HIV Diagnosis, Management and Prognosis

HIV: Its Effects and Treatment

Antiretroviral therapy (ART) is one of the most significant success stories of modern medicine. It has saved millions of lives and prevented countless HIV transmissions. The advent of effective ART in 1996 was to herald a new phase of hope in the fight against HIV. Treatment brings about suppression of HIV, which in turn allows the immune system to function normally without being impeded by viral replication. CD4 cells, part of the immune system and the main target for HIV, are destroyed when HIV uses their internal machinery to produce new copies of itself, thereby destroying the host’s own immune cells and ensuring the propagation of new viral particles to infect other CD4 cells. This process is repeated until eventual destruction of all CD4 cells.

In uncontrolled HIV, it thought that up to ten billion copies are produced each day which slowly depletes the level of functioning CD4 cells, leading to profound immunosuppression and increased susceptibility to opportunistic infections which a competent immune system would neutralise easily. On average, the process of being immunocompetent to becoming immunosuppressed takes approximately ten years, but this time frame
can be highly variable. Use of ART and restoration of immune function are key to living well with HIV. Opportunistic infections can be fatal or leave patients with significant morbidity, especially if the central nervous system is affected. This is entirely avoidable with early diagnosis and treatment.

Of course, access to treatment is not equitable with some groups having decreased levels of engagement with specialist HIV services which can lead to poorer outcomes in the long term. Adherence to ART has improved for most as the side effect profiles of the newer drugs are infinitely better than the first generation of drugs from the 1990s. Looking ahead, injectable ART and sub-dermal implants of HIV drugs will supplant the need to take tablets every day further improving adherence and lessening the impact that HIV has on one’s life.

**HIV Virology and Patient Prognosis**

HIV is an extremely effective virus. Not only does it manage to evade our immune system, one of the most sophisticated on the planet, but it also uses our very own cells to replicate and produce more viruses. This leads to the double whammy of destroying our immune system that usually protects us from viruses whilst releasing billions of copies of new HIV every day to infect other cells, if untreated. The resulting immunosuppression leaves us open to numerous opportunistic infections—ranging from mild, transient symptoms to often fatal conditions with significant morbidity if one does survive.

HIV cannot replicate on its own. It needs to utilise the host’s cellular machinery to be able to replicate and establish infection. HIV must first circumnavigate the immune defence on the exposed mucosal surface, be it anal or vaginal mucosa if sexually transmitted. However, in cases where immune cells are present, they can also be used as an entry point for the virus. These ‘target cells’ for HIV are then presented to the immune system in preparation to be destroyed. HIV overwhelms the immune cells and causes an acute infection (otherwise known as seroconversion) which is discussed later.

There are many different types of immune cell, some of which kill pathogens immediately (natural killer cells); some which ‘present’
pathogens to other parts of the immune system for subsequent destruction (T cells); and some which have memory for previous infections (B cells) and if re-challenged produce antibodies—protective proteins that help to reduce repeat infections of the same pathogen. The main immune cells involved in HIV are CD4 cells, a T lymphocyte: CD standing for ‘cluster of differentiation’, named after its specific receptor on its surface to differentiate it from the 400 or so other cells within the immune system.

The viral capsid, or outer coat, of HIV is rich in glycoproteins which are perfectly adapted to attach to our CD4 receptors on the T-cell’s surface. To gain entry into the CD4 cell, however, another co-receptor is needed. The co-receptor is usually CCR5, but the CXCR4 receptor is used if HIV infection has been established for many years.

Once the HIV is attached to the CD4 cell, it leads to fusion of the HIV capsid to the T-cell injecting the ‘core virus’ into the cytoplasm. The viral RNA is then translated into DNA by the reverse transcriptase enzyme (Jayappa, Ao, & Yao, 2012). The DNA then moves into the nucleus of the cell where it integrates or splices with our DNA and begins its replication cycle.

After this, the viral RNA is configured into new virions as the outer shell or viral capsid is constructed around the viral RNA by cellular processes from the host cell—HIV cannot complete this without the help of the host cell’s own machinery. The outer layer of the virion is a lipid layer taken directly from the host’s CD4 cell as it buds from the cell. These immature virus-like particles are then processed further to form an infectious new virion, which is then capable of infecting other CD4 cells. The CD4 cell does not survive this process. The process repeats until CD4 counts are eventually depleted.

**Innate Immunity**

Approximately 1% of the world population are naturally immune to HIV. As discussed, two receptors are needed for HIV to enter a CD4 cell, the CD4 receptor and either a CCR5 or CXCR4 receptor. If a mutation occurs in the CCR5 receptor, this does not allow HIV to enter the cell—rendering oneself immune. Nearly every mammal has two matching
chromosomes, one from each parent; the two different genes on each chromosome are called alleles. If the alleles are the same, we are homozygous for that gene, if different, heterozygous. The gene that codes for the CCR5 receptor sometimes has a deletion (or a mutation) which changes the shape of the receptor and does not allow HIV to enter the CD4 cell. When just one of the alleles contains the mutation and the other is normal (heterozygous), only some of the receptors will allow HIV to enter, reducing the risk of HIV entering the CD4 cell. If both chromosomes contain the same mutation (homozygous), one is essentially immune to HIV as all of the receptors are mutated and do not allow HIV to enter the CD4 cell. The prevalence of this mutation is related directly to geographical latitude with those in higher latitude countries (i.e. Scandinavian countries) having higher levels of mutations of the CCR5 receptor and therefore a lower risk of HIV infection (Ni, Wang, & Wang, 2018). The role of the CCR5 receptor is not fully understood, but it is thought to interact with other immune cells involved in the regulation of immune response. Interestingly, this mutation is the reason why two patients have been ‘cured’ following bone marrow transplants—after destruction of the immune system by chemo- and radiotherapy, the transplanted immune cells were selected to have CCR5 mutation, residual HIV was unable to infect the new immune cells, and a total cure was achieved. Given the dangerous nature of bone marrow transplants, this is unlikely to become a practical modality for HIV cure in the future.

HIV Seroconversion

Seroconversion, or very early infection, appears about two to four weeks after initial exposure to the virus and is characterised by a flu-like illness, sore throat, muscle aches and rash—not unlike many other common viral exanthems such as COVID-19, making it difficult to distinguish clinically. During early infection, the immune system is overwhelmed by HIV, and levels of virus in the blood are extremely high as the immune system tries to control its unrelenting replication. A corollary of this is that these patients are also extremely infectious during this period. Once the seroconversion period is over, a ‘viral set point’ is established with
equilibrium between the immune system and HIV itself, that is, the immune system manages to gain control over HIV replication. The higher the viral set point, the quicker it takes to overwhelm the immune system leading to earlier onset immunosuppression (Geskus et al., 2007).

After seroconversion, viral replication continues, but at a more controlled rate than early infection. The viral set point is a good indicator of how long it will take to develop AIDS—defined as a CD4 <200 cells/mm³ plus an AIDS-defining illness (Stein, Korvick, & Vermund, 1992). AIDS-defining illnesses are still commonly seen by HIV doctors who work on medical wards caring for hospitalised patients—the most commonly seen infections are PCP (or PJP as it now called), TB, progressive multifocal leucoencephalopathy (PML) and toxoplasmosis. One of most well-known of the AIDS diagnoses was the pathognomonic violaceous skin lesions of Kaposi’s sarcoma—a skin cancer driven by a HSV that often heralded a diagnosis of AIDS in the early days. These lesions are still seen today and can affect skin and other organs needing intensive chemotherapy with unfavourable long-term side effects.

Most of us will have a CD4 count of above 500 cells/mm³ and this constitutes a ‘normal’ immune system. This can be transiently increased by exercise but also temporarily depleted by any viral illness, such as the common cold or influenza. When we talk about immunocompromise, clinicians usually refer to a CD4 count of less than 200 cells/mm³ where the chances of acquiring an opportunistic infection are greatly increased.

Patients may have up to ten years of latent infection before the CD4 count drops to dangerously low levels—with some never progressing to full immunosuppression, so-called elite controllers. The rate at which the CD4 count drops is variable, but observational data suggest that this can range from 35 to 60 cells/mm³ per year (Verma, 2014; Wolbers et al., 2010). The slope of the decline does not accurately predict the time taken to progress to AIDS, but being older and having a lower CD4 count at diagnosis (especially <350 cells/mm³) are associated with a more rapid decline in immune function.

In clinical practice, CD4 counts were the main HIV surrogate marker and were measured at every clinic visit (2–3 times a year for stable patients). These days HIV viral load (the amount of virus in each drop of blood) is a much better marker of whether treatment is working. CD4
counts are now only measured once a year, or less, if people are stable on treatment. CD4 count was used as a marker to determine when to initiate treatment with ART, as directed by the relevant national guidelines. For the UK, the British HIV Association (BHIVA) guidelines are the gold standard and updated regularly to reflect new data and commissioning changes.

Nowadays, HIV treatment is started immediately after diagnosis, regardless of CD4 count. However, this was not always the case. Generally, a CD4 count of between 200 and 350 cells/mm$^3$ was the recommended starting point for many years in most guidelines across the world. This involved waiting for the patient’s immune system to reach these levels if they had a high CD4 at diagnosis. Exceptions were fairly common and those who were symptomatic, diagnosed with an AIDS-defining illness, or had a strong desire to start treatment were initiated on ART outside the recommendations of the guidelines. However, this began to change as new data from the SMART and START studies indicated that early initiation of ART, even while CD4 counts were still relatively high, yielded better clinical outcomes.

The SMART study (Strategies for Management of Antiretroviral Therapy (SMART) Study Group et al., 2006), funded by the National Institute of Allergy and Infectious Diseases in the US, sought to address the impact of drug holidays (the voluntary cessation of ART to minimise side effects) in a clinical trial focusing on continuous versus intermittent treatment. The trial was halted early as it was shown that intermittent treatment was associated with a 2.6 higher chance of dying than when taking continuous therapy. Unexpectedly, rates of non-AIDS-defining illnesses, such as cardiovascular, renal and hepatic complications were also greatly increased in those taking intermittent treatment. The assumption before the trial was that, given the sometimes harmful side effects of ART, health complications would reduce with drug holidays—the opposite was found to be true indicating a very strong protective factor when taking continuous ART.

Nine years later, the START study (INSIGHT START Study Group et al., 2015) further demonstrated the benefits of effective ART by comparing ART initiation at a CD4 of over 500 cells/mm$^3$ and less than 350 cells/mm$^3$. Incredibly, immediate treatment (i.e. CD4 >500 cells/mm$^3$)
led to a 57% reduction in all serious illnesses, whether related to AIDS or not. There was also a reduction in serious AIDS-related events of over 70%. These significant reductions in adverse outcomes has meant that the START study was one of the most influential HIV treatment studies in the last decade and that it has transformed how clinicians deliver HIV care. Prior to this, many physicians would wait for the CD4 count to fall, but on publication of these data, national guidelines were updated almost immediately and recommended immediate treatment for all, regardless of CD4 count.

With these data came the realisation that HIV not only depletes CD4 cells but that unchecked viral replication was causing widespread inflammation. The heart, kidney and brain especially were being subjected to damage from the very mechanism designed to protect the body from such assaults. Clinicians soon began to start HIV treatment immediately for all patients, challenging the central dogma of using CD4 thresholds as a treatment guide. This also led to a novel pathway for HIV research, namely, chronic inflammation caused by replicating virus.

**Chronic Inflammation and HIV**

Data from both the SMART and START trials demonstrated that cardiovascular and renal complications were much lower in those taking ART due to lower levels of immune activation in this cohort. It is now known that even this level of immune activation can lead to severe complications through a variety of mechanisms (Zicari et al., 2019).

For example, during the seroconversion illness, a large proportion of the memory immune cells, which usually reside in specialised tissue in the gastrointestinal tract (gastrointestinal-associated lymphoid tissue), are destroyed by HIV. This usually occurs within the first six months of infection and cannot be restored by ART, even if initiated relatively early. The destruction of this specialised gut tissue leads to a disruption in how pathogens are processed and leads to higher levels of inflammation within the gut with important consequences for the microbiome of the gut, or the balance of ‘good’ and ‘bad’ bacteria. Disruptions of the microbiome of the gut have been linked to depression, anxiety and a whole host of
other conditions. There are significant differences in the microbiome of the gut in those living with HIV versus the general population (Dillon, Frank, & Wilson, 2016).

Some studies have shown increased levels of pro-inflammatory bacteria, such as *Prevotella* spp., which can lead to gut inflammation and overall increased immune activation (Dillon et al., 2014). Destruction of the gut immune tissue also impairs the ability to stop microbes from entering the bloodstream. This microbial translocation from the gut to the bloodstream activates the immune system, which recognises them as invaders and releases cytokines, or inflammatory messengers, into the blood leading to prolonged chronic inflammation which begins to exhaust the immune system over time. Known effects of this are a reduction in the function of the thymus gland (where T-cells are matured and released) and sclerosis of the immune lymphatic system. It also affects the intricate blood clotting system leading to the release of pro-coagulants which increases the risk of thrombosis, or blood clots, leading to higher rates of myocardial ischaemia (i.e. heart attacks) and strokes. Treatments that counter this unchecked inflammation have so far been inconclusive. Much hope is being placed on the anti-inflammatory effects of statins, anti-cholesterol drugs in a large trial called REPREIVE which aims to reduce inflammation seen in HIV and reduce negative health outcomes associated with chronic inflammation, such as cardiac events.

**HIV Treatment**

A key responsibility of HIV clinicians is to construct an effective combination of antiretroviral drugs, which is tailored to individual patients. Relevant factors to consider when starting ART include the patient’s age, gender, immune status, renal function and the presence of other chronic diseases. Clinicians must also consider bone health, other medicines being taken, recreational drugs, HIV viral load, side effect profiles of each drug and the size of the tablets themselves (some are quite large and can be difficult to swallow). A key *psychological* factor is whether the patient is actually ready and willing to initiate life-long ART—if this part of the puzzle is missing, successful treatment is hard to maintain over long periods.
The patient’s lifestyle is a key consideration. For example, a young gay man who participates in chemsex would not be initiated on a protease-based ART regimen, as ritonavir may boost (or potentiate) the drugs used in chemsex (i.e. GHB). Indeed, there have been many cases of HIV-positive gay men (on a protease-based regimen) who have taken GHB and ended up in hospital in a life-threatening condition. Another example is the patient who works night shifts regularly who may find that efavirenz (a commonly used non-nucleoside reverse transcriptase inhibitor [NNRTI]) gives him severe dizziness and disorientation during his night shift.

Moreover, clinicians must be mindful of the costs associated with particular ART regimens. The prescription of more expensive drugs is monitored closely, and they are often saved for those who are treatment-experienced with multiple class resistance (i.e. resistance to multiple classes of ART). In London, there is a collaboration of clinicians, policymakers and academics who publish guidance on the most cost-effective treatments available at the time. For more expensive drugs to be prescribed, regimens must be discussed on a case by case basis at local team meetings to rationalise the use of expensive drugs.

It is useful to discuss briefly the drugs by class with common considerations given as to why they would be used.

**Nucleoside Reverse Transcriptase Inhibitors**

Nucleoside reverse transcriptase inhibitors (NRTI or ‘nukes’) are the backbone of the vast majority of HIV drug regimens. The basic building blocks of DNA, and therefore life, are the bases adenosine, cytosine, thymidine and guanine. These are the smallest building blocks in DNA which go on to form proteins, ubiquitous organic molecules vital to human function and survival. Each NRTI drug is essentially a copy of the corresponding base and similar to the naturally occurring bases found in almost all cells.

AZT is a copy (or analogue) of thymidine, tenofovir to adenosine, abacavir to guanine and so on. Structurally, they are nearly indistinguishable from naturally occurring bases (apart from a few extra phosphate
groups), so will be fully utilised by HIV enzymes to form the DNA needed to produce new virus. Once these mimics are incorporated into the viral DNA, the added phosphate group leads to inhibition of the enzyme reverse transcriptase and stops further HIV replication by not allowing further bases to be added to the genetic material.

Each NRTI has its own side effect and pharmacological profile. However, this class of ART is generally well tolerated in most people. When HIV drugs were in their infancy, the older NRTIs did have significant longer-term side effects, such as lipodystrophy (abnormal accumulation of fat tissue), one of the most visible side effects for those living with HIV in the 1990s and early 2000s. Other side effects attributed to the early NRTIs are pancreatitis, neuropathy, lactic acidosis, hepatic steatosis and cardiomyopathy—all very unpleasant and some life-threatening.

Side effects result from damage to the mitochondria, energy-producing organelles that power all living cells. The number of mitochondria differs greatly depending on how much energy is needed, but on average there are between 100 and 1000 per cell. Mitochondrial DNA (mtDNA) and the enzyme that builds it (DNA polymerase $\gamma$) are affected by NRTIs. This inhibition of DNA polymerase $\gamma$ and damage to mtDNA lead to cellular dysfunction, increasing levels of waste product and leading to eventual death. As mtDNA is ubiquitous in the body, the side effect profile of NRTIs is very diverse (Gerschenson & Brinkman, 2004).

Older NRTIs were particularly malign, with ddI (didanosine) and d4T (stavudine), two of the earliest NRTI, causing peripheral nerve damage in a third of patients exposed to these drugs (Simpson & Tagliati, 1995). The longer-term complications of nerve damage are unpleasant, painful and difficult to treat, often necessitating large doses of drugs to suppress nerve conduction (such as amitriptyline or gabapentin), which in turn can have their own fatigue-inducing side effects.

Another debilitating and sometimes life-limiting side effect of these early NRTI is liver disease. People who were exposed to ddI or d4T are, on average, 30–40% more likely to develop liver disease, and thus, these drugs have been discontinued in nearly all developed countries (Ryom et al., 2016).

One newer NRTI, abacavir, deserves special consideration here as its history is chequered, although it remains one of most commonly used
Abacavir is one of the first drugs to have its side effect profile clarified in relation to the genetics of the person taking it. It is one of the first drugs personalised to one’s genetic code. When abacavir was first used in 1998, it was found that between 5 and 8% of patients developed an unusual array of symptoms which mimicked a severe immune reaction (nausea, diarrhoea, rash, fatigue, fever, cough, shortness of breath) and when abacavir was restarted after a break, patients had severe anaphylactic reactions (Escaut, Liotier, Albengres, Cheminot, & Vittecoq, 1999; Walensky, Goldberg, & Daily, 1999). Initially it was unclear as to why only certain patients developed these side effects until 2002 when it was first linked to the gene HLA B*5701 (Mallal et al., 2002). Human leucocyte antigens (HLA) are a complex mechanism for recognising one’s own proteins versus those from virus or bacteria, and they are expressed on most cells in the body. It was discovered that those with certain genetic codes (HLA B*5701 positive) recognised abacavir as a potential pathogen and inadvertently triggered and stimulated the immune system in an effort to destroy it. This gave rise to the abacavir hypersensitivity reaction manifesting as the symptoms described above (Illing, Purcell, & McCluskey, 2017).

Guidelines are now unanimous in relation to the testing of HLA B*5701 in all patients who would need to take abacavir. In certain instances, it can be given before the test is done if there is an urgent need, but with caution in higher-risk populations as the allele is much more likely in White patients (Cao et al., 2001; Hughes et al., 2004) than those with African heritage.

**Non-nucleoside Reverse Transcriptase Inhibitors**

Non-nucleoside reverse transcriptase inhibitors (NNRTI or ‘non-nukes’) have been the most popular third choice of drug since their development (with the other two drugs, or the ‘backbone’, usually being NRTIs). The most commonly used NNRTIs have been efavirenz and nevirapine, with efavirenz being one of the most commonly used agents globally to date.

NNRTIs still inhibit the reverse transcriptase enzyme but do not mimic the bases themselves. Instead, they directly interact with the reverse transcriptase enzyme to stop its activity. Three dimensionally, the enzyme
looks like a hand, palm up; when it is active the thumb and index finger come together, a little like the universally recognised ‘ok’ sign with the genetic material being made in the space between thumb and index finger. NNRTI irreversibly binds to reverse transcriptase to induce a conformational change to the protein—it keeps the active sites apart (the thumb and index finger cannot touch) so the enzyme is unable to function.

Efavirenz has been a first-line treatment for HIV since 1998 and was used as a gold standard of treatment for over a decade. It has high potency against HIV, can be co-formulated easily, only needs to be taken once a day, is safe in pregnancy and has a more favourable safety profile than its main counterpart, nevirapine. It was also part of the first single-tablet regimen in the UK and US, Atripla.

The release of Atripla—the first ever one-tablet once-a-day regimen—in 2007 was a huge step forward not only for drug adherence but also for normalising HIV treatment. It proved popular among patients who may have previously struggled with taking up to 25 tablets a day and was a firm favourite for clinicians across the globe. Nowadays we have a wide array of single-tablet regimens for the treatment of HIV, but at the time this co-formulation was a pharmaceutical success with three separate drug companies (Gilead Sciences, Merck and Bristol-Myers Squibb) in an unusual but successful collaboration. To date, Atripla continues, in its generic formulation, to be one of the most popular single-tablet regimens to date globally.¹

As with most medicines, however, it is not without its side effects. Up to a third of those taking efavirenz will experience dizziness and other neuropsychiatric effects such as anxiety, vivid dreams and poor sleep (Ford et al., 2015). These symptoms do eventually settle but can be persistent in many. Efavirenz use is avoided in those who work night shifts (it is taken before bed to reduce these side effects) and those with mental health issues.

Interestingly, it has been shown that these side effects may be related to the genetics of the person taking it. The CYP2B6 is a pathway in the liver which metabolises certain drugs. Those with certain mutations

¹ https://apps.who.int/iris/bitstream/handle/10665/179532/9789241509152_eng.pdf;jsessionid=E0A1B30C508A16A7C461F87779762440?sequence=1.
metabolising efavirenz more slowly leading to higher concentrations and side effects (Gounden, van Niekerk, Snyman, & George, 2010). Another example of how genetics influences drug delivery and future dosing (many of those with the less efficient alleles can tolerate a dose reduction of efavirenz with fewer side effects and no loss in viral suppression).

A metabolite (8-hydroxyefavirenz) has been subject to much research as it has been shown that, in the central nervous system, the concentration can be high enough to cause direct neuronal damage possibly explaining why patients have such side effects from this drug (Tovar-y-Romo et al., 2012). To date, it is still used in developing countries but has largely been replaced by newer drugs with cleaner side effect profiles in resource-rich healthcare systems.

**Protease Inhibitors**

Protease inhibitors (PIs) remain an extremely useful and popular third-line agent for those who need HIV treatment. The HIV protease enzyme is used late in the HIV replication cycle when the immature HIV virion is being assembled within the CD4 cell. The protease helps to develop its outer shell and starts to mature into an infective virion ready to be released and infect other cells. If this enzyme is inhibited, HIV replication is halted.

The first PI to begin clinical trials in 1989 was saquinavir. It was shown to have potent anti-HIV activity making it very durable, especially if the HIV had been exposed to previous drugs and had developed resistance. This is the case with all PIs. This group of medicines was the final piece of the puzzle in highly active ART and heralded a new phase in sustained HIV suppression that came about in 1996 when effective ART was discovered.

PIs, however, are not without their drawbacks. Early PIs often had severe gastrointestinal side effects, mainly diarrhoea and nausea, with many patients stopping due to the unbearable side effects. This was in addition to marked changes in body fat distribution which gave patients hollow cheeks and had a significant psychological impact on those experiencing these side effects. Given the short half-life of the early PIs, they
were often given three times a day with little forgiveness for poor adherence—virological failure was common. Ritonavir, a very early PI, was found to have potent inhibitory activity against the pathway that metabolises many medicines (cytochrome P450 [CYP] 3A4), thereby ‘boosting’ other PIs and is still used today.

This ‘boosting’ mechanism constituted an important development as it reduced doses of other toxic PIs and therefore side effects. However, a significant number of other drugs are affected leading to very complicated interactions if taken with other medicines. Often they are avoided as balancing these drug effects can be very difficult.

A common and sometimes serious interaction is with inhaled corticosteroids (such as those taken for asthma, especially fluticasone) which increase levels of the steroid when taken with ritonavir. The high levels of steroid from the inhalers, potentiated by ritonavir, can lead to Cushing syndrome, which is an endocrinological syndrome leading to swelling of the face and abnormal fat distribution. If the inhalers are stopped abruptly, this can result in an Addisonian crisis, an endocrinological emergency, which can be fatal if not treated early. Most HIV clinicians will have seen cases in their clinical practice which can be challenging to manage.

Patients who have poor adherence to ART are at risk of adverse health outcomes, and a careful history concerning concomitant medicines and attitudes towards HIV should be explored so that the clinician can challenge any myths or anxieties the patient may have. Often, reasons for not taking medicines may mask underlying acceptance issues in relation to the HIV diagnosis itself with the pills being a daily reminder of their status. Psychological support is key to overcoming this.

Integrase Strand Transfer Inhibitors

INSTIs (or integrases) were developed later than other classes of ARVs, with the first, raltegravir, being approved in 2007. It was given accelerated approval from the FDA due to its high effectiveness, especially in treatment-experienced patients who were resistant to other drugs. The
enzyme, integrase, splices HIV DNA into the host cell’s genome. Once this enzyme is inhibited, further replication of HIV is stopped.

Clinically, integrases have been an extremely useful addition to the HIV treatment armamentarium. Those who developed resistance to other classes of drugs exhibited a good response to this class of drugs providing a lifeline of effective treatment for those who would otherwise have struggled to remain virologically suppressed. Also, the rate of viral decline in those taking INSTIs is much quicker when compared to other agents (Messiaen et al., 2013; Rockstroh et al., 2013). It is therefore extremely effective for treating patients with very high viral loads, such as those who are seronconverting to reduce onward transmission.

INSTIs remain a popular choice for many patients due to a relatively clean side effect profile with only a minority of patients experiencing sleep disturbance and insomnia. They have few interactions with other medicines allowing effective viral suppression for those with other health conditions who may be taking a pharmacopeia of other medicines. In clinical practice, they have also proven to be very useful for those engaging in chemsex given the few interactions with recreational drugs with less inadvertent overdose—a lethal phenomenon seen in those taking enzyme inhibitors such as ritonavir.

The newer INSTI, dolutegravir, has proven very popular due to a high genetic barrier to resistance and excellent tolerability for patients. Two recent studies, TANGO (van Wyk et al., 2020) and GEMINI (Cahn et al., 2019), have both shown that dolutegravir plus either lamivudine (a NRTI) or rilpivirine (a NNRTI) as dual therapy is just as effective as taking the standard three-drug regimen, with few side effects and almost no viral resistance. This is an important step in challenging the dogma of three-drug regimens allows less drug exposure to NRTIs, the usual backbone for ART, and also significant cost savings (3TC is amongst the cheapest of ARVs due to it being off patent for a number of years). Dual therapy may be the future of modern ART as newer medicines have improved genetic barriers to resistance with favourable pharmacokinetic profiles.
CCR5 Receptor Inhibitors

Since the discovery of the CCR5 receptor on immune cells and its necessity for HIV entry into CD4 cells, it has long been a target for drug therapy. Maraviroc, currently the only licensed CCR5 receptor inhibitor for use in HIV, was developed at astonishing speed with only six years between identification in 2001 and approval in 2007.

Maraviroc is unique in that the target—the CCR5 receptor—is an external protein on the cell surface rather than an intracellular viral protein, the target of nearly all other HIV drugs. Maraviroc is a small molecule that causes a physical structural change to the CCR5 receptor which makes it impossible for HIV to enter the CD4 cell.

Maraviroc is used when a novel mechanism of HIV inhibition is needed, usually in the context of multi-drug-resistant HIV, and is an effective part of many salvage regimens for heavily treatment-experienced patients. Care must be taken to ensure the HIV is CCR5-tropic, that is, the HIV population uses CCR5 as its co-receptor, and not CXCR4, as this renders maraviroc useless.

HIV Treatment Failure and Resistance

Despite the huge advances in ART, treatment can still fail. Of those taking ART, 5–10% per year fail (Jose et al., 2016). The rate of failure differs by group, with gay men having the lowest rates of treatment failure when compared to heterosexual patients. Within the gay demographic, there are also variations in failure rates with those aged over 45 years having estimated failure rates of only 1% per year (O’Connor et al., 2017). Starting ART after 2008 is also a protective factor against treatment failure; this is likely due to the less toxic side effects and better adherence to the newer drugs.

Of those who do fail, some will go on to develop drug resistance rendering their current regimen inactive against their HIV. This is familiar territory for the HIV physician and testing for resistance should occur in
this setting. The results of these resistance tests then allow a new and more effective regimen to be given to help re-suppress HIV replication.

HIV replicates quickly and efficiently once infection is established. Between 1 and 10 billion viruses are produced each day in an individual who is not on treatment. Reverse transcriptase, as with other enzymes, often makes random errors in the transcription of the genetic code. Therefore the daughter copy may differ from its parent which can result in HIV with new properties. Some mutations confer an advantage, most do not. These quasispecies, or sub-populations, which are related to the original but slightly different, may go on to become the dominant viral species.

Drug resistance arises when drugs exert pressure on those viruses whose mutation confers resistance to that particular drug. Commonly, these are the result of having sub-therapeutic drug levels within the body (i.e. the drug is present in the body, but not at high enough levels to fully suppress the virus). These low levels of drugs in the body can result from missing doses of ART or drug interactions with other medicines. Once this mutated family is resistant to a drug, it then becomes the main species of HIV and treatment failure occurs.

Generics

The annual budget in England for HIV care, provided by NHS England, is just over half a billion pounds per year, with the majority being spent on ART. The cost of these drugs is dictated by pharmaceutical companies and market forces. Often, new HIV drugs (especially single-tablet regimens containing two or more drugs) can cost over £500 per month.

Pharmaceutical companies spend vast sums of money on identifying and developing effective drugs that are safe, effective and tolerable. Companies have exclusivity of the drugs they develop for 20 years after approval. During this time, the pharmaceutical company can set whatever price it sees fit. Significant discounts can be given for large orders of drug, as seen in the HIV London Consortium who bulk buy all ARVs for those receiving care in London, saving millions of pounds in the process.
By 2033, nearly all of the HIV drugs we see on the market today will be beyond the patent period and can then be manufactured by any company at a fraction of the cost, often as low as 10% of the original price. For now, only some of the older HIV drugs are now available as generics, but the huge price reduction has led to a shift in prescribing the cheaper generic versions. A study led by Public Health England (Ong et al., 2019) showed that if clinicians switched all patients to generics as they are released, it could save the NHS up to £7 billion by 2033, allowing more funding to be directed towards HIV prevention and research into effective vaccines and a possible cure for HIV.

Choosing an Effective HIV Treatment Regimen

The HIV clinician has to choose an effective regimen for those newly diagnosed or who have failed on their previous treatment, for whatever reason. A number of factors must be taken into consideration before starting, giving different weight to each factor depending on the patient sitting in front of them. Often, patients are started on the accepted first- or second-line combinations (usually a Kivexa or Truvada backbone with the third agent as NNRTI, INSTI or PI) with the best virological efficacy and a side effect profile that is acceptable to both clinician and, more importantly, the patient. Some of the considerations associated with choosing an effective HIV treatment regimen are discussed below.

HIV Viral Load

The viral load at initiation of therapy can vary significantly and is dependent on the stage of infection, with those who are seroconverting or diagnosed very late often having very high viral loads which may need to be reduced quickly. Several factors must be considered, such as, if the person has an HIV-negative partner, if their occupation requires them to have undetectable virus (i.e. surgeon or dentist) or the need for urgent surgery. Also, some drugs may have unacceptable failure rates when the viral load
is too high. Abacavir, for example, has been shown in some studies to have less efficacy for viral loads of over 100,000 copies/ml (Sax et al., 2009). This does not mean that patients cannot have this medicine, but that the HIV clinician will have to be very vigilant to ensure viral decay is progressing at the expected rate with a low threshold for intensifying or switching, if needed. There has been a recent shift to giving a standard three-drug regimen plus an INSTI to help bring the viral load down quickly. The INSTI can then be stopped once viral suppression is achieved.

**Renal Function**

Kidneys filter the blood and excrete waste products in the urine, including the majority of drugs, antiretrovirals included. Older age naturally leads to a decline in kidney function which may be worsened by other common conditions such as hypertension or diabetes. As one’s ability to clear waste products and drugs decreases, there may be a risk of drug accumulation in the body, which may be toxic in extreme cases. Therefore, if renal function is impaired, some drug doses need to be reduced in order to avoid damaging toxicities. Also, some drugs may have a direct toxic effect on the kidneys, especially tenofovir disfumarate, which is avoided in patients with kidney impairment to slow the rate of further decline.

**HLA B*5701**

This genetic test is part of the baseline set of tests for all new diagnoses in the UK, with approximately 6% of Caucasian patients having the genes that make it dangerous to administer abacavir (Hughes et al., 2004). In certain settings (mainly emergencies and with no other options), abacavir can be given without the test results, but with extreme caution and a high level of suspicion for the abacavir sensitivity reaction if the patient becomes unwell.
Co-infections

In the UK, approximately 6.7% and 10.7% of people living with HIV are co-infected with hepatitis B (HBV) and C (HCV), respectively (Thornton, 2015). This is much higher than the general population (around 0.5–1.0% for each) and is reflective of high rates of sexually acquired infections (fisting, for example, has been shown to be a risk factor for HCV infection). Injecting drug use, as seen in some gay men who participate in chemsex, is also problematic despite robust needle exchange programmes available in the UK.

Treatment of HBV and HIV often overlap, particularly tenofovir and 3TC/FTC. Therefore, solutions of regimens containing both these drugs can simultaneously treat both infections with minimal drug exposure. For those diagnosed with HCV, the treatment now often includes oral therapy for 12 weeks but interactions between these and HIV drugs, especially PIs, can lead to unacceptable toxicities and modification of ART is required. TB is also much more common in those living with HIV, as HIV is in fact a risk factor for TB infection. The treatment for TB can often involve rifampicin, a potent anti-TB agent but an even more potent liver enzyme inducer which reduces the levels of many concomitant medications. Careful liaison with pharmacists and drug databases is required to ensure all drug levels are at an effective concentration.

Concomitant Medicines

A careful drug history from all patients is required at every visit for the HIV clinician—the frequency of drug interactions is high and almost every clinic requires a discussion with pharmacy colleagues to help decipher the often complicated interactions. This can even apply to over-the-counter medications, herbal remedies and recreational drugs.
HIV Resistance Test

All newly diagnosed patients in the UK should have baseline resistance patterns. The rates of transmitted drug resistance in the UK are between 6 and 10% of all new infections (Tostevin et al., 2017). However, this is decreasing with the advent of newer PI and INSTI. If drug mutations are found, this can influence the decision about which ART to prescribe as certain classes of drugs (or more than one in some cases) may not be effective in suppressing HIV. Discussion at team meetings at most HIV centres allows clinicians to reach an agreement about the best regimen for that patient.

Patient Acceptability

Most patients allow their HIV clinician to decide their most appropriate treatment regimen, although some will have expectations about what they will find acceptable or will have completed research (online or through friends who have HIV) before the consultation. This can significantly influence the decision-making process of the clinician, and conversations about the patient’s expectations are helpful.

Some patients will not want to start ART immediately for a variety of reasons as they do not, for instance, feel ready to start medication for the rest of their life or they may be worried about the side effects or a household member finding their tablets. These decisions must be respected. However, it is also important to explore any possible factual errors and patients’ fears and anxieties. Clinicians should be sensitive and responsive to patients’ concerns in relation to their treatment—if patients are not fully invested in the process, adherence can be poor, resulting in treatment failure, a poor patient experience and opportunistic infections if treatment is not adhered to.
Social Background

All clinicians should be able to construct a fairly accurate idea of the social background for most patients (i.e. education level, occupation) as this may affect the treatment regimen given. For those who work nights, efavirenz should be avoided given the dosing at night and potential dizziness. Those who may have unstable social settings, such as those with substance use issues or who are homeless, HIV may not be their main priority and adherence can be poor. For these groups of patients, treatment with PIs or newer INSTI drugs are often preferred as they allow more doses to be missed without resultant treatment failure.

Future of HIV Treatment

Future treatment modalities for HIV seem promising, with the eventual goal of an HIV cure, either functional cure where viral suppression is achieved without ART, or a total cure where HIV is removed from all host cells.

The ultimate goal of future HIV treatment is the elusive cure. This has proved much more complicated than anyone first imagined. The challenge is that, once the HIV DNA is incorporated into the CD4 cells as part of its replication cycle, it is very difficult to ‘splice’ out the infected DNA portion. Indeed, the integration of viral DNA into our own is sometimes called the ‘fatal step’ as this then allows the creation of a reservoir of HIV that is difficult to treat even with the most effective ART (Wiegand et al., 2017). On treatment these latently infected cells escape immune recognition, and when ART is stopped, they begin producing HIV virus, and an HIV viral rebound ensues.

This reservoir is the cornerstone to HIV cure research. It is thought that the majority of the reservoir is created during the early stages of infection. Research has shown that those treated with effective ART during seroconversion, that is, very early on in the infection, have a reduced reservoir (and therefore have low levels of latently infected cells). Indeed, some patients who had treatment started during seroconversion
and continued for a number of years, remained undetectable when treatment was stopped some years after starting—a so-called functional cure (Sáez-Cirión et al., 2013). This is in contrast to a sterilising cure, whereby all cells with HIV are eradicated, such as the two patients who were successfully ‘cured’ subsequent to being given new immune cells without CCR5 receptors, which inhibited the re-establishment of HIV, as part of a bone marrow transplant. For those who do subsequently develop haematological cancers, this approach may become standard practice in the future. This may have the advantage of a sterilising cure through the use of new cells that are immune to HIV (due to a lack of the CCR5 receptor).

Two of the most significant unanswered questions include how to precisely measure the size of the reservoir and how to activate the latent cells so they can be treated with ART, a therapeutic vaccine or broadly neutralising antibodies or a combination of all three—the ‘kick and kill’ theory. The standard of measurement is called a viral outgrowth assay (Bruner et al., 2016) where a concentration of T-cells is stimulated to produce HIV and measured quantitatively. However, this approach is costly, time-consuming and not all of the HIV produced is viable. Work continues on refining this process which has not yet been perfected to yield a cure (Bruner et al., 2019).

The activation of latent CD4 cells can be achieved with medications called histone deacetylation inhibitors. One of these drugs, vorinostat, was used with a therapeutic vaccine in the recent RIVER study (Fidler et al., 2020) but was shown to have no discernible effect on the HIV reservoir. Despite this disappointing result, the data produced will allow new avenues of research with a view to achieving a functional cure. Perhaps more effective histone deacetylation inhibitors with a combination of vaccines, ART and broadly neutralising antibodies may hold the key.

In the meantime, we are reliant on advances in drug technology. Recently, a successful combination of two long-acting medications has been used as an injection every two months negating the need for daily tablets. Patients find this therapeutic approach acceptable, but there remain questions about patient adherence and how the approach might
fit into some patients’ lifestyles. The ultimate goal would be for once or twice yearly treatments (either long-lasting injections or sub-dermal implants delivering sufficient drug to suppress HIV replication) which are now a very real possibility. This approach, coupled with advances in vaccine science or the addition of broadly neutralising antibodies, points to a future of much simpler and more effective treatment options.

The functional cure would revolutionise the lives of those living with HIV, with reduced clinic visits and potentially less HIV stigma. If this were coupled with a workable HIV vaccine received before sexual debut (not unlike the human papillomavirus vaccine administered to school children), HIV truly may well be consigned to the history books. When this becomes a reality—however distant this reality may be—equity of access and ensuring patient safety will be the primary concerns for the clinician.

Overview

It is safe to say that the development of effective ART has had the most profound impact on the course of HIV. A terrifying diagnosis, which often led to an undignified death for many gay men in the 1980s and 1990s, is now a manageable, long-term chronic condition. The drugs themselves have also evolved into extremely effective agents of viral suppression with relatively few serious side effects. The efficacy and tolerability of orally administered ART will continue to increase until we enter a world where an injection every three to six months, or a sub-dermal implant slowly releasing drug to suppress the virus for years at a time, will be the next standard of HIV care. The shape of HIV care will change dramatically over the next few decades as treatments improve, less monitoring from specialised HIV clinics is required, and HIV stigma is eradicated.

Another seminal moment in HIV treatment was the realisation that being on effective medication renders the patient uninfectious. Many patients living with HIV describe a torturous fear of passing the virus onto lovers, partners and children—a heavy burden for many. The U = U message (undetectable = transmittable) has been revolutionary for those
who are HIV-positive and their partners, resulting in less anxiety, less stigma and a more enjoyable sex life.

Many patients in a clinical setting mention the hope for a cure and this is certainly possible in the future. A cure where all traces of HIV are eliminated from the body is the ultimate goal. However, treatment that can kill enough HIV to stop it replicating at high levels without ART is potentially the next logical step—a so-called functional cure. Cure research is the key to another stage in the fight against HIV, whereby gay men are slowly ‘cured’ and weaned off their medication. This will not be easy to achieve, and we must ensure equity of access to these services once they do become available.

A world without new HIV diagnoses and a functional cure for those who have been affected is the ideal future that is certainly attainable in the next phase of HIV treatments provided there is sufficient scientific innovation, political engagement and psychological readiness. Any milestones in drug development must be made available to all, and not just to those fortunate enough to live in developed nations. With the networks of treatment and care now more firmly established and the rapid rate of scientific development, there is a very real possibility of winning the battle against HIV.

References

Bruner, K. M., Murray, A. J., Pollack, R. A., Soliman, M. G., Laskey, S. B., Capoferri, A. A., … Siliciano, R. F. (2016). Defective proviruses rapidly accumulate during acute HIV-1 infection. *Nature Medicine, 22*(9), 1043–1049.

Bruner, K. M., Wang, Z., Simonetti, F. R., Bender, A. M., Kwon, K. J., Sengupta, S., … Siliciano, R. F. (2019). A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. *Nature, 566*(7742), 120–125.

Cahn, P., Madero, J. S., Arribas, J. R., Antinori, A., Ortiz, R., Clarke, A. E., … Ustianowski, A. (2019). Dolutegravir plus lamivudine versus dolutegravir plus tenofovir disoproxil fumarate and emtricitabine in antiretroviral-naive adults with HIV-1 infection (GEMINI-1 and GEMINI-2): Week 48 results from two multicentre, double-blind, randomised, non-inferiority, phase 3 trials. *The Lancet, 393*(10167), 143–155.
Cao, K., Hollenbach, J., Shi, X., Shi, W., Chopek, M., & Fernández-Viña, M. A. (2001). Analysis of the frequencies of HLA-A, B, and C alleles and haplotypes in the five major ethnic groups of the United States reveals high levels of diversity in these loci and contrasting distribution patterns in these populations. *Human Immunology, 62*(9), 1009–1030.

Dillon, S. M., Lee, E. J., Kotter, C. V., Austin, G. L., Dong, Z., Hecht, D. K., … Wilson, C. C. (2014). An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. *Mucosal Immunology, 7*(4), 983–994.

Dillon, S. M., Frank, D. N., & Wilson, C. C. (2016). The Gut microbiome and HIV-1 pathogenesis: A two way street. *AIDS, 30*(18), 2737–2751.

Escaut, L., Liotier, J. Y., Albengres, E., Cheminot, N., & Vittecoq, D. (1999). Abacavir rechallenge has to be avoided in case of hypersensitivity reaction. *AIDS, 13*(11), 1419–1420.

Fidler, S., Stöhr, W., Pace, M., Dorrell, L., Lever, A., Pett, S., … Murray, T. (2020). Antiretroviral therapy alone versus antiretroviral therapy with a kick and kill approach, on measures of the HIV reservoir in participants with recent HIV infection (the RIVER trial): A phase 2, randomised trial. *The Lancet, 395*(10227), 888–898.

Ford, N., Shubber, Z., Pozniak, A., Vitoria, M., Doherty, M., Kirby, C., & Calmy, A. (2015). Comparative safety and neuropsychiatric adverse events associated with efavirenz use in first-line antiretroviral therapy: A systematic review and meta-analysis of randomized trials. *Journal of Acquired Immune Deficiency Syndromes, 69*(4), 422–429.

Gerschenson, M., & Brinkman, K. (2004). Mitochondrial dysfunction in AIDS and its treatment. *Mitochondrion, 4*(5), 763–777.

Geskus, R. B., Prins, M., Hubert, J.-B., Miedema, F., Berkhout, B., Rouzioux, C., … Meyer, L. (2007). The HIV RNA setpoint theory revisited. *Retrovirology, 4*(1), 65. https://doi.org/10.1186/1742-4690-4-65

Gounden, V., van Niekerk, C., Snyman, T., & George, J. A. (2010). Presence of the CYP2B6 516G> T polymorphism, increased plasma Efavirenz concentrations and early neuropsychiatric side effects in South African HIV-infected patients. *AIDS Research and Therapy, 7*, 32. https://doi.org/10.1186/1742-6405-7-32

Hughes, A. R., Mosteller, M., Bansal, A. T., Davies, K., Haneline, S. A., Lai, E. H., … on behalf of the CNA30027 and CNA30032 study teams. (2004). Association of genetic variations in HLA-B region with hypersensitivity to abacavir in some, but not all, populations. *Pharmacogenomics, 5*(2), 203–211.
Illing, P. T., Purcell, A. W., & McCluskey, J. (2017). The role of HLA genes in pharmacogenomics: Unravelling HLA associated adverse drug reactions. *Immunogenetics, 69*(8–9), 617–630.

INSIGHT START Study Group, Lundgren, J. D., Babiker, A. G., Gordin, F., Emery, S., … Neaton, J. D. (2015). Initiation of antiretroviral therapy in early asymptomatic HIV infection. *The New England Journal of Medicine, 373*(9), 795–807.

Jayappa, K. D., Ao, Z., & Yao, X. (2012). The HIV-1 passage from cytoplasm to nucleus: The process involving a complex exchange between the components of HIV-1 and cellular machinery to access nucleus and successful integration. *International Journal of Biochemistry and Molecular Biology, 3*(1), 70–85.

Jose, S., Quinn, K., Dunn, D., Cox, A., Sabin, C., & Fidler, S. (2016). Virological failure and development of new resistance mutations according to CD4 count at combination antiretroviral therapy initiation. *HIV Medicine, 17*(5), 368–372.

Mallal, S., Nolan, D., Witt, C., Masel, G., Martin, A. M., Moore, C., … Christiansen, F. T. (2002). Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet, 359*(9308), 727–732.

Messiaen, P., Wensing, A. M. J., Fun, A., Nijhuis, M., Brusselaers, N., & Vandekerckhove, L. (2013). Clinical use of HIV integrase inhibitors: A systematic review and meta-analysis. *PLoS ONE, 8*(1). [https://doi.org/10.1371/journal.pone.0052562]

Ni, J., Wang, D., & Wang, S. (2018). The CCR5-Delta32 genetic polymorphism and HIV-1 infection susceptibility: A meta-analysis. *Open Medicine, 13*, 467–474.

O’Connor, J., Smith, C., Lampe, F. C., Johnson, M. A., Chadwick, D. R., Nelson, M., … Delpech, V. (2017). Durability of viral suppression with first-line antiretroviral therapy in patients with HIV in the UK: An observational cohort study. *The Lancet HIV, 4*(7), e295–e302. [https://doi.org/10.1016/S2352-3018(17)30053-X]

Ong, K. J., van Hoek, A. J., Harris, R. J., Figueroa, J., Waters, L., Chau, C., … Delpech, V. (2019). HIV care cost in England: A cross-sectional analysis of antiretroviral treatment and the impact of generic introduction. *HIV Medicine, 20*(6), 377–391.

Rockstroh, J. K., DeJesus, E., Lennox, J. L., Yazdanpanah, Y., Saag, M. S., Wan, H., … STARTMRK Investigators. (2013). Durable efficacy and safety of
raltegravir versus efavirenz when combined with tenofovir/emtricitabine in treatment-naive HIV-1-infected patients: Final 5-year results from STARTMRK. *Journal of Acquired Immune Deficiency Syndromes, 63*(1), 77–85.

Ryom, L., Lundgren, J. D., De Wit, S., Kovari, H., Reiss, P., Law, M., … D:A:D Study Group. (2016). Use of antiretroviral therapy and risk of end-stage liver disease and hepatocellular carcinoma in HIV-positive persons. *AIDS, 30*(11), 1731–1743.

Sáez-Cirión, A., Bacchus, C., Hocqueloux, L., Avettand-Fenoel, V., Girault, I., Lecouroux, C., … Rouzioux, C. (2013). Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI study. *PLoS Pathogens, 9*(3). https://doi.org/10.1371/journal.ppat.1003211

Sax, P. E., Tierney, C., Collier, A. C., Fischl, M. A., Mollan, K., Peeples, L., … AIDS Clinical Trials Group Study A5202 Team. (2009). Abacavir-lamivudine versus tenofovir-emtricitabine for initial HIV-1 therapy. The New England Journal of Medicine, 361(23), 2230–2240.

Simpson, D. M., & Tagliati, M. (1995). Nucleoside analogue-associated peripheral neuropathy in human immunodeficiency virus infection. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology: Official Publication of the International Retrovirology Association, 9*(2), 153–161.

Stein, D. S., Korvick, J. A., & Vermund, S. H. (1992). CD4+ lymphocyte cell enumeration for prediction of clinical course of human immunodeficiency virus disease: A review. *The Journal of Infectious Diseases, 165*(2), 352–363.

Strategies for Management of Antiretroviral Therapy (SMART) Study Group, El-Sadr, W. M., Lundgren, J. D., Neaton, J. D., Gordin, F., Abrams, D., … Rappoport, C. (2006). CD4+ count-guided interruption of antiretroviral treatment. *The New England Journal of Medicine, 355*(22), 2283–2296.

Thornton, A. C. (2015). Viral hepatitis and HIV co-infection in the UK collaborative HIV cohort (UK CHIC) study [Doctoral, UCL (University College London)]. In Doctoral thesis, UCL (University College London). UCL. Retrieved from https://discovery.ucl.ac.uk/id/eprint/1473437/

Tostevin, A., White, E., Dunn, D., Croxford, S., Delpech, V., Williams, I., … UK HIV Drug Resistance Database. (2017). Recent trends and patterns in HIV-1 transmitted drug resistance in the United Kingdom. *HIV Medicine, 18*(3), 204–213.

Tovar-y-Romo, L. B., Bumpus, N. N., Pomerantz, D., Avery, L. B., Sacktor, N., McArthur, J. C., & Haughey, N. J. (2012). Dendritic spine injury induced by the 8-hydroxy metabolite of efavirenz. *The Journal of Pharmacology and Experimental Therapeutics, 343*(3), 696–703.
van Wyk, J., Ajana, F., Bisshop, F., De Wit, S., Osiyemi, O., Portilla, J., … Smith, K. Y. (2020). Efficacy and safety of switching to dolutegravir/lamivudine fixed-dose two-drug regimen versus continuing a tenofovir alafenamide-based three- or four-drug regimen for maintenance of virologic suppression in adults with HIV-1: Phase 3, randomized, non-inferiority TANGO Study. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America.* https://doi.org/10.1093/cid/ciz1243

Verma, R. (2014). Decline in CD4 counts in HIV patients. *Medical Journal, Armed Forces India, 70*(3), 301. https://doi.org/10.1016/j.mjafi.2014.06.012

Walensky, R. P., Goldberg, J. H., & Daily, J. P. (1999). Anaphylaxis after rechallenge with abacavir. *AIDS, 13*(8), 999–1000.

Wiegand, A., Spindler, J., Hong, F. F., Shao, W., Cyktor, J. C., Cillo, A. R., … Kearney, M. F. (2017). Single-cell analysis of HIV-1 transcriptional activity reveals expression of proviruses in expanded clones during ART. *Proceedings of the National Academy of Sciences of the United States of America, 114*(18), E3659–E3668. https://doi.org/10.1073/pnas.1617961114

Wolbers, M., Babiker, A., Sabin, C., Young, J., Dorrucci, M., Chêne, G., … on behalf of the CASCADE Collaboration. (2010). Pretreatment CD4 cell slope and progression to AIDS or death in HIV-infected patients initiating antiretroviral therapy—The CASCADE collaboration: A collaboration of 23 cohort studies. *PLOS Medicine, 7*(2), e1000239. https://doi.org/10.1371/journal.pmed.1000239

Zicari, S., Sessa, L., Cotugno, N., Ruggiero, A., Morrocchi, E., Concato, C., … Palma, P. (2019). Immune activation, inflammation, and non-AIDS comorbidities in HIV-infected patients under long-term ART. *Viruses, 11*(3). https://doi.org/10.3390/v11030200