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HIV cure research: a formidable challenge

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Abstract
The ultimate goal of HIV cure research is to allow HIV-infected individuals to be free of disease in the absence of antiretroviral therapy. We discuss current directions and future opportunities aimed at achieving sustained virological remission, and possibly eradication. A multidisciplinary approach to HIV cure research will be important, and ethical, social and behavioural research should be conducted in parallel with basic and clinical research.

The ultimate goal of HIV cure research is to allow HIV-infected individuals to be free of disease in the absence of antiretroviral therapy. This can potentially be achieved in a number of ways. The most obvious and most difficult approach would be to eliminate all HIV-infected cells capable of producing HIV – that is, to achieve eradication of replication–competent virus from an HIV-infected individual. Today, Timothy Ray Brown (the Berlin patient) remains the only person in the world who appears to have been cured. The measures that cured him were extreme and necessary to treat his cancer, although not his HIV infection. Extensive chemotherapy and total body irradiation decreased the number of HIV-infected cells; he received a stem cell transplant with cells that lacked CCR5 (RS) HIV-1 cellular entry co-receptors, and thus, were resistant to HIV [1,2]. Moreover, the HIV quasispecies present in the patient before transplant were CCR5-dependent for viral replication, thereby eliminating the possibility for HIV propagation using the CXCR4 co-receptor [3]. This regimen has very limited applicability, even for those HIV-infected individuals with cancer, as reported CCR5-Δ32/Δ32 prevalence is no more than 1%, and frequently much rarer. Therefore, locating an HLA-matched CCR5-Δ32/Δ32 donor is a substantial challenge.

Cancer treatment and stem cell transplantation without CCR5-Δ32/Δ32 donor cells are not curative for HIV, as shown in the two ‘Boston patients’ [4]. Efforts to screen and store CCR5-Δ32/Δ32 cord blood stem cells that require less stringent HLA match are being pursued by several groups [5,6]. The low cell numbers in each cord blood unit limits their use in adults, although not necessarily in children. Indeed, it has been suggested that a paediatric recipient of such stem cells may have been a second ‘cured’ patient. Unfortunately, the child died of graft-versus-host disease shortly after transplantation, and autopsy revealed no PCR-detectable HIV (Timothy Schacker, personal communication). Reduction of CCR5 expression by gene-editing therapy using zinc finger nucleases has been pursued in HIV-infected individuals without cancer who are receiving antiretroviral therapy and have suppressed viral loads. This approach has resulted in decreased proviral DNA levels and increased CD4+ T cell counts; however, HIV re-emerged in the blood soon after antiretroviral therapy was interrupted [7]. Research in this area is now directed towards improving engraftment of CCR5-modified cells with preconditioning chemotherapy and infusing higher numbers of modified cells. In addition, attempts are being made to use additional genetic modifications, removing CXCR4 expression [8] or including a suicide gene to kill target cells. The latter has been done successfully for cancer [9].

Perhaps a more exciting prospect for HIV eradication is suggested by a recent non-human primate study. In this study, rhesus macaques were vaccinated with a replicating cytomegalovirus (CMV) vector vaccine that expressed simian immunodeficiency virus (SIV) gag, nef, env and pol genes prior to inter-rectal challenge with a highly pathogenic strain of SIVmac239. All of the macaques were infected; however, half went on to have SIV apparently eradicated from all organs [10,11]. The replicating CMV vector vaccine generated ongoing production of SIV-specific effector memory CD8+ T cell responses in blood and tissues. Unlike natural immune responses, viral escape did not occur, probably because strikingly broad CD8+ T cell responses induced by the vaccine did not target the HLA class I-restricted immunodominant epitopes that mutate readily, thereby enabling the virus to evade the immune response. This study provides compelling evidence that immune–based therapy targeting CD8+ responses could be key in eradicating HIV. It also suggests that vaccine regimens could target subdominant epitopes, at least for T cell based approaches [12].

Are there other ways to generate persistent, effective T cell responses to eliminate HIV-infected cells? In this regard, the field of HIV research could be informed by recent successes in the field of cancer immunotherapy. Today, chemotherapy-resistant acute lymphoblastic leukaemia has a 90% long-term remission and, possibly, cure rate after infusion of autologous T cells transduced with a CD19-directed chimeric antigen receptor via a lentiviral vector [13]. In HIV-infected individuals, CD8+ T cells with chimeric antigen receptor-expressing CD4 infused with a CD3 zeta signalling domain have been shown to persist for a decade [14] with the ability to reduce HIV plasma viraemia and home in rectal tissue [15,16]. The engineered CD8+ T cells express CD4, bind HIV gp120 on infected cells, and kill them. It remains unclear if, and how, this approach can clear latently infected cells that, by definition, do not express HIV. Other genetically engineered cell-based therapies that could enhance effector T cell function are artificial T cell receptors that control affinity to certain epitopes, or polyclonal T cells that recognise multiple epitopes including subdominant epitopes [17].

Sustained viral remission in absence of therapy: a more realistic goal?

An alternative approach to absolute eradication of all replication competent virus in achieving a ‘cure’ of HIV infection is the induction of sustained virological remission following discontinuation of antiretroviral therapy. The recent HIV viral rebound in the Mississippi child who was thought possibly to have
been cured after receiving early antiretroviral therapy instituted at 30 hours of life was sobering [18,19]. This case forced the field to reconsider that a feasible goal of a 'cure' effort for HIV infection may not necessarily be eradication of virus but could be sustained virological remission following discontinuation of antiretroviral therapy in the absence of absolute eradication. Since rebound of HIV viraemia upon discontinuation of antiretroviral therapy emanates from the, often latent, reservoir of HIV, greater understanding of the nature, characteristics, cell types and anatomical distribution of the body's HIV reservoirs is critical. Early initiation of antiretroviral treatment currently appears the most effective way to attenuate the size of latent reservoirs of HIV [20]. However, this approach has not been shown to eradicate the virus, most likely due to the very early establishment of reservoirs in long-lived memory CD4+ T cells soon after infection [21]. Indeed, rapid viral rebound was observed in two children after 3 years of antiretroviral treatment even though therapy was initiated within the first 24 hours of life [22,23]. These two cases provide clues to possible predictors of viral rebound. In contrast to the Mississippi child, these children had evidence of ongoing viral replication with detectable cell-associated HIV RNA, HIV-specific T cells or immune activation. The cases also show that initiating antiretroviral therapy within 24 hours after birth (probably at the time of onset of infection), was not early enough. This observation provides a basis for evaluating therapy initiated even sooner – immediately after birth – particularly in babies at high risk for HIV infection (i.e. those born to mothers who received no antiretroviral treatment) to prevent HIV reservoir seeding. This approach of initiation of therapy to the newborn immediately after birth would be most relevant for babies infected perinatally; however, it would theoretically be less effective for babies who may have been infected weeks earlier in utero. The lack of detectable proviral DNA and replication-competent HIV prior to treatment interruption in these cases [22,23] illustrate the limitation of current assays in detecting low numbers of HIV-infected cells [24]. Moreover, the bulk of replicating HIV persists in tissues such as the lymph node and gut where antiretrovirals have limited penetration; it will be important in the future to more carefully examine these sites [25,26]. In this regard, improving measurements of the HIV reservoir in peripheral blood and tissues is a research area under intense investigation. Recent studies in non-human primates underscore the rapidity with which the lentiviral reservoir is established and the inadequacy of combination antiretroviral therapy alone in sterilising viral reservoirs [27].

Early initiation of antiretroviral therapy is a critical step in any pathway towards an HIV cure. In addition, novel therapeutic approaches may be needed. In particular, some have advocated approaches that activate latent reservoirs, thereby allowing them to be destroyed. Clinical trials of such latency-reversing classes of drugs such as histone deacetylase inhibitors suggest that they can induce some expression of latent HIV although not its clearance [28]. Other drug classes, such as activators of protein kinase C or toll-like receptors, may be more effective [29]. Given potentially large HIV reservoirs [30,31], the extent to which these agents could provide adequate awakening of latent virus is unclear. Their potential role, if any, would most likely be together with an immune-based therapy to kill the re-activated cells. Recent work has shown that broadly neutralising antibodies can clear cell-free virus and infected cells that express HIV [32]. Proviral DNA was reduced in macaques treated with these antibodies [33,34]; however, the extent of the ability of antibodies to clear latently infected cells is not well understood. Several human studies are planned to evaluate the effects of passively infused antibodies on the HIV reservoir. Immune checkpoint blockers such as anti-programmed cell death-1 antibody could improve function and persistence of effector T cells [35]. Targeted cytotoxic therapy using immunotoxin is another potential approach [36]. Although therapeutic HIV vaccines have had little success in the past, newer vaccines such as the prime boost adenovirus26/modified vaccinia Ankara vaccine in macaques have shown promise in blunting plasma viraemia and achieving persistent viral control [37].

What are our opportunities going forward? The latest developments in HIV cure research were recently discussed at the US National Institute of Allergy and Infectious Diseases’ Strategies for an HIV Cure meeting in October 2014 (www.blsmmeetings.net/hivcuremeeting2014). It became clear at that meeting that a concerted effort towards an HIV cure is being made on many fronts.

Are we on the right track? Only time will tell. We are making progress and many approaches to a cure have a sound scientific rationale. However, basic and clinical science are not enough; we must also consider important ethical, social and behavioural research questions in parallel [38]. Many of the interventions pose significant health risks with modest (at best) benefit to trial participants. Treatment interruption of antiretroviral therapy is the ultimate test for HIV remission. Still, it presents medical risk without close monitoring and prompt resumption of antiretroviral therapy if a patient’s HIV viral load rebounds. Most of the trial participants so far are young white men. Equity in trial participation for gender, transmission risk groups, age and race remain important issues [39]. Low- and middle-income countries, where the majority of people living with HIV reside, should be encouraged to participate in HIV cure research. This is particularly important for interventions for which efficacy is HIV clade-dependent, such as broadly neutralising antibodies and therapeutic HIV vaccines. The initial trials for HIV cure modalities are generally not longer than 1–2 years. Post-trial access to these experimental interventions may become an important consideration if prolonged HIV remission could be maintained with repeat administrations. The concepts of HIV eradication and remission may be confusing to trial participants. False hope and misperception could lead to uninformed trial participation. Behavioural research on patient expectations, and willingness and decision making to participate in cure research will guide interactions with trial volunteers and the community along with adoption of approaches from normative bodies such as the UNAIDS/AVAC Good Participatory Guidelines [40]. With the rapidly moving science of HIV cure research and its high risk and uncertainty for success, stakeholder engagement should be done early to facilitate its sustainability and should include national and international regulatory and funding agencies, the scientific community, clinicians, the pharmaceutical industry and civil society.

Ultimately, for HIV cure research to have significant public health impact, the interventions must be effective, simple, safe and scalable [6]. We are still in the discovery phase of research toward a cure, and there will be many disappointments. Moving forward it will be critical to put forth our best efforts, collaborate widely and be guided by the best science and highest ethical standards.

**Conflicts of interest**

The authors declared no potential conflict of interest relevant to this work.
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The views expressed are those of the authors and should not be construed to represent the positions of the US National Institutes of Health, the Department of Health and Human Services, the US Army or the Department of Defense.

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Towards the elimination and eradication of hepatitis B

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Abstract

Despite the introduction of vaccination, chronic hepatitis B remains a major cause of liver-related morbidity and mortality including cirrhosis, decompensated cirrhosis and hepatocellular carcinoma. Maintenance antiviral therapy is required for most people, as low rates of cure occur. The stated aim of therapy presently is HBV DNA suppression; effective suppression of viral replication is associated with significant reductions in morbidity from end-stage liver failure and to an extent, hepatocellular carcinoma. Unfortunately, major barriers to cure, such as a reservoir of episomal covalently closed circular DNA (cccDNA) (the HBV minichromosome), and a dysfunctional immune response, pose challenges. These barriers will need to be overcome to ensure higher rates of cure than can be achieved presently. Quantitative and diagnostic testing for HBV DNA is not generally available, hampering effective monitoring and treatment in low-income countries. The majority of patients in resource-constrained countries are not identified before the onset of cirrhosis. Without coordinated action, and transfer of new diagnostic technologies and treatments to low-income countries, recent therapeutic advances will have little effect on the global burden of disease. A shift to curative treatment for the majority would be a major advance in the elimination of hepatitis B. New and improved molecular therapeutics and immunological strategies for the treatment of chronic hepatitis are emerging, however. A number of promising lines of development are in progress. A curative regimen may require a combination of viral suppression via nucleoside analogue therapy to prevent cccDNA amplification and viral propagation, safe selective cccDNA inhibitors to deplete, silence or degrade cccDNA, agents to block the entry of HBV into the hepatocyte plus compounds to prevent capsid assembly and cccDNA interactions. Targeted immune activation could restore the exhausted immune cell repertoire.

Keywords: Hepatitis, chronic hepatitis B, antiviral therapy, nucleoside analogues, interferon

Introduction

Type B hepatitis is caused by the hepatitis B virus (HBV), a small, enveloped DNA. HBV infection can be either acute or chronic, and can range in severity from being inapparent and asymptomatic to severe or fulminant. The chronic disease may be asymptomatic, until progressive and ultimately fatal illness occurs. Acute hepatitis B is defined as a self-limiting disease marked by acute inflammation, and hepatocellular necrosis in association with a transient HBV infection. Chronic hepatitis B is defined as persistent HBV infection accompanied by evidence of hepatocellular injury, inflammation and fibrosis. The diagnosis of chronic hepatitis B is based upon the finding of abnormal concentrations of serum aminotransferases (ALT) and hepatitis B surface antigen (HBsAg) in serum for 6 months or more.

The identification of HBV led to the development of recombinant DNA-derived vaccines that are widely used throughout the world. However, despite the introduction of vaccination, chronic hepatitis B remains a major cause of liver-related morbidity and mortality including cirrhosis, decompensated cirrhosis and hepatocellular carcinoma (HCC). Some countries have developed effective national plans and the World Health Organization (WHO) has developed a Framework for Global Action based on specific interventions ranging from raising awareness to increasing access to care and treatment [1].

Treatment has been restricted to interferon, pegylated interferon or five nucleoside analogues: lamivudine, adefovir, telbivudine, entecavir and tenofovir. Maintenance therapy is required for most people, as low rates of cure occur. In many regions treatment is governed by international guidelines. Long-term suppression of HBV DNA is the achievable endpoint for most patients. Hepatitis B e antigen (HBeAg)-positive patients may lose HBeAg and subsequently HBsAg, but HBsAg loss occurs in a minority. In anti-HBe-positive persons, sustained low levels of replication are induced by nucleoside analogue therapy. Thus, the stated aim of therapy presently is HBV DNA suppression; effective suppression of viral replication is associated with significant reductions in morbidity from end-stage liver failure and to an extent, hepatocellular carcinoma (HCC).

Unfortunately, major barriers to cure such as a reservoir of stable episomal covalently closed circular DNA (cccDNA) and a dysfunctional immune response, pose challenges. These barriers will need to be overcome to ensure higher rates of cure than can be achieved presently. New and improved molecular therapeutics and immunological strategies for the treatment of chronic hepatitis are emerging, however.

Epidemiology and prevention

Over 400 million people are chronically infected with hepatitis B virus. HBV is thus a major cause of liver-related morbidity. Most persons who acquire chronic HBV have been infected at birth or in early childhood, usually during the first 5 years of life [2–4]. Worldwide, up to 650,000 people die from the complications of chronic HBV, cirrhosis and HCC each year [5]. The incidence of HCC and cirrhosis is low before the age of 35, but rises in mid- and later life [6]. Although, in Africa a higher incidence of HCC has been reported in young male adults.

WHO recommends that all infants receive hepatitis B vaccine as soon as possible after birth, preferably within 24 hours, and that the birth dose is followed by two or three subsequent doses [7]. The vaccine is effective in 95% of infants and children but protection may fail in infants born to highly viraemic mothers. By 2012, 183 countries vaccinated infants against hepatitis B as part of primary vaccination schedules [8]. Unfortunately new infections are still occurring and vaccination, while effective in reducing incident chronic disease in endemic regions, will not have the desired impact on the rates of end-stage liver disease and HCC.
in the extant, chronically infected population. Thus, a policy to identify and treat persons at risk with chronic HBV is important.

Transmission

Most persons who are infected at birth or in the first 5 years of life develop chronic hepatitis B [2–4]. HBsAg has been found in blood and in various body fluids (e.g. saliva, menstrual and vaginal discharges, seminal fluid, colostrum and breast milk, serous exudates) and these have been implicated as vehicles of transmission of infection. Transmission of the infection may result from accidental inoculation of minute amounts of blood or fluid contaminated with blood during medical, surgical and dental procedures; immunisation with inadequately sterilised syringes and needles; intravenous and percutaneous drug abuse; tattooing; body piercing; acupuncture; laboratory accidents; and accidental inoculation with razors and similar objects that have been contaminated with blood. The virus may be infective by mouth. There is considerable evidence for the transmission of hepatitis B by intimate contact and by the sexual route. Sexually promiscuous unvaccinated individuals, particularly men who have sex with men, are at high risk; however, infection in adulthood leads to chronic HBV in <5% of cases.

Viraemic mothers, who are seropositive for HBeAg, almost invariably transmit the infection to their infants at the time of, or shortly after, birth in the absence of prophylaxis. Individuals infected at an early age exhibit high levels of vireaemia, and a limited or dysfunctional immune response, and may remain viraemic for decades. Perinatal transmission has been an important factor in maintaining the reservoir of the infection in some regions, particularly in China and Southeast Asia. The mechanism of perinatal infection is uncertain, but it probably occurs during or shortly after birth. However, mother-to-infant transmission only accounts for half of the infections in children and horizontal transmission between children is an important, if poorly defined, route of infection. The probability of a childhood infection becoming persistent declines with age, from around 90% in neonates to less than 5% if infection is acquired in adolescence or later.

A proportion of infants born to HBsAg mothers acquire hepatitis B despite prophylaxis. Research has suggested that the babies most likely to become infected are those born to mothers with very high viraemia, defined as >10^7 IU/mL [9,10], but estimates of the risk of transmission despite HBV vaccination and HBIG vary [11].

Virology

The HBV genome is 3,200 base pairs in length. Analysis of protein coding reveals four conserved open reading frames (ORFs) encoding: HBsAg; HBeAg; the viral polymerase; and the HBx protein. Identification of animal viruses resembling human HBV has led to the characterisation of the replication cycle of the hepadnaviruses. The genomes of a variety of isolates of hepatitis B virus have been cloned and the complete nucleotide sequences determined. There is some variation in sequence (up to 12% of nucleotides) between these isolates and up to nine genotypes (A to I) have been described on the basis of more than 8% nucleotide sequence divergence. HBV replicates largely in the liver. The hepadnaviruses are unique among animal DNA viruses in that they replicate through an RNA intermediate. HBV exists as a 42-nm, double-shelled particle found in serum. HBV has an outer envelope component of HBsAg and an inner nucleocapsid component of HBeAg. In addition to complete virions, incomplete viral particles, 20-nm spheres and tubules, which consist entirely of HBsAg without HBeAg or nucleic acid, are present in serum and outnumber virions. HBeAg can be detected in the liver. The nucleocapsid, HBeAg, is not found free in serum, but within HBV virions and can be detected by histochemical staining in the liver. HBV DNA can be detected in serum and is used to monitor viral replication. HBeAg, unlike HBsAg and HBeAg, is not partuculate, but rather is detectable as a soluble, 17-kDa protein in serum. The HBV surface proteins (HBs) are composed of three proteins: large, middle and small surface proteins, and include the preS1, preS2 and S regions. The large HBs includes the preS1, preS2 and S regions; the middle HBs comprises the preS2 and S; and the small HBs comprises the S region [12,13]. The large envelope glycoprotein on the surface of HBV (and HDV) particles has been shown to play a pivotal role in virus entry. Virus entry incorporates binding of virions to heparan sulphate proteoglycans at the hepatocyte surface and subsequent binding of the myristoylated N-terminal preS1-domain of the L-protein to the sodium taurocholate co-transporting polypeptide – the recently identified HBV (and HDV) entry receptor [13].

On infection of the hepatocyte, the viral DNA is uncoated and converted to a covalently closed circular (supercoiled) form in the nucleus (cccDNA), which is the template for transcription of the viral RNAs including the pre-genomic RNA. There are at least four viral promoters; mRNAs for the synthesis of HBeAg, HBeAg, the viral polymerase and progeny genomes have been identified. Binding of the polymerase to a secondary structure at the 5′ end (epsilon signal, ε) of the pre-genome leads to packaging into immature viral cores in the cytoplasm. The amino terminal domain of the viral polymerase acts as the primer for minus strand DNA synthesis. Minus strand synthesis proceeds by reverse transcription of the pre-genome by the viral polymerase with concomitant degradation of the template. The capsid of HBV is formed by copies of the 22-kDa core protein, and assembled into core particles. HBV capsids (core) are coated with HBsAg to form mature virus particles [14]. HBV DNA is integrated into the genome of hepatocytes and HBsAg can be produced following transcription of integrated viral DNA [15]. Expansion of clones of such cells may be a stage in progression to neoplasia, but integration of the viral genome is not believed to be required for replication of the virus. Integrations of HBV DNA into the host genome seem to be random but ostensibly contribute to the development of HCC.

A number of naturally occurring mutations in the pre-core region preventing HBeAg synthesis have been identified in HBeAg-negative carriers. These variants of hepatitis B virus are found in serum of anti-HBe and HBV DNA-positive persons who have HBeAg in hepatocytes and histological evidence of active chronic hepatitis but lack HBeAg in serum. The C gene has two initiation codons upstream of two regions (pre-core and core) and two potential molecular forms (HBeAg and HBeAg) can be produced. Initiation of translation at the first site (nucleotide 1814) produces a 312-amino acid polypeptide (p25) that has a signal peptide directing it to the endoplasmic reticulum. There a signal piece is removed by signal peptidase to cleave the N-terminal 19 amino acid residues as well as the C-terminal 34 residues; the resultant polypeptide of 150 amino acids is secreted as HBeAg (p15–18), a soluble protein that is the product of 10 residues coded by the pre-core region and 149 residues coded by the C gene. Translation from the second initiation codon (nucleotide 1901) results in unprocessed polypeptides (p23, 183 amino acids), which are assembled into core particles within the liver (p21). Amplification by polymerase chain reaction and subsequent sequencing of DNA from virions in serum of patients lacking
HBeAg has revealed one or more nucleotide substitutions in the pre-core region of the HBV genome and an HBV variant with a G→A mutation at nucleotide 83 in the pre-core region accounts for most cases of HBeAg-negative hepatitis B. A point mutation from G to A creating an in-frame TAG stop codon, with or without additional point mutations in succeeding codons, has been described. This mutation induces a Trp at codon 28, and explains the serological absence of HBeAg. During disease, HBeAg-defective virus may be selected for by immune selection pressure. There is an influence of HBV genotype on the prevalence of pre-core mutations. For example, the pre-core mutation is relatively uncommon in HBsAg persons of North American and Western European origin who are infected with genotype A of HBV and who carry a cytosine at position 1858 (rather than a thymine (uracil) at this position). Uracil at position 1858 may form a base pair with either G or A in nucleotide 1896, but a cytosine at position 1858 cannot pair with the G→A mutation in nucleotide 1896, and this reduces the efficiency of encapsidation and replication.

In the areas where HBV genotype C is common, particularly East Asia, perinatal transmission accounts for more than half the cases of chronic HBV in unvaccinated infants of HBsAg-positive mothers. However, in areas where genotype C is rare, such as Africa, the Middle East and Europe, serocconversion from HBeAg to anti-HBe occurs more frequently in childbearing women. Higher rates of HCC have been found in persons infected with genotypes C and F compared with those infected with genotypes B or D. HCC occurs at a younger age in patients infected with genotypes F and in those infected with subtypes of genotype A found in southern Africa, although aflatoxin exposure may play a role in parts of sub-Saharan Africa [8]. Fortunately, both HBV nucleoside analogue therapy and HBV vaccine are equally effective against all HBV genotypes.

Natural history

The natural history of HBV is complex, not linear and is incompletely understood. Older patients in endemic regions frequently present for the first time with complications of cirrhosis, or even HCC. Several phases of chronic hepatitis B are recognised. The terms ‘immune tolerant,’ ‘immune active,’ ‘immune escape’ and ‘inactive carrier’ phases have been commonly used to describe sequential stages of the disease (Table 1), but it is increasingly recognised that these descriptions are not fully supported by immunological data [16]. Typically, in early childhood and young adults, serum HBsAg and HBeAg are detectable; serum HBV DNA levels are high (usually greater than 10^6 IU/mL) and serum ALT may be normal or only minimally elevated. High levels of HBsAg are found. This pattern is common in young individuals who are infected in the neonatal period and whose infection may last for 10–30 years afterwards. This phase has been referred to as the ‘immune tolerant’ phase, although the concept of true immune tolerance is being challenged. In HBeAg-positive patients, progression to cirrhosis occurs at an annual rate of 2–5.5% with a cumulative 5-year incidence of progression of 8–20%. The high replicative phase may be followed by a phase of active inflammatory disease when symptoms of hepatitis may be present, and serum ALT may become elevated. During this HBeAg-positive phase, exacerbations in serum ALT are observed, accompanied by variable and fluctuating changes in HBV DNA concentrations. Severe histological hepatitis and fibrosis may ensue during this ‘inactive carrier’ phase.

A proportion of patients with chronic hepatitis B may undergo spontaneous seroconversion from HBeAg to anti-HBe (the ‘inactive carrier’ state). The inactive phase is characterised by prolonged and persistent normalisation of ALT and suppression of HBV DNA levels to under 2,000 IU/mL. Lower quantities of HBsAg are present in serum. Some of these patients will eventually lose HBsAg at a rate of 0.5–2.0% per year resulting in a decreased risk of complications but there will still be a risk of HCC. A spontaneous remission in disease activity may occur in approximately 10–15% of HBeAg-positive persons per year. The prognosis for these patients, if stable and without pre-existing advanced disease, is good. HBeAg seroconversion rates are higher in those with raised serum aminotransferases and in patients with genotype D and (in Asia) genotype B infection. Once HBeAg is cleared, the disease can remit and serum aminotransferases become normal. This may confer a good prognosis if seroconversion occurs at a young age, prior to the onset of significant liver disease.

Active chronic hepatitis can occur in HBsAg-positive, HBeAg-negative, anti-HBe-positive persons with serum HBV DNA concentrations >2,000 IU/mL and raised aminotransferases (the previously termed ‘immune escape’ phase). HBeAg is undetectable in these persons because of selection for HBV virions not expressing HBeAg (pre-core mutant HBV). Individuals with anti-HBe-positive chronic hepatitis B tend to be older, and may present with progressive necro-inflammatory change or cirrhosis. HBsAg-negative chronic hepatitis has a variable course, often with fluctuating serum aminotransferases and HBV DNA levels. Severe exacerbations may occur. Progression to cirrhosis is generally more rapid in anti-HBe-positive disease and occurs at an annual rate of 8–20%. Levels of HBsAg are generally 1 log lower than in HBeAg-positive patients.

The immunological profile underlying hepatic inflammation is not fully explained. The concept that the establishment of persistent infection and induction of ‘immune tolerance,’ that is the inability to mount a virus-specific immune response, are linked is being challenged. Studies suggest that young adolescents exhibit a normal Th1 T cell response and harbour hepatitis B-specific T cells that are functionally active. It is possible that this phase is triggered by hepatitis B-specific CD8+ T cells but the hepatic inflammation is not proportional to the quantity of hepatitis B-specific CD8 T cells [17,18]. Other factors such as chemokines and natural killer cell activation may be important. Exacerbations in serum ALT may be observed accompanied by variable decreases in HBV DNA concentrations and can be followed by HBeAg to anti-HBe seroconversion.

Recent retrospective studies have examined survival in compensated cirrhosis due to hepatitis B. The reported yearly incidence of hepatic decompensation is about 3% with a 5-year cumulative incidence of 16%. In a European multicentre longitudinal study to assess the survival of 366 cases of HBsAg-positive compensated cirrhosis, death occurred in 23% of patients mainly due to liver failure or HCC. The cumulative probability of survival in this cohort was 84% and 68% at 5 and 10 years, respectively [19–22]. The worst survival was in HBeAg-negative but HBV DNA-positive subjects. Chinese patients remaining HBeAg positive are more likely to develop HCC.

Diagnosis and pathology

Acute or chronic hepatitis B is usually diagnosed by the detection of HBsAg in serum. Many persons can be detected through routine screening for HBsAg or the presence of abnormal serum aminotransferases. Detection of viral DNA is the optimal method of quantitating hepatitis B viraemia and standardised quantitative
assays are valuable for monitoring virus loads during antiviral therapy [23]. Quantitative assays for HBV DNA were previously limited by a lack of standardisation but a WHO standard has been developed [23]. Unfortunately HBV DNA testing is not widely available in low-income countries.

HBeAg is a marker of viraemia but anti-HBe does not necessarily indicate clearance of virus replication. The levels of aminotransferases may fluctuate with time. Usually, the levels of ALT are higher than those of aspartate aminotransferase (AST). However, with progression of the disease to cirrhosis, the AST/ALT ratio may be reversed. Elevation of these enzymes may be the only abnormality to be found in individuals with asymptomatic and anicteric infections. A progressive decline in serum albumin concentrations and prolongation of the prothrombin time are characteristically observed after decompensated cirrhosis has developed.

Single measures of ALT do not indicate disease status in a disease as dynamic as hepatitis B, and there is a controversy regarding the level below which HBV DNA concentrations are indicative of ‘inactive’ disease, or provide a threshold for initiating treatment [24]. Longitudinal measures, over at least a few months are required. A full staging of the disease includes measures of serum albumin, platelet count, prothrombin time, and assessment of cirrhosis, including measures to determine the presence or absence of oesophageal varices. Ultrasonography is usually used to screen patients for HCC, as part of regular surveillance for HCC.

Alpha-fetoprotein (AFP) is usually monitored in patients with cirrhosis. Non-invasive methods are supplanting liver biopsy and have been validated in hepatitis B. Markers for fibrosis, including APRI and FIB-4 as well as commercial markers can be performed or a FibroScan, to ascertain for advanced fibrosis. Their validation should be encouraged in resource-poor regions [25–27]. Liver ultrasound has fair specificity but low sensitivity for cirrhosis, but may be helpful. Moderate fibrosis may be more difficult to detect by any non-invasive test.

Many clinicians would consider a liver biopsy helpful for ascertaining the degree of necro-inflammation and fibrosis. Hepatic morphology can assist the decision to treat. There are several established methods of scoring histology, measuring activity (necro-inflammation) separately from stage (fibrosis). There are, however, several limitations of biopsy including sampling error, subjectivity and reproducibility, and of course costs, risks and discomfort to the patient, and lack of training opportunities and infrastructure in low-income countries. The activity of hepatitis B can vary over time but ultimately the degree of hepatic fibrosis determines the prognosis. Assessment of fibrosis measures how far the disease has progressed. Progression of disease in hepatitis B is not linear, but is influenced by episodic activity and injury to the liver.

The pathological features of chronic hepatitis B depend upon the stage of the disease, the host immune response and the degree of virus replication. In chronic hepatitis B with mild activity, only rare piecemeal necrosis is seen. Characteristic hepatocytes with eosinophilic ground-glass cells are relatively common in anti-HBe-positive patients with low levels of virus replication. Lobular hepatitis is more common in patients with active virus

<table>
<thead>
<tr>
<th>Table 1. Commonly defined phases of HBV disease</th>
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<tr>
<td>Stage</td>
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<tr>
<td>Immune tolerant</td>
</tr>
<tr>
<td>HBeAg positive</td>
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<tr>
<td>HBeAg positive</td>
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<tr>
<td>High levels of HBV replication (high HBV DNA concentrations)</td>
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<tr>
<td>Minimal histological disease</td>
</tr>
<tr>
<td>Stage seen in many children</td>
</tr>
<tr>
<td>Interferon generally ineffective</td>
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<tr>
<td>Maintenance therapy nucleosides required</td>
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<tr>
<td>Benefit if HBeAg loss at early stage</td>
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<tr>
<td>Obviates progression</td>
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<tr>
<td>Direct inhibition of HBV replication?</td>
</tr>
<tr>
<td>Immune escape</td>
</tr>
<tr>
<td>HBeAg negative, anti-HBe positive</td>
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<tr>
<td>HBeAg-negative disease</td>
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<tr>
<td>HBeAg negative, anti-HBe positive</td>
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<tr>
<td>Ongoing HBV replication</td>
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<tr>
<td>HBV DNA &gt;20,000 IU/mL</td>
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<tr>
<td>Exacerbations of ALT</td>
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<tr>
<td>Older persons</td>
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<tr>
<td>Progressive disease</td>
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<tr>
<td>Nucleoside analogue treatment</td>
</tr>
<tr>
<td>Less commonly, interferon</td>
</tr>
<tr>
<td>Direct inhibition</td>
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<tr>
<td>Immune activation feasible</td>
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<tr>
<td>Reactivation of acute or chronic hepatitis</td>
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<tr>
<td>HBeAg positive or negative</td>
</tr>
<tr>
<td>HBV DNA elevated</td>
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<tr>
<td>Serum ALT elevated</td>
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<tr>
<td>Seroreversion to HBeAg can occur if HBeAg negative</td>
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<tr>
<td>High risk of decompensation if cirrhosis</td>
</tr>
<tr>
<td>Can be precipitated by immunosuppression</td>
</tr>
<tr>
<td>Nucleoside analogue treatment required</td>
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<tr>
<td>Curative treatment would prevent</td>
</tr>
<tr>
<td>Urgent intervention required</td>
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<tr>
<td>Inactive carrier</td>
</tr>
<tr>
<td>HBeAg negative anti-HBe positive</td>
</tr>
<tr>
<td>HBV DNA &lt;2,000 IU/mL</td>
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<tr>
<td>Risk of cirrhosis declines</td>
</tr>
<tr>
<td>HCC risk lower</td>
</tr>
<tr>
<td>Can develop anti-HBe-positive disease</td>
</tr>
<tr>
<td>Monitoring only</td>
</tr>
<tr>
<td>Functional cure</td>
</tr>
<tr>
<td>HBsAg loss obviates frequent monitoring</td>
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replication, and raised serum aminotransferases. CD8+ cells predominate in areas of piecemeal necrosis. HBsAg and HBcAg can be detected by immunoperoxidase staining in routinely fixed liver biopsy sections. Patients with high levels of viraemia may have minimal hepatitis [28].

Co-infections with hepatitis B

HBV and hepatitis delta virus (HDV)

Delta hepatitis (HDV) was first recognised following detection of a novel protein delta antigen, HDAg, by immunofluorescence staining in the nuclei of hepatocytes from patients with hepatitis B [29]. HDV is now known to require HBsAg for its propagation and transmission. HDV is coated with HBsAg, which is required for release from hepatocytes and for entry and propagation. The virus consists of a particle measuring 35–37 nm in diameter with an internal nucleocapsid comprising the genome surrounded by the delta antigen and envelope composed of HBsAg. The genome consists of a single standard circular RNA of around 1,700 nucleotides; the delta antigen is encoded by anti-genomic RNA [30,31].

Two major forms of delta hepatitis infection are known. In the first, a susceptible individual is co-infected simultaneously with both HBV and HDV, which can lead to a more severe form of acute hepatitis. In the second, an individual infected chronically with HBV becomes superinfected with HDV. This may accelerate the course of the chronic disease and cause overt disease in asymptomatic HBsAg carriers. HD antigen has been observed in individuals post liver transplant as HDV replication persists in isolated hepatocytes.

Limited studies indicate a worldwide distribution of hepatitis D infection. The infection is important in southern Europe, Turkey, the Middle East, Japan, Taiwan and parts of Africa including the horn of Africa, West Africa and Saudi Arabia. The disease is encountered in South America. It has been estimated that 5% of HBsAg–positive carriers worldwide, approximately 15 million people, are co-infected with HDV. In areas of low prevalence those at risk of hepatitis B, particularly intravenous drug users, are also at risk of HDV infection. There are eight reported genotypes. Genotype 1 is prevalent worldwide. Genotype 2 is found in Japan, Taiwan and Russia. Genotype 3 is common in the Amazon basin with genotype 4 being found in Taiwan and Japan. Genotypes 5–8 have been detected in Africans [31].

The range of clinical presentation is wide, varying from mild disease to fulminant liver failure. The prevalence of HDV is increasing in northern and central Europe because of immigration. A test for antibody to hepatitis delta is mandatory in all HBsAg–positive persons. Specific serological tests detect antibody to HDV (anti-HD IgM and anti-HD IgG) as well as HDV RNA. Hepatitis D antigen can be detected by histochemical staining. Superinfection of HBV carriers with HDV frequently results in persistent HDV infection. Hepatitis D viraemia is followed by anti-HD IgM and subsequently IgG anti-HD. Markers of HBV replication may be suppressed during chronic hepatitis D.

Treatment of HDV is with pegylated interferon α; however, response rates are poor. Novel therapeutic targets for chronic viral hepatitis are urgently required. The mainstay of treatment of chronic HDV remains long–term pegylated interferon. newer agents such as prenylation or HBV entry inhibitors may prove useful [32]. Patients with decompensated liver disease are candidates for transplantation. Prevention and control measures of HDV are similar to those for HBV: immunisation with hepatitis B vaccine protects against HDV infection.

HIV and HBV

HIV and HBV co-infection is problematic in resource-poor settings. HBV has little effect on the natural history of HIV infection. However, HIV, and its treatment, profoundly affects the natural history of HBV. Appropriate management of both diseases is required. In high-income countries therapy is supported by appropriate diagnostic tests. Antiretroviral therapy in high-income countries usually encompasses tenofovir, which effectively suppresses both HIV and HBV. Monitoring has been simplified. Unfortunately the prior use of lamivudine, previously a key drug in first–line HIV treatment regimens, has led to high rates of resistant hepatitis B in low–income countries. HBV resistance occurred in more than 90% of co–infected individuals after 4 years of lamivudine therapy [33].

Routine testing for HBsAg in HIV–positive individuals has not been widely applied and thus the prevalence of co–infection is not fully established in Asia or sub-Saharan Africa. A test for HBsAg in all HIV–positive individuals is justified and should be mandated. It has become apparent that in many parts of the world, persons with HIV have better access to HBV treatment than HIV–negative HBV mono–infected persons.

In some countries 6–13% of persons with HIV are co–infected with HBV [34,35]. Persons with HIV co–infection can experience a more rapid progression to cirrhosis. Persons in the inactive phase of HBV can experience reactivations in the setting of HIV co–infection with a decline in CD4 cell count [36,37]. Furthermore, when reconstitution of the immune system occurs in patients treated with antiretroviral therapy, flares of hepatitis can occur with elevation of ALT and even fulminant hepatitis if ART therapy does not adequately cover both HIV and HBV [38].

Recent longitudinal cohort studies have found that co–infection with HBV also can lead to progression of AIDS–related outcomes and death in HIV–infected persons [37,39]. The all–cause mortality has been found to be higher in co–infected individuals. The increase in mortality has followed a large decrease in AIDS–related mortality, which declined after the introduction of HAART. The highest rates of liver–related mortality have been observed in persons co–infected with HIV and HBV. Co–infected men are eight times more likely to have died from liver disease than those infected with HIV alone. Thus, comprehensive management of both diseases is required [38].

ALT elevations in co–infected patients may be the result of opportunistic infections, HAART hepatotoxicity, mitochondrial toxicity, HBV clearance, immune reconstitution, emergence of drug resistance, reactivation after withdrawal of therapy, or superinfection with HDV, HAV or HCV. Other general causes of active disease include alcohol or drugs.

HBV and HCV

A European concerted action study provided a comprehensive analysis of hepatitis B and C infections in primary liver cancer in Europe. A high prevalence of co–infection between hepatitis B and C infections has been found in patients with HCC. Persons with HBV who are also co–infected with HCV have a much higher risk of developing HCC in several cohort studies [40]. However, the role of co–infection to progression to cirrhosis is less clear since, in many instances, HCV is the dominant virus and may suppress levels of HBV DNA [41]. Persons with HCV and HBV infection may be more likely to develop an infiltrating and aggressive HCC and are younger than those with nodular HCC, perhaps suggesting accelerated hepatocarcinogenesis [42]. Although hepatitis B virus plays a predominant role in the aetiology of HCC in Africa, co–infection with both viruses may increase the risk [43,44].
Treatment of HBV

Although the disease can be prevented by vaccination, chronic hepatitis B is still a cause of considerable morbidity. Morbidity is linked to ongoing HBV replication. Thus, treatment of existing carriers forms an important part of the control of the disease. There are international, national and association guidelines [45–56] for the treatment of hepatitis B. Some controversies and discordant decisions remain.

The choice of therapy depends upon a number of factors. The major goals of therapy are to prevent disease progression to cirrhosis and to prevent end-stage liver disease or HCC. If HBV replication can be suppressed, the accompanying reduction in histological chronic hepatitis reduces the risk of cirrhosis and HCC. Extrahepatic manifestations of hepatitis B such as glomerulonephritis or polyarteritis nodosa require treatment. In general, treatment should be targeted at patients with active disease and viral replication, preferably before the signs and symptoms of cirrhosis or significant injury have occurred.

Treatment rates have improved over the past three decades with the advent of interferon α and more recently of nucleoside analogues. Interferon α has remained a benchmark therapy for chronic hepatitis B. Interferon α is a naturally occurring intracellular signalling protein that induces an antiviral state in hepatocytes and human liver tissue specimens [57]. Lucifora et al. have recently demonstrated that interferon α can induce specific degradation of the nuclear viral DNA; they proposed that interferon α and lymphotoxin β receptor activation upregulated APOBEC3A and APOBEC3B cytidine deaminases, respectively, in HBV-infected cells, primary hepatocytes and human liver tissue specimens [57].

The main advantages of treatment with interferon α over nucleoside analogues are the absence of resistance and the possibility of immune-mediated clearance of hepatitis B. Pre-treatment factors predictive of response to interferon α have been identified. These include low virus load, high serum ALT levels and increased activity scores on liver biopsy and shorter duration of infection. A number of relative and absolute contraindications to interferon exist and these include Child’s B or C cirrhosis and hypersplenism, autoimmune hepatitis, severe coronaary artery disease, renal transplant disease, pregnancy, seizures, concomitant drugs, retinopathy, thrombocytopenia or leukopenia. Side-effects of interferon are common. Pegylated interferon add-on to entecavir therapy may result in greater suppression of HBsAg than entecavir alone [58]. Combinations of, or sequential therapy with, pegylated interferon and nucleoside analogues probably induce HBsAg synthesis but the effects on cure are marginal. The efficacy of interferon α is restricted: only a proportion will respond. However, sustained suppression of viral replication can be achieved and HBsAg or even HBsAg seroconversion can be attained. Thus, finite courses of interferon α can be successful.

HBV DNA and HBsAg levels can guide treatment although the positive predictive value of HBsAg concentrations at treatment week 12 is suboptimal. HBsAg concentrations of HBsAg >20,000 IU/mL at treatment week 12 have a high negative predictive value irrespective of HBV genotype and can be used to stop therapy in HBsAg-positive patients [59]. Side-effects of treatment with interferon α preclude its use in a proportion of patients and its utility in resource-poor countries is restricted. Eradication is only possible in a minority of patients.

Nucleoside analogues have similar structures to the natural nucleotides and compete at the HBV polymerase catalytic site during synthesis of HBV DNA. They prevent the formation of a covalent bond with the adjoining nucleotide causing chain termination of the elongating DNA. Although all nucleotide analogues act on HBV polymerase, their mechanism differs. Adefovir inhibits the priming of reverse transcription. Lamivudine, emtricitabine and tenofovir inhibit the synthesis of the viral minus strand DNA. Entecavir inhibits three major stages of HBV replication. Nucleosides are less effective against cccDNA formation and thus residual viral replication persists during antiviral treatment [60]. Nucleoside analogues act to inhibit HBV DNA strand synthesis via inhibition of priming or inhibition of chain elongation and are thus effective inhibitors of HBV replication but seldom result in cure, as they have little effect on cccDNA formation or maintenance and the HBV DNA minichromosome. They may have some effect on the immune response after prolonged suppression. Thus, low cure rates and loss of HBsAg are observed. The long-term effects of nucleoside analogues for hepatitis B are unknown.

New treatments

Tenofovir alafenamide fumarate (TAF) is an orally bioavailable phosphonoamidate prodrug of tenofovir. By comparison with tenofovir, TAF enables enhanced delivery of the parent nucleotide and its active diphosphate metabolite into lymphoid cells and hepatocytes. The enhanced delivery is attributed to an improved plasma stability and differential intracellular activation mechanism for TAF relative to tenofovir. TAF is in phase 3 trial and may offer effective and safer treatment for HIV and HBV, and offer economic advantages, but is unlikely to enhance rates of cure [61–63]. Other agents in the pipeline include besifovir [64]. Newer pro-drugs are in development that may bypass the non-productive first phosphorylation step. It is proving possible to deliver potent antiviral drugs encapsulated within biodegradable organic nanoparticles. Early compounds have exhibited a significantly prolonged blood circulation time, decreased plasma elimination rate, and enhanced area under the curve.

Higher rates of cure are being sought by new treatment approaches. Cure of hepatitis B will require several steps for eradication or functional cure in the host. Some of the problems that prevent cure are the maintenance of capsid RT–cccDNA interaction and assembly, and maintenance of HBV replication during nucleoside analogue therapy. The S protein may play a role in tolerance resulting in a poor antibody response. A dysfunctional T cell response and T cell exhaustion also permit chronic infection. Thus, a cure might require prolonged suppression of HBV replication, degradation or silencing of cccDNA and restoration of the innate and adaptive immune response.

New molecules under investigation include entry inhibitors and short interfering RNA (siRNAs), or capsid inhibitors [65]. The sodium taurocholate co-transporting polypeptide has been identified as the HBV (and HDV) receptor [13]. A synthetic acylated pre-S peptide derived from the large protein of HBV that blocks the entry of HBV in susceptible cells (Myrcludex B) is being studied in both chronic HBV and HDV infection [66]. A formulation for subcutaneous application has been developed and initial, promising phase 1 studies with nucleosides and interferon are in progress.

Cai and co-workers have identified two structurally related disubstituted sulphonamides (DSS), CCC-0975 and CCC-0346, which act as inhibitors of cccDNA production [67–69]. Epigenetic regulation may also limit transcription from cccDNA. Current models of chromatin indicate that transcription from cccDNA...
could be reduced by post-translational modification of histones; and acetylation, phosphorylation, methylation and ubiquitylation reactions could be targeted. Two groups of enzymes, histone deacetylases (HDACs) and histone acetyltransferases (HATs), determine the acetylation status of histones, relaxed chromatin is associated with activation of gene expression whereas compacted chromatin is associated with repression of gene expression. The acetylation status of the HBV minichromosome (cccDNA-bound H3 and H4 histones) regulates HBV transcription replication and is reflected in viral load. It may also be possible to mediate degradation of cccDNA in infected hepatocytes. Lucifora et al. have proposed that lymphotixin β receptor activation upregulated APOBEC3A and APOBEC3B cytidine deaminases [57].

It is not clear whether all cccDNA chromatin would need to be cleared for a cure of hepatitis B or whether low threshold levels would result in slowing of the disease (as may be the case in inactive carriers). Can ‘epigenetic’ and immunological control achieve the same effect? Unfortunately there are no proven surrogates for cccDNA that can be studied in early phase experiments, and trials in patients may require liver tissue and validated tools to quantify cccDNA. HBsAg quantitation may serve this purpose but phase 1 dose-finding studies would probably require quantitation of cccDNA with liver tissue.

Methylation of HBV DNA may influence high and low replication phenotypes of HBV. Recent studies suggested that cccDNA contains methylation-prone CpG islands, and that the minichromosome structure of cccDNA is epigenetically regulated by DNA [67]. Epigenetic silencing of cccDNA transcriptional activity could prove an antiviral strategy in cccDNA eradication or silencing [70–73].

The HBV nucleocapsid may be a crucial target because of the interaction between the HBV capsid and cccDNA. HBV core protein mediated the interaction with nuclear cccDNA, an interaction that resulted in cytidine deamination, and cccDNA degradation; thus lymphotixin receptor activation could prove a therapeutic target.

Mutational inactivation is another avenue being explored. Mobile genetic elements in bacteria are neutralised by clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated (Cas) proteins. The CRISPR/Cas9 system is potentially a powerful tool for site-specific cleavage of HBV DNA targets, after direction by a synthetic guide RNA (gRNA) potentially a powerful tool for site-specific cleavage of HBV DNA. The CRISPR/Cas9 system could disrupt HBV in vivo.

Nucleic acid technologies

Several new antisense, siRNA targeting technologies are being examined. These include targeting with ARC 520, which is in phase 2 trial. ARC 520 is an RNA interference (RNAi)-based, liver-targeted antiviral molecule. The compound comprises an equimolar combination of two cholesterol-conjugated siRNA molecules (AD0009 and AD0010) that target HBV RNA transcripts with the aim of triggering a sequence-specific downmodulation of gene expression, reducing viral load and viral proteins [76–79].

HBV replication depends upon the assembly of the core particle composed of the capsid protein polymerase and pre-genomic RNA. A number of new compounds could inhibit or dysregulate encapsidation; capsid disassembly, impeding the entry of RC DNA into the nucleus, could inhibit conversion of rcDNA to cccDNA. Pre-packaging inhibitors of packaging with compounds active against the core are in development, for example BAY41–4109 a heteroaroyldihydropyrimidine compound. Other compounds include phenylpropenamide derivatives. Control of HBsAg secretion could potentially reduce tolerance owing to high HBsAg levels, and several compounds are being investigated.

Regulation of immunity

Restoration of adaptive immunity may be difficult. Chronic hepatitis B is associated with functional exhaustion of HBV-specific CD8 T cells, the result of prolonged exposure to large quantities of HBsAg and HBeAg. The antigen–specific cells express inhibitory molecules such as PD-1, and thus lose their effector function. Blocking these immune regulatory receptors, which may be driving T cell dysfunction, could restore functional T cell activity to exhausted T (and B) cells. In a woodchuck study, blockade of the PD-1 pathway with woodchuck PD-L1 antibody, therapeutic DNA vaccination and treatment with entecavir enhanced virus-specific T cell immunity and led to the resolution of chronic infection in some woodchucks.

Experimental toll-like receptor agonists suggest that HBV replication can be controlled by the activation of innate immune responses in the liver. The effects of immune activation with CS–9620, a selective orally active small molecule agonist of toll-like receptor 7 in chimpanzees with chronic HBV infection, has been investigated. CS–9620 administered to chimpanzees thrice weekly for 4 weeks resulted in a 2-log reduction in HBV DNA, but levels of HBsAg in serum were not altered. The molecule also induced production of interferon α and other cytokines. These pharmacodynamic effects are being further studied in humans [80].

Other strategies

Other new therapeutic strategies and novel immunological therapies include the possible application of therapeutic vaccines to boost HBV-specific T cell responses or offset the dysfunctional immune response and restore an intrahepatic innate immune response. A tasmogen, CS4774, is a genetically modified yeast expressing HBV antigens to activate T cells. Other therapeutic vaccines including adenovirus fusion proteins are in human trials. A full review of potential curative strategies is beyond the scope of this article. However, cure of hepatitis B is the next goal of therapy of hepatitis B. Cure could be considered if patients are HBsAg negative and have undetectable HBV DNA in blood and in liver, have no relaxed circular DNA, no detectable (or functionally silent) cccDNA and are HBeAg negative.

A number of promising lines of development are in progress. A curative regimen may require a combination of viral suppression via nucleoside analogue therapy to prevent cccDNA amplification and viral propagation; safe selective cccDNA inhibitors to deplete, silence or degrade cccDNA; immune activation to activate an immune response or restore an exhausted T cell repertoire; and agents to block the entry of HBV into the hepatocyte, cell spread, or compounds to prevent capsid assembly and cccDNA interactions.

Conclusions

Universal vaccination has fortunately limited the transmission of hepatitis B and limited the incidence of chronic infections in previously high-prevalence regions. In low-income countries diagnostic testing has lagged behind, and therapy has been
limited. Treatment will need to form part of the control of the disease. HBV DNA suppression is effective in preventing progression to cirrhosis and to hepatic decompensation and can reverse advanced fibrosis if present. Treatment may (to a degree) reduce the incidence of hepatocellular carcinoma. However HBsAg loss is infrequent, and the prospect of lifelong maintenance suppressive therapy deters policymakers. Quantitative and diagnostic testing for HBV DNA is not generally available, hampering effective monitoring and treatment. Without coordinated action, and transfer of new diagnostic technologies and treatments to low-income countries, recent therapeutic advances will have little effect on the global burden of disease. A shift to curative treatment for the majority would be a major advance in the elimination of hepatitis B. The relationship between intrahepatic cccDNA and viral replication, and immunological control during chronic hepatitis B is being actively studied; new cell lines that support the entire life cycle of hepatitis B virus are being developed and will provide powerful tools for study. Broadly curative antiviral strategies are the next goal for the worldwide management of hepatitis B.

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HIV-1 resistance to dolutegravir: update and new insights

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Abstract

Integrase strand transfer inhibitors (INSTIs) are the latest class of potent anti-HIV drugs. Currently, three INSTIs have been approved by the US Food and Drug Administration: raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG). Resistance mutations to RAL and EVG emerge rapidly, and significant cross-resistance between these compounds has been documented. In addition, limited cross-resistance has been observed among DTG, a newer INSTI, and RAL and EVG even though clinical resistance to DTG, or mutations associated with DTG resistance in treatment-naive patients, has not yet been observed. This review summarises progress in studies on understanding resistance to DTG, mechanisms of possible resistance to DTG, and reasons for the absence of DTG-associated resistance mutations when the drug has been used in first-line therapy.

Introduction

Antiretroviral therapy (ART), which commonly includes at least three different drugs to maximally suppress HIV viral replication, has led to a decrease in HIV-related morbidity and mortality. Currently, 29 anti-HIV drugs in six different classes have been approved by the US Food and Drug Administration (FDA) and are available for HIV therapy. However, HIV can develop resistance to almost all drugs on the basis of mutations that are usually located within the coding regions of the enzymes that serve as drug targets. Integrase strand transfer inhibitors (INSTIs), which block the integration of the HIV viral DNA into host chromosomal DNA, are the latest class of anti-HIV drugs. To date, there are three approved INSTIs: raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG). Although both RAL and EVG are highly effective in treatment of HIV-positive patients, both drugs have a low-to-moderate genetic barrier to resistance. Indeed, HIV resistance to RAL and EVG can evolve fairly rapidly both in vitro and in patients on the basis of single mutations or combinations of mutations within the HIV integrase [1–4]. Cross-resistance between RAL and EVG has also been observed. However, DTG, a newer INSTI, seems to possess a resistance profile that is different from those of both RAL and EVG. For one thing, DTG often retains activity against RAL- and EVG-resistant viruses and it is the only anti-HIV drug against which HIV has not yet developed resistance mutations in patients who have received treatment with this compound in first-line therapy [5]. This review focuses on the latest findings on resistance mutations to DTG, the underlying mechanisms of possible resistance, as well as reasons for the absence of resistance to DTG and the compounds with which it has been co-administered when these drugs are used in first-line therapy.

Resistance patterns involving DTG

In the case of RAL, primary mutations at positions Y143, Q148 and N155 within the active site of integrase are involved in three major resistance pathways. For EVG, significant primary mutations include T66I, E92Q, N155H and Q148H/K/R. Cross-resistance between RAL and EVG is observed on the basis of mutations at positions 155 and 148. DTG is not often compromised by mutations at N155 but is affected by mutations at position Q148 (Table 1) [6].

Several mutations that are potentially involved in resistance to DTG have been identified either in culture or in the clinic, and these substitutions have occurred at positions F121, S153, G118, E138 and R263 [7,8]. These mutations, alone or in association with secondary mutations, can influence susceptibility to DTG and/or impair viral replicative fitness to varying extents (Table 2). It has been shown, for example, that the R263K mutation in integrase confers low-level resistance to DTG (fold change, FC=2.3-fold) [8]. However, this mutation also impairs integrase strand transfer activity and diminishes viral replication capacity.

M50I was identified as an accessory mutation in association with R263K and was selected under pressure with DTG. Usually, secondary mutations, in combination with primary mutations, increase drug resistance while also restoring viral replication fitness. The natural polymorphism M50I alone does not impair either strand transfer activity or viral replication capacity. Unusually, the addition of M50I to R263K increases resistance to DTG by ~15-fold but it does not restore viral infectivity and replication capacity [9]. A combination of H51Y with R263K increases resistance to DTG by roughly 10-fold, but it also dramatically decreases viral replication capacity by approximately 90%, and is accompanied by a near 80% decrease in enzyme strand transfer activity [3]. Recent studies have shown that the addition of E138K to R263K, while modestly increasing resistance to DTG in cell culture (FC=4.3), slightly increased susceptibility to DTG in cell-free strand-transfer assays from FC=3 to FC=4.4. The combination of E138K and R263K decreased integrase strand transfer activity to about 60% of that obtained with a wild-type (WT) enzyme and also failed to restore viral infectivity (~two-fold decrease) or replication capacity [10].

Mutations in integrase at positions R263K, G118R, H51Y and E138K have been characterised as conferring low-level resistance to DTG. A recent study tested the ability of DTG-resistant viruses harbouring either R263K or G118R together with H51Y to develop further resistance against reverse transcriptase inhibitors such as lamivudine or nevirapine in tissue culture selections. In the presence of lamivudine, WT viruses developed the M184V/I mutation resistance to lamivudine in as little as 6 weeks. The H51Y mutation alone had little or no effect on the speed with which M184V/I occurred, but viruses harbouring R263K were delayed in regard to the appearance of M184V by several weeks. Similarly, the V106A mutation that confers resistance to nevirapine was detected after 6 weeks in the case of WT virus but only appeared between weeks 11 and 14 in selections performed with viruses carrying R263K. G118R- and H51Y/G118R-containing viruses did not develop relevant

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resistance mutations to lamivudine or nevirapine over longer than 25 weeks. These results clearly show that the R263K or G118R mutations, alone or in combination with H51Y, can delay the emergence of mutations responsible for resistance to both nevirapine and lamivudine. It may be that these delays have been caused by the decreased viral replication capacity associated with DTG resistance mutations [11], since no compensatory mutation has been identified for DTG in tissue culture selection experiments that have continued for more than 4 years [5,12]. Interestingly, combinations of R263K with primary resistance mutations for RAL and EVG at positions 143, 148 and 155 have resulted in vastly diminished enzymatic activity that may be incompatible with viral survival, which helps to explain why R263K has never been observed in the clinic together with primary RAL or EVG mutations [13].

As stated above, no virological failure in the context of development of resistance to DTG in treatment-naïve individuals has been reported [5]. This may be partially explained by the fact that the presence of mutations that confer resistance to DTG can impair the ability of HIV to develop further resistance against other drugs such as lamivudine and nevirapine. Thus, DTG-containing regimens may not lead to virological failure if the R263K mutation emerges. This hypothesis will eventually be verified by ultrasensitive sequencing of the integrase gene in residual plasma viral RNA and from the DNA of lymphocytes of patients who have been successfully treated with DTG [11].

Since no treatment-naïve patient treated with DTG has yet developed resistance to DTG, and since the R263K mutation confers low-level resistance to DTG in tissue culture, it may be expected that DTG should play a role in limiting HIV persistence. To study the impact of the R263K mutation on HIV replication capacity and the ability of HIV to establish or be reactivated from latency and/or spread through cell-to-cell transmission, a series of experiments that have continued for more than 4 years [5,12].

Interestingly, combinations of R263K with primary resistance mutations for RAL and EVG at positions 143, 148 and 155 have resulted in vastly diminished enzymatic activity that may be incompatible with viral survival, which helps to explain why R263K has never been observed in the clinic together with primary RAL or EVG mutations [13].

Obviously, baseline sequences in integrase might affect any given mutation in terms of susceptibility to DTG. Indeed, DTG can remain effective against RAL-resistant variants that contain mutations at positions N155H, Y143C, N155H/Y143C and G140S/Q148H in different cells, including C8166, human primary monocyte-derived macrophages (MDMs), and peripheral blood mononuclear cells (PBMCs) [15]. Similarly, it has been shown that DTG is effective against patient-derived RAL-resistant variants that contain either Y143 or N155 mutations in both macrophages and CD4+ T cells, with the exception of Q148H/R-bearing variants that display reduced susceptibility (FC=5.5–19) [16]. In addition, a RAL-treatment-experienced patient harbouring the N155H mutation resistant to RAL, was switched to a DTG-containing regimen for about 10 months. Subsequently, mutations at positions T97A and E138K in integrase developed and displayed 37-fold resistance to DTG. After use of DTG for 10 more months, the sequential acquisition of mutations at A49P, L68FL and L234V led to further resistance to DTG (FC=63-fold). Of the foregoing substitutions, A49P and L234V are novel. It was further observed that the serial acquisition of DTG-resistance mutations was associated with deficits in viral replicative capacity (=41%) relative to levels observed (101%–187%) prior to the use of DTG [17].

The G118R mutation was also detected in a patient harbouring a subtype CRF02_AG virus and for whom treatment with RAL was failing. In addition, an F121Y mutation was detected alongside other mutations in another patient harbouring subtype B and for whom RAL treatment was failing. Phenotypic susceptibility analyses in cell culture showed that the G118R and F121Y

<table>
<thead>
<tr>
<th>Table 1. Resistance pathways for each of RAL, EVG and DTG</th>
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<tr>
<td>Mutational pathways</td>
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<td>Y143 pathway</td>
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<tr>
<td>Y143C</td>
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<td>Y143R</td>
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<td>R263K/H51Y</td>
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Considerable progress has been made on mechanisms of HIV resistance to DTG. Generally, it is accepted that DTG is effective against many RAL- and EVG-resistant variants because binding of DTG to integrase–DNA complexes is much stronger than that of either RAL or EVG [4,12,16,19]. Biochemical studies have shown that a systematic residue interaction network analysis revealed that P145 in the 140S loop (G140–G149) of the intasome can have strong hydrogen bonds with WT complexes [18]. These findings provide valuable information on the mechanisms of resistance to INSTIs and will hopefully be useful for the structure-based design of novel INSTIs with better cross-resistance profiles.

**Mechanisms of HIV resistance to DTG**

Considerable progress has been made on mechanisms of HIV resistance to DTG and other INSTIs. Generally, it is accepted that DTG is effective against many RAL- and EVG-resistant variants because binding of DTG to integrase–DNA complexes is much stronger than that of either RAL or EVG [4,12,16,19]. Biochemical studies have shown that the R263K mutation in integrase, which confers low-level resistance to DTG, results in decreases in strand transfer activity but does not affect 3′-processing activity [20]. Recent computational analysis of the G118R and F121Y mutants demonstrated that communications between residues in resistant mutants are increased compared with those of the HIV-1 intasome. In addition, the chelating ability of the oxygen atoms in INSTIs (e.g. RAL and EVG) to Mg2+ at the active site of resistance mutations was reduced due to conformational change; this is most probably responsible for cross-resistance [21]. More recently, a computational analysis of the G118R and F121Y mutations, conferring high resistance to RAL, EVG and DTG, showed that the G118R and F121Y mutations in integrase were associated with reduced binding affinities to each of the INSTIs studied, due to a decreased number of hydrogen bonds compared with WT complexes [18]. These findings provide valuable information on the mechanisms of resistance to INSTIs and will hopefully be useful for the structure-based design of novel INSTIs with better cross-resistance profiles.

**Perspectives**

Patients receiving DTG-containing regimens in first-line therapy have achieved high rates of success with up to 90% of individuals showing a drop in viral load to below 50 copies of viral RNA per mL. Even though therapy has failed for some patients, no resistance mutations to either DTG or to the nucleoside and nucleotide drugs with which it has been co-administered have been identified in patients who had been treatment naive. Moreover, the selection of resistance to DTG in cell culture has yielded only two mutations that confer low-level resistance, but in association with decreased viral replication capacity. Furthermore, no secondary compensatory mutations that might augment resistance and restore viral replication capacity have been observed in tissue culture selection experiments over longer than 4 years. These results may be explained by the fact that the viruses containing DTG-resistant mutations are relatively replication impaired and may be unable to replicate efficiently in patients. Therefore, we have speculated that the development of low-level resistance to DTG in first-line therapy might not have adverse clinical consequences and that DTG might be a useful agent to employ in treatment as prevention (TasP) protocols, since its use might obviate the problem of drug resistance while reducing viral load on a population level; such a strategy could

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Virus</th>
<th>Cell type</th>
<th>Susceptibility to INST (Fold change)</th>
<th>RC (Fold change)</th>
<th>STA (Fold change)</th>
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<tr>
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<td>G118R/E138K</td>
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<td>0.13</td>
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RC: replication capacity; STA: strand transfer activity; WT: wild-type

**Table 2.** Effects of mutations in integrase on resistance to INSTIs, viral replication capacity and strand transfer activity

mutations conferred broad cross-resistance to all three currently used INSTIs. In fact, higher levels of resistance were reported for clinical isolates than were observed for site-directed mutants containing the same substitutions in an NL43 backbone (Table 2) [18]. These results suggest that DTG should be used with caution in INSTI salvage therapy for patients for whom RAL–containing regimens have failed and/or that such patients may require that the DTG be protected by other active drugs in this setting.
eventually result in vastly diminished rates of HIV transmission [3,12].

This hypothesis could be tested in a study in which DTG is employed as a monotherapy in treatment-naïve patients. In such a situation, resistance mutations to DTG in both the RNA of patient plasma samples as well as in the DNA of patient lymphocytes would need to be intensively monitored by ultrasensitive sequencing methods. Our hypothesis would be partially validated if the results showed an absence of resistance mutations to DTG, similar to the findings of the phase III clinical trials on the use of DTG triple therapy to treat first-line HIV-infected subjects. Moreover, a number of cycles of DTG monotherapy might help to convert HIV in latent reservoirs to impaired forms in the aftermath of activation of such reservoirs, if compensatory mutations were unable to develop. This concept could be tested in animal models such as rhesus macaques that are infected by simian immunodeficiency virus (SIV) or in humanised mice that are infected by HIV [12]. Of course, the fact that viral evolution may be significantly impaired in the case of R263K/H51Y-containing viruses, as shown in the studies on resistance to lamivudine and nevirapine cited above, may also mean that changes in relevant viral antigens may also be much less likely to occur. In this context, anti-HIV immune responsiveness may remain durable over very long periods of time and also limit the likelihood of viral rebound.

Further characterisation of the resistance profile of DTG in both tissue culture and the clinic is essential. More sensitive assays such as next-generation sequencing for the detection of tissue culture and the clinic is essential. More sensitive assays such as next-generation sequencing for the detection of low-level viraemia and minority resistant variants will be such as next-generation sequencing for the detection of ultrasensitive sequencing methods. Our hypothesis would be partially validated if the results showed an absence of resistance mutations to DTG, similar to the findings of the phase III clinical trials on the use of DTG triple therapy to treat first-line HIV-infected subjects. Moreover, a number of cycles of DTG monotherapy might help to convert HIV in latent reservoirs to impaired forms in the aftermath of activation of such reservoirs, if compensatory mutations were unable to develop. This concept could be tested in animal models such as rhesus macaques that are infected by simian immunodeficiency virus (SIV) or in humanised mice that are infected by HIV [12]. Of course, the fact that viral evolution may be significantly impaired in the case of R263K/H51Y-containing viruses, as shown in the studies on resistance to lamivudine and nevirapine cited above, may also mean that changes in relevant viral antigens may also be much less likely to occur. In this context, anti-HIV immune responsiveness may remain durable over very long periods of time and also limit the likelihood of viral rebound.

Further characterisation of the resistance profile of DTG in both tissue culture and the clinic is essential. More sensitive assays such as next-generation sequencing for the detection of low-level viraemia and minority resistant variants will be important. The development of new classes of anti-HIV drugs with high genetic barriers for resistance that do not show cross-resistance with current drug classes is still needed [22].

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Animal models in HIV cure research
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Abstract
Current HIV antiretroviral therapy (ART) successfully inhibits viral replication in the majority of HIV-infected individuals. However, ART is not curative and lifelong adherence is required. Despite the undisputed benefit of ART, long-lived latently infected cells that carry HIV-integrated DNA remain. Hence, upon ART interruption, HIV-infected subjects experience viral rebound. Interestingly, similar disease course occurs in the well-characterised animal model of SIV-infected non-human primates. Using these animal models to investigate the mechanisms involved in the generation of latently infected cells, define the phenotypic and anatomical nature of persistent viral reservoirs, and test novel interventions for viral eradication, is critical for strengthening our understanding of HIV persistence and developing novel therapeutics aimed at curing HIV. In this review, we discuss the current animal models used in AIDS cure research, with a particular focus on non-human primates, and outline the experimental strategies explored in the quest for virus eradication.

Keywords: Non-human primates, SIV, ART, residual inflammation, viral persistence, immune-based interventions, immune checkpoint blockers

Introduction
AIDS was first identified in the early 1980s and remains one of the most devastating infectious diseases in recent history [1–3]. AIDS is caused by HIV, of which there are two known forms: HIV-1, responsible for the pandemic, and HIV-2 [4]. The high rates of mortality initially attributed to HIV/AIDS have been significantly reduced as a result of the development and success of antiretroviral therapy (ART). Current ART regimens have the ability to reduce or fully suppress virus replication, increase peripheral CD4 T cell counts in the blood, and improve the health of HIV-infected persons overall; thus, adherence to ART can significantly prolong life expectancy in the majority of infected individuals [5,6]. Nevertheless, despite its success in reducing viral burden, ART alone is unable to cure HIV infection. One of the major limitations of ART is its inability to eliminate replication-competent, latently infected cells – the HIV reservoir – from infected individuals. As a result, within weeks of treatment interruption, patients experience viral rebound [7]. Mathematical modelling of ART suppression in HIV-infected individuals estimated that ART alone would take nearly 80 years to sufficiently eradicate HIV infection [8–10]. ART is also limited in its ability to fully restore immune system function to pre-infection levels, including full antiviral CD8 T cell functionality and CD4 T cell reconstitution. Furthermore, many ART-suppressed patients experience chronic low-level inflammation that has been consistently associated with end-organ disease and early mortality for individuals receiving effective ART [11,12]. Recent findings support a model in which residual inflammation critically contributes to HIV persistence during ART by several mechanisms, including driving the infection of susceptible cells, upregulating the expression of immune checkpoint blockers (ICBs), and limiting the function of HIV-specific immune responses that could potentially clear the virus [13]. This suggests a close connection between inflammation, immune dysfunction and HIV persistence. As such, there is a strong consensus that a cure for HIV infection will not be achieved through ART intensification alone, and that novel approaches aimed at limiting residual inflammation and enhancing antiviral responses in combination with ART are needed.

Optimising animal models for studies of HIV eradication and cure
The design and testing of novel interventions aimed at curing HIV infection necessitates the use of animal models, particularly, the non-human primate (NHP) and humanised mouse systems. NHP models have become powerful tools for the study of HIV transmission, pathogenesis, immune responses and vaccine. The best characterised NHP models for HIV infection are those of rhesus macaques (RMs) infected with either SIVmac251 (quasispecies) or related clone SIVmac239. Infection of RMs with either isolate yields nearly identical clinical features of pathogenic HIV-1 infection including: rapid depletion of mucosal CD4 T cells; chronic immune activation; and AIDS-related opportunistic infections [14]. Furthermore, naturally occurring NHP genetic variation in HLA alleles seems to play a role in infection outcome as it does in the human disease state. Despite their extensive use in understanding the immunobiology of HIV infection and transmission, NHPs have only recently been used for HIV eradication studies [15].

In developing animal models to be used in HIV cure research, it is imperative for the models to recapitulate the current state of suppression in HIV-infected individuals, that is, to fully suppress plasma viraemia in the majority of infected individuals, but also to fill in the information gaps where human studies are limited. Historically, a key limitation to studying sources of persistent reservoirs and latently infected cells in SIV-infected RMs was the lack of an effective ART regimen that fully and consistently suppressed virus replication. Recent studies, including work from our laboratory, have developed a suppressive ART regimen (<50 copies/mL; limit of detection of the standard HIV viral load assay) in the pathogenic RM model [15–19], which has yielded numerous advantages in the study of viral persistence, such as the ability to experimentally control the acquisition of infection and ensure compliance to the ART regimen. Yet, similarly to humans, there is still no experimental evidence of viral clearance, as latently infected cells harbouring replication-competent virus persist in the host that, upon ART interruption, are the major contributors to viral rebound. Another advantage of using NHP models for HIV cure studies is the ability to perform longitudinal collections from both blood as well as lymphoid and mucosal tissues, thus informing both the seeding of the viral reservoir, as well as its immunophenotype and anatomical location – two key unanswered questions in the field of HIV persistence. These data
have traditionally been very difficult to obtain in HIV-infected humans, in which only limited tissue samples can be obtained. Finally, the ability to quantify the latent viral reservoir has recently been optimised for NHPs, including the development of assays measuring cell-associated and tissue-associated SIV-DNA and RNA, high sensitivity viral load (limit of detection of 1 copy SIV-RNA/mL), and the viral outgrowth assay [20]. With the optimisation of assays that are able to accurately represent the levels of cell-associated, and, more importantly, replication-competent virus in the NHP system, the SIV/RM model is well suited to become an essential animal model for testing novel therapies aimed at reducing and/or eliminating persistent virus, particularly in the case of risky immune-based interventions in vivo. Nevertheless, NHP studies are currently limited by high costs.

An additional animal model currently being established to study HIV is the humanised bone marrow/liver/thymus (BLT) mouse [21]. BLT mice are irradiated and reconstituted with donor CD34+ haematopoietic progenitors so that human haematopoietic cells are present in all tissues [22]. The result is that these mice are then susceptible to HIV infection via oral, rectal, vaginal and intravenous routes [23,24]. This in vivo small animal model of HIV infection provides important benefits, including the ability to infect with the ‘true’ HIV-1 virus, as well as the potential cost reduction and usage of larger cohorts for statistical power. However, each individual BLT mouse requires the procedure for ‘humanisation’ and is unique. Moreover, humanised mice typically have shorter life spans than their common inbred laboratory peers, are severely immunocompromised, and lack some haematopoietic cell types, yielding immune responses that are not entirely representative of HIV-infected humans. Nevertheless, establishment of HIV latency was recently demonstrated in humanised mice in which a three-drug ART regimen was able to suppress HIV replication with subsequent viral rebound upon ART discontinuation [25]. At present, an important drawback of the humanised mouse model in addressing key questions of HIV eradication strategies is the low sensitivity of the viral load assay available (750 copies/mL) compared to the standard clinical assay (50 copies/mL), due primarily to the amount of plasma that can be obtained during blood draws.

In the next sections, we review current uses of animal models for the study of HIV eradication, and their use in understanding three critical areas in HIV cure research: namely, the phenotype and anatomical location of the latent viral reservoir; the causes of HIV persistence and residual immune dysfunction during ART; and the design of therapies to eliminate reservoirs and achieve full immune recovery. The development of fully suppressive ART therapy for SIV-infected RMs has afforded the opportunity to address these key questions for HIV cure, as well as to investigate ART-additive, single or combined immune-based interventions to achieve a functional cure. Because most of our current understanding is derived from NHP models, these are the focus of our review.

Animal models to define the seeding and phenotype of the HIV reservoir

A major obstacle in the quest for a cure is our incomplete understanding of the kinetics, anatomical compartmentalisation, and phenotype of the persistent HIV reservoir. To properly investigate these features of the HIV reservoir, it is essential to have access to infected individuals and/or start ART in the first days following infection, in combination with accessing multiple anatomical compartments. These tasks are very difficult to perform in HIV-infected humans; thus, this is one area of research in which the NHP model of HIV infection can critically contribute to fill in the information gaps. In this context, key advantages of the SIV/RM model include: (i) the ability to experimentally control the acquisition of infection and ensure compliance to the ART regimen; (ii) characterising viral reservoirs in several tissues that can be monitored longitudinally; and (iii) the ability to deplete in vivo specific cell subsets (CD4 T cells, monocytes/macrophages, CD8 T cells, B cells, etc.) in order to investigate their direct (as viral targets) or indirect (as key players for the antiviral immune response) contribution to HIV persistence.

Recent studies employed the SIV/RM model to understand the early events of reservoir seeding and persistence during ART. Whitney et al. demonstrated that the SIV reservoir is seeded rapidly following intra-rectal SIV infection and before detectable plasma viremia [17]. To determine if early ART is able to prevent establishment and/or long-term maintenance of viral reservoirs, RMs were treated during a 3–14-day window post mucosal challenge with SIV. Even the most aggressive treatment regimen, initiated at only 3 days post infection, did not eradicate viral reservoirs, as demonstrated by SIV rebound upon ART interruption. This study provides compelling evidence that SIV reservoirs are established rapidly upon mucosal infection, and early ART is not sufficient to prevent or eliminate latently infected cells. Furthermore, these findings demonstrate the great clinical challenge of initiating ART prior to the seeding of the reservoir if HIV latency is established similarly to the SIV/RM model. Evidence of rapid establishment of HIV latency was observed in the clinical case of the ‘Mississippi baby’ [26]. This infant born to a woman who was HIV positive began receiving ART 30 hours after birth, owing to high-risk exposure, and ART was continued when detection of HIV DNA and RNA were confirmed. Therapy was then discontinued when the child was 18 months of age. Levels of plasma HIV RNA, proviral DNA in PBMCs, as well as HIV antibodies remained undetectable in the child through the next 27 months off-ART, thus generating the hope that the child was cured. Unfortunately, later examinations revealed viral rebound in this child. Thus, parallel to the experimental animal model, early ART initiation was insufficient to avoid the establishment of a latent viral reservoir [17].

In addition to understanding the kinetics of viral reservoir establishment, it is also paramount to define the cellular and anatomical nature of the viral reservoir. Indeed, a phenotypic characterisation that investigates anatomical compartments where the virus persists during ART has become indispensable for the design of targeted interventions able to eradicate HIV. Ongoing studies from different laboratories aim to define the phenotype, transcriptome, and localisation of persistent HIV reservoirs through the use of the SIV/RM animal model. Building on findings generated in HIV-infected humans that support Programmed cell Death protein-1 (PD-1)-expressing CD4 T cells as an important source of HIV persistence [27], several groups are using the SIV/RM model to understand whether the combined expression of immune checkpoint blockers, including, among others, PD-1, on CD4 T cells is associated with increased SIV-DNA content, and a critical source of latent virus.

While we are still in the early phases of these studies and further work is necessary to elucidate the source(s) of viral reservoirs, it is important to highlight that cutting-edge techniques, such as single cell transcriptome and whole body immunoPET/CT scan imaging based on radiolabelled anti-SIV antibodies, are currently successfully applied to ART-treated, SIV-infected NHPs (P Johnson and F Villinger, personal communication).
Animal models to test novel therapeutic approaches for HIV cure

A second key area in which the NHP model of HIV infection can critically contribute in the quest for an HIV cure is in the design and testing of novel therapeutic approaches. In particular, several studies are exploring immunomodulatory interventions to be combined with ART in an effort to improve the reconstitution of the CD4 T cell compartments, augment the quality of antiviral immune responses, reactivate and/or eradicate latently infected cells, and/or reduce persistent, residual inflammation (Table 1); these are summarised in this section.

Interventions targeting mucosal immunity and residual immune activation

The gastrointestinal (GI) tract has been shown to play an important role in HIV infection because it is a primary site of viral replication and exhibits profound immune dysfunction [28,29]. Pathogenic SIV infection in RMs recapitulates human disease course in the GI tract, by generating high levels of virus replication as well as a severe depletion of intestinal CD4 T cells, including CD4 T cell subsets critical for mucosal immunity, such as Th17 and Th22 cells [30]. This loss of CD4 T cells associates with impaired mucosal barrier integrity and translocation of microbial products from the GI tract to extra-intestinal sites [30,31]. Owing to the high frequency of infected cells and the high levels of immune activation, the GI tract is also considered an important site of HIV/SIV persistence [32–34]. Therapeutic interventions that target mucosal homeostasis in ART-treated, SIV-infected NHPs have been the focus of several recent studies. Klatt et al. [35] have shown that administration of symbiotic probiotics and prebiotics (PP) in ART-treated SIV-infected pigtail macaques resulted in increased frequencies of intestinal antigen-presenting cells, enhanced reconstitution and functionality of colon CD4 T cells, and decreased fibrosis of lymphoid follicles in the colon [35]. This study provides evidence that PP treatment as a supplement to ART may improve restoration of mucosal immunity and reduce inflammatory complications of HIV infection.

Our group recently conducted a study in which interleukin-21 (IL-21) was administered in SIV-infected RMs in conjunction with a potent three-class, five-drug ART regimen that sustained total body irradiation [36]. Compared to ART-treated SIV-infected RMs (controls), ART+IL-21-treated animals showed improved restoration of intestinal Th17 and Th22 cells as well as a faster and more pronounced reduction in the levels of activated (HLA-DR+CD38+) and proliferating (Ki-67+) T cells in the rectum and blood [16]. Intriguingly, ultrasensitive PCR (limit of detection <3 copies/mL) and tissue-associated viral-DNA assays revealed that plasma SIV-RNA and rectal SIV-DNA contents were significantly reduced between an early and late experimental time-point on ART only in IL-21-treated RMs. Finally, IL-21-treated RMs maintained significantly lower levels of T cell activation compared to controls up to 8 months after ART-interruption, which were associated with an increased CD4:CD8 T cell ratio in blood [16].

Taken together, these studies support the existence of a molecular link between mucosal immunity, inflammation and HIV persistence. This molecular link provides rationale to include immune-based interventions that supplement ART, specifically those that target mucosal immunity, as a key component of combined interventions aimed at curing HIV. To this aim, further exploration into the mechanisms which continually drive residual inflammation, despite viral suppression, will be essential in better designing therapies that can achieve a functional cure.

Interventions targeting immune checkpoint blockers

Chronic infection often results in T cell exhaustion with antigen-specific T cells expressing high levels of one or more ICBS, such as PD-1. Interventions that reinvigorate exhausted antiviral immune functions have become a major focus of HIV cure research, with the rationale that these reinvigorated HIV-specific CD8 and CD4 T cells will have a renewed capacity to suppress virus replication, thereby preventing disease progression even upon ART interruption. In addition to improving antiviral immune responses, the rationale to block the PD-1/PD-L1 pathways comes from studies in HIV-infected humans indicating that cells expressing PD-1, and potentially other ICBS, are enriched in proviral DNA [27].

Several groups have shown that blockade of inhibitory pathways in the natural history of infection can partially restore T cell function and impact viral replication [36]. More recent studies on ART-treated, SIV-infected RMs suggest that blockade of PD-1 or its ligand PD-L1 can delay viral rebound following ART interruption [37–39]. In particular, Mason et al. [37] demonstrated that PD-L1 blockade in conjunction with fully suppressive ART resulted in a delayed viral rebound following ART interruption in half of the SIV-infected RMs when compared to controls. Amancha et al. [38] found that SIV-infected RMs treated with both rPD-1–Fc and ART (PMPA/racivir) showed

### Table 1. Summary of the major ART-additive intervention studies in non-human primates aimed at understanding and/or impacting HIV latency, immune function and eradication

<table>
<thead>
<tr>
<th>Reference</th>
<th>NHP species</th>
<th>Virus</th>
<th>Additive intervention</th>
<th>Duration of ART (months)</th>
<th>Main effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klatt et al. [35]</td>
<td>Pig-tail macaques</td>
<td>SIVmac239</td>
<td>Administration of probiotics/prebiotics</td>
<td>5</td>
<td>Improved reconstitution of intestinal CD4 T cells and lower frequency of those expressing Ki-67</td>
</tr>
<tr>
<td>Micci et al. [16]</td>
<td>Rhesus macaques</td>
<td>SIVmac239</td>
<td>Administration of interleukin-(21)</td>
<td>7</td>
<td>Reconstitution of intestinal Th17 cells and reduction of systemic and intestinal residual inflammation, reduced cell-associated SIV-DNA in the GI tract during ART</td>
</tr>
<tr>
<td>Mason [37]</td>
<td>Rhesus macaques</td>
<td>SIVmac251</td>
<td>Administration of anti-PD-L1</td>
<td>10</td>
<td>Improved control of immune activation, viral replication, and CD4 homeostasis following ART interruption</td>
</tr>
<tr>
<td>Del Prete et al. [19]</td>
<td>Rhesus macaques</td>
<td>SIVmac239</td>
<td>Administration of SAHA</td>
<td>up to 12</td>
<td>Increased histone acetylation and cell-associated SIV–vRNA–vDNA ratio post-SAHA administration</td>
</tr>
<tr>
<td>Mavixner et al. [58]</td>
<td>Rhesus macaques</td>
<td>RT–SHIV TC</td>
<td>Autologous haematopoietic stem cell transplant post total body irradiation</td>
<td>up to 4</td>
<td>Rapid viral rebound post-ART interruption despite successful HSC in two out of three animals</td>
</tr>
</tbody>
</table>
slower rebound in plasma viral loads as compared to ART-treated RMs alone. Vargas-Inchaustegui et al. [39] found slightly different responses based on treatment strategy. In this study, chronically SIV-infected RMs that had received PD-1 blockade during ART administration experienced viral rebounds upon ART interruption similar to those treated with ART alone; however, animals who continued to receive PD-1 blockade after ART interruption experienced reduced rebound viremia. Therefore, in both studies, PD-1 blockade resulted in improved T cell function and a delayed viral rebound upon ART interruption. Differently from Mason et al., the Amanca and Vargas-Inchaustegui studies did not achieve full suppression as evidenced by ongoing viremia, and thus, interpretation of these results in the context of HIV cure is more complicated. Indeed, a lack of full viral suppression by ART prior to its interruption may have contributed to persistent antigen exposure and T cell exhaustion despite ART. The more recently developed humanised mouse model has not yet been used for studies involving ART treatment in tandem with immune-based interventions. Nevertheless, consistent with outcomes from similar NHP studies [36], PD-1 blockade in HIV-infected humanised mice, in the absence of ART, also resulted in decreased viral replication and increased CD4 T cell levels [40,41].

Targeting multiple mechanisms involved in T cell exhaustion is an attractive strategy to more broadly reinvoke the antiviral immune response in the setting of HIV infection. Apart from PD-1, several other ICBs have been shown to regulate T cell responses, and be expressed at elevated levels, during chronic viral infections, including TIM-3, LAG-3, CD160, and 2B4 [42–44]. Furthermore, CD4 T cells that express a combination of these markers may be key contributors to the HIV reservoir. Therefore, future studies that test the efficacy of dual and triple co-inhibitory molecule blockades in vivo will be critical to determine if targeting multiple molecular networks can better re-equip the immune system with effective antiviral activity while also purging latent viral reservoirs.

Studies of HDAC inhibition to reactive latent reservoirs in animal models

Another approach that has been examined in ART-treated SIV-infected RMs is the administration of the histone deacetylase inhibitor (HDACi) suberoylanilide hydroxamic acid (SAHA; Vorinostat). The aim of this approach, named ‘shock and kill’, is to purge latent HIV reservoirs during ART, thus blocking de novo infection of uninfected cells, using the assumption that the viral reservoir from which the virus is reactivated will be killed by the antiviral immune response and/or the cytopathic effects of the reactivated HIV [45]. Thus, the combination of purging latent virus and blocking infection of new target cells with ART may progressively reduce viral reservoirs. Treatment of latently infected cells with SAHA and other HDACi compounds has been shown to increase histone acetylation as well as induce viral transcription and virus production in several in vitro models of HIV latency [46–48] and in primary CD4 T cells from cART-suppressed patients [49,50]. Furthermore, the levels of cell-associated HIV-RNA in resting CD4 T cells transiently increased in ART-suppressed patients following a single oral administration of SAHA, demonstrating the potential for this strategy to perturb viral latency in vivo [51]. In a study conducted by Del Prete et al. [19], repeated administration of SAHA in ART-suppressed SIV-infected RMs resulted in increased histone acetylation and cell-associated SIV vRNA:vDNA ratio, a correlate of viral transcription, in peripheral blood mononuclear cells. However, in vivo viral RNA and DNA were detectable in all animals at the end of the SAHA treatment course, suggesting limited effects on the persistent virus pool. Similar results were more recently observed in ART-treated HIV-infected patients receiving multiple doses of vorinostat [52], thus confirming the relevance and high translational value of the SIV/RM model.

Future studies that increase the duration of SAHA administration or that combine the use of multiple reactivation agents should be performed, since these strategies may enhance the extent of reactivation of the latent HIV reservoir. Interestingly, several recent findings question one of the main postulates of the ‘shock and kill’ strategy, i.e. that HIV reactivation from latently infected cells will result in the death of those cells [53]. As such, interventions that combine latency reversing agents with immune checkpoint blockades (to improve T cell cytoxicity) would be helpful to better understand the potential of HDACi in clearing the HIV reservoir.

Autologous haematopoietic stem cell transplant in rhesus macaques:

Only a single case of ‘cured’ HIV infection has been reported [54,55]. Timothy Brown, known as the ‘Berlin patient’, was diagnosed as HIV positive and, later in life, with acute myeloid leukemia (AML). He underwent a haematopoietic stem cell transplant (HSCT) from a donor who carried a homozygous 32-base pair deletion of the CCR5 gene (delta32 mutation) [54], frequently found in Northern Europeans and reported to enhance resistance to HIV and progression to AIDS. As a result, he has lived in the absence of ART for 6 years and remains free of detectable viral RNA and DNA; therefore, he is considered cured. More recently, the effects of a reduced-intensity conditioning, allogeneic HSCT from donors with wild-type-CCR5+ cells have been studied in two HIV-infected patients with lymphoma [56]. In these patients, HIV DNA was undetectable from the peripheral blood and the rectal mucosa for extended periods of time following HSCT (2.6 and 4.3 years, respectively). Furthermore, no replication-competent HIV was recovered from co-culture assays involving a large number of purified CD4 T cells from either patient. Plasma HIV-RNA and cell-associated HIV DNA remained undetectable until 12 and 32 weeks after ART cessation in the two patients. Unfortunately, this suppression was not sustained and both patients experienced viral rebound [57].

Recent work by Mavigner et al. used autologous HSCT in ART-treated simian/human immunodeficiency virus (SHIV)-infected RMs [58]. This was a small-scale study to demonstrate the feasibility of HSCT in the NHP model and to understand the effects on viral persistence. SHIV-infected RMs were treated with ART (plasma viremia <100 copies/mL), and autologous haematopoietic stem cells (HSCs) were banked prior to infection. Between 37 and 54 days on ART, three out of six SHIV-infected RMs received myeloablative total body irradiation (TBI) in which 94–99% of circulating CD4 T cells were depleted. Following TBI, autologous HSCs were successfully engrafted. Between 40 and 75 days post transplant, ART was interrupted and plasma viremia was monitored. Two out of three treated RMs exhibited rapid viral rebound (that interestingly was even higher than the rebound found in controls), while the third exhibited undetectable plasma viremia and SHIV DNA in peripheral blood up to 2 weeks post-ART interruption. However, this animal had to be euthanised due to its poor clinical condition, and upon further analysis, it was determined that SHIV DNA was detectable in tissues at the time of necropsy, though at lower levels when compared to controls. This study has been an important proof of concept that autologous HSCT is feasible in ART-treated SHIV-infected RM, and provides a novel experimental method of investigating interventions to eradicate HIV.
Furthermore, this study suggests that the conditioning treatment (at least that used in this study) is insufficient to achieve HIV eradication.

Conclusion

In the past few years, great efforts have been dedicated to the development of animal models for HIV cure research. Although optimisation is still in progress, NHP and humanised mouse models are becoming powerful tools to address critical questions on the mechanisms of HIV latency as well as on the cellular and anatomical nature of the HIV reservoir. As the mechanisms of HIV persistence and the phenotype of latently infected cells become more clear, these animal models will become increasingly important for evaluating the safety and efficacy of novel therapeutic strategies targeting viral persistence in vivo.

The latest data discussed here highlight how complex the task of effectively targeting the HIV reservoir is. Furthermore, they underscore the need to start to design and test ‘next-generation’ combined interventions that synergistically target the main contributors of HIV persistence, including latency, residual inflammation, ineffective antiviral immune response, and the upregulation of ICBs that increase the longevity of infected cells. Although still in their initial phases and thus not discussed in this review, studies that supplement ART with therapeutic vaccines or the recently identified Env-specific monoclonal antibodies (that possess potent HIV neutralising capabilities) are actively being investigated in SIV-infected rMs (L. Picker and D. Barouh, personal communication), and may prove to be critical components of an HIV cure strategy. Determining the optimal combination of these interventions, in addition to scheduling and dosing, should be a top priority in future animal studies. In conclusion, the studies summarised in this review highlight the utility, relevance, and translational potential of the NHP model to inform clinical trials aimed at HIV functional cure or eradication.

Acknowledgements

We gratefully thank all those individuals whose studies are discussed above, and others we were unable to discuss here, for their continual dedication and scientific contributions aimed at achieving a cure for HIV infection.

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Hepatitis E virus in developed countries: 
one of the most successful zoonotic viral diseases in human history?

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Abstract

Until recently, hepatitis E was thought to be largely confined to hyperendemic areas in Asia, Africa and Mexico. Over the last 10 years it has become clear that this is not the case, as it is surprisingly common in developed countries. In these settings, it is caused by HEV genotypes 3 and 4, and is a porcine zoonosis. It causes a range of human illness including acute and chronic hepatitis, and a spectrum of neurological injury. HEV RNA has been found in donated blood from an increasing number of countries, and in some locations with a very high incidence. The clinical phenotype and burden of disease in humans is still emerging. In contrast to previous ‘received wisdom’, zoonotically transmitted HEV may be one of the most successful zoonotic viral infections in human history. How did we, as a scientific community, get this so badly wrong? This review considers this question from a largely clinical perspective, explores the places HEV has been ‘hiding’ and the emerging clinical phenotype in humans.

Keywords: hepatitis E virus (HEV), hepatitis, zoonosis, neurological injury, blood products

Hepatitis E in developing countries

For many years hepatitis E virus (HEV) was thought to be largely confined to the developing world where it is a major health issue often causing large outbreaks. In such settings hepatitis E is caused by HEV genotypes 1 and 2, which are obligate human pathogens spread orofaecally via contaminated water supplies [1,2]. It mainly affects young adults with a self-limiting illness, except in pregnant women where fulminant hepatic failure can occur, with a high mortality rate [3].

A recent study has estimated that in nine of 21 global burden of disease (GBD) regions, there are 3.4 million symptomatic cases of hepatitis E each year, with 70,000 deaths and 3,000 stillbirths [4]. This is almost certainly an underestimate of the GBD, as there may be up to 1,000 HEV-related maternal deaths per annum in Bangladesh alone [5]. In addition, recent studies suggest that anti-HEV seroprevalence estimates (on which the GBD was partly calculated) may have lacked sensitivity, and underestimated the true seroprevalence by 50% (unpublished observations).

Hepatitis E in developed countries

Following the discovery of HEV in the 1980s in developed countries, hepatitis E was thought to be a disease seen only in travellers returning from endemic areas in the developing world. Hepatitis E was considered exceedingly rare and of little relevance. Conceptually, the virological and hepatological features of HEV were considered analogous to hepatitis A virus (HAV): both being orofaecal hepatotropic RNA viruses causing acute self-limiting hepatitis. These misguided notions unfortunately remained the ‘received wisdom’ for the best part of 20 years [6].

Acute hepatitis E

We have now carefully documented over 100 cases of acute autochthonous hepatitis E [26]. All were caused by HEV genotype 3, when sequencing was possible. Remarkably, and uniquely, acute hepatitis E appears to have a predilection for middle-aged/elderly males [1], with a male:female ratio of 3.2:1 and a median age of 63.5 years [26]. Acute hepatitis E caused by genotype 3 (and genotype 4 in China, Japan and a few recently described clusters in Europe) [27,28] has been found in
every single developed country in which it has been sought. All these studies have described very similar demographics, with an excess of cases in older men (13,14,29–33). The reasons for these observations are uncertain.

The symptoms of hepatitis E infection are similar to those seen in any form of viral hepatitis (Table 2) [26], except in a minority of patients who present with a primarily neurological illness (see later). A number of other extra-hepatic manifestations have been described (Table 3) [20,26,34–37]. In contrast to HEV genotype 1, excess mortality in pregnant women is not seen with genotype 3, and the few women who have been described in the literature have all survived [38]. The majority of patients have a self-limiting illness, with clinical and biochemical recovery within a few weeks. A minority have a more severe hepatitis, and some patients (3.8% in our recent series) [26] die from subacute liver failure [16,19,21,39]. Such patients usually have underlying chronic liver disease [19,40]. Studies of acute HEV genotype 1 infection in patients with chronic liver disease in Asia show a mortality rate of up to 70% [41]. A prospective UK/French study of 372 patients with decompensated chronic liver disease and HEV genotype 3 showed a mortality rate of 27% (Blasco-Perrin et al., personal communication). The incidence of hepatitis E in the context of chronic liver disease varies significantly by geographical location, and was much more common in southwest France (7.9%) than in the UK (1.1%). There appears to be no clinical or laboratory clues to diagnosis at presentation, and so patients with decompensated chronic liver disease should be considered for routine HEV testing, particularly in high incidence areas. An early diagnosis is important, as patients with chronic liver disease who have decompensated due to HEV infection have been successfully treated with ribavirin [19].

Chronic hepatitis E

The field of hepatitis E was changed for ever by the description of chronic infection in transplant recipients in two side-by-side papers from southern France published in the New England Journal of Medicine in 2008 [42,43]. Typically patients have no symptoms, they are not jaundiced and their alanine aminotransferase (ALT) runs between 200 and 300 IU/L. Chronic infection occurs in approximately 60% of solid organ transplant recipients exposed to HEV genotype 3 infection [44]. Progressive liver disease is common and is usually more rapid than that seen in chronic infection with HBV or HCV and 10% of recipients with chronic hepatitis E infection are cirrhotic within 2 years [44,45]. The prevalence of chronic hepatitis E in the European transplant...
population averages between 1% and 2% (46,47). The figure is much higher in the transplant centres in southwest France [44]. Chronic hepatitis E infection can also occur in other immunosuppressed groups, including patients with haematological malignancy [48] and in individuals with HIV infection [12]. In the latter group, HAV/HEV chronic co-infection is uncommon, and is only seen in patients who are profoundly immunosuppressed with CD4 cell counts < 250 cells/µL. So far chronic infection has only been described with HEV genotype 3 [49].

Treatment and prevention

Case reports and case series show that chronic hepatitis E infection can be treated with the antiviral agents ribavirin and/or interferon. Most published data concern treatment of chronically infected solid-organ transplant recipients with ribavirin. The suggested treatment algorithm is shown in Figure 3 [50]. Acute infection generally requires no treatment, as it is usually a self-limiting illness. A few cases of severe hepatitis have been treated successfully with ribavirin (see above) [21].

A safe and effective vaccine has now been licensed for use in China [51]. It is not known whether it will be licensed for use in other countries.

Epidemiology

The incidence of hepatitis E infection varies between and also within countries. The incidence in the USA is 0.7% [52], the Netherlands 1.1% [53] and in southwest France it is as high as 3.2% [54]. In the UK, the incidence is 0.2% [2]. As in France, where infection is more common in the south of the country, significant regional variation in the UK also occurs. There appears to be far more circulating virus in England than Scotland, reflected by anti-HEV IgG seroprevalence rates in blood donors of 12% and 4.6%, respectively [55,56] and congruently higher rates of viraemic blood donors in England [57]. Why there is such a regional variation within these countries is not known.

Each year it is estimated that in England there are 100,000 infections with HEV [57]. In 2013 there were just 691 laboratory confirmed cases [58], suggesting that most infections are either asymptomatic or unrecognised (Figure 4). In contrast with HEV genotype 1 where infection is more common in the south of the country, significant regional variation in the UK also occurs. There appears to be far more circulating virus in England than Scotland, reflected by anti-HEV IgG seroprevalence rates in blood donors of 12% and 4.6%, respectively [55,56] and congruently higher rates of viraemic blood donors in England [57]. Why there is such a regional variation within these countries is not known.

It has been known for some years that humans in developed countries can become re-infected with HEV genotype 1. Data from China suggest that 20% of infections with genotype 4 are re-infections, which appear to be more common in females and cause a milder hepatitis than primary infection [1]. Typically patients are IgM negative but IgG and PCR positive, so it can be difficult to establish a diagnosis. Until very recently in Europe, scant attention has been paid to the concept of re-infection. A recent study from Toulouse, France has shown that re-infections are surprisingly common in the transplant population [59]. In this setting, anti-HEV IgG antibody levels < 7 WHO units/mL did not protect against re-infection. Now that we appreciate that there are large amounts of circulating HEV genotype 3 in Europe, the issue of re-infection merits further study.

HEV has been found in an increasingly diverse range of animals, most of which have no consequence for human infection [60,61]. HEV genotypes 3 and 4 have been found in pigs worldwide, and the pig is considered the primary host [6]. HEV has been found in all stages of the human food chain [62] and one established route of transmission from pigs to humans is via undercooked or uncooked pig meat products [63,64]. However, there are other possible ways this virus can be transmitted to humans; HEV has been found in soft fruits such as strawberries [65], watercourses [66] and the sea, and in shellfish [67]. It has also been found in the blood supply (see later). The relative importance of these other routes of transmission is not completely understood. Unlike HAV, household outbreaks have not been seen with zoonotic HEV. However, there have been reports of family members being asymptotically infected, possibly from a common food source [14]. How frequently this occurs is unknown, and is the subject of ongoing study.

Places where HEV hides

It is now clear that autochthonous hepatitis E is extremely common in developed countries, where it has been described as an ‘emerging infection’ [6]. This is not strictly true, as HEV biological time–clock studies show that HEV diverged into its four genotypes several hundred years ago [68]. It would be more accurate to describe locally acquired hepatitis E in developed countries, as a disease that is ‘emerging in human consciousness’. How long has zoonotic HEV been causing human disease? No one knows for sure, but possibly hundreds of years [2]. During this time, HEV has very successfully evaded human scrutiny, due to its ability to ‘hide’ in diverse places.

Populations

Many early studies estimated the anti-HEV IgG seroprevalence in developed countries at < 5% [69,70]. As a result, hepatitis E was thought not to be a health issue in these geographical settings. Some years ago we compared a commonly used commercial

---

**Figure 3.** Treatment algorithm for chronic HEV infection in the transplant population

- Chronic HEV infection in the transplant population (defined as infection >3 months)
- If possible, reduce immunosuppression to promote spontaneous viral clearance (1/3 will clear the virus with this method) [44]
- NO
- YES
- Treat with ribavirin therapy for 3 months’ duration (SVR 6 months 78%) [50]
- Main side effect found is anaemia requiring reduction in ribavirin dose in 29%, erythropoietin treatment in 54% and blood transfusion in 12% [50]
- No further treatment required

**Figure 4.** The clinical spectrum of infection with HEV genotype 3. Most cases are asymptomatic, however, many are symptomatic, but not recognised

- Symptomatic but not currently recognised as HEV infection
- Asymptomatic HEV infection
- Symptomatic infection

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anti-HEV IgG assay (Genelabs) with an assay developed in China (Wantai), against a bank of acute and convalescent (up to 7 years post-infection) sera from PCR proven cases from southwest England [10]. The Chinese assay had a sensitivity of 98%, compared to 56% for the Genelabs assay. When applied to a population of blood donors the Genelabs assay underestimated the seroprevalence by a factor of four. The Chinese assay was subsequently applied to a population of blood donors in southwest France and the seroprevalence estimate increased from 16% to 52% with the more sensitive assay [71].

Sceptics have argued that a seroprevalence of 52% in Toulouse blood donors cannot possibly be correct, and that the result is due to lack of assay specificity. This is very unlikely to be true, as the seroprevalence in children aged 2–4 from the same population is low (2%) [71]. In addition, a seroprevalence of 52% is entirely congruent with the high incidence of primary and re-infections with hepatitis E documented in this community, both in transplant recipients [53] and asymptomatic blood donors [72]. These data suggest that much of the early literature is flawed, as assays of poor sensitivity have grossly underestimated the true seroprevalence, so enabling HEV to ‘hide’ at population level. More recent studies with the Chinese assay have shown much higher estimates than previously, with seroprevalence rates that are compatible with rates of HEV viraemia in asymptomatic blood donors (Table 4) [53,55–57,69–81].

Transplant recipients

Chronic hepatitis E infection in transplant recipients is clinically ‘silent’, as patients have no symptoms. The only clue to the diagnosis is a very modest elevation in serum ALT (100–300 IU/L) [71]. It is a diagnosis that is easily overlooked. How long has HEV been ‘hiding’ in transplant patients? This is unknown, but probably since the advent of transplantation in the late 1960s.

Drug-induced liver injury (DILI)

HEV can also masquerade as drug-induced liver injury (DILI). Some years ago we studied patients with criterion-referenced DILI and found that in six of 47 patients (13%) we had made a diagnostic error, as their illness was not due to DILI, but infection with HEV genotype 3 [18]. This is an easy diagnostic error to make, as both DILI and HEV genotype 3 infection are common in the elderly.

Neurological illness

Over recent years there have been quite a few case series and reports describing HEV-associated neurological illness. There appears to be a very wide spectrum of reported neurological injury that includes Bell’s palsy, encephalitis, vestibular neuritis, small fibre peripheral neuropathy, Guillain–Barré syndrome and brachial neuritis [20,26]. In some cases HEV RNA has been found in the cerebrospinal fluid. The pathogenic mechanisms are unknown.

Guillain–Barré syndrome is a post infectious immune-mediated polyradiculopathy triggered by *Campylobacter* in 35% of cases but with an unknown aetiology in 50% of cases. In a prospective longitudinal study in 100 patients in the mid-1990s, the Dutch Guillain–Barré Study Group, found that 30% of patients had unexplained mildly abnormal liver function tests (LFT) at the start of the neurological illness [82]. A recent case control study of 201 patients with Guillain–Barré syndrome from the Netherlands showed that 5% of patients (n=10) had evidence of hepatitis E infection at the start of their neurological illness [83] and of these, three patients (1.5%) were viraemic with HEV genotype 3 at presentation. This has raised the question of whether these patients might benefit from early treatment with ribavirin therapy.

What about the other 25% of patients with Guillain–Barré syndrome and abnormal LFTs? Could these cases have also been triggered by HEV? One possible explanation could be that these cases might have been triggered re-infection with HEV. If the re-infection with HEV was 1 or 2 months before the neurological symptoms started, it would be very difficult to make a diagnosis. Such patients would be IgM negative (typical of re-infection), HEV PCR negative (the viraemic ‘window’ lasts only a few weeks), but IgG positive. Thus, the only way of distinguishing recent re-infection from distant past infection would be to demonstrate a rising IgG. This is problematic in this cohort of patients, as many are treated with intravenous immunoglobulin, which may well interfere with the IgG result on the convalescent blood sample.

A further Anglo-Dutch cohort study of 47 patients with brachial neuritis showed that 10% (n=5) had evidence of hepatitis E infection at the onset of neurological symptoms [84]. In contrast to other triggers of brachial neuritis, HEV–associated cases had bilateral neurological symptoms, sometimes with phrenic nerve involvement. Following this study, a brachial neuritis registry has been established in southwest England, with several further HEV–associated cases documented in just a few months (unpublished observations). A similar registry is being established in the Netherlands.

In both the above studies, neurological symptoms and signs dominated the clinical picture: patients were anicteric, the ALT was only mildly elevated (typically <600 IU/L), and occasionally

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### Table 4. HEV viraemia and seroprevalence in blood donors

<table>
<thead>
<tr>
<th>Country</th>
<th>Blood donors</th>
<th>HEV RNA sero-prevalence (%)</th>
<th>Assay</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW France¹</td>
<td>1:1595</td>
<td>52.5</td>
<td>Wantai</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td>1:4525</td>
<td>16</td>
<td>Adaltis</td>
<td>[73]</td>
</tr>
<tr>
<td>France²</td>
<td>1:2218</td>
<td></td>
<td></td>
<td>[72]</td>
</tr>
<tr>
<td>Germany</td>
<td>1:1200</td>
<td>29.5</td>
<td>Wantai</td>
<td>[74]</td>
</tr>
<tr>
<td></td>
<td>1:2848</td>
<td>18.0</td>
<td>Mikrogen</td>
<td>[75]</td>
</tr>
<tr>
<td></td>
<td>1:4525</td>
<td>4.5</td>
<td>MP diagnosis</td>
<td>[76]</td>
</tr>
<tr>
<td>Netherlands</td>
<td>1:2671</td>
<td>27.0</td>
<td>Wantai</td>
<td>[53]</td>
</tr>
<tr>
<td></td>
<td>1:2848</td>
<td>1.1</td>
<td>Abbott</td>
<td>[69]</td>
</tr>
<tr>
<td>England</td>
<td>1:7000</td>
<td>12.0</td>
<td>Wantai</td>
<td>[77]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.3*</td>
<td>Abbott</td>
<td>[70]</td>
</tr>
<tr>
<td>Sweden</td>
<td>1:7986</td>
<td>9.2*</td>
<td>Abbott</td>
<td>[78]</td>
</tr>
<tr>
<td>Scotland</td>
<td>1:4520</td>
<td>4.7</td>
<td>Wantai</td>
<td>[56]</td>
</tr>
<tr>
<td>USA</td>
<td>Nil</td>
<td></td>
<td></td>
<td>[75]</td>
</tr>
<tr>
<td></td>
<td>Nil²</td>
<td></td>
<td></td>
<td>[79]</td>
</tr>
<tr>
<td>Japan</td>
<td>1:1781</td>
<td>3.7</td>
<td>Wantai</td>
<td>[80]</td>
</tr>
<tr>
<td>China³</td>
<td>1:1493</td>
<td>32.6</td>
<td>Wantai</td>
<td>[81]</td>
</tr>
</tbody>
</table>

* Data shown are from blood donors with the exception of those marked *, which are from healthy adults. HEV RNA was genotype 3 in all cases, except where stated.

¹ Deconstructed solvent-detergent treated mini-pools.

² Only 1,939 donors tested.

³ Of those with HEV viraemia, 57% were genotype 1 and 43% genotype 4.
normal. This led the lead neurologist to pose the following question: ‘Is it possible that hepatitis E has been misnamed? These patients have profound neurological illness, but not much of a hepatitis!’ This is an interesting question. The first step in the process of addressing this issue is about to start: a UK-Dutch-French multinational study of all patients with non-traumatic neurological injury who will be systematically tested for HEV at presentation. The results are awaited with interest.

Current diagnostic testing algorithms

In the UK, and in most of the rest of Europe, current diagnostic testing algorithms suggest that patients presenting with acute hepatitis should first be tested for HAV, HBV and HCV. If these tests are negative, then testing for HEV should be considered [85]. This approach is outdated, and means that the diagnosis of hepatitis E is either delayed, or missed altogether.

As hepatitis E is the commonest cause of acute viral hepatitis (Table 1, Figure 2) [24,25], it would make much more sense to first test all patients for HEV, and if this is negative then consider testing for HAV, HBV and HCV. How should we define ‘hepatitis’? Preliminary data from southwest England suggests that testing patients with an ALT >400 IU/L or with an ALT/alkaline phosphatase ratio >6 times the upper limit of normal has quite high sensitivity and specificity for HEV diagnosis. Patients with Guillain–Barré syndrome and brachial neuritis should be tested for HEV irrespective of the ALT result. In addition, clinicians should have a low threshold for testing patients with unexplained neurological symptoms and abnormal LFTs. Immunosuppressed patients with persistently abnormal LFTs should be tested for HEV to exclude chronic infection. This should include PCR as well as serology, as the latter is less accurate in the immunosuppressed.

Blood supply

One of the potentially most worrisome places that HEV has been ‘hiding’ is in human blood products used for transfusion. Given that zoonotically acquired hepatitis E is very commonly asymptomatic, it comes as no surprise that HEV has found its way into the blood supply. What has astonished some observers is the very high incidence of HEV viraemia in the donor population (Table 4) [53,55–57,69–81]. Transmission of HEV via blood products is currently occurring as donors are not screened, and there are increasing numbers of reports of both acute and chronic infection in recipients (Table 5) [57,86–97].

In southeast England, a recent study demonstrated HEV RNA-positive plasma pools in 0.04% of donor samples, with one in 2,848 donors having HEV (genotype 3) viraemia at the time of donation [57]. Retrospective analysis showed that blood components were given to 60 patients, 43 of whom were available for follow-up: the overall transmission rate of HEV was 42%, and infection was significantly more common from high-viral-load donations and plasma-based blood products, and less likely if the donor sample contained anti-HEV antibodies. ‘Classical’ post-transfusion hepatitis was uncommon in infected recipients, with only one in 18 developing clinically apparent post-transfusion hepatitis. This is not so different from the ‘clinical attack rate’ seen in individuals infected orofaecally. Seven of 10 immunocompromised patients infected were viraemic at 3 months following exposure, which is the working definition of chronic infection with HEV (see above). Two were treated with antiviral agents. There were four deaths, three of which were from unrelated causes. With a prevalence of one in 2848 in this study, a projection across England leads to an estimated 100,000 infections and a total of 7,200 HEV-contaminated transfusion events in the year of the study [57].

Table 5. Transfusion-transmitted HEV infection

<table>
<thead>
<tr>
<th>Country, Year</th>
<th>Ref</th>
<th>Number of infections / exposed</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK, 2014</td>
<td>[57]</td>
<td>18 / 43</td>
<td>Retrospective study of 43 transfusion episodes available for investigation, 18 patients developed HEV</td>
</tr>
<tr>
<td>France, 2014</td>
<td>[86]</td>
<td>5</td>
<td>Immunocompetent liver transplant recipients. Outcome not detailed</td>
</tr>
<tr>
<td>France, 2014</td>
<td>[87]</td>
<td>2</td>
<td>Immunocompetent liver transplant recipients treated with plasmapheresis with Intercept-treated plasma. Both developed asymptomatic chronic infection requiring ribavirin therapy</td>
</tr>
<tr>
<td>Japan, 2014</td>
<td>[88]</td>
<td>1</td>
<td>Immunocompetent patient, received HEV-contaminated platelet transfusion and developed post-transfusion acute hepatitis</td>
</tr>
<tr>
<td>Japan, 2014</td>
<td>[89]</td>
<td>1</td>
<td>Immunocompetent patient, received HEV-contaminated platelet transfusion and developed post-transfusion acute hepatitis</td>
</tr>
<tr>
<td>France, 2013</td>
<td>[90]</td>
<td>1</td>
<td>Immunocompetent patient, received HEV-contaminated packed red cell transfusion. Developed post-transfusion acute hepatitis. HEV cleared with ribavirin</td>
</tr>
<tr>
<td>Germany, 2013</td>
<td>[91]</td>
<td>1</td>
<td>Six blood products identified from one donor. Retrospective study. Three patients died (no follow up). One immunocompromised patient – chronic infection. One immunocompetent child – probably acute infection. One immunocompromised patient did not contract HEV</td>
</tr>
<tr>
<td>France, 2012</td>
<td>[92]</td>
<td>1</td>
<td>One immunosuppressed patient, on prednisolone then cyclosporine, developed chronic hepatitis. Cleared virus when immunosuppression stopped. Died from underlying condition</td>
</tr>
<tr>
<td>Japan, 2008</td>
<td>[93]</td>
<td>1</td>
<td>Retrospective study. Platelet transfusion with HEV 4. Recipient developed clinical acute hepatitis</td>
</tr>
<tr>
<td>Japan, 2007</td>
<td>[94]</td>
<td>1</td>
<td>Immunocompetent patient with T-cell lymphoma on chemotherapy. Chronic infection after HEV-contaminated red cell transfusion</td>
</tr>
<tr>
<td>France, 2007</td>
<td>[95]</td>
<td>1</td>
<td>7-year-old immunosuppressed child, on chemotherapy. Acute post-transfusion hepatitis</td>
</tr>
<tr>
<td>UK, 2006</td>
<td>[96]</td>
<td>1 / 2</td>
<td>Two patients received HEV-contaminated blood products. Immunocompetent recipient did not develop HEV. Second recipient was immunosuppressed (lymphoma on chemotherapy) and developed acute post-transfusion hepatitis</td>
</tr>
<tr>
<td>Japan, 2004</td>
<td>[97]</td>
<td>1</td>
<td>Acute post-transfusion hepatitis following receipt of HEV-infected fresh frozen plasma</td>
</tr>
</tbody>
</table>

In all the above studies, HEV was genotype 3 where sequencing data were available, unless otherwise stated.
There is particular concern about the use of plasmapheresis, which uses pooled plasma, often from thousands of donors. Current treatment methods appear not to remove or inactivate HEV from pooled plasma, and plasmapheresis has been shown to transmit HEV to transplant recipients in France [87].

**Should we screen blood donors for HEV?**

There is currently a very lively debate about whether blood donors should be screened for HEV [98]. This has been brought to a head by the recent Lancet paper by Hewitt and colleagues discussed above [57]. The authors concluded that: ‘on a clinical basis alone, the resulting minimal burden of disease does not signal a pressing need for donation screening at this time’. This statement is however reminiscent of the initial reluctance to consider screening for HCV over 20 years ago [98]. Protagonists of screening would argue that, in common with any retrospective analysis, data collection in the Hewitt study was far from complete and there were no follow-up data at all on 17 HEV-infected recipients. Also, our understanding of the places HEV ‘hides’ is still developing and the clinical phenotype of hepatitis E is still emerging. Nucleic acid amplification testing will begin in Europe in 2015 of pooled plasma processed with solvent detergent [72]. The issue of safety of plasma-derived medicinal products is also under active review by the European Medicines Agency.

If blood donors should be screened for HEV, how should this be done? The answer to this is uncertain, as most viraemic donors have a normal ALT, and often have absent anti-HEV antibodies [72,75]. Nucleic acid amplification testing will probably be the technique of choice, but this remains to be determined.

**Conclusions**

Zoonotic HEV is a remarkably successful virus. It has several niches in animals, and appears in various ways in humans. It causes acute and chronic hepatitis. It causes a range of neurological injury. It has found its way into the blood supply. Like other successful RNA viruses, such as HIV and HCV, it has niches in animals, and appears in various ways in humans. It

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Cervical cytological abnormalities and HPV infection in perinatally HIV-infected adolescents

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Abstract

Background: Behaviourally HIV-infected adolescent females are at higher risk for abnormal cervical cytology and HPV infection compared to those who are uninfected, but data on perinatally HIV-infected adolescent females are lacking.

Methods: Cervical cytology, HPV infection and E6/E7 mRNA were assessed in sexually active 12–24-year-old adolescent females: perinatally HIV-infected (group 1, n=40), behaviourally HIV-infected (group 2, n=10), and HIV-uninfected (group 3, n=10).

Results: Median age was lower in group 1 (18 years) than in groups 2 (24 years) and 3 (20.5 years) (P<0.001), and median time since sexual debut was shorter: 2 vs 5 vs 4 years (P<0.001). More trial participants in group 1 than group 2 were on antiretrovirals (90% vs 70%; P<0.001). Abnormal cervical cytology (atypical squamous cells of undetermined significance and higher) was observed in 30% (group 1), 40% (group 2) and 30% (group 3) (P=0.92), whereas high-risk HPV infection was observed in 45%, 45% and 40%, respectively (P=1.00). Positive E6/E7 mRNA was found in 28% of group 1, but not in other groups. High-risk HPV infection predicted abnormal cytology in all groups (OR 6.77, 95% CI 1.16–153.06; P=0.04). Additionally, plasma HIV RNA ≥50 copies/mL (OR 13.3, 95% CI 1.99–23.0; P=0.001). Additionally, plasma HIV RNA ≥50 copies/mL (OR 1.99–23.0; P=0.001). High-risk HPV infection predicted abnormal cytology in all groups (OR 13.3, 95% CI 1.99–23.0; P=0.001). High-risk HPV infection predicted abnormal cytology in all groups (OR 13.3, 95% CI 1.99–23.0; P=0.001). High-risk HPV infection predicted abnormal cytology in all groups (OR 13.3, 95% CI 1.99–23.0; P=0.001). High-risk HPV infection predicted abnormal cytology in all groups (OR 13.3, 95% CI 1.99–23.0; P=0.001). High-risk HPV infection predicted abnormal cytology in all groups (OR 13.3, 95% CI 1.99–23.0; P=0.001). High-risk HPV infection predicted abnormal cytology in all groups (OR 13.3, 95% CI 1.99–23.0; P=0.001). High-risk HPV infection predicted abnormal cytology in all groups (OR 13.3, 95% CI 1.99–23.0; P=0.001). High-risk HPV infection predicted abnormal cytology in all groups.

Conclusions: Despite the younger age and shorter time since sexual debut, cervical cytological abnormalities and HPV infection were as common in perinatally HIV-infected as in behaviourally infected and uninfected adolescents. HPV vaccination, pre-cancer screening and antiretroviral treatment in HPV-infected female adolescents should be implemented to minimise the risk of cervical cancer.

Keywords: perinatal HIV, HPV, cervix, adolescents, Pap smear

Introduction

Worldwide, more women lose their lives to cervical cancer than any other cancer [1], and persistent infection with high-risk types of human papillomavirus (HPV), the most common sexually transmitted disease (STD), is the cause. HIV infection increases a woman’s risk for developing cervical cancer by up to 20-fold [2,3]. Prevalence and incidence of HPV infection among young adults who are sexually experienced is high [4,5], especially in resource-limited settings like Thailand, where HPV vaccination is not routinely available. Almost 25% of women aged younger than 20 years old worldwide are infected with HPV [4].

Despite the younger age and shorter time since sexual debut, cervical cytological abnormalities and HPV infection were as common in perinatally HIV-infected as in behaviourally infected and uninfected adolescents. HPV infection and abnormal cervical cytology amongst female adolescents who were perinatally HIV-infected, behaviourally HIV-infected and HIV-uninfected female adolescents were lacking.

Methods

Study design and population

This was a feasibility study to explore the prevalence of HPV infection and abnormal cervical cytology amongst female adolescents who were perinatally HIV-infected, behaviourally HIV-infected and HIV-uninfected. A convenience sample of sexually active female adolescents was enrolled and assigned to among HIV-infected patients. Longer duration of HIV, high HIV RNA level and low CD4 cell count are risk factors for HPV progression to cancer, and the probability of HPV clearance in HIV-infected patients increases with rising CD4 cell counts [7,9,10]. Therefore, it is possible that adolescents with longer duration of HIV infection (i.e. perinatally infected) will be at higher risk for HPV infection and progression to cancer than those infected more recently via sexual transmission of HIV (i.e. behaviourally infected). However, previous research has concentrated on behaviourally HIV-infected adolescents and data in perinatally infected adolescents are sorely lacking [7,8,11–13]. In this study, we aimed to determine the prevalence of cervical cytological abnormalities, HPV infection and E6/E7 oncogenic mRNA in perinatally HIV-infected compared to behaviourally HIV-infected and HIV-uninfected female adolescents. We hypothesised that perinatally HIV-infected adolescents would have the highest prevalence of these abnormalities compared to the other two groups.

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one of three groups. Group 1 consisted of perinatally HIV-infected adolescents, who were enrolled at the HIV-Netherlands Australia Thailand (HIV-NAT) paediatric HIV research clinic in Bangkok (n=16) and the paediatric HIV clinic at Chiangrai Prachanukroh Hospital in Northern Thailand (n=24). Group 2 consisted of behaviourally HIV-infected adolescents and group 3 included HIV-uninfected adolescents. Both groups 2 (n=10) and 3 (n=10) were recruited from the clients who came for HIV testing at the Thai Red Cross Anonymous clinic, one of the largest HIV voluntary counselling and testing clinics in Bangkok, Thailand.

Patients were eligible if they were female, aged 12–24 years old, had a history of vaginal intercourse with a male, and fulfilled the HIV status criteria as follows.

Group 1: documented positive HIV enzyme immunoassay (EIA) or nucleic acid testing (NAT) at any time and history of maternal HIV infection.

Group 2: documented positive EIA or NAT at any time after sexual debut without history of maternal HIV infection.

Group 3: documented negative HIV EIA and NAT.

Patients aged ≥18 years gave their consent. For patients aged 12–17 years consent was required from both themselves and their parents. The study was approved by the ethics committees of Chulalongkorn University in Bangkok and Chiangrai Prachanukroh Hospital in Chiang Rai.

At recruitment, patients were asked for their medical history and any information relevant to HIV infection, such as antiretroviral therapy (ART) use. CD4 cell count and HIV RNA data were obtained from medical records. All patients underwent physical examination and cervical sample collection in liquid-based cytology fluid (Liqui-PREP fluid, LGM International, Inc., Florida, USA) for cytology. Adolescents with abnormal cervical cytology results were invited back for colposcopy and biopsy. The cytology liquid was also used to determine HPV subtype and E6/E7 oncogenic messenger RNA (mRNA) quantification. An Audio Computer-assisted Self Interview (ACASI) was used to collect general demographic data, as well as sensitive information on sexual behaviour and behavioural risks. At the Bangkok site, patients were asked to return for a 12-month follow-up visit, at which the same procedures were performed.

**Cervical cytology**

Cervical sample collection was done by an experienced nurse or physician using a combined spatula and cytobrush technique. The cytology slides were read at the Cytology and Pathology Unit at the Department of Obstetrics and Gynecology, Chulalongkorn University by experienced cytotechnicians [10]. Results were classified according to the 2001 Bethesda system as: normal, atypical squamous cell of undetermined significance (ASC-US); atypical squamous cells – cannot exclude high-grade squamous intraepithelial lesion (ASC-H); low-grade squamous intra-epithelial lesion (LSIL); high-grade squamous intra-epithelial lesion (HSIL); or squamous cell carcinoma. All abnormal cytology slides and a randomly selected 10% of normal cytology slides were reviewed by a senior pathologist, who confirmed the final results.

**HPV typing**

Stored liquid-based cytology fluid was used for HPV typing by LINEAR ARRAY (LA HPV GT, Roche Molecular Systems, Inc., New Jersey, USA) to identify 37 HPV DNA genotypes: 13 high-risk genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), and 24 other HPV genotypes that are either possibly carcinogenic, non-carcinogenic or of unknown carcinogenicity (6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73 [MM9], 81, 82 [MM4], 83 [MM7], 84 [MM8], IS39 and CP6108).

**Quantification of intracellular HPV E6/E7 mRNA**

An aliquot from the liquid-based cytology fluid was used for intracellular HPV E6/E7 mRNA flow cytometric analysis using HPV OncoTect E6, E7 mRNA Kit (IncellDx, Menlo Park, CA, USA) [14]. The kit covers the detection of E6/E7 mRNA from HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. Flow cytometry was performed using 3-colour analysis on Beckman Coulter Cytomix FC500. A sample with ≥2% of cells exhibiting E6/E7 mRNA was considered positive. HPV E6/E7 oncoproteins mediate cancer development, and their overexpression can be measured by E6/E7 mRNA.

**HPV vaccination**

During the conduct of this study, HPV vaccination was offered to adolescents at the two clinics. Two products were used: Cervarix, that protects against HPV 16 and 18 (GlaxoSmithKline Biologicals, Rixensart, Belgium), and Gardasil, that protects against HPV 6, 11, 16 and 18 (Merck & Co, Inc, Whitehouse Station, NJ, USA). The information on uptake of vaccination and number of vaccines received was collected. All adolescents who elected to receive the vaccine had it after completing the baseline visit assessment.

**Statistical methods**

Demographic and relevant disease-related characteristics of the patient cohort were described and comparisons of categorical covariate across groups were made using a Chi-squared test, or Fisher’s exact test, as appropriate; continuous covariates across groups were analysed using a Kruskal–Wallis or Mann–Whitney U test, depending on whether comparisons were between all subject groups or among only HIV-infected subjects. Prevalence of abnormal cervical cytology, HPV infection, and E6/E7 mRNA positivity were calculated. The proportion with any abnormal cytology, or differing degrees of abnormal cytology between female adolescents in the three groups were compared using Fisher’s exact test. Changes in abnormal cytology and high-risk HPV genotypes over time in a subset with month 12 follow-up were described. Logistic regression models were used to assess predictors of abnormal cervical cytology at baseline in all adolescents; predictor covariates included HPV subtypes, E6/E7 mRNA positivity, sexual and social behaviour. Further models were developed for HIV-infected adolescents to assess the influence of HIV-related characteristics including HAART use, CD4 cell count, HIV RNA suppression, HIV acquisition method, HPV infection and E6/E7 mRNA positivity. Multivariate models were developed including covariates with P<0.1 in univariate models, and P values in multivariate models were calculated using Wald tests.

**Results**

**Demographic, behavioural and HIV-related characteristics**

A total of 60 female adolescents was recruited for the study, 40 of whom were perinatally HIV-infected (group 1), 10 were behaviourally HIV-infected (group 2), and 10 were HIV-uninfected (group 3). The demographic characteristics for all adolescents and sexual behavioural characteristics for 58 adolescents who answered the ACASI questionnaire are shown in Table 1. The perinatally infected patients were younger and had a shorter time since sexual debut. They were more likely to...
Table 1. Demographic, sexual and HIV-related characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Overall</th>
<th>Group 1 (perinatally HIV-infected females)</th>
<th>Group 2 (behaviourally HIV-infected females)</th>
<th>Group 3 (HIV-negative females)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>60</td>
<td>40</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Median age (IQR), yrs</td>
<td>19 (17–21)</td>
<td>18 (17–19)</td>
<td>24 (20–24)</td>
<td>20.5 (19–23)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median age (IQR) at sexual debut, yrs</td>
<td>16 (15–17)</td>
<td>16 (15–17)</td>
<td>17 (16–18)</td>
<td>15.5 (15–18)</td>
<td>0.34</td>
</tr>
<tr>
<td>Median (IQR) time since sexual debut, yrs</td>
<td>3 (1–4)</td>
<td>2 (1–3)</td>
<td>5 (4–7)</td>
<td>4 (3–6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lifetime sexual partners n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>14 (23.3)</td>
<td>13 (22.5)</td>
<td>0 (0)</td>
<td>1 (10.0)</td>
<td>0.06</td>
</tr>
<tr>
<td>2–3</td>
<td>23 (38.3)</td>
<td>16 (40.0)</td>
<td>4 (40.0)</td>
<td>3 (30.0)</td>
<td></td>
</tr>
<tr>
<td>≥4</td>
<td>19 (31.7)</td>
<td>9 (22.5)</td>
<td>4 (40.0)</td>
<td>6 (60.0)</td>
<td></td>
</tr>
<tr>
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<td>4 (6.7)</td>
<td>2 (5.0)</td>
<td>2 (20.0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Number of partners in the last 3 months n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td>No partner</td>
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<td>3 (7.5)</td>
<td>2 (20.0)</td>
<td>1 (10.0)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>47 (78.3)</td>
<td>33 (82.5)</td>
<td>6 (60.0)</td>
<td>8 (80.0)</td>
<td></td>
</tr>
<tr>
<td>2–3</td>
<td>6 (7.7)</td>
<td>2 (5.0)</td>
<td>1 (10.0)</td>
<td>1 (10.0)</td>
<td></td>
</tr>
<tr>
<td>≥4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
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<td>Did not answer/missing</td>
<td>3 (5.0)</td>
<td>2 (5.0)</td>
<td>1 (10.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Frequency of sexual intercourse per month in previous 3 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>0–5</td>
<td>30 (50.0)</td>
<td>19 (47.5)</td>
<td>8 (80.0)</td>
<td>3 (30.0)</td>
<td></td>
</tr>
<tr>
<td>5–10</td>
<td>17 (28.3)</td>
<td>12 (30.0)</td>
<td>1 (10.0)</td>
<td>4 (40.0)</td>
<td></td>
</tr>
<tr>
<td>≥10</td>
<td>13 (3)</td>
<td>5 (12.5)</td>
<td>0 (0)</td>
<td>3 (30.0)</td>
<td></td>
</tr>
<tr>
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<td>3 (8.3)</td>
<td>2 (5.0)</td>
<td>1 (10.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Types of contraception n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condoms</td>
<td>33 (55.0)</td>
<td>27 (67.5)</td>
<td>4 (40.0)</td>
<td>2 (20.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Hormonal contraception</td>
<td>3 (5.0)</td>
<td>2 (5.0)</td>
<td>0</td>
<td>1 (10.0)</td>
<td>0.70</td>
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<tr>
<td>Morning-after pill</td>
<td>3 (5.0)</td>
<td>0</td>
<td>2 (20.0)</td>
<td>1 (10.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>Contraceptive implant</td>
<td>13 (21.7)</td>
<td>6 (15.0)</td>
<td>3 (30.0)</td>
<td>4 (40.0)</td>
<td>0.15</td>
</tr>
<tr>
<td>Abstinence</td>
<td>2 (3.3)</td>
<td>1 (2.5)</td>
<td>1 (10.0)</td>
<td>0</td>
<td>0.56</td>
</tr>
<tr>
<td>Sterilisation</td>
<td>6 (10.0)</td>
<td>4 (10.0)</td>
<td>1 (10.0)</td>
<td>1 (10.0)</td>
<td>0.33</td>
</tr>
<tr>
<td>Injection contraception</td>
<td>9 (15.0)</td>
<td>7 (17.5)</td>
<td>1 (10.0)</td>
<td>1 (10.0)</td>
<td>1.0</td>
</tr>
<tr>
<td>No contraception</td>
<td>9 (15.0)</td>
<td>4 (10.0)</td>
<td>2 (20.0)</td>
<td>3 (30.0)</td>
<td>0.23</td>
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<tr>
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<td>1 (2.5)</td>
<td>1 (10.0)</td>
<td>0</td>
<td>0.56</td>
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<tr>
<td>Condom use n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Always</td>
<td>15 (25.0)</td>
<td>13 (22.5)</td>
<td>2 (20.0)</td>
<td>0</td>
<td>0.27</td>
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<tr>
<td>Almost always</td>
<td>9 (15.0)</td>
<td>6 (15.0)</td>
<td>2 (20.0)</td>
<td>1 (10.0)</td>
<td></td>
</tr>
<tr>
<td>Sometimes</td>
<td>27 (45.0)</td>
<td>15 (37.5)</td>
<td>4 (40.0)</td>
<td>8 (80.0)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>7 (11.7)</td>
<td>5 (12.5)</td>
<td>1 (10.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Did not answer/missing</td>
<td>2 (3.3)</td>
<td>1 (2.5)</td>
<td>1 (10.0)</td>
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<tr>
<td>Ever had an STD n (%)</td>
<td>5 (8.3)</td>
<td>2 (5.0)</td>
<td>2 (20.0)</td>
<td>1 (10.0)</td>
<td>0.20</td>
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<tr>
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<td>1 (2.5)</td>
<td>1 (10.0)</td>
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<tr>
<td>Sex for money/goods n (%)</td>
<td>6 (10.0)</td>
<td>2 (5.0)</td>
<td>0</td>
<td>4 (40.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Did not answer/missing</td>
<td>2 (3.3)</td>
<td>1 (2.5)</td>
<td>1 (10.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Smoking cigarettes n (%)</td>
<td>49 (81.7)</td>
<td>37 (92.5)</td>
<td>7 (70.0)</td>
<td>5 (50.0)</td>
<td>0.003</td>
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<tr>
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<td>2 (3.3)</td>
<td>1 (2.5)</td>
<td>1 (10.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ever used alcohol n (%)</td>
<td>33 (55.0)</td>
<td>25 (62.5)</td>
<td>3 (30.0)</td>
<td>5 (50.0)</td>
<td>0.25</td>
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<tr>
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<td>2 (3.3)</td>
<td>1 (2.5)</td>
<td>1 (10.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ever used illicit drugs n (%)</td>
<td>6 (10.0)</td>
<td>3 (7.5)</td>
<td>1 (10.0)</td>
<td>2 (20.0)</td>
<td>0.42</td>
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<td>1 (2.5)</td>
<td>1 (10.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HIV-related parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>50</td>
<td>40</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>On HAART at month 0 n (%)</td>
<td>43/50 (86.0)</td>
<td>36/40 (90.0)</td>
<td>7/10 (70.0)</td>
<td>n/a</td>
<td>0.13</td>
</tr>
<tr>
<td>Median (IQR) duration on HAART before month 0, yrs (n=43)</td>
<td>6.9 (2.2–8.3)</td>
<td>7.4 (3.3–8.5)</td>
<td>0.6 (0.5–2.2)</td>
<td>n/a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median (IQR) CD4 on month 0 cells/μL (n=50)</td>
<td>542 (263–771)</td>
<td>559 (359–832)</td>
<td>343 (222–565)</td>
<td>n/a</td>
<td>0.07</td>
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<tr>
<td>Median (IQR) HIV RNA on month 0, log10 copies/mL (n=50)</td>
<td>1.60 (1.60–1.74)</td>
<td>1.60 (1.60–1.60)</td>
<td>1.74 (1.60–5.45)</td>
<td>n/a</td>
<td>0.02</td>
</tr>
<tr>
<td>HIV RNA &lt;50 copies/mL n (%)</td>
<td>35 (70)</td>
<td>32 (80)</td>
<td>0</td>
<td>3 (30)</td>
<td>0.001</td>
</tr>
<tr>
<td>Disclosed HIV to last partner before engaging in sex n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td>‘He knows’ (yes)</td>
<td>26 (52.0)</td>
<td>23 (57.5)</td>
<td>3 (30.0)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>‘He doesn’t know’ (no)</td>
<td>17 (34.0)</td>
<td>13 (32.5)</td>
<td>4 (40.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Not sure’</td>
<td>5 (10.0)</td>
<td>3 (7.5)</td>
<td>2 (20.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did not answer/missing</td>
<td>2 (4.0)</td>
<td>1 (2.5)</td>
<td>1 (10.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbreviations: STD: sexually transmitted disease; HAART: highly active antiretroviral therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
use condoms and less likely to use emergency contraception (i.e. morning after pills). The HIV-uninfected adolescents were more likely to have sex in exchange for money or goods and to smoke cigarettes. More perinatally infected adolescents were on HAART and the duration on treatment was longer compared to those who were behaviourally infected. As a result, group 1 had higher HIV RNA suppression rates \( (P=0.004) \) and tended to have higher CD4 cell counts \( (P=0.07) \) than group 2.

**Cervical cytology, HPV infection and E6/E7 mRNA**

Overall, 19 of 60 adolescents (31.7%) had abnormal cervical cytology (defined as ASC-US or higher, referred to hereafter as ASC-US+). Prevalence of ASC-US+ were similar among the three groups: 12 perinatally infected (30%), four behaviourally infected (40%) and three HIV-uninfected adolescents (30%), \( P=0.92 \) (Table 2). LSIL was only observed in perinatally and behaviourally infected adolescents, and no one had HSIL or cancer. We offered colposcopy and biopsy to all adolescents with abnormal cytology with ASC-US+. However only four of 12 perinatally infected adolescents underwent this procedure (three with ASC-US and one with LSIL), while none in the other two groups did (0/4 in group 2 and 0/3 in group 3). Of the three with ASC-US, one had a biopsy result that could not exclude cervical intra-epithelial neoplasia (CIN) so she underwent a loop electrosurgical excision procedure, and the pathology of the tissue showed no CIN. For the other two adolescents with ASC-US, one had no CIN and one had CIN 1 on cervical biopsy. In the last adolescent with LSIL, the biopsy showed CIN 1.

The overall prevalence rates of cervical infection with any HPV and high-risk HPV were 60% and 43%, respectively, and these were not statistically different between groups (Table 2). Of perinatally HIV-infected adolescents, 58% had any HPV infection, compared with 80% of behaviourally HIV-infected and 50% of HIV-uninfected adolescents \( (P=0.32) \). Furthermore, 45% of perinatally HIV-infected adolescents had high-risk HPV infection, compared to 40% in the other two groups. The frequencies of HPV types did not differ between groups (data not shown), and high-risk HPV types other than those included in preventive vaccines (non-HPV 16, 18) featured prominently in all groups. Of the adolescents with abnormal cytology, 9/12 (75%), 3/4 (75%) and 2/3 (66%) of perinatally infected, behaviourally infected and HIV-negative females had infection with a high-risk HPV type. Infection with two or more high-risk HPV types was observed in about 15% of adolescents, without significant differences between groups. Figure 1 displays the prevalence of HPV types in 40 perinatally HIV-infected adolescents. HPV-16 was most common high-risk subtype (20%), followed by HPV-58 (17.5%) and HPV-51 and HPV-52 (both 7.5%). HPV-18 infection was observed in 2.5%. E6/E7 mRNA positivity was observed in 27.5% (95%CI 14.6–43.8%) perinatally HIV-infected, compared to 0% (95%CI 0–30.8%) in both behaviourally HIV-infected and HIV-uninfected adolescents (Table 2).

**Predictors for abnormal cytology**

Logistic regression analyses were performed to identify factors associated with abnormal cervical cytology (ASC-US+) at baseline among the 60 subjects in the study (Table 3). The only factor associated with abnormal cytology was cervical infection with high-risk HPV (OR 6.77, 95% CI 1.99–23.0; \( P=0.001 \)). Further models were developed for the 50 HIV-infected adolescents to assess the influence of HAART use, CD4 cell count, plasma HIV RNA ≥50 copies/mL, mode of HIV acquisition, HPV infection and E6/E7 mRNA positivity. In the univariate analyses, infection with a high-risk HPV type, HAART use, lower CD4 counts and plasma...
HIV-RNA ≥50 copies/mL were all associated with abnormal cytology. In a multivariate model, after adjusting for duration of HAART use and baseline CD4 cell count, cervical infection with high-risk HPV (OR 11.23, 95% CI 1.73–72.97; P=0.01) and a HIV RNA of ≥50 copies/mL (OR 13.31, 95% CI 1.16–153.06; P=0.04) were risk factors for abnormal cytology.

**Twelve-month follow-up**

Forty adolescents at the Bangkok site were asked to return for a 12-month follow-up, and 13/20, 8/10 and 6/10 in groups 1, 2 and 3 did. Three of 27 adolescents had LSIL at baseline (one in group 1 and two in group 2), and by month 12, one had persistent LSIL, one regressed to ASC-US and the other to normal. Seven had ASC-US at baseline. At month 12, the ASC-US lesion had regressed to normal in five adolescents, whereas two, both perinatally infected, progressed to LSIL. Cytology remained normal in 14/17 with normal cytology at baseline. However, two perinatally infected adolescents had progression to either LSIL or ASC-US, and one who was HIV-uninfected had progression to ASC-US. All adolescents who had progression of lesions and the adolescent with persistent LSIL had at least one prior dose of vaccine prior to the 12-month follow-up visit; two of seven adolescents who had regression of lesions had no doses of vaccine, and the remaining five had at least one dose. Of the 27 adolescents with baseline and 12-month cytology results, 10 were infected with ≥1 high-risk HPV subtype at baseline, and of the majority (n=7/10) had persistent infection of at least one high-risk subtype at month 12 (3/4 in group 1, 2/3 in group 2, 2/3 in group 3). Five acquired a new high-risk subtype at month 12 (three in group 1, and one each in groups 2 and 3). Among 17 with no HPV or no high-risk HPV infection at baseline, eight acquired a new high-risk HPV infection at month 12 (6/9 in group 1, 1/5 in group 2, 1/3 in group 3). In these adolescents, two of those in group 1, and one in group 3 acquired HPV 16 and/or 18 at month 12. Five of six from group 1 had received three doses of vaccine and one received two doses before month 12; the adolescents in groups 2 and 3 received one and two doses of vaccine before month 12, respectively.

<table>
<thead>
<tr>
<th><strong>Table 3. Logistic regression models for factors associated with abnormal cytology (ASC-US+) on Pap smear at baseline</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1: All subjects</strong></td>
</tr>
<tr>
<td><strong>Unadjusted OR (95% CI)</strong></td>
</tr>
<tr>
<td><strong>HPV subtypes</strong></td>
</tr>
<tr>
<td>No HPV or low-risk subtypes</td>
</tr>
<tr>
<td>High-risk subtypes</td>
</tr>
<tr>
<td>E6/E7 mRNA positive</td>
</tr>
<tr>
<td><strong>Demographics</strong></td>
</tr>
<tr>
<td>Age at sexual debut &lt;17 yrs</td>
</tr>
<tr>
<td>Smokes cigarettes</td>
</tr>
<tr>
<td>Ever used alcohol</td>
</tr>
<tr>
<td>Ever used illicit drugs</td>
</tr>
<tr>
<td>Subject group</td>
</tr>
<tr>
<td>Perinatally HIV-infected</td>
</tr>
<tr>
<td>Behaviourally HIV-infected</td>
</tr>
<tr>
<td>HIV-uninfected</td>
</tr>
<tr>
<td><strong>Sexual behaviour</strong></td>
</tr>
<tr>
<td>Lifetime number of sexual partners</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2–3</td>
</tr>
<tr>
<td>4 or higher</td>
</tr>
<tr>
<td>Number of partners in last 3 months</td>
</tr>
<tr>
<td>None or one</td>
</tr>
<tr>
<td>2–3</td>
</tr>
<tr>
<td>Ever diagnosed with an STD</td>
</tr>
<tr>
<td>Condom use</td>
</tr>
<tr>
<td>Always condom use</td>
</tr>
<tr>
<td>Sometimes/almost always</td>
</tr>
<tr>
<td>Never</td>
</tr>
<tr>
<td><strong>HIV-related characteristics</strong></td>
</tr>
<tr>
<td>CD4 count at baseline (cells/μL) &gt;350</td>
</tr>
<tr>
<td>200–349</td>
</tr>
<tr>
<td>0–199</td>
</tr>
<tr>
<td>Baseline HIV RNA ≥50 copies/mL</td>
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<tr>
<td>Duration used HAART before enrolment</td>
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<tr>
<td>&lt;3 yrs</td>
</tr>
<tr>
<td>≥3 yrs</td>
</tr>
</tbody>
</table>

*Since apart from HPV grouping, all factors meeting criteria for entry into a multivariate model related to HIV, the adjusted model presented is relevant to the HIV-infected subjects only.

Abbreviations: STD: sexually transmitted disease; HAART: highly active antiretroviral therapy.
Uptake of HPV vaccination

All 60 adolescents in the study were offered HPV vaccination after the first visit, and 52 (87%) received at least one dose of the vaccine. At time of study closure, 41 had completed all three doses and the remaining 11 are scheduled to complete their doses. No adolescent to date has failed to return for scheduled doses. Of the eight adolescents who did not agree to vaccination, two each were in groups 1 and 2, and four were in group 3.

Discussion

There is a paucity of data on cervical HPV infection and cytology among sexually active, perinatally HIV-infected female adolescents [15,16]. Although conducted in a small cohort, this report is, to our knowledge, among the first. In our adolescents, HPV infection of any type was observed in 58%, including 45% having high-risk HPV infection. This is higher than a US study of non-sexually active perinatally infected children and adolescents, where only 35% of females >12 years of age had any anogenital HPV infection (i.e. vulvar +/- perianal) [17]. We also found that 30% of our perinatally HIV-infected adolescents had abnormal cervical cytology, which was lower than the rate of 47% reported in sexually active perinatally infected adolescents enrolled in the Pediatric AIDS Clinical Trials Group protocol 219C [15]. HPV infection and abnormal cervical cytology were highly prevalent in behaviourally HPV-infected and in HIV-uninfected adolescents, at similar rates. The rate of E6/E7 oncogenic mRNA positivity was 27.5% amongst the perinatally infected adolescents; no adolescents in the other two groups exhibited E6/E7 mRNA positivity, although this should be interpreted with caution due to small sample sizes of these latter groups. Furthermore, infection with at least one high-risk HPV type and detectable plasma HIV-RNA were important predictors of cervical cytological abnormalities.

In our study, the younger and less sexually experienced perinatally infected group had similarly high rates of cervical cytological abnormalities and high-risk HPV infection as the behaviourally infected and uninfected adolescents. This is despite also having higher CD4 cell count and lower HIV RNA than the behaviourally infected adolescents. In addition, in those who returned for their 12-month follow-up, a fair number had persistent high-risk HPV infection and/or acquired new HPV infection. In contrast to previous studies [9,18], the persistence of immune suppression enables persistence of HPV, which in turn is necessary for the development of neoplastic lesions. It is also possible that hormonal surges around puberty can reactivate latent HPV infection, further contributing to the risk of HPV persistence [17].

ASC-US and LSIL were common among adolescents from all three groups in our study (30%, 40% and 30%, respectively), and none had HSIL or cervical cancer. The rate of ASC-US in our study was similar to that of the Reaching for Excellence in Adolescent Care and Health (REACH) cohort in the US (30%) of behaviourally HIV-infected and -uninfected adolescents. However, REACH had higher rates of LSIL (35%) and HSIL (5%) [7]. Strikingly, the behaviourally HIV-infected adolescents had a much higher rate of HSIL at 22% compared with 5% among HIV-uninfected adolescents (P<0.01) despite the two groups having similar rates of high-risk HPV infection and low-grade cytological abnormality [11]. These data suggest that HIV-infected adolescents are more likely to progress to high-grade lesions, and routine cytological screening in these adolescents should be a priority.

Persistent infection with high-risk HPV is a precursor to cervical cancer [7–9,18]. In our study, the rates of infection with high-risk HPV type were also similar among perinatally infected, behaviourally infected and uninfected adolescents, in spite of the HIV-uninfected group having somewhat greater sexual risk behaviours (e.g. number of life-time partners, frequency of sex, sex in exchange for goods or money). These HIV-uninfected adolescents were clients who walked in for HIV testing at the Thai Red Cross Anonymous Clinic, and probably represent a group at higher risk for HIV infection than the general Thai population. The rates of infection with any type of HPV (60%) or high-risk HPV type (43%) in our study are similar to published reports in behaviourally HPV-infected and HIV-uninfected adolescents, highlighting the importance of this infection across settings [7,8,11,19–22].

We evaluated the E6/E7 oncogenic mRNA, an early marker of high-risk HPV-mediated cell transformation, among the three groups of adolescents, and observed a positivity rate of about 30% in only the perinatally HIV-infected adolescents. This rate is higher than the 11% observed in HIV-uninfected adolescents of similar ages in a US study [22]. Studies have shown E6/E7 mRNA positivity to be superior to HPV testing in predicting abnormal cervical cytology [23], and a better predictor for abnormal pathology on biopsy than cytology screening [24]. However, in this study, we did not observe a significant association between E6/E7 mRNA positivity and abnormal cytology.

According to the American College of Obstetricians and Gynecologists, HIV-uninfected adolescents should begin screening for cervical cancer at age 21, regardless of the onset of sexual debut. It is advisable that behaviourally HIV-infected adolescents should have cervical cytology screening twice in the first year after HIV diagnosis and annually thereafter [25]. Although there are no specific guidelines for perinatally HIV-infected adolescents, our data suggest that abnormal cytology can occur within a few years after sexual debut; therefore, these adolescents should begin cervical cytology screening shortly after sexual debut. UK guidelines on the HYPNet website advise annual screening for perinatally infected young people from a year from coitarche with baseline colposcopy [26].

The best way to prevent HPV infection and subsequently abnormal cervical cytology is to immunise against HPV infection prior to sexual debut. Currently, HPV vaccination in the US is advised for females from the ages of 9 to 26 years old for the bivalent vaccine, and 11 to 26 years old for the quadrivalent vaccine [27–30]. The rates of uptake vary widely between settings. However, despite the low uptake in the US, a marked decrease in HPV prevalence in females aged 14–19 years has been seen [31]. In Thailand, HPV vaccine is not yet part of routine immunisation, and it costs approximately US$100 per course,
which is unacceptable for most Thais. We were able to offer HPV vaccination to all participants in this study because of vaccine donations, and the uptake was high. As the age at sexual debut is becoming younger and HPV prevalence is the highest shortly after sexual debut [19], government healthcare costs associated with diagnosis and treatment of cases with abnormal cytology, could be significant and outweigh the costs of prevention. In HIV-infected adolescents at high risk for cervical cancer, routine HPV vaccination would be a prudent investment, even for developing countries such as Thailand [32,33]. Cervical pre-cancer screening programmes using cytology and possibly the addition of other biomarkers such as HPV DNA or E6/E7 mRNA remain essential for early diagnosis and management of cervical cancer.

There are several limitations of our study. First, the small sample size precluded robust statistical comparisons of the prevalence rates of abnormal cytology, E6/E7 oncoprotein and HPV prevalence rates between groups; however, recruitment for the study was difficult, particularly for subjects aged under 18 who may not have wanted their parents to know they were sexually active. Secondly, the longitudinal follow-up rates were low. Nevertheless, these data highlight the high prevalence of cervical cytological abnormalities in adolescent females, and show that high-risk HPV infection in perinatally HIV-infected adolescents can be similar to their behaviourally HIV-infected and uninfected peers, despite being younger and having a shorter time since sexual debut.

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HIV–NAT 139 Study group


Conflict of interest

All authors declare no conflict of interest.

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References


HIV self-testing among key populations: an implementation science approach to evaluating self-testing

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Abstract

Objectives: To review methods for measuring HIV self-testing (HIVST) among key populations, including both conventional approaches and implementation science approaches.

Methods: We reviewed the literature on evaluating HIVST among key populations.

Results: Simple HIV self-tests have already entered markets in several regions, but metrics required to demonstrate the benefits and costs of HIVST remain simplistic. Conventional measurements of sensitivity, specificity, acceptability, and behavioural preferences must be supplemented with richer implementation science measurement tools and innovative research designs in order to capture data on the following components: how self-testing affects subsequent linkage to confirmatory testing, preventive services and onward steps in the HIV continuum of care, how self-testing can be marketed to reach untested subpopulations; and how self-testing can be sustained based on overarching organisational and financial models. We outline an implementation science research agenda that incorporates these components, drawing from evaluation study designs focused on HIVST and testing in general.

Conclusion: HIVST holds great promise for key populations, but must be guided by implementation research to inform programmes and scale up.

Keywords: HIV, self-testing, implementation science, evaluation, metric, testing

Background

Achieving the global goal of having 15 million individuals on antiretroviral therapy by 2015 [1] will require substantial expansion of HIV testing and counselling (HTC) because over half of HIV-infected individuals are unaware of their serological status [2]. Suboptimal awareness of HIV serostatus is especially problematic among key populations [3–5]. Key populations are defined as vulnerable and most-at-risk populations [6] and have a higher risk of acquiring and transmitting HIV infection. Key population HTC is a key component of comprehensive HIV service provision [5]. Delayed testing is associated with increased mortality and morbidity [7]. Despite the known importance of HTC, there are many systems level barriers to expanding key population HTC services. Fear of testing [8], test–associated and other stigma [9], concerns about confidentiality [8,10], and inadequate follow-up services [11] delay key population testing.

HIV self-testing (HIVST) may help decrease some of these barriers associated with HTC in key populations. We use the definition of self-testing that specifies the collection, performance, and interpretation in private by the individual who wants to know their serological status. HIVST does not confer knowledge of serological status or provide a definitive diagnosis [12]. Self-testing could help decentralise testing, safeguard confidentiality, and make HIV service delivery systems more responsive to key populations [13]. Data from key populations suggest that willingness to HIVST may be high [14,15]. Technological advances in point-of-care technology have also facilitated the shift towards diagnostic testing outside centralised facilities [16]. There are a variety of approaches to implementing HIVST that differ based on the level of support (supervised or unsupervised), level of access (restricted by health services, semi-restricted, open access), and venues for distribution. HIVST has been piloted at facility-based clinical sites [15], emergency departments [17,18], mobile clinics, non-governmental organisations [19], pharmacies, vending machines [20], street-based testing [21], and home testing [15,22,23]. As HIVST is implemented at a wide range of sites, there is an increasing need to develop comprehensive evaluation measures.

Implementation research is a useful framework for evaluating the advantages and disadvantages of HIVST among key populations. Here we define implementation science as the study of methods to improve the uptake, implementation, and translation of research findings into routine and common practices [24]. Implementation science is often conceived as research necessary to bridge the ‘know-do’ or ‘evidence to programme’ gaps [25]. This commentary examines current measurement of HTC impact and then considers how an implementation science perspective can enrich this monitoring and evaluation among key populations.

Current measurement of HTC

Most HIV testing evaluation examines test kit sensitivity, test kit specificity, health professional acceptability and preferences among key populations [15]. These are all critical variables that are necessary, but alone insufficient, to inform comprehensive evaluation of HIV testing strategies. HIVST evaluations need to clarify study design, reasons for testing, and the local HIV testing policy guideline. First, there is no established randomised control trial-like gold standard study design for evaluating testing strategies because testing is a node in a clinical decision tree and not a novel therapeutic intervention. While there is no placebo condition for a test, there are several relevant counterfactuals.
worth considering such as delayed testing [26], never HIV tested [26–28], and algorithm-based testing (based on clinical symptoms versus screening asymptomatic individuals). Earlier research evaluated test uptake, knowledge of HIV status, and effects on sexual behaviours [29]. Second, there are multiple potential test functions, including testing for triage, screening asymptomatic individuals, diagnosis, confirmation, surveillance, and blood safety. Testing for triage refers to an initial test that can help to expedite subsequent referral, confirmatory testing and prevention services [5,30]. It is important to note that HIVST are not typically used for definitive diagnosis and should be situated within the local guidelines for HIV testing. Differentiating the test function is important for moving beyond test kit evaluation and towards testing algorithm and strategy evaluation. Research trials need to clearly specify the function of testing under evaluation in order to clearly understand the broader implications. Third, understanding local HTC guidelines is necessary for interpreting the baseline testing characteristics of the key population.

New approaches to evaluating HIVST

The general challenges of HIV testing evaluation and the more specific context of evaluating HIVST highlight the need for new models of evaluation that could be used by researchers and/or public health agencies. We first introduce an implementation science approach to evaluating HIVST. Then we use this approach to consider the influence of self-testing on engagement within the HIV care continuum, the role of self-testing in reaching and retaining untested populations, and the potential for self-testing to catalyse new organisational and financial models. The term HIV care continuum or cascade refers to the series of services required for HIV-infected individuals to achieve complete viral suppression [31].

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Using implementation science to evaluate HIVST

Unlike clinical trials, which test the efficacy of interventions, implementation science aims to evaluate the effectiveness and efficiency of interventions in real world settings. This involves examining the entire cycle, including the following stages (Figure 1): identifying gaps in existing HTC service provision, developing new HIVST interventions, implementing and disseminating interventions, measuring effectiveness and efficiency, and reviewing data to inform improved service provision. In particular, evaluation of implementation research projects focuses on two main components: evaluation of implementation fidelity during implementation and dissemination stages, and outcomes evaluation that assesses intervention effectiveness [32]. Measurement of implementation fidelity is the measurement of the degree to which organisations responsible for service delivery adhere to the intervention. This includes intervention content, frequency, duration and coverage. Implementation fidelity or adherence can be affected or moderated by a range of factors: (1) intervention characteristics including complexity, design quality and packaging, and costs; (2) outer setting including target population’s needs, peer pressure, and external policies and incentives; (3) inner setting such as an organisation’s structural characteristics, its networks and culture, which directly impact quality of delivery of an intervention; and (4) characteristics of the target population including knowledge and beliefs about the intervention, self-efficacy, and individual identification with organisation [33]. All of these implementation fidelity characteristics have implications for the evaluation of HIVST. Incorporating evaluation of these implementation fidelity characteristics is therefore important to the overall measurement of HIVST programs.

Outcome evaluation is the second key aspect of implementation evaluation. Alternative research designs dedicated to measuring intervention effectiveness are important because the focus is on external validity and practical issues in addition to efficacy [34,35]. While a detailed discussion is beyond the scope of this paper, some suggested designs include simulation modelling, pragmatic trials, rapid learning studies, and integrative studies combining community data and public health data [34]. Data from mathematical models were essential in the original approval of the HIVST by the US Food and Drug Administration [13] and remain useful in understanding the potential influence of self-testing [36]. Pragmatic trials are distinguished by their focus on real-life contexts in order to expand the generalisability of the results [37–39]. Pragmatic trials have been used to evaluate HIV interventions [40–42], but have not yet been applied to the case of HIVST. Rapid learning studies refer to generating real-time feedback for organisations that is then used to guide subsequent implementation [43]. These study designs have been used to help extend HIV treatment access [44]. Integrative evaluation that draws together community-based data as well as clinical data from public health organisations may also be useful for self-testing. The expanding capacity of HIV community-based organisations related to HTC suggests new opportunities for incorporating these data sources into implementation science evaluations.

HIVST and engagement in the HIV care continuum

Evaluating HIVST will require data on key population testing, linkage to care and retention in care within the HIV care continuum. The receipt of an HIV test used to be considered the ‘end’ of the evaluation, but the results of HPTN 052 [45] now suggest that it is really only the beginning of a complex set of services that require measurement. Self-testing may increase first-time testers and testing frequency [13] and subsequently increase key population engagement across the HIV care
continuum. However, demonstrating this will require not only data about testing experience and frequency, but also reliable data on previous key population interactions with health facilities, non-facility testing sites, mobile clinical services and other testing sites. Tracking individuals through the HIV system (testing, confirmation, linkage, retention) is fundamentally about implementation within the health system. Such evaluation may be more feasible within smaller systems (e.g. Denmark), more centralised systems (e.g. China), more unified payer systems (e.g. United Kingdom) and systems where unique health identifiers have been implemented (e.g. global north, China, Thailand). HIVST removes the opportunity for a formal, structured clinic visit that more easily collects identifying information for evaluating linkage to care. Comparing linkage and retention between individuals who enter the HIV care continuum through an HIVST test of triage compared to individuals who enter the HIV care continuum using an HIV facility-based test will be important, especially in light of the challenges in tracking self-testers. Evaluating individuals who enter the HIV care continuum through HIVST provides a strong foundation for identifying gaps in service provision.

**HIVST and social marketing**

Social marketing is the systematic application of commercial marketing concepts and techniques to the analysis, planning, execution and evaluation of programmes designed to influence the voluntary behaviour of target audiences in order to improve their personal welfare [46]. Social marketing is a potentially powerful tool to increase demand for HIVST and organise evaluation programs. A systematic review found that social marketing campaigns increased HIV testing uptake significantly among MSM populations [47]. Social marketing campaigns to promote testing are sometimes branded [48,49], similar to how companies brand individual products with distinctive packaging, tag lines and promotion materials. Similarly, branding HIVST would also be feasible through unique packaging and promotional materials, but would need to be sustained over time. Social marketing campaigns are usually delivered through multimedia platforms, with or without incentives [50], at venues where key populations congregate. These campaigns are often specific enough to aid in evaluation programs [49]. For example, we can ask key populations if they have seen the promotional materials associated with the campaign. In this respect, social marketing provides a new denominator of individuals exposed to a behavioural intervention promoting HIVST, enhancing capacity to measure effectiveness. Understanding the social marketing messages that are most effective in promoting HIV testing among specific key populations can help scale up HIVST services among those populations.

**HIVST and organisational/financial models**

The effectiveness of HIVST may depend on organisational and financial characteristics of implementation. Organisational and financial models play a large role in determining whether HIVST programs are sustained over time. From an organisational perspective, there are several different organisational models for HIVST, including the following: home-based testing; community-based organisation testing; mobile clinic testing; facility-based clinic testing, pharmacy testing and online testing [13]. Each of these organisational structures has advantages and disadvantages in terms of scaling up testing and they are not mutually exclusive within comprehensive HIV control strategies. More detailed data about testing for triage as HIV-infected individuals receive their confirmatory tests could help better understand those individuals in care, but more information is also needed about the period between test for triage and confirmatory testing. From a financial perspective, the price of self-testing and the financial model will be critical for testing sustainability in the long term. Our empirical data from South China suggest at least two financial models may be feasible to support HIVST [19]: the social enterprise model [51] and the social franchise model.

**Social enterprise model**

Social enterprise refers to organisations that apply entrepreneurial strategies to maximise improvements in human well-being rather than maximising profits for shareholders [52]. In the context of HIVST, social enterprises could charge modest fees for supervised self-testing services (testing, counselling and referral services) in order to generate revenues that would be re-invested in the organisation and ensure sustainability as public sector support decreases.

**Social franchise model**

Social franchising refers to using a commercial franchising system for social purposes instead of generating profit [53]. This model would allow individual community-based organisations to join into a franchise network to provide HIVST in accordance with quality and other standards. Both social enterprises and social franchises have advantages and disadvantages that need to be considered in the context of HIVST. New organisational and financial models may expand the limits of HIVST implementation.

**HIVST limitations**

Several limitations of HIVST are worthy of further consideration. First, oral HIV tests have a lower sensitivity than blood-based HIV tests [54]. This will lead to false negative HIV test results for key populations that could be particularly worrisome among acutely infected individuals who have some of the greatest potential for onward HIV transmission. The potential for false negative HIVST to contribute to behavioural disinhibition should be considered as well. Second, the gradual decentralisation of HIV services as HIV is integrated into local health systems introduces many challenges, including individuals receiving services at multiple sites, non-clinical sites and from multiple nurses or physicians. This makes it challenging to differentiate first-time testers from re-testers [5], which is critical for evaluating HIVST. Third, tracking individuals following self-testing as they link or delay care is a fundamental issue within HIVST. Data from a systematic review suggesting that community-based HIV testing compared to facility based testing resulted in higher uptake among men who have sex with men and female sex workers and comparable linkage to care rates is encouraging [11], but further research tracking retention in care over time will be important. Finally, we use the term key populations, but there is much more data available from HIVST studies among MSM and sex workers compared to transgender individuals and people who inject drugs.

**Summary**

HIVST may be a promising intervention as part of a comprehensive HIV control strategy. Rigorous evaluation is needed to address how self-testing can be scaled up in several contexts. In particular, heterogeneous legal/regulatory environments, social and cultural testing norms (e.g. family-based testing and couples-based testing), policy environments (e.g. human rights frameworks), regulatory and guidance environments (e.g. norms established by professional institutions.
References


Recruitment and ethical considerations in HIV cure trials requiring treatment interruption

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Abstract

Introduction: Relative to antiretroviral treatment (ART), early HIV cure-related trials (HCRTs) carry limited therapeutic benefits and unknown risks. In HCRTs requiring treatment interruption (TI) the health risks and burdens may create a barrier to study enrolment and increase the possibility for unintentional ethical violations in recruitment.

Methods: An online survey was administered to over 2,000 HIV-positive ART users in the US. Using multivariable ordinal regression we assessed effects of research participation attitudes, health and demographic traits on willingness to participate in treatment interruption studies (WtP-TI).

Results: WtP-TI was greatest among those who were highly motivated to participate in research studies for the benefit of science, society and, to a lesser extent, personal benefit. Personal benefit was less of an influence on WtP-TI among persons with higher viral loads or a history of multiple ART regimens. WtP-TI was greater among respondents who were more likely to consider personal health in making decisions about trial participation. WtP-TI had no association with perceptions of the importance of compensation to research participation. After accounting for attitudes, health status and demographic traits were generally not significantly related to WtP-TI. Notable exceptions included viral suppression status and race/ethnicity.

Conclusion: Recruitment strategies in TI studies can benefit from a focus on the long-term scientific and social benefits of study participation. Strategies targeted to particular demographic groups may have little impact on accrual, and in some cases will need to be accompanied by strategies to improve the quality of researcher–community relationships. Findings also suggest that informing communities about the health impacts of trial participation may positively impact participation decisions. However, more research is needed to interpret the impact of health messaging on recruitment and therapeutic expectations. Future work should explore the implications of altruism-based expectations on the strategic and ethical appropriateness of TI study recruitment efforts.

Keywords: HIV cure, clinical research, treatment interruption, patient attitudes, willingness to participate, altruism

Introduction
Antiretroviral treatments (ARTs) have substantially improved the health and wellbeing of persons living with HIV (PLWHs) [1–8]. Nevertheless, inadequate ART access, substantial viral resistance, long- and short-term side-effects, imperfect adherence and persistent high-risk behaviours all lead to incomplete viral suppression and demonstrate the growing need to identify an effective cure for HIV [9]. Cure strategies currently being explored may emphasise the complete elimination of HIV from the body, viral suppression and maintenance of HIV in the absence of ART and immunity from future HIV infection [10–14]. Early HIV cure-related trials (HCRTs) are likely to have limited therapeutic benefits while increasing susceptibility to severe or unknown health risks [11]. Moreover, most HCRTs will probably involve some degree of treatment interruption (discontinuation of ART use for a specified time period) to support proof of concepts, assess HIV clinical progression, and evaluate the safety and efficacy of novel modalities. Given the well-documented health benefits of ART adherence – including reduced drug resistance, decreased immune activation, fewer co-morbidities such as cardiovascular disease, lower mortality and less onward transmission [6–8,15–24] – HCRT studies involving treatment interruption (TI studies) will have a less favourable therapeutic risk–benefit profile compared to current treatment standards, particularly for virally suppressed PLWHs. The potentially high-risk profile of TI studies could present a significant obstacle to study accrual. The challenge to recruitment for TI studies may also be compounded by recent reports in mainstream media on viral rebound after ART discontinuation in persons previously considered to be ‘cured’ of HIV (e.g. the ‘Boston patients’ and ‘Mississippi baby’) [25,26]. Targeted or novel outreach approaches may be required to improve recruitment efficiency, equity and success.

Recruitment efforts may be enhanced by targeting outreach to particular study-eligible groups, or by working to improve attitudes about TI studies among harder-to-engage populations [27–33]. One goal of the present study is to provide preliminary evidence on associations between demographic and health traits, and willingness to participate in TI studies (WtP-TI). Targeted and non-targeted recruitment strategies may appeal to relevant concerns and attitudes about the benefits, burdens and risks of participation [27–33]. We focus on two classes of attitudes that may influence participation decision-making. One class of motivational attitudes reflects expectations about the immediate or long-term outcomes of trial participation. We refer to these as outcome attitudes, and these include attitudes about the personal, social or scientific benefits of participation. The latter two benefits are commonly considered altruistic motivations. A second class of motivational attitudes we call resource attitudes reflect perspectives about potential burdens of participation on important resources, with an emphasis on factors affecting daily functioning. Participation may impact therapeutic resources such as one’s health, or non-therapeutic resources such as income, employment or social relationships. A second goal of our study is to explore the relative potential for these different attitudes to influence WtP-TI, and to assess

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whether personal (demographic and health) traits moderate the attitudinal effects.

Our study is also concerned with ethical considerations in TI studies; in particular, unrealistic expectations, therapeutic misconception, coercion and exploitation [34–38]. Participation in high-risk, low-benefit trials is not necessarily unethical, even when effective alternatives (i.e. ART) are widely available [38,39]. However, phrases such as ‘cure’ can lead to unrealistic expectations, or therapeutic misconceptions about the benefits of participation. Coercion and exploitation in clinical trials reflect both intentional/unintentional behaviours of researchers (or influential others), and perceptions of potential volunteers [37,40]. Resource disadvantage may compel people to participate in a trial they otherwise would not have joined in the hopes of acquiring needed supports. Such motivations can increase the potential for exploitation or coercion in recruitment of volunteers. Although our study does not directly explore these ethical issues, we intend our analyses to highlight potential factors for future explorations of ethical recruitment strategies in TI studies.

Methods

Sample and recruitment

From December 2011–January 2012, 2,262 HIV-positive individuals completed an uncompensated online survey exploring HCRT participation attitudes. Participants were recruited through popular HIV educational and community websites and list-serves. Eligibility was restricted to those who were HIV-positive and over the age of 16. Survey participants were provided with a brief overview of HCRTs. Our analyses are restricted to respondents who reported current ART use (n=2,100), and who had non-missing data on the measured variables (n=2,094; 92% of original respondent sample).

WtP-TI measure

Our primary outcome measure is the willingness of respondents to participate in treatment interruption studies (WtP-TI). This was assessed through a single item: ‘If a study would require you to go off of your HIV medication for a period of time, which might carry health risk, how willing would you be to participate?’ A 4-point Likert response scale was used with responses ranging from ‘not at all willing’ to ‘very willing’.

Outcome attitudes

In this study attitudinal measures reflect attitudes about participation in HCRTs in general and are not limited to TI studies specifically. Among our outcome attitude measures, the personal benefit item asked respondents ‘Assuming that entering a study might pose health problems and other risks, how much would the chance to benefit yourself by participating in a study motivate you to join the study?’ Social benefit was measured with the statement: ‘Assuming that entering a study might pose health problems and other risks, how much would the chance to benefit others by participating in the study motivate you to join the study?’ Scientific benefit was measured as: ‘Assuming that entering a study might pose health problems and other risks, if you were aware that you would probably not benefit from a new drug or procedure being studied, but that your participation in the study might advance the field of HIV research, how willing would you be to participate?’ All items were rated on a 4-point Likert scale with responses ranging from ‘not at all motivated’ to ‘very motivated’. The statement ‘potential health risks and other harms’ included in each of the above measures does not specify precise harms or risks. Thus, individuals may differ in how they interpret this statement (see Discussion below).

Resource attitudes

Resource attitude measures focus on the importance or influence of a given resource to participation decisions. Similar to outcome attitude measures, our resource attitude measures focus on participation in HCRTs in general. We focus on two resource attitudes: perceived health influence (‘How much would your current health affect your willingness to participate in studies that may eventually lead to a cure for HIV?’) and the importance of compensation to trial participation (‘Assuming that entering a study might pose health problems and other risks, how important would it be to compensate you for your time and discomfort?’). Both items were rated on 4-point scales ranging from ‘very reluctant’ to ‘very motivated’ (health influence), or ‘not at all important’ to ‘very important’ (compensation importance).

Health traits

HIV diagnostic measures were self-reported and include current viral load (<50 copies/mL, i.e. suppressed, 50+ copies/mL, or ‘Don’t know’(DK)), and CD4 cell count (>500, 351–500, <351, or ‘Don’t know’). HIV experience includes ‘years HIV positive’ (categorical) and the ‘number of ART regimens since first diagnosis’ (categorical). Perceived current health was rated on a 4-point response scale ranging from ‘very poor’ to ‘excellent’.

Demographic traits

Gender was reported as male, female, transgender [separately for male-to-female (MTF) and female-to-male (FTM)], or transitioning (separately by MTF and FTM). Very few respondents (<1%) identified as transgender or transitioning (and only as MTF). We include these women within the ‘female’ designation. Latino/Hispanic ethnicity was asked separately from racial identity. We combined these variables to obtain the following racial/ethnic designations: white (not Latino), Latino (alone or in combination), black and ‘other race’. We also measured age, annual income, employment status and highest level of educational attainment.

Analyses

In addition to standard univariate descriptive analyses, we conducted bivariate and multivariate analyses. First, we conducted chi-squared analyses exploring differences in the distributions of outcome and resource attitudes about HCRT participation. Second, the health and compensation resource attitude items reflect the importance of these resource considerations to HCRT participation decision-making. We conducted supplemental multivariable ordinal regression analyses of these resource attitudes to assess associations between (a) health resource attitudes and health traits, and (b) compensation resource attitudes and demographic traits. These supplemental analyses are intended to highlight personal traits that might influence responsiveness to resource attitude-based recruitment strategies. Third, we employed ordinal logistic regression to estimate the effects of our attitudinal, health and demographic factors on WtP-TI. This analysis provides insight into the potential impact of trait-targeted or attitude-focused recruitment strategies on TI study accrual. We also tested a series of interaction terms between the significant trait and attitudinal measures in our model in order to assess the extent to which the relative importance of attitudinal motivators of WtP-TI differ by personal traits. Proportional odds models included a logit link and unstructured thresholds, and Wald-type confidence intervals were estimated. Analyses were conducted in the R statistical software and proportional odds models were estimated using the Ordinal package [41,42].
Results

Sample demographic and health traits

The sample largely comprised older, college-exposed white men with low to moderate income. Men accounted for 83% of the sample, and 74% of the sample identified as white. Only about 10% of the sample identified as Latino (alone or in combination) and 10% identified as black. Of respondents, 44% were over the age of 50, and 34% were between the ages of 41 and 50. Nearly 50% of respondents reported income below $25,000 per year (22% <$10,000, 27% $10,000–24,999), 27% had income between $25,000 and $49,999, 15% had income between $50,000 and $74,999, and roughly 20% had income at or above $75,000. Respondents were generally either employed full time (48%) or on disability (28%), and 55% had at least a college degree compared to 15% with only a high school degree/equivalent.

With respect to self-reported health, 36% of respondents stated that they were in excellent health, and an additional 45% stated that their health was ‘good’. Nearly 87% of respondents reported suppressed viral loads (<50 copies per ml), and a small majority (55%) had CD4 cell counts above 500 cells/μL. For both the viral load and CD4 cell items, roughly 2% of the sample (n=45 and 48, respectively) were unaware of their current status. We make the assumption that persons reporting unknown HIV diagnostics are not likely to be engaged in regular HIV monitoring and include them with those reporting unsuppressed viral loads and lower CD4 cell counts. Roughly 30% of respondents were on their first regimen, 46% were on second, third or fourth regimens, and 23% were on their fifth or higher regimen. Over half of respondents (57%) received an HIV diagnosis over 10 years prior, and very few (17%) had been diagnosed in the previous 3 years.

Decision-making outcome and resource attitudes

We hypothesise that willingness to participate in TI studies will vary according to perceived motivation to participate in any HIV cure-related trials. We explored five potential motivators: personal benefit; social benefit; scientific benefit; health influence; and financial compensation. Figure 1 highlights the distribution of responses to each of these motivators. For each item, the 4-point motivational attitude responses ranged from low (e.g. ‘not at all motivated’ to participate for personal benefit) to high (e.g. ‘very motivated’). Most respondents stated that they were motivated or very motivated to participate in any HCRTs for personal (62%) or social (56%) benefits, whereas less than half of the sample was motivated or very motivated to participate for scientific (45%) benefit. Current health was reported as an important influence on participation, with 42% of respondents stating that their current health was a significant motivator (‘very motivated’), and another 35% stating that it was ‘somewhat’ of a motivator. Only 23% of respondents expressed reluctance to participate due to current health. Although 34% of respondents noted that financial compensation was ‘very important’ to HCRT participation, the remaining respondents were nearly equally likely to state that compensation was not important (17%), somewhat important (24%) or important (24%).

We conducted chi-squared tests of homogeneity to assess the degree of difference in the distributions of the attitudinal variables presented in Figure 1. All chi-squared analyses were significant at P<0.001 (Table 1). We classify distributions as meaningfully different if they lie above the median $\chi^2$ of 293. Respondents were much more likely to be motivated to participate in HCRTs for personal as opposed to scientific benefits ($\chi^2=338$). Social benefit motivations mirrored perspectives on both personal ($\chi^2=47$) and scientific ($\chi^2=168$) benefit. Respondents’ views on the relevance of health and compensation considerations to participation decisions most closely reflected personal benefit motivations ($\chi^2<248$) and differ substantially from social or scientific benefit motivations.

In order to better interpret the distributional meanings of health and compensation resource attitudes we regressed these items on health and demographic traits, respectively. For brevity we present only those variables demonstrating significance in each of the models in Table 2. The relationship between personal traits and resource attitudes about trial participation is complex and multidimensional. Persons reporting greater influence of health considerations to trial participation (Table 2a) had higher viral loads and lower CD4 cell counts compared to others, but (perhaps paradoxically) rated their self-reported health as ‘excellent’. Years HIV positive and number of ART regimens since diagnosis were unrelated to health resource attitudes. Persons reporting greater importance of compensation to trial participation (Table 2b) were black or Latino, and had low incomes (<$10,000). However, unemployed individuals were far less likely to state that compensation was important to their decision-making. Age, sex and education were not significantly related to compensation importance.

Determinants of willingness to participate in treatment interruption studies (WTP-TI)

Most respondents expressed ambivalence about participating in TI studies. Only a third (34%) of respondents stated that they would be ‘willing’ or ‘very willing’ to participate in HCRTs that involve TI, compared to 34% who agreed that they would be
Additionally, among persons who reported moderately low viral loads (>50/DK) had lower WtP-TI scores [interaction (a) in Table 3]. HCRTs for personal benefit, those with higher viral loads were more likely than others to express higher WtP-TI. However, among persons who had a history of two or more ART regimens were significantly more likely to express high levels of WtP-TI compared to peers who did not consider health as important to participation decision-making. As noted in Table 2 (panel a) whether these derive from challenges to managing HIV (e.g. unsuppressed viral load) or feeling healthy enough to participate in such studies remains to be seen. The absence of interaction effects between health resource attitudes and health traits further complicates interpretation. With respect to health traits, persons with un-suppressed (>50/DK) viral loads, and persons with a treatment interruption history of two or more ART regimens were significantly more likely to express high levels of WtP-TI. However, among persons who reported high levels of motivation to participate in HCRTs for personal benefit, those with higher viral loads (>50/DK) had lower WtP-TI scores [interaction (a) in Table 3]. Additionally, among persons who reported moderately low personal benefit motivations for trial participation, those who had a history of two or more ART regimens were less likely than their counterparts to express high levels of WtP-TI. 

### Discussion

Our findings suggest that appealing to scientific altruism for TI study participation may have the highest impact on accrual, irrespective of demographic or health traits. Perhaps paradoxically, we found that persons for whom health resource considerations were highly relevant to study participation were more likely to express support for TI study participation.

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### Table 2. Adjusted relative odds ratios for significant associations between health and demographic traits and resource attitudes about HCRT participation (n=2,094)

<table>
<thead>
<tr>
<th>Variable</th>
<th>αROR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Health resources attitudes in HCRT participation decision-making</td>
<td></td>
</tr>
<tr>
<td>Viral load (copies/μL)</td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>ref</td>
</tr>
<tr>
<td>50+/DK</td>
<td>1.32* (1.04, 1.69)</td>
</tr>
<tr>
<td>CDF</td>
<td></td>
</tr>
<tr>
<td>&gt;500</td>
<td>ref</td>
</tr>
<tr>
<td>351–500</td>
<td>0.98 (0.81, 1.19)</td>
</tr>
<tr>
<td>&lt;351/DK</td>
<td>1.28* (1.04, 1.59)</td>
</tr>
<tr>
<td>Health</td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>ref</td>
</tr>
<tr>
<td>Good</td>
<td>0.69† (0.58, 0.83)</td>
</tr>
<tr>
<td>Fair/Poor/Very poor</td>
<td>0.48† (0.38, 0.61)</td>
</tr>
<tr>
<td>(b) Compensation resource attitudes in HCRT decision-making</td>
<td></td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>ref</td>
</tr>
<tr>
<td>Latino</td>
<td>1.54† (1.17, 2.02)</td>
</tr>
<tr>
<td>Black</td>
<td>2.21† (1.66, 2.94)</td>
</tr>
<tr>
<td>Other</td>
<td>1.19 (0.83, 1.71)</td>
</tr>
<tr>
<td>Income</td>
<td></td>
</tr>
<tr>
<td>&lt;$10,000</td>
<td>ref</td>
</tr>
<tr>
<td>$10,000–24,999</td>
<td>0.71† (0.52, 0.96)</td>
</tr>
<tr>
<td>$25,00–49,999</td>
<td>0.47* (0.34, 0.64)</td>
</tr>
<tr>
<td>$50,000–74,999</td>
<td>0.32† (0.22, 0.46)</td>
</tr>
<tr>
<td>$75,000–100,000</td>
<td>0.27‡ (0.18, 0.41)</td>
</tr>
<tr>
<td>&gt;$100,000</td>
<td>0.18† (0.12, 0.26)</td>
</tr>
<tr>
<td>Employment status</td>
<td></td>
</tr>
<tr>
<td>Full time</td>
<td>ref</td>
</tr>
<tr>
<td>Part time</td>
<td>0.91 (0.69, 1.20)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>0.54† (0.54, 0.97)</td>
</tr>
<tr>
<td>Disability (temporary or permanent)</td>
<td>0.96 (0.76, 1.22)</td>
</tr>
</tbody>
</table>

* P<0.05, †P<0.01, ‡P<0.001.

---

### Table 3. Relative odds ratios (ROR) from final ordinal logistic regression model of willingness to participate in treatment interruption studies (WtP-TI) (n=2,094)

<table>
<thead>
<tr>
<th>Variable</th>
<th>αROR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal benefit motivation</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>ref</td>
</tr>
<tr>
<td>Moderate–low</td>
<td>2.87† (1.38, 5.98)</td>
</tr>
<tr>
<td>Moderate–high</td>
<td>4.68‡ (2.38, 9.98)</td>
</tr>
<tr>
<td>High</td>
<td>4.69‡ (2.25, 9.77)</td>
</tr>
<tr>
<td>Social benefit motivation</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>ref</td>
</tr>
<tr>
<td>Moderate–low</td>
<td>1.57* (1.05, 2.37)</td>
</tr>
<tr>
<td>Moderate–high</td>
<td>2.38‡ (1.52, 3.75)</td>
</tr>
<tr>
<td>High</td>
<td>3.53‡ (2.16, 5.78)</td>
</tr>
<tr>
<td>Scientific benefit motivation</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>ref</td>
</tr>
<tr>
<td>Moderate–low</td>
<td>3.76‡ (2.08, 6.82)</td>
</tr>
<tr>
<td>Moderate–high</td>
<td>5.00‡ (2.78, 9.89)</td>
</tr>
<tr>
<td>High</td>
<td>6.69‡ (3.72, 12.03)</td>
</tr>
<tr>
<td>Viral load (copies/μL)</td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>ref</td>
</tr>
<tr>
<td>50+/DK</td>
<td>4.10† (1.63, 10.32)</td>
</tr>
<tr>
<td>ART regimens</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>ref</td>
</tr>
<tr>
<td>2</td>
<td>3.17* (1.20, 8.40)</td>
</tr>
<tr>
<td>3</td>
<td>3.51† (1.35, 9.13)</td>
</tr>
<tr>
<td>4</td>
<td>4.13* (1.37, 12.48)</td>
</tr>
<tr>
<td>5+</td>
<td>3.42* (1.28, 9.09)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>ref</td>
</tr>
<tr>
<td>Latino</td>
<td>1.36* (1.02, 1.83)</td>
</tr>
<tr>
<td>Black</td>
<td>0.70* (0.52, 0.93)</td>
</tr>
<tr>
<td>Other</td>
<td>0.80 (0.55, 1.16)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>&lt;31</td>
<td>ref</td>
</tr>
<tr>
<td>31–40</td>
<td>0.99 (0.67, 1.44)</td>
</tr>
<tr>
<td>41–50</td>
<td>1.01 (0.70, 1.44)</td>
</tr>
<tr>
<td>51–60</td>
<td>0.81 (0.56, 1.17)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>0.54† (0.35, 0.82)</td>
</tr>
<tr>
<td>Significant interaction</td>
<td></td>
</tr>
<tr>
<td>(a) Personal benefit =High/Viral load=50+/DK</td>
<td>0.32* (0.11, 0.87)</td>
</tr>
<tr>
<td>(b) Personal benefit =Mod–low/Viral load=5+</td>
<td>0.34* (0.12, 0.99)</td>
</tr>
<tr>
<td>=ART regimen=2</td>
<td>0.22* (0.07, 0.72)</td>
</tr>
<tr>
<td>(c) Personal benefit =Low/Viral load=5+</td>
<td>0.25† (0.09, 0.69)</td>
</tr>
<tr>
<td>(d) Personal benefit =Low/Viral load=ART regimen=5+</td>
<td></td>
</tr>
</tbody>
</table>

1 Other variables tested but not significant: importance of financial compensation, current CD4, years HIV positive, perceived current health; gender; income; employment, and educational attainment.

2 Adjusted relative odds of expressing greater support for participation in HIV cure related trials (HCRTs).

* P<0.05, †P<0.01, ‡P<0.001.
participation compared to others, even after accounting for health traits and personal benefit motivators. We also found an ambiguous relationship between health resource attitudes about participating in HCRTs and self-reported health traits, which suggests that the health risks and benefits of participation may be complexly related to decision-making. Moreover, to the extent that our findings support demographic-targeting in TI recruitment strategies to increase accrual or diversity, they suggest that developing positive researcher–community relationships may be more important than identity-focused marketing. Among black respondents, in particular, the lower levels of WtP-TI and greater importance of compensation to participation decision-making irrespective of attitudes, health, and economic considerations may reflect greater distrust for high-risk/low-benefit trial participation among blacks. Demographically targeted TI recruitment strategies will likely only succeed where the legacy of government- and academic-sponsored abuses based on identity and group affiliations are openly addressed, and direct researcher–community trust is actively promoted.

Our results suggest that future ethical research should explore the implications of recruitment strategies that heavily appeal to scientific and social altruism or health resource attitudes. These considerations have a strong impact on WtP-TI such that unrealistic expectations or therapeutic misconceptions may unduly sway participation decision making. Appeals to personal benefit, while affecting WtP-TI, did not carry as much weight as the other motivational attitudes.

The absence of WtP-TI associations with compensation resource attitudes, gender, income, employment and education may either be a consequence of the sample recruited for the study, or indicate that these factors do not substantially influence TI participation decision making. More in-depth qualitative and quantitative work is needed to explore influence of structural factors such as social organisation, cultural/community norms, and differential access on participation decision making.

Among our study limitations, survey recruitment and completion was conducted entirely online through diverse yet select websites. Thus, our sample excludes PLWHs who never or rarely access these sites. Survey respondents did not receive financial compensation, which may have affected motivations to complete the survey. However, lack of compensation would also reduce the incentive for respondents to complete these anonymous surveys multiple times, or provide socially desirable responses. In some cases (especially our attitudinal and WtP items) the complexity of survey wording may have limited full understanding of items and responses. Future work should continue to assess facilitators of risk, benefit, burden and procedural understanding among PLWHs interested in participating in TI studies.

Conclusion

Our results indicate that support for participation in TI studies will probably be predicated on altruistic and health resource considerations, irrespective of therapeutic risks, personal benefits, and health and demographic traits. Future work should explore these considerations in greater detail. Such work can aid HCRT TI researchers in their goal to improve effective outreach, raise awareness, community support and gaps, and increase accrual rates and diversity. Additionally, more research is needed to understand the ethical implications of these studies for persons who decide to participate. These ethical considerations will require attention to social, cultural, and structural components of altruistic motivations for participation. Qualitative and quantitative work in this area should better explicate and calibrate the importance of specific study designs, risks, burdens, and benefits to WtP-TI. The views of providers, educators and advocates should also be explored as these may be essential to promoting participation and developing acceptable research and engagement strategies for HCRTs. Future work should explore the ethical implications of altruism-based recruitment strategies on participation expectations, and of researcher–community trust on coercion and exploitation in demographically targeted research efforts.

Acknowledgements

We would like to thank members of the International AIDS Society (IAS) Psychosocial Study Section for their feedback and discussion of previous analyses of this data, and Dr Joseph Turner for his review of earlier versions of our manuscript. Most importantly, we would like to thank the thousands of individuals who completed the uncompensated survey. The study received approval through the Institutional Review Board at Fred Hutchinson Cancer Research Center (Seattle, WA).

Authors’ contributions

DE and NV led survey creation and dissemination and data collection. MA conducted analyses and led manuscript development. All authors reviewed and edited the final manuscript.

Competing interests

No competing interests exist for the authors.

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DE and NV led survey creation and dissemination and data collection. MA conducted analyses and led manuscript development. All authors reviewed and edited the final manuscript.

Competing interests

No competing interests exist for the authors.

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Community participation in HIV cure research: perspectives from Thailand

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1Thai Red Cross AIDS Research Centre, Bangkok, Thailand, 2SEARCH, Bangkok, Thailand, 3AIDS Access Foundation, Bangkok, Thailand, 4HIV-NAT, Bangkok, Thailand

Abstract

Thailand aims to end its AIDS epidemic by 2030, and key strategies to effect this include an increase in HIV testing coverage to 90% for key populations (i.e. men who have sex with men, sex workers, people who inject drugs and partners of people living with HIV) and antiretroviral treatment (ART) initiation for all, regardless of CD4 cell count. Thailand is now focusing its national HIV strategic plan on the recruit-test-treat-retain cascade. In order to recruit more key populations into HIV testing, offer immediate ART and retain both HIV-negative people for regular HIV testing and HIV-positive people for continued ART service, effective communication to the community about the clear benefits of early HIV diagnosis and early ART, including the possibility for HIV cure, has become more important than ever. We discuss the need for more innovative ways of communicating.

Community participation in HIV research in Thailand started more than a decade ago. Widespread concerns over people in the community being used and treated unfairly like guinea pigs in HIV research studies, HIV vaccine trials in particular, have driven strong demands from the community to be effectively engaged in the entire research life-cycle from study design to result dissemination. Participation occurred naturally with the demand from the community to understand more about the research to be conducted and to share their views and concerns with the researchers. Meetings, forums and demonstrations have been used for that purpose until the community advisory board (CAB) has become widely accepted locally and globally as an official way of obtaining community participation when doing research. CABs have been established in many research settings in Thailand as protocol-specific, institution-based or key population-based CABs. In general, HIV prevention research causes more concern to the community than therapeutic research. Common concerns include sufficient provision of comprehensive HIV prevention packages to participants in HIV prevention trials, and post-trial access to HIV prevention products or procedures for the participants and the country as a whole. Good participatory practice (GPP) guidelines for biomedical HIV prevention trials have recently been introduced as systematic guidance on how to engage stakeholders effectively in the development, planning, implementation and conclusion of a trial, including dissemination of trial results [1]. GPP has also been adapted for use in other settings outside HIV prevention research [2].

GPP proposes the use of as many formal and informal stakeholder advisory mechanisms as possible to acquire community participation in the research. These mechanisms include stakeholder meetings, local events, ongoing dialogue with community-based organisations (CBO), focus group discussions, talk radio/TV shows, CABs, and non-governmental organisation (NGO) advisory and participant groups. The GPP has been used actively in many completed and ongoing HIV vaccine, pre-exposure prophylaxis and other HIV prevention studies in Thailand [3]. According to GPP, stakeholders include trial participants, community stakeholders (CBOs, participants’ family, friends, schools, colleagues, peers, trial site staff, local religious institutions, traditional leaders, community advisory boards and local health service providers), broader stakeholders (NGOs, local policy makers, local media and medical professionals), as well as national (parliamentarians, Ministry of Health, media, regulatory bodies, ethical review committees, funders, sponsors) and international stakeholders (international NGOs, international organisations, networks, sponsors and funders).

For HIV cure research in particular, media attention has become both a strength and a weakness for community participation. Currently, ART can reduce HIV viral burden and infectiousness although it does require strict adherence to daily medications. What could an HIV cure offer beyond this? In the medical community, a cure could be HIV remission (living with HIV without HIV RNA detected in the blood), or HIV eradication (no HIV in the body and completely HIV negative). However, it is not well understood what HIV cure means to those within the community who are at risk of HIV but have never been tested or still test negative, to those who are currently living with HIV with and without ART, and to those who are diagnosed early or late. The word ‘cure’ certainly has different meanings for different people: from living with HIV without the risk of transmitting the virus to others, to living without any traces of HIV in the body [4].

Recent news regarding a possible HIV cure case in Thailand [5] demonstrates an example of large variations in reactions among the Thai community, from very cautious to ready to believe. Alongside real HIV cure news, the Thai community has experienced fake HIV cure news before [6]. Without a good communication and issue management plan that follows the GPP [1], these news stories have the potential to send out wrong or pre-emptive messages to those people living with HIV on ART who may risk stopping their ART to see whether their HIV has been cured. On the other hand, media attention could be used as one of the most robust tools to educate and communicate correct messages around HIV cure to the community.
Basic questions around HIV may again be coming back in this era of cure research. What is HIV? What does HIV do to you? How does HIV medicine work? Basic concerns around being involved in research studies have also become more specific and in a manner that is different from those asked to be involved in HIV prevention or other HIV treatment studies. Who is participating in such HIV cure research? What are the risks of participating, and what will one get out of this research? Asking people with acute HIV infection with their uniquely low reservoir to participate in HIV cure research using an intervention without ample data on effectiveness and safety, means that he/she will risk reseeding the reservoir and probably losing options for future trials with better interventions. People may want to wait for a better marker of viral control or a more effective intervention to be available before joining such a study. In addition, health service providers and their clients are now asking how soon after an exposure should one test for HIV. This is a question around the window period, which differs from one setting to another depending on the HIV testing methods. Knowing that diagnosing HIV within the first 2–4 weeks of acquiring HIV infection might give a better chance of a future cure, may well affect willingness to test initially as well as to test quickly after any further potential exposure. The community is also demanding that the benefits and risks of early ART be communicated clearly to them.

How should researchers promote community participation in HIV cure research? Simply ask this question to the children and adults around you, and the answers may include invitations to visit homes for further discussions, make booklets, create videos and post on YouTube, do public presentations, do surveys, or hold public meetings where people can come and share ideas. All these fit well with what is recommended in GPP but the question is: are we doing enough?

In Thailand, as one of the countries with the most advanced research studies in HIV cure, owing to the successful establishment of the SEARCH 010/RV254 study at the Thai Red Cross AIDS Research Centre in Bangkok [7] and the RV217 study at the ECHO Center in Pattaya, community participation in HIV research could be strongly promoted in the following ways. First, the community should be updated on the steps towards HIV cure, from identifying HIV at its earliest stage, treating as early as possible after HIV diagnosis in order to limit HIV replication and reservoir size, identifying interventions to enhance the body’s immune system and/or killing residual HIV, and interrupting ART. Secondly, the community should be encouraged to voice their concerns and advise on how we should move together through the next stages, not only at a research setting level but at a national level. HIV cure research should not be seen as a separate issue from the recruit-test-treat-retain cascade, but rather as an ideal subsection in which the complete cascade can be demonstrated. Finally, looking into the future, all stakeholders should work together using the lessons already learned to maintain and then increase the momentum for early testing and early treatment. Community participation through CAB has been an important foundation but researchers and community stakeholders must not be complacent and now need to build on that input and make use of current and innovative consultation methods as outlined in GPP to further improve communication.

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What motivates participation in HIV cure trials?
A call for real-time assessment to improve informed consent

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2Department of Social Medicine, University of North Carolina School of Medicine, Chapel Hill, NC, USA

Abstract
HIV cure research, a diverse set of studies aimed at eradicating or greatly reducing HIV in latent reservoirs, has become a strategic priority for global AIDS research. However, in early-phase HIV cure research there are ethical challenges related to the uncertainty around potential risks and the risk–benefit balance. Similar to clinical trials in other disease areas, these concerns may impact clinical trial participants’ comprehension and decision making. Here we suggest attention to the terminology used to describe HIV cure research that may promote therapeutic misconception, and exploration of the decision-making influences and processes of those who accept and decline participation in HIV cure trials. These data will facilitate efforts to improve protocols and informed consent based on an understanding of participant preferences and needs.

Keywords: HIV cure, decision making; clinical trials

‘I believe that everybody expects to be cured of HIV, at least in 10 years. But the definition of HIV cure and expectations are different for everyone.’
Young adult Thai male with HIV [1]

Curing HIV has become a strategic priority for global AIDS research [2]. The NIH Clinical Trials database includes more than one hundred current or completed early phase clinical trials devoted to some aspect of HIV cure research [3]. These highly diverse studies employ a variety of approaches to eradicate HIV in latent reservoirs, including gene editing, therapeutic vaccines, ART intensification studies, latency reversing strategies and combination designs. Each features very different study designs and types and sources of potential risks. Many require participants to have demonstrated long-term viral suppression. Some introduce ‘structured treatment interruption,’ where ART is withdrawn under controlled conditions to examine the impact of a particular intervention [3].

The exciting prospect of clinical research aiming to cure HIV has also generated numerous commentaries on ethical aspects of these studies including developing an acceptable risk–benefit balance, and in some cases, focusing on how much risk and uncertainty is acceptable before a clinical trial can proceed [4–7]. Commenting on the potential for direct medical benefit for participants, Dubé and colleagues [8] describe HIV ‘cure’ trials as ‘proof-of-concept studies designed to evaluate novel paradigms to reduce persistent HIV-1 reservoirs, without any expectation of medical benefit.’ Evans [7] focuses on trial-related risk for participants who are relatively healthy, and Eyal and Kuritzkes [9] ask, ‘Is it ethical to invite patients to volunteer for studies that replace safety with great uncertainty?’

Such commentaries focus attention on how participants in early-phase HIV cure research may balance perceived risks and benefits and manage uncertainty. The HIV clinical research community must grapple with these concerns, and address such practical questions as: within the constraints of applicable regulations, how much individual risk should be allowed in clinical trials for individuals who are ‘healthy’ on ART, especially when there may be major public health implications? How are benefits characterised and valued [10]? To offset risks for participants, what chance (if any) for direct medical benefit is sufficient? What about indirect benefits, such as enhanced care access (real or perceived), improved social support, and psychological benefits from participation? How should altruistic motivations and aspirational benefits to future patients be valued? How can research teams and institutional review boards make informed decisions when so much uncertainty exists about potential harms, benefits and participant preferences?

These issues are not, of course, unique to HIV. The ethics of early phase clinical trials, including the implications of how risks and benefits are presented and understood, has long been a focus of both conceptual and empirical bioethics literature (for examples, see [10–12]). Concerns about insufficient participant comprehension undermining informed consent have been studied in many clinical areas (see [13]). These concerns are especially relevant for early phase trials and for clinical areas with limited treatment options and a severe disease manifestation or progression. A phenomenon raising particular challenge to participant comprehension of trial risks and benefits is therapeutic misconception, first described by Appelbaum and colleagues in 1982 [14], and defined subsequently as when, ‘…individuals do not understand that the defining purpose of clinical research is to produce generalizable knowledge, regardless of whether the subjects enrolled in the trial may potentially benefit from the intervention under study or from other aspects of the clinical trial’ [12]. Therapeutic misconception is orientated around deficiencies in understanding and knowledge of the research that may stem from the participant, the informed consent materials, and/or the clinical trial team. A contrasting framework is therapeutic optimism, which refers to a research participant’s optimism for the best personal outcome, and does not necessarily compromise the decision-maker’s autonomy or stem from a misunderstanding or lack of information [15,16]. In addition, trials have been shown to present a valued opportunity for participants to express optimism for better outcomes for themselves and others with the disease [17,18].

The potential impact of therapeutic misconception and optimism on clinical trial decision making deserves (and receives) continued attention (for example [19–21]). In HIV cure research, existing ethical concerns about acceptable risk–benefit balance, and uncertainty regarding levels of risk and benefit, will naturally lead
to important questions about participants’ clinical trial decision making and the informed consent process. And yet, determinations about how clinical trial participants ‘should’ make their decisions are fraught with challenges, notably that such recommendations may not be informed by evidence about the decision-making influences and processes of the population in question, and may assume rational, cognitively based decision making, when a large body of research suggests otherwise (as widely publicised in recent best-sellers such as Kahneman’s Thinking, Fast and Slow [22]).

If efforts to improve informed consent processes are to be applied to best effect, there is a need to understand what motivates people to participate in HIV cure research and when and how they make decisions. Here we suggest addressing an area of ‘low hanging fruit’ regarding the terminology used to describe HIV cure research, and then present a longer-term need to explore decision making so the HIV cure community can make judgements and develop interventions based on an understanding of participant preferences and needs.

Attend to terminology that may promote therapeutic misconception

Decision-making influence may come in unexpected ways. Isles and Pearn’s clinical trial commentary discusses the impact of descriptors and acronyms used to describe a range of clinical trials, where the use of positive descriptors may exert undue influence on the perception of potential participants [23]. In HIV, several authors discuss the potential for the word ‘cure’ itself to create misunderstanding and raise unrealistic expectations for potential personal benefit from participation in trials. Tucker and colleagues [24] consider three conceptual frameworks to replace ‘cure’ in describing current research: sterilising/functional cure, sustained virological response (SVR), and clinical remission. They opt for ‘clinical remission,’ a term long familiar in cancer research and clinical care, which appropriately ‘denotes improvement with some uncertainty’ [24]. Dubé and coauthors [8] concur that ‘language used to describe clinical research represents a powerful opportunity to educate volunteers,’ recommending the term ‘experiment’ as more appropriate than HIV ‘studies’ or ‘clinical trials.’ Finally, Volberding [25] points to the broader, potentially negative impact of over-hyped media attention, where inappropriately positive terminology is used to describe very preliminary trial results.

Because clinician scientists are not immune to therapeutic misconception and highly optimistic beliefs about clinical trials [12,26,27], the need to clarify and revise the HIV cure language extends to professional use as well as when describing these trials to patients and communities. The input of community leaders and advocacy organisations about language preferences should be highly prioritised, and replacement terms should be explored with patients to evaluate their acceptability.

Explore trial decision making

There are well-established decision-making and health behaviour theories that provide a systematic framework to conceptualise and explore health-related behaviours relevant to clinical trial decision making. For example, the Health Belief Model (HBM) [28,29] is a commonly used conceptual framework to understand why individuals do or do not engage in health-related actions. Dimensions of the HBM include perceived susceptibility (a perception of personal vulnerability or risk), illness severity and burden, perceived benefits of the health-related behaviour, and perceived barriers to achieving the desired health outcome. The model includes cues to action (e.g. the offering of trial participation) that might spur a health-related decision, together with influences of social, demographic and personality factors. Several resources that describe social science relevant to decision studies are shown in the Resources panel.

The use of decision frameworks and theoretical models can inform studies nested within clinical trials to better understand the processes of decision making, influences on decisions that are made, and post-trial decision satisfaction. Furthermore, it may be useful to explore potential participants’ hopes and expectations as distinct decision-making influences [30]. Specific examples of decision-making topics are provided in Table 1.

We encourage clinical trial teams to collaborate with social scientists to integrate decision-making studies in their trials. In contrast to studying responses to hypothetical scenarios or retrospective studies of trial participants, the current situation in HIV cure research offers a unique opportunity to investigate participants’ experiences as they are unfolding, in real time. Concurrently, collaborative research teams can determine how to ensure that a nested decision study provides important feedback to the clinical trial team while taking care not to disrupt or threaten the clinical trial process [31].

In developing decision-making studies in these early days of HIV cure research, community engagement is especially vital to ensure that the decision study focuses on domains and asks specific questions that are most relevant to the participant experience. Longitudinal or comparative studies that follow participants from consent to trial end will provide especially important information on perceptions of ‘cure’ over time and address ongoing, real-world ethical concerns [12,32]. Such studies can also be used to inform decision-making interventions and generate (and later test) hypotheses related to study adherence and maintenance of participation. In addition, exploring perspectives of individuals who qualify for a trial, but decline participation provides valuable input into trial design and recruitment.

Panel 1. Resources

|---------------------------------------------------------------|
Table 1. Aspects of clinical trials decision-making for exploration

<table>
<thead>
<tr>
<th>Decision-making topic</th>
<th>Relevant questions</th>
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| Decision-making processes | 1. Who is involved in making the decision? Are there cultural and societal norms that play a role in decision making?  
2. How is the decision made, i.e. how is the ‘evidence’ (information and/or emotion) weighted? How is uncertain information regarding benefits, risks and burden internalised?  
3. When is the decision made? At time of the primary informed consent encounter, before, or after? |
| Influences on decision making | 1. What is the impact of the person’s experience with HIV on his/her trial decisions?  
2. What does the participant expect will happen during the clinical trial, in terms of logistics, benefits, burden, and harms? What are the information source(s) and motivations that underpin those expectations?  
3. What does the participant hope might happen during the clinical trial, what are the influences of emotion and optimism? What are the information source(s) and motivations that underpin those hopes?  
4. What would participants consider as meaningful benefits and harms? How does this compare with investigators? |
| Decision satisfaction during and after the trial | 1. How is decision satisfaction related to prior decision-making influences, if at all?  
2. Does the participant express decisional regret? In what areas?  
3. Is satisfaction and/or regret associated with the trial meeting the participants’ expectations? The trial outcome? Individual benefits, perceived or real? |

Better understanding the perspectives, experiences, and decision making of clinical trial participants and decliners will not make the ethical challenges any less challenging. It should, however, lead the HIV cure community to more informed choices about how to address the challenges that face us as we aim to enhance the potential societal benefits of cure research while best protecting participating and patient communities. It is vital to protect the public trust in research on HIV cure so that any future interventions arising from it are not tainted with negative reputation that could undermine effectiveness [9].

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References

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