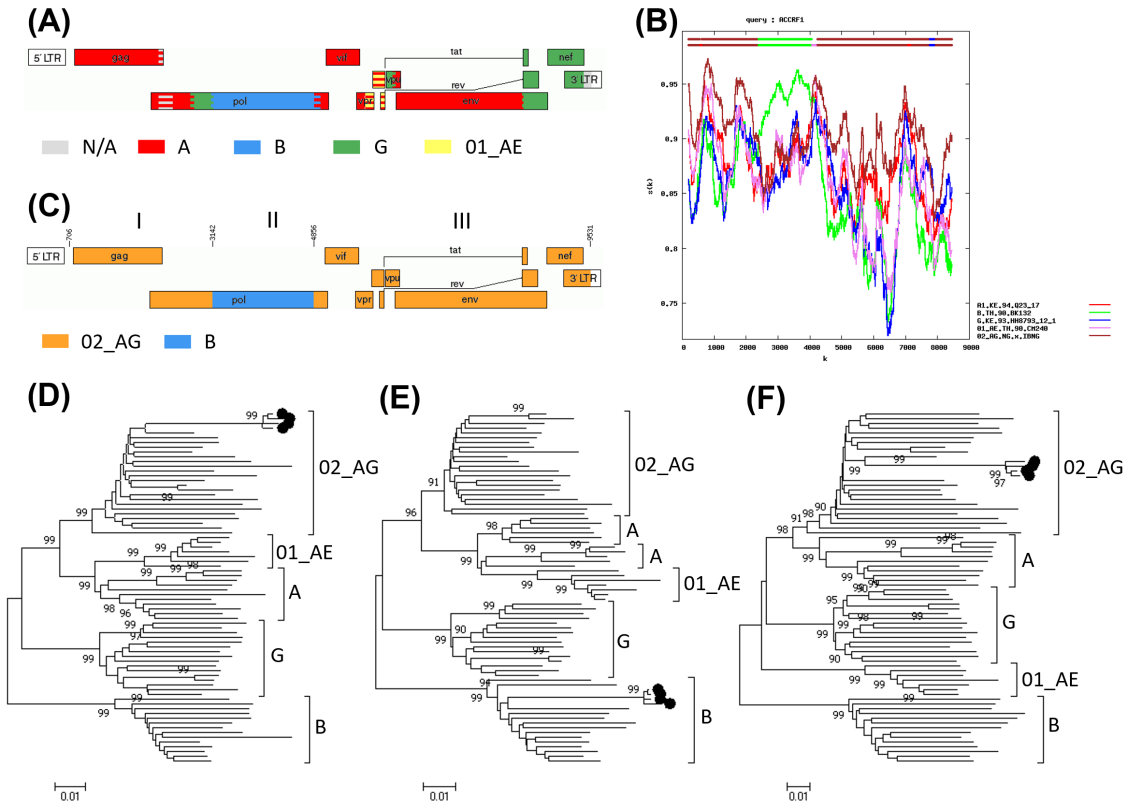

Figure 1. Analysis of recombination in CRF139_02B. (A) Recombination pattern of ACCRF1 analyzed by jpHMM; **(B)** Recombination pattern of ACCRF1 analyzed by RIP; **(C)** Recombination pattern of ACCRF1 depicted by Recombinant HIV-1 Drawing Tool; **(D)** Phylogenetic tree analysis of region I (HXB2 position: 706-3141); **(E)** Phylogenetic tree analysis of region II (3142-4855); **(F)** Phylogenetic tree analysis of region III (4856-9531). The closed circle showed CRF139_02B in Japan. The number of nearby nodes showed a bootstrap score ≥ 90.

1 identified in Japan, to our knowledge. Previously identified 2 CRFs of HIV-1 in Japan (CRF69_01B (*15*) and CRF76_01B (*16*)) were composed of 2 major subtypes in Japan (B and CRF01_AE) (*14*). While, CRF139_02B was composed of subtype B and CRF02_AG, the latter was a minor subtype (1.2%) in Japan (*14*). All variants of CRF139_02B did not have any anti-HIV drug resistant mutation in Protease, Reverse transcriptase, Integrase, and Capsid region. Additionally, these viral tropism of coreceptor usage were CCR5.

To search for a similar sequence to CRF139_02B, we used Nucleotide BLAST with 2 regions; region IV was protease and reverse transcriptase (HXB2 position: 2253-3509) and region V was integrase (4230-5093) (Figure 2A). There were 3 reasons why we chose these regions. First, a part of CRF139_02B genome might have a similar sequence, though we could not find any similar sequence to NFLG of CRF139_02B. Second, substantial reference sequence data were available because drug resistance testing of HIV-1 had been conducted in all over the world. Third, these regions contained the breakpoint of recombination in CRF139_02B. The top 15 sequences of max score in BLAST were added to phylogenetic tree analysis in each region. In region IV, 9 sequences from the UK (*28,29*) and 1 sequence from Nepal (*30*) were found near CRF139_02B sequences with 100 bootstrap score (Figure 2B). In region V, 7 sequences from the UK (*28*) were found near CRF139_02B sequences with 100 bootstrap score (Figure 2C). It turned out that some sequences in region IV and region V were obtained from the same PLWH (*e.g.* MT571106 and MT570777) (*28*). Thus, we united paired sequences and analyzed recombination patterns with RIP (Figure 3). It turned out that 6 cases (MT570597, MT571106, MT571148, MT571077, MT571085, and MT571086) had the 2 same breakpoints as CRF139_02B. Additionally, 2 cases (MZ538466 and MT570773) had the same breakpoint as CRF139_02B, though only one region sequence data was available in each case. It suggested that these 8 cases can be CRF139_02B, though their NFLG sequences were not available. The other 3 cases (MT571265, MF109637, and MT571025) also had the same breakpoint as CRF139_02B in protease and reverse transcriptase region, at the same time, however, they had subtype F recombination. Thus, these 3 sequences were not CRF139_02B, but were a related recombinant to CRF139_02B. According to Yebra G, *et al.*, a part of the MF109637 genome derived from subtype F was close to the pure subtype F sequence observed in the UK, suggesting that MF109637 was generated in the UK (*29*).

To search for the origin of CRF139_02B, MCC tree analysis was performed. To eliminate the effect of

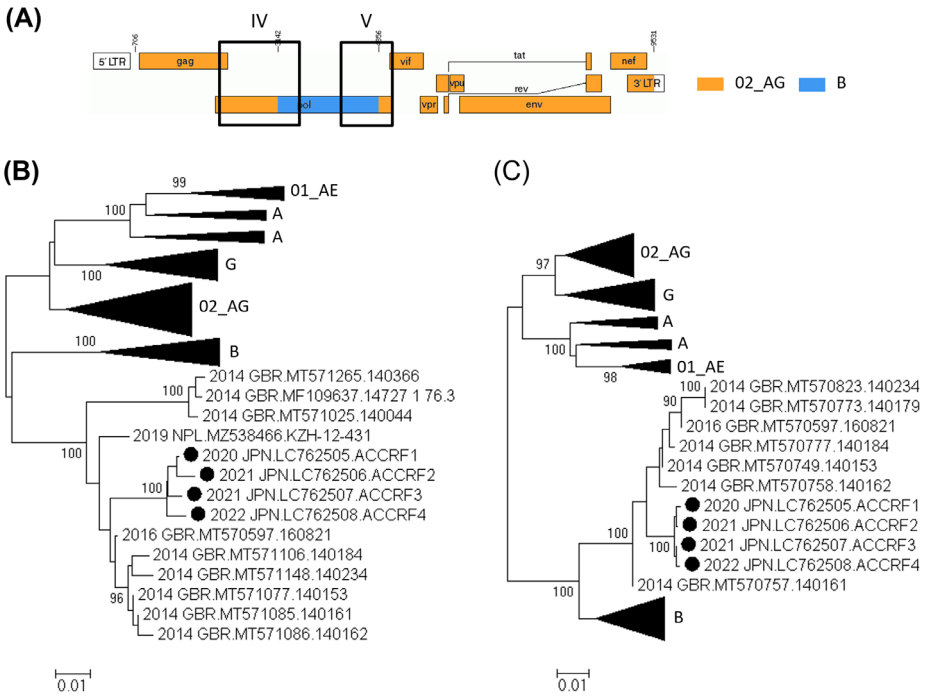

Figure 2. Search for similar sequences to CRF139_02B. (A) Two regions were used for BLAST; **(B)** Phylogenetic tree analysis of region IV (HXB2 position: 2253-3509); **(C)** Phylogenetic tree analysis of region V (4230-5093). The closed circle showed CRF139_02B in Japan. The number of nearby nodes showed a bootstrap score ≥ 90.

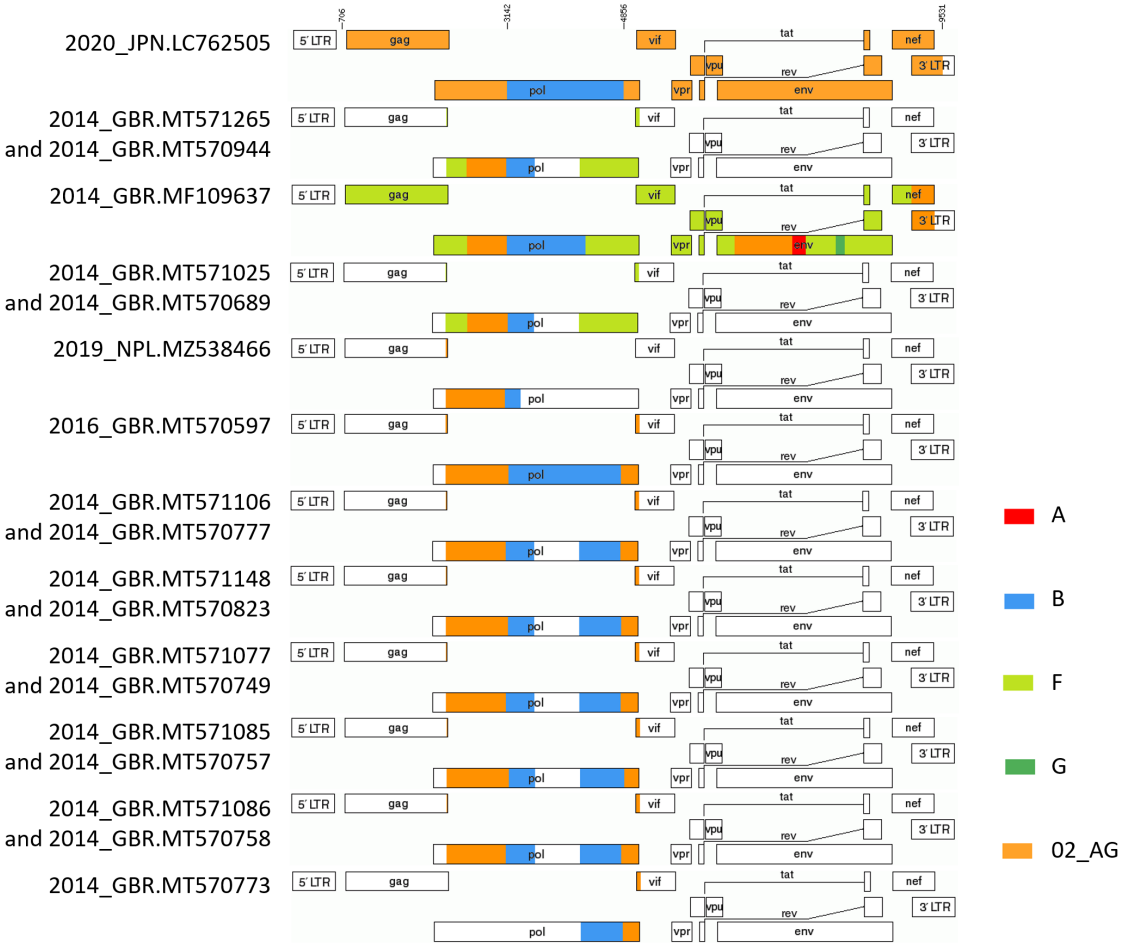

Figure 3. Recombination pattern of similar sequences to CRF139_02B. Each recombination pattern was analyzed by RIP and depicted by Recombinant HIV-1 Drawing Tool. LC762505 (at the top) was ACCRF1.

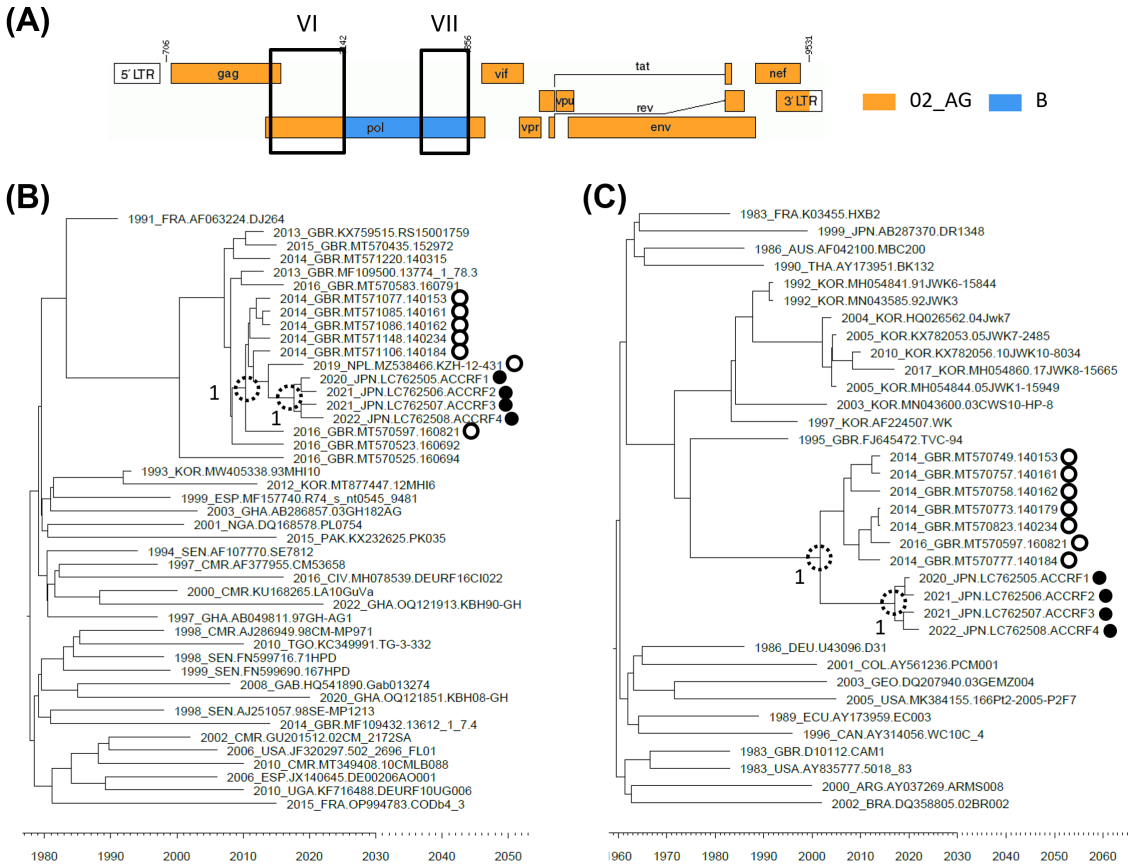

Figure 4. Search for the origin of CRF139_02B. (A) Two regions were used for maximum clade credibility (MCC) tree analysis; **(B)** MCC tree analysis of region VI (HXB2 position: 2253-3141); **(C)** MCC tree analysis of region VII (4230-4855). The closed circle showed CRF139_02B in Japan. The open circle showed CRF139_02B in the UK or Nepal. The dotted circle showed a node of the common ancestor. The number near the dotted circle showed posterior probability.

recombination, we used regions composed of a single subtype or CRF; region VI (HXB2 position: 2253-3141), and region VII (4230-4855) (Figure 4A). MCC tree analysis was performed with only CRF02_AG or CRF139_02B sequences in region VI (Figure 4B). Four sequences of CRF139_02B in Japan formed a cluster, and tMRCA was 2017.6 (95% highest posterior density (95% HPD) interval: 2015.9-2019.3). At the upstream, CRF139_02B sequences in the UK and Nepal were located and made a cluster with CRF139_02B sequences in Japan, and tMRCA was 2010.4 (2007.8-2012.5). Further upstream, pure CRF02_AG sequences in the UK were located. These results revealed that CRF139_02B in Japan, the UK and Nepal had the same origin. Furthermore, the origin of CRF139_02B could be in the UK. In Japan, CRF139_02B seemed to be circulating recently and domestically, rather than migrating independently from elsewhere to Japan. This suggestion did not conflict with the clinical information of the participants at (or nearby) the first visit; their CD4 counts were maintained, and 3 of 4 participants had an HIV negative history recently (Table 1). In the same way, MCC tree analysis was performed with only subtype B or CRF139_02B sequences in region VII (Figure 4C). Four sequences of CRF139_02B in Japan formed a cluster, and tMRCA was 2017.2 (2014.2-2019.7). Upstream, CRF139_02B sequences in the UK were located and made clusters with CRF139_02B sequences in Japan, and tMRCA was 2001.7 (1992.3-2009.7). However, no close sequence was found further upstream. These results revealed that CRF139_02B in Japan and the UK had the same origin, though no information was found about the parental subtype B strain of CRF139_02B. That may be the reason why tMRCA of CRF139_02B in region VII had a longer 95% HPD interval than that in region VI.

Our study has 4 limitations to be mentioned. First, this study was conducted in a single center. We had no more information about current circulation of CRF139_02B in Japan. Second, the number of study participants was small. The clinical or virological feature of CRF139_02B was unclear. Third, no epidemiological link among the participants was observed. We could not confirm epidemiological migration or circulation of CRF139_02B in Japan. Fourth, the putative parental subtype B of CRF139_02B was unknown, which weakens our hypothesis that CRF139_02B originated in the UK.

In conclusion, we newly identified HIV-1 CRF139_02B from 4 PLWH in Japan. It turned out that CRF139_02B has existed in at least 8 PLWH outside of Japan. Furthermore, the origin of CRF139_02B could be in the UK. Because there is a possibility that

further international circulation of CRF139_02B may be observed in the near future, continuous monitoring of HIV-1 molecular epidemiology will be needed.

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Sexual health care barriers and HIV/STI prevention for transgender people in Japan

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Abstract: Primary care and sexual health services for transgender people in Japan are lacking. We surveyed 233 transgender patients (57 [24.5%] assigned male at birth [AMABs] and 176 [75.5%] assigned female at birth [AFABs]) at the Personal Health Clinic to collect data on sexually transmitted infections (STI) and human immunodeficiency virus (HIV) risk, as well as lifestyle, stigma, and health literacy. Among respondents, 55% reported a sexual intercourse history, and 7.6% noted a previous STI. Only 62.2% underwent free STI testing. Current smoking rates were 19.3% higher in AFABs. Hospital visit hesitation was reported by 59.6%, and 83.3% experienced daily mental struggles. Awareness of post-exposure prophylaxis and pre-exposure prophylaxis was low. Our findings highlight the urgent need for improved primary care and sexual health services for transgender people in Japan, emphasizing the necessity to increase sexual health care facilities, reduce primary care access barriers, and improve knowledge among health care providers.

***Keywords*:** sexually transmitted infection screening, pre-exposure prophylaxis, sex education, transgender people, transgender health

Introduction

By 2022, 12,000 transgender people with Japanese citizenship had undergone a legal gender change. While the actual number is unknown, as many transgender people do not legally change their gender. Primary care and sexual health care for transgender people in Japan are poorly developed. Health providers may lack knowledge about transgender people due to shortcomings in medical education (*1*). Consequently, transgender people who are ill may hesitate to seek care because physicians often do not understand the concept of gender.

We conducted a survey on sexual health care and examined lifestyle, stigma, and health literacy in the transgender population.

Current situation in Japan and overseas regarding transgender medical care

Few physicians in Japan have received specialized training in transgender-affirming care, and data on primary or sexual health care for transgender people remain limited (*1*). One gap of particular concern is the lack of comprehensive data on the risk of sexually transmitted infections (STI) and human immunodeficiency virus (HIV) infection among transgender individuals. Previous research showed that transgender individuals in Asia, including Japan, face stigma and poor understanding among health care providers. Additionally, the number of certified specialists in gender incongruence remains low in Japan (*2*). According to the World Professional Association for Transgender Health Standards of Care, there is a pressing need to improve gender-affirming care globally, including primary care and sexual health (*3*). To address this gap, we conducted a survey on sexual health care, and examined lifestyle, stigma, and health literacy in the Japanese transgender population.

Several countries have already implemented specific measures to provide comprehensive transgender-affirming health care. For example, the Fenway Institute in Boston has developed a successful, integrative model of transgender healthcare that includes primary care, mental health services, and gender-affirming treatments. Their approach, which emphasizes patient-centered care and provider training, has significantly improved health outcomes and reduced disparities among transgender patients (*4*). Japan could follow these successful models to improve access to transgender-affirming care.

Questionnaire survey

This study conforms to the provisions of the Declaration of Helsinki (as revised in 2013). We surveyed 233 transgender patients who visited the gender outpatient department at the Personal Health Clinic between June 2023 and April 2024 (10 months). The main purpose of the clinic visits was treatment consultation and gender-affirming hormone therapy (GAHT). At the initial visit, patients whose informed consent was obtained were given free STI/HIV testing and surveyed using online questionnaires.

Response options to the survey questions were yes, no, don't know, and refuse to answer. Only patients who answered with a yes or no response and were able to complete the online questionnaire were included in the study. We excluded respondents who did not answer eight mandatory questions. Sex assigned at birth was a required response; respondents were divided into transgender individuals assigned male at birth (AMABs) and transgender individuals assigned female at birth (AFABs). Gender identity was freely reported: responses included transgender female or male, male to female, female to male, non-binary, and genderqueer.

Characteristics of survey respondents

Of 233 respondents, 57 (24.5%) were AMABs and 176 (75.5%) were AFABs. The mean age of transgender patients was approximately 32.7 years. The mean age of AMABs was 34.5 years, with 27 (47.4% of AMABs) under age 30 years, 13 (22.8%) between age 31 and 40 years, and 17 (29.8%) over age 41 years. The average age of AFABs was 32.1 years; 76 (43.2% of AFABs) were under age 30 years, 80 (45.5%) were between age 31 and 40 years, and 20 (11.4%) were aged 41 years or older.

Table 1 shows the rate of yes responses and the yes/no ratio among AMABs and AFABs. Among 211 respondents who responded regarding their history of sexual intercourse, 55% answered yes, including 66.7% of AMABs and 49% of AFABs; a higher rate of sexual intercourse experience was observed in older respondents. Regarding STI history, 197 respondents gave valid answers (yes or no), with a surprisingly low proportion noting a previous STI history (7.6%).

All study participants (n = 233) gave valid answers about accepting free STI testing and current smoking. Only 62.2% of respondents underwent free testing for STI (syphilis, gonorrhea, chlamydia, hepatitis B virus, and HIV): 31.6% of AMABs and 72.2% of AFABs. More AFABs than AMABs underwent free STI testing in all age groups (≤ 30, 31–40, and ≥ 41 years), indicating that AFABs may be more aware of their own STI risk. Total smoking rate was 19.3% in the total: 8.8% of AMABs and 22.7% of AFABs, with higher rates among AFABs in all age groups.

A total of 194 and 205 transgender people gave valid answers regarding hesitation to visit a hospital and mental struggles in daily life, respectively. Specifically, 58.8% of AMABs and 61.0% of AFABs said they were hesitant about visiting a hospital when ill; 82.4% of AMABs and 84.1% of AFABs reported mental struggles in daily life. Higher proportions of AFABs reported these problems across all age groups.

As shown in Table 2, we surveyed respondents regarding their awareness about post-exposure prophylaxis (PEP) and pre-exposure prophylaxis (PrEP); 87 transgender people (23 AMABs and 64 AFABs) gave valid answers to both questions. Among them, 20.7% and 10.3% of respondents knew about PEP and were interested in PrEP, respectively. A higher proportion of AMABs knew about PEP and were interested in PrEP compared with AFABs in all age groups. In particular, younger AMABs seemed to have greater awareness about PEP and interest in PrEP.

Experience of sexual intercourse

Few studies have investigated the rates of sexual intercourse among transgender individuals in Japan using comprehensive definitions of gender and

Table 1. Sexual health literacy, medical care accessibility, and life difficulty among transgender people

Characteristics			Transgender							
			AMABs				AFABs			
	n	Yes responses (%)	Average	Age (y)			Average	Age (y)		
				≤ 30	31–40	≥ 41		≤ 30	31–40	≥ 41
Sexual intercourse experience	211	55.0	66.7	56.0	76.9	85.7	66.7	56.0	76.9	85.7
STI history	197	7.6	6.0	4.5	15.4	0.0	6.0	4.5	15.4	0.0
Accepts STI check	233	62.2	31.6	29.6	30.8	35.3	31.6	29.6	30.8	35.3
Current smoker	233	19.3	8.8	3.7	7.7	17.6	8.8	3.7	7.7	17.6
Hesitates to visit a hospital	194	58.8	52.1	47.8	63.6	50.0	52.1	47.8	63.6	50.0
Struggles mentally in daily life	205	82.4	77.1	82.6	63.6	78.6	77.1	82.6	63.6	78.6

AMABs, assigned male at birth; AFABs, assigned female at birth; STI, sexually transmitted infection.

Table 2. PEP and PrEP awareness among transgender people

Characteristics			Transgender							
			AMABs				AFABs			
				Age (y)				Age (y)		
	n	Yes responses (%)	Average	≤ 30	31–40	≥ 41	Average	≤ 30	31–40	≥ 41
Knows about PEP	87	20.7	39.1	37.5	50.0	20.0	14.1	16.1	15.4	0.0
Interested in PrEP	87	10.3	13.0	25.0	10.0	0.0	9.4	9.7	11.5	0.0

AMABs, assigned male at birth; AFABs, assigned female at birth; PEP, post-exposure prophylaxis; PrEP, pre-exposure prophylaxis.

sexuality. Available data often do not distinguish between cisgender and transgender populations (*1*). One survey reported that the age-standardized prevalence of never having had heterosexual intercourse among adults aged 18–39 years was 24.6% for "women" and 25.8% for "men" (*5*); however, it is unclear whether these categories were based on gender identity or sex assigned at birth. Overall, approximately 70% of respondents said they had had sexual intercourse. The rate of sexual intercourse among transgender people in our study was only 55%. Some transgender people may also have misconceptions about what constitutes sexual intercourse. It may be helpful to explore patients' understanding of sexual intercourse to address any potential misconceptions.

Here, we identified one chlamydia-positive person among those who requested free STI/HIV testing, despite this individual claiming to never have had sexual intercourse. Without proper awareness, STI/HIV infection rates among transgender people may increase undetected. Thus, measures to raise awareness about transgender-specific STI/HIV testing are urgently needed. In addition, Safer *et al.* (2016) discuss the barriers transgender individuals face in healthcare systems, noting that healthcare providers' lack of knowledge can be a significant challenge in STI/HIV care (*6*). The lack of guidelines addressing transgender-specific risks may contribute to these barriers, potentially limiting the provision of adequate healthcare.

Acceptance of free STI/HIV testing

Free STI/HIV testing is not mandatory for patients. In our study, slightly over half (62.2%) of participants sought free STI/HIV testing, mostly AMABs (31.6%). Reasons for not wanting to be tested included claims of never having had sexual intercourse, no possibility of being infected with an STI, and being uncomfortable discussing sexual matters.

International studies indicate a high prevalence of HIV and STI among transgender people globally (*7*). Physiologically, the risks for HIV and STI among AFABs who retain a vagina compared with those who do not remain unclear. Additionally, the distinct biological risks for HIV and STI among AMABs who have undergone penile inversion or sigmoid colostomy vaginoplasty require further investigation. There are scarce STI studies on transgender men, but the risk of STI and HIV among transgender men who have sex with men (MSM) is considered as high as that among cisgender gay men (*8*). Although the proportion of our study participants who underwent free STI/HIV testing was low, the close relationship between transgender people and STI/HIV suggests the need for regular screening, along with patient education.

Smoking rates among transgender people

The overall smoking rate in 2019 in Japan was 16.7%, with 27.1% in men and 7.6% in women (*9*). In this study, the overall transgender smoking rate was 19.3%, higher than the overall smoking rate in Japan, and the AFABs smoking rate (22.7%) was higher than the AMABs rate. Worldwide, smoking rates are reportedly high among transgender people, especially AFABs (*10*). Health providers should consider the higher burden of smoking-related health morbidities and mortality faced by AFABs when providing lifestyle guidance.

Barriers to health and sexual health care for transgender people

Many transgender people live on the margins of society, facing stigma, discrimination, exclusion, violence, and poor primary care (*11*). Barriers to accessing medical and health care are high for transgender people, and many do not visit a hospital if they have symptoms of illness (*6*). This may contribute to the exacerbation of health conditions and spread of STI/HIV infections. In this survey, more than half of all respondents felt hesitant to visit a hospital, and more than 80% said they struggle mentally each day. The term "struggle" here refers to problems or barriers experienced in daily life because of gender. Transgender people have a high suicide rate, and suicidal ideation is predicted by experiences of discrimination and struggle among transgender people (*12*). Improving access to care for transgender people therefore requires a multi-pronged approach, including

clinician education in gender-affirming care, policy changes in primary care institutions, and advocacy to address social determinants of health. Health providers must have competent knowledge of transgender health problems and address these to provide truly equitable care for transgender people (*13*).

PrEP and PEP awareness among transgender people

PrEP and PEP research in transgender men is limited, but recent studies in transgender women reveal alarmingly high rates of HIV infection. According to a recent international analysis, transgender women are 49 times more likely to be HIV-positive than the general population. Although transgender men are less likely to be HIV-positive than transgender women, their infection rates still exceed those of the general population (*14*).

Transgender MSM do not have easier access to primary care than cisgender MSM, and barriers to HIV testing and PrEP intake are higher. Hence, there is an urgent need for HIV prevention education for transgender MSM (*15*).

Although there is a strong link between transgender people and HIV worldwide, policies to address this issue are lacking. Increasing the number of facilities that provide sexual health care along with GAHT for transgender people, reducing barriers to clinic visits, and improving health care providers' understanding of transgender people are issues to be resolved. Transgender people have the right to understand their own risk of HIV infection, as well as preventive methods such as PEP and PrEP.

Conclusion and suggestions

This study underscores the urgent need for improved primary care and sexual health services for transgender people in Japan. Comprehensive data on the health of this population, particularly concerning STI and HIV, remains scarce. Our survey highlights gaps in sexual health awareness, testing uptake, and overall primary care access.

Enhancing health care services for transgender people involves increasing the availability of primary care and sexual health care, reducing barriers to health care access, and improving knowledge among health care providers regarding transgender health issues. Future research should include more comprehensive data, particularly focusing on high-risk groups, to better inform and implement effective health care policies and practices. Raising awareness about the high risk of STI/HIV infection among transgender people and promoting the use of PEP and PrEP are critical steps toward achieving equitable health care for all.

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