

The impact of HIV-1 drug escape on the global treatment landscape

Collier DA¹, Monit C¹, Gupta RK^{2*}

¹ Division of Infection and Immunity, UCL, UK

² Department of Medicine, University of Cambridge

* Corresponding author

Address for correspondence:

Professor Ravindra Gupta

Department of Medicine
University of Cambridge
Cambridge
CB2 0XY
Email: Rkg20@ucl.ac.uk

Abstract

The rising prevalence of HIV drug resistance (HIVDR) could threaten gains made in combating the HIV epidemic and in reaching the 90-90-90 target to achieve virological suppression in 90% of all persons receiving antiretroviral therapy (ART) by the year 2020. HIVDR has implications for the persistence of HIV, the selection of current and future ART drug regimens and strategies of vaccine and cure development. Focusing on drug classes that are in clinical use, this review critically summarises what is known about the mechanisms the virus utilizes to escape drug control. Armed with this knowledge, strategies to limit the expansion of HIVDR are proposed.

Keywords: HIV, drug, resistance, mechanism, antiretroviral

Introduction

Enormous gains have been made since the advent of combined antiretroviral therapy (ART), resulting in reduced HIV-associated mortality and morbidity. 36.9 million people are living with HIV (PLWH), of whom 21.7 million receive

ART (UNAIDS, 2018). Over the past two decades, non-nucleoside reverse transcriptase inhibitor (NNRTI) based triple combination therapy has been the predominant first-line treatment. NNRTIs were initially combined with nucleoside/tide reverse transcriptase inhibitors (NRTIs), including lamivudine (3TC), zidovudine (AZT) or stavudine (D4T). The thymidine analogues were subsequently replaced with tenofovir (TDF) (World Health Organisation, 2013).

The rate of virological failure (VF) amongst individuals in low and middle-income countries (LMICs) on first-line ART is around 20% (Boender et al., 2015). Although poor adherence is implicated as the initial cause for VF, resuppression can occur on the same regimen even with drug resistance mutations (DRMs) (Gupta et al., 2014). HIV drug resistance (HIVDR) accumulates as VF continues necessitates a treatment switch to second-line, protease inhibitor (PI) based triple combination therapy (Boender et al., 2016; Goodall et al., 2017). This acquired drug resistance (ADR) in individuals failing first-line ART is high in various regions of the world. Up to half of patients in sub-Saharan Africa failing TDF based treatment have resistance to all three drugs in the regimen (TenoRes Study, 2016) (Gregson et al., 2017).

Transmission of these drug resistant HIV strains has inevitably resulted in PLWH presenting with transmitted drug resistance (TDR) (Gupta et al., 2018; Gupta et al., 2012; Rhee et al., 2015). In LMICs where drug resistance testing is not available routinely, pre-treatment drug resistance (PDR) is associated with an increased risk of VF (Avila-Rios et al., 2016; Hamers et al., 2012), cessation of treatment (World Health Organisation, 2017a) and death (Cambiano et al., 2013; Pinoges et al., 2015). Surveillance reveals that the prevalence of PDR is rising and NNRTI resistance has exceeded 15% in a number of countries (Gupta et al., 2018; Gupta et al., 2012; World Health Organisation, 2012, 2017b). HIVDR has now been recognised as a threat to combating the HIV epidemic and an impediment to the Joint United Nations Programme on HIV/AIDS 90-90-90 by 2020 target to achieve virological suppression in 90% of all persons receiving ART by the year 2020 (UNAIDS, 2014).

In this perspective, we 1) examine how HIVDR is acquired under drug pressure, exploring the mechanisms of drug escape for all drug classes currently in clinical use 2) explore the contribution of HIV persistence and anatomical compartments to

HIVDR 3) discuss what the rise of HIVDR means for future treatment options and the landscape of HIV infections and 4) suggest next steps to achieve HIVDR control and to limit the expansion of HIVDR to even further lines of treatment.

Molecular aspects of HIV-1 resistance to antiretrovirals in clinical use

HIV-1 evolution is driven by the need to evade host immunity. HIV has vast genetic diversity within and between individuals (Kearney et al., 2009). This is driven by a high replication rate, the absence of a proof-reading mechanism during reverse transcription (Mansky and Temin, 1995) and a high recombination rate that also propagates drug resistance mutations (DRMs) and contributes to HIV-1 persistence and immunopathogenesis (Song et al., 2018). HIV-1 recombination among parent viruses that are each resistant to a single drug can generate multidrug resistant variants (Rawson et al., 2018). Additionally, genetic diversity is broader in the presence of ART compared to drug naïve individuals. A cross-sectional study investigating HIV genetic sequences found greater diversity in ART treated participants compared with ART naïve individuals (Haddad et al., 2000). This inherent diversity has implications for the persistence of HIV, the selection of drug resistance mutations (DRMs) and strategies of vaccine and cure development.

Resistance to nucleoside/tide reverse transcription inhibitors (NRTIs)

NRTIs are purine/pyrimidine analogues and therefore competitive inhibitors of incoming dNTPs that constitute the viral nucleic acid (NA). Most NRTIs lack a 3'-OH and act as chain terminators when they are incorporated into viral DNA by reverse transcriptase (RT). RT has two enzymatic activities, a DNA polymerase that can copy either a DNA or RNA template, and a RNase H that cleaves RNA when complexed with DNA. RT is a heterodimer with 2 subunits; p66 and p51 (Figure 1a). The NA binding cleft or binding pocket is formed primarily by the p66 fingers, palm, thumb, connection and RNase H subdomains of p66. The connection and thumb subdomains of p51 form the floor of the binding cleft. Within the p66 subunit is the DNA primer grip, which helps to position the 3'-OH end of the primer strand at the polymerase active site (Sarafianos et al., 2001). Also within the polymerase active site in the p66 palm subunit is the YMDD loop which coordinates the catalytic carboxylates (D110, D185, and D186) that bind to divalent metal ions required for catalysis (Hsiou et al., 1996; Rodgers et al., 1995).

Mechanisms of resistance:

- 1) *Exclusion*- These mutations are in the finger or palm of RT and affect the binding of an incoming dNTP. M184V and K65R increase discrimination between the dNTP and NRTIs, thereby reducing incorporation of the NRTI into the growing DNA chain. M184V/I selectively reduces the incorporation of 3TC and emtricitabine (FTC) by steric hindrance(Gao et al., 2000; Sarafianos et al., 1999). K65R reduces the rate of both nucleotide and TDF incorporation(Gu et al., 1995; White et al., 2006) but also increases susceptibility to AZT by decreasing AZT excision(Parikh et al., 2007; White et al., 2005). L74V causes resistance by disrupting the hydrogen-bonding network that involves 3-OH and is specific for didanosine (ddI) and abacavir (ABC)(Deval et al., 2004; Martin et al., 1993; Miranda et al., 2005).
- 2) *Excision*- This resistance mechanism involves pyrophosphorolysis, a hydrolysis reaction that removes the chain-terminating residue and enables reverse transcription and DNA synthesis to resume. Thymidine analogue mutations (TAMs) enhance removal of drug from its attachment at the end of the DNA chain (Table 1). Combinations of these mutations give rise to high levels of resistance to AZT and also resistance to other NRTIs(Boyer et al., 2001; Meyer et al., 1999).
- 3) *Connection domain mutations*- The connection domain is one of five subdomains of RT and acts as a tether between polymerase and RNase H. Connection domain (CN) mutations in the context of TAMs increase resistance to AZT by 500 fold(Nikolenko et al., 2007).
- 4) *RNase H mutations*- These mutations decrease RNase H activity and RNA template degradation, allowing more time for AZT-MP to be excised from the terminated primer (Nikolenko et al., 2005).

Resistance to non-nucleotide reverse transcription inhibitors (NNRTIs)

NNRTIs are allosteric inhibitors of RT. Although the NNRTIs in clinical use; Nevirapine (NVP), Efavirenz (EFV), Etravirine (ETR), and Rilpivirine (RPV), are chemically diverse, they all bind the same hydrophobic pocket, distinct from the polymerase active site(Das et al., 2005) (Figure 1b). Structural studies show that RT

bound to NVP causes a conformational change in the primer grip within the p66 thumb subunit, leaving it in a locked, hyperextended position. This alters the alignment of the DNA primer with the polymerase active site and impairs polymerase activity(Hsiou et al., 1996). In addition, binding of RT with NNRTI distorts the YMDD loop of the polymerase active site such that its catalytic carboxylates cannot bind the metal cofactors, which affects translocation of the nucleic acid that normally occurs after the incorporation of each nucleotide(Das et al., 2007). NMR studies demonstrate that the NNRTI-binding site is highly plastic in the ligand-free enzyme. However, this conformational plasticity is reduced upon RT/ NNRTI binding, with the NNRTI-binding pocket becoming more rigid(Sharaf et al., 2016). Drug resistant variants- K103N, V108I and E138K modulate this conformational plasticity, with K103N having the biggest effect(Sharaf et al., 2016).

Mechanisms of resistance:

- 1) *Loss/change of key hydrophobic interactions*- Y181C, Y188L and F227L cause loss of the aromatic ring interactions with NNRTIs(Das et al., 1996; Ren et al., 2001; Ren et al., 2004). These residues are in the hydrophobic core of the NNRTI binding pocket(Kohlstaedt et al., 1992). and result in high levels of resistance to first generation NNRTIs.
- 2) *Steric Hindrance*- K100I alters the pocket shape due to a change from a gamma-branched to a beta-branched amino acid(Ren et al., 2004). G190A introduces a bulge in the binding pocket(Das et al., 2004). The diarylpyrimidine inhibitors (ETR and RPV) bind in a horseshoe formation and thus are unaffected by the G190A mutation(Das et al., 2004).
- 3) *Pocket Entrance Mutations*- K103N and K101E are located at the rim of the binding pocket. These mutations interfere with the entry of NNRTIs into the pocket(Hsiou et al., 2001; Ren et al., 2007).

Resistance to protease inhibitors

HIV protease enzymes are symmetrical dimers with a central core that binds the peptides that are modified by the enzyme (Figure 2). The active site is formed at the dimer interface and contains two conserved aspartic acid residues, one from each monomer. This active site is covered by two beta-hairpin flaps(Collins et al., 1995). Protease cleaves viral gag and gag-pol polyproteins, yielding the structural proteins

and enzymes. Gag cleavage allows formation of a mature, infectious viral particle (Konvalinka et al., 2015). The protease flaps adopt an open conformation in the absence of substrate but a closed conformation when bound (Freedberg et al., 2002; Katoh et al., 2003).

Protease inhibitors (PIs) are competitive inhibitors of the protease enzyme and bind at the catalytic site with high affinity and thereby block its activity. Inhibition of HIV protease enzymes results in immature and non-infectious viral particles (Wensing et al., 2010). PIs have a high barrier to resistance, as multiple mutations are likely necessary to overcome their inhibition. Darunavir (DRV) in both inhibiting protease activity and dimerization leads to high barrier to resistance (Hayashi et al., 2014).

Mechanism of resistance:

1. *Protease mutations*- Resistance to protease inhibitors occurs primarily due to mutations within or proximal to the catalytic binding site of the drug. These reduce affinity of the protease to the PI. Consequently, the accumulation of multiple PI mutations is required for clinically relevant resistance (Table 1). D30N, G48V, I50L/V, V82A/F/T and I84V mutations located near the protease binding site, are initially selected upon PI exposure (Nijhuis et al., 2007a). They enlarge the binding cleft, thus reducing the affinity of the PI. Structural analyses of the V82A mutant protease, revealed that this residue is important for PI recognition but not the protease's natural substrate- gag (Prabu-Jeyabalan et al., 2003). The flaps covering the active site are critical for protease ligand binding (Deshmukh et al., 2017; Karthik and Senapati, 2011) and mutations in the protease flaps affect the flap's mobility thereby reducing PI binding. Molecular dynamic studies revealed that a mutant protease with flap residues G48 and I50 was unable to adopt the close conformation when bound to PIs but was able to when bound to a natural substrate (Appadurai and Senapati, 2017). Structural studies of multidrug resistant HIV variants have revealed that mutational clusters at the binding cleft and distal to the binding cleft act synergistically to alter flap dynamics, resulting in weaker PI binding (Agniswamy et al., 2016). Minor mutations affect PI susceptibility through complementary mechanisms. They occur later than major mutations and do not directly affect PI resistance. Minor mutations to the three PIs currently in clinical use are listed in Table 1.

2. *Gag mutations*- Gag is the natural substrate of protease and the ordered cleavage of gag is essential to produce mature, infectious virions(Deshmukh et al., 2015). Mutations in gag can both overcome the reduced proteolytic cleavage of gag and gag-pol in the presence of PIs(Fun et al., 2012; Nijhuis et al., 2007a) but also independently increase resistance to PIs(Nijhuis et al., 2007b). Full-length gag has been associated with reduced susceptibility to ATV, LPV and DRV, in HIV isolates derived from individuals failing treatment with PIs(Gupta et al., 2010; Parry et al., 2009; Sutherland et al., 2015). The most common gag mutations reported are cleavage site mutations, including A431V, K436E, I437T/V in the NC/SP2 site and L449F in the SP2/p6 site. (Carrillo et al., 1998; Nijhuis et al., 2007b). Enhanced processing of these mutated gag proteins by wild-type protease is the mechanism leading to PI resistance(Nijhuis et al., 2007b). Additionally, non-cleavage site mutations, particularly in matrix are selected upon PI exposure(Parry et al., 2009). Although the mechanisms leading to PI resistance are poorly understood, a transient interaction between protease and globular domains of gag – matrix, capsid and nucleocapsid, occur in order to guide protease to the gag cleavage sites(Deshmukh et al., 2016). Disruption to these interactions may enhance the efficiency of gag cleavage in the presence of PIs.
3. *Envelope mutations*- Mutations in gp41, a subunit of the viral envelope, contributes to drug resistance in a proportion of patients who had failed PI containing regimens both with and without major protease mutations(Rabi et al., 2013). However, the mechanism is unclear.

Resistance to integrase strand transfer inhibitors (INSTIs)

Integrase strand transfer inhibitors (INSTIs) inhibit HIV-1 integrase (IN). IN is encoded by the *pol* gene and is cleaved from the pol polyprotein by HIV protease enzyme. It is a 32KDa tetramer with 3 domains: 1) the N-terminal domain (NTD), which contains a zinc-binding motif HHCC involved in IN oligomerization(Cai et al., 1998), 2) the catalytic core domain (CCD), which contains the active site residues-D64, D116, and E152 (the catalytic triad)(Goldgur et al., 1998) involved with Mn²⁺ or Mg²⁺ ions, which are co-factors in the catalysis of IN(Marchand et al., 2003; Mazumder et al., 1996) and 3) the C-terminal domain (CTD), which is the DNA

binding site(Lodi et al., 1995) (Figure 3). HIV IN has two main functions; 1) 3' -end processing (3'EP) that excises a 3' terminal dinucleotide at both ends of HIV-1 DNA 2) strand transfer (ST) that integrates the proviral DNA into the host's genome(Pommier et al., 2005). INSTIs selectively inhibit this second function.

Raltegravir (RAL) and Elvitegravir (EVG) are first generation INSTIs. Three resistance pathways have been described and characterized for RAL: E92QV/N155H, T97A/Y143CHR, and G140CS/Q148HKR(Malet et al., 2008). While the G140S alone does not impact susceptibility to RAL, Q148H caused a 2 to 3-fold increase in IC_{50} and the double mutant G140S and Q148H caused 43-fold increase in IC_{50} compared to wild type(Metifiot et al., 2010). Cross-resistance of these mutants to EVG has been demonstrated(Metifiot et al., 2010). These mutations are in the flexible loop, adjacent to the CCD and are required for catalytic activity. A possible mechanism of E138K/Q148K double mutant to RAL and EVG maybe a conformation rearrangement of HIV-1 intasome active site leading to a decrease in the chelating ability of INSTIs to Mg^{2+} in the active site of the intasome(Mbisa et al., 2011; Xue et al., 2013).

Dolutegravir (DTG) and bictegravir (BIC) are second-generation INSTI with a high genetic barrier to development of resistance mutations (Cahn et al., 2013; Oliveira et al., 2018; Stellbrink et al., 2013). DTG and BIC select for the S153F/Y and R263K mutations (Oliveira et al., 2018; Quashie et al., 2012). The emergence of R263K to DTG has also been reported in multiple clinical cases and clinical trials(Ahmed et al., 2019; Taiwo et al., 2018; Wijting et al., 2017). The R263 residue is within the CTD domain and adjacent to K264, K266 and K273 residues, which are important in the pre-integration complex. They are involved in post-translational modification by cellular histone acetyltransferase (HAT) enzymes that enhance DNA binding(Cereseto et al., 2005; Terreni et al., 2010). Inhibition of HAT enzymes can reduce the IC_{50} of WT HIV in the presence of DTG but not the R236K mutant(Anstett et al., 2017). Additionally, the infectivity of the R263K mutant was enhanced by inhibiting endogenous HAT enzymes, suggesting that acetylation regulation may have a role in the mechanism of HIV resistance to INSTIs(Anstett et al., 2017).

G118R substitution in IN has emerged in patients exposed to DTG (Aboud et al., 2019; Brenner et al., 2016). The pathway to this mutation in non-B subtypes is due to a polymorphism at position 118 with a GGA motif that requires only a single mutation to transition to AGA (Brenner et al., 2016). This DRM diminishes the formation of the HIV-1 intasome and therefore the strand transfer step of integration (Munir et al., 2015; Quashie et al., 2013).

Although resistance in IN is rare, resistance in other areas of the genome of HIV such as the 3'-polypurine tract (3' PPT) (Malet et al., 2017) and 4 terminal bases of the LTR (Dicker et al., 2007) have been implicated in INSTI resistance. The 3'-PPT is a hybridization site for the RNA primer for plus-strand DNA synthesis during reverse transcription. Removal of these RNA primers defines the end of the linear proviral DNA for integration (Malet et al., 2017; Rausch and Le Grice, 2004). Mutations in 3' PPT will therefore impact polymerisation and integration. Mutations in the 4 terminal bases of the LTR leads to decreased binding of INSTI to integrase, while the strand-transfer activity of integrase remains relatively intact (Dicker et al., 2007). Recently mutations in Env protein were found following in vitro studies with HIV-1 clones cultured in DTG. These clones developed partial resistance to DTG with the emergence of env mutations- E209K, A539V, and H641Y. The mechanism of the decreased DTG sensitivity is thought to be mediated by effective cell-to-cell transmission (Van Duyne et al., 2019).

Resistance to entry inhibitors

Two classes of entry inhibitors are on clinical use; fusion inhibitors and CCR5 co-receptor antagonists. They target the envelope (Env) protein and the CCR5 HIV co-receptor respectively. HIV Env protein consists of trimers gp41 and gp120 subunits. Gp120 is responsible for virion attachment to target cells via the CD4 receptor, whilst gp41 mediates fusion between the virus and host cell membranes (Merk and Subramaniam, 2013). Binding of gp120 to CD4 leads to structural rearrangements that exposes the CCR5 co-receptor binding sites and secondary binding to CCR5 (Dragic et al., 1996) (Figure 4b). Gp41 consists of 2 sets of heptad repeat regions (HR1/ N-terminal heptad repeat and HR2/ C-terminal heptad repeat), which together form a stable post-fusion six-helix bundle (Harrison, 2008) (Figure 4a).

Fusion inhibitors selectively inhibit the function of the gp41. They are peptide mimetics of the HR2 that bind to HR1 and prevent the formation of the six-helix bundle. This interrupts fusion between gp41 and the host cell plasma membrane (Pang et al., 2009). Enfuvirtide (ENF) is the only fusion inhibitor currently in clinical use but it requires subcutaneous administration, limiting its clinical use. Mechanism of resistance: In vitro selection studies as well as sequencing of *env* from individuals with VF in ENF clinical trials show emergence of DRMs in the HR1 region of gp41 (Figure 4a). (Lalezari et al., 2003; Lazzarin et al., 2003; Rimsky et al., 1998) (Greenberg and Cammack, 2004) (Table 1).

CCR5 co-receptor antagonists are allosteric inhibitors of CCR5, binding hydrophobic pockets within the transmembrane helices of CCR5, thereby blocking the gp120–CCR5 interaction (Briz et al., 2006). The drug induces a conformational change in the second extracellular loop of CCR5, which the V3 stem loop of gp120 HIV-1 cannot recognise (Dragic et al., 2000). Maraviroc (MVC) is the only CCR5 co-receptor antagonist in clinical use, although others are in development. These drugs are only effective against R5 tropic viruses, limiting clinical use and mandating prior co-receptor testing in patients.

Mechanism of resistance: Multiple mutations within different regions of HIV gp120 account for the drug-resistant phenotype (Anastassopoulou et al., 2011). Although it has been reported that tropism shift from R5 to CXCR4 is the primary mechanism of MVC resistance, it is believed that this only occurs in patients with pre-existing CXCR4 virus variants (Westby et al., 2006). In addition, VF was associated with reduced mean percentage inhibition but not change in IC_{50} , consistent with the virus being able to bind to the drug-bound form of CCR5. Mutations in the V3 loop were observed but were inconsistent amongst the resistant viruses (Table 1) (Flynn et al., 2017; Lewis et al., 2018).

HIV-1 reservoirs and drug resistance

It is estimated that 10% of infected cells persist during ART (Besson et al., 2014). Different HIV quasispecies have been identified in different cellular compartments including memory and naive CD4 T cells and CD14 monocytes (Delobel et al., 2005). Long-lived cells such as macrophages and neurons in anatomical sanctuary

privileged sites such as the central nervous system (CNS), gut and male genital tract may harbour replication competent HIV-1 (Collier et al., 2018; Ganor et al., 2019). Proviruses may lie dormant in long-lived cells and reactivate later (Sigal and Baltimore, 2012). There is also evidence that viruses in these anatomical or tissue reservoirs can continue to replenish the compartment and perhaps the peripheral blood (Chun et al., 2005).

HIV can also adapt to replicate in different anatomical compartments and cell types. This evolution may be in the form of CCR5 to CXCR4 shift, or shift from R5-T cell tropic to R5-macrophage tropic HIV (Arrildt et al., 2015). Microglial cells (tissue macrophages) and other macrophages in the central nervous system (CNS) have lower levels of CD4 and are the targets for R5-Mac-tropic HIV which has adapted to infect cells with low levels of CD4 (Joseph et al., 2015) by the cell free route. Macrophages can become highly permissive to HIV by the deactivation of the major post entry restriction factor-SAMHD1 following G0 to G1 transition (Mlcochova et al., 2017). HIV reservoir sanctuary sites can aid HIVDR in the presence of ART. Persistence in tissue reservoirs where there is poor drug penetration may allow the virus to sequester and replicate, such as occurs in the CNS (Beguelin et al., 2016; Canestri et al., 2010; Collier et al., 2018; Peluso et al., 2012). Persistence of drug resistant HIV in latent reservoirs is not only a challenge for HIV-1 eradication but more immediately for deciding on subsequent regimens following VF.

What does rising resistance mean for the future HIV-1 treatment landscape?

The rising prevalence of HIVDR globally in both treated and untreated populations has implications for future treatment options. Where viral load (VL) monitoring and genotypic testing is readily available, it is possible to individualise treatment. However, in resource limited settings (RLS), where VL monitoring and genotypic testing are not readily available, the composition of HIV treatment regimens is decided at a population level and motivated by the balance of drug efficacy, public health priorities, cost and logistics. The rise in PDR levels, particularly to NNRTIs, has led WHO treatment guidelines (Table 2) (17) now recommending DTG-based combination ART for first-line treatment (World Health Organisation, 2018). Although DTG has a highly barrier to resistance when compared with NNRTIs, there are

concerns that without adequate VL monitoring and drug resistance testing, resistance to DTG will emerge and expand as has resistance to NNRTIs in SSA.

Development of new drugs that can circumvent the resistance mechanisms previously described are in the pipeline. 4'-ethynyl-2-fluoro-2'-deoxyadenosine (EFdA), a novel nucleoside reverse transcriptase translocation inhibitor (NRTTI), has been designed to be active against TDF resistant HIV, and is undergoing phase 2b clinical trials. Although this molecule has a 3'-OH, it induces immediate chain termination by inhibition of primer translocation thereby blocking further addition of nucleotides and preventing ongoing polymerisation, followed by delayed chain termination which prevents nucleotide excision from occurring (Michailidis et al., 2009). A next generation NNRTI doravirine (DOR) was designed to be active in the face of classical NNRTI DRMs selected in particular by NVP and EFV (Colombier and Molina, 2018). Long-acting injectable antivirals such as cabotegravir and rilpivirine, have demonstrated efficacy in the phase 3 ATLAS and FLAIR clinical trials in treatment experienced (Swindells et al., 2019) and naïve participants respectively (Orkin et al., 2019). In addition, new drug classes will be needed for patients with multidrug resistant HIV. Ibalizumab, a humanized IgG4 monoclonal antibody against the extracellular domain 2 of the CD4 receptor has shown promise (Emu et al., 2018); Fostemavir: an attachment inhibitor that binds to gp120 of HIV envelope of the HIV and blocks attachment to host CD4+ T-cell appears effective, though *env* diversity may be a potential barrier (ViiV Healthcare, 2018). The capsid inhibitor- GS-6207 has shown promise and is undergoing phase 1b trials in HIV infected patients (Gilead, 2019).

Conclusion

The advent of combination ART has led to reduction in HIV-associated mortality and morbidity. Despite gains made, the use of ART has been constantly challenged by the emergence of both acquired and transmitted DRMs. An understanding of the mechanisms by which HIV evades antiviral drugs is key not only for optimising use of existing drugs, but also the development of novel inhibitors that overcome these resistance pathways. A multi-pronged approach centred around combination antiretroviral therapy continues to be the means to achieving sustainable control of the HIV epidemic.

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Figures Legend

Figure 1a. HIV-1 reverse transcriptase (coloured from dark blue to light green representing N-terminal to C-terminal residues) bound to AZT (grey) and ATM (AZT/DNA complex; black). The subdomains of the p66 subunit are labelled and p51 subunit is shown as grey spheres in the background. A detailed look at the nucleic-acid binding cleft is shown in the enlarged image where alpha carbons of the NRTI drug resistant mutation site residues are shown as spheres and labelled. A divalent Mg^{2+} is shown in the polymerase active site. AZT; zidovudine, ATM; 3'-azido-3'-deoxythymidine-5'-monophosphate. Determined using X-ray crystallography by Das et al 2012; PDC ID: 5U1C(Das et al., 2012).

Figure 1b. HIV-1 reverse transcriptase shown as in Figure 1, including non-nucleotide reverse transcription inhibitor (NNRTI) binding pocket containing a bound Nevirapine (NVP) molecule. This is adjacent to the polymerase active site containing a bound ATM (AZT/DNA complex; 3'-azido-3'-deoxythymidine-5'-monophosphate) molecule. Alpha carbons of NNRTI drug resistance mutation site residues are shown as spheres. Determined using X-ray crystallography by Das et al 2012; PDC ID: 5U1C(Das et al., 2012).

Figure 2. Dimeric HIV-1 protease bound to darunavir (DRV; blue) at the protease active site. Protease subunits are shown as yellow and bronze cartoons. Alpha carbons of residues associated with protease inhibitor resistance mutations are shown as spheres and labelled for a single monomer. Determined using X-ray crystallography by Lockbaum et al 2019; PDB ID: 6DGX(Lockbaum et al., 2019).

Figure 3. HIV-1 tetrameric integrase strand transfer complex. Residues of a single subunit are coloured from dark blue (N terminus) to light green (C terminus) and labelled by domain, while other subunits are coloured various shades of grey; DNA strands are shown as grey or black wires and a magnesium ion is shown as a grey sphere. Alpha carbons of residues associated with integrase inhibitor resistance mutations are shown as spheres. Determined using cryo-electron microscopy by Passos et al 2017; PDB ID: 5U1C(Passos et al., 2017).

Figure 4a. Ectodomain of HIV-1 gp41-GCN4 recombinant, showing the heptad repeat 1 (HR1) in yellow, HR2 in blue and trimeric GCN4 coiled coil in grey. Fusion inhibitors such as enfuvirtide mimic HR2 and block its association with HR1. Alpha carbons of residues associated with enfuvirtide resistance mutations are shown as spheres. Determined using X-ray crystallography by Wiseenhorn et al 1997; PDB ID: 1ENV(Weissenhorn et al., 1997).

Figure 4b. HIV-1 gp120 (blue and yellow cartoon) in complex with CD4 (gray spheres) and CCR5 (green cylinders). The V3 loop of gp120 of is shown in yellow; the CCR5 inhibitor maraviroc binds CCR5 at the same position. Alpha carbons of residues associated with maraviroc resistance mutations are shown as spheres. Determined using cryo-electron microscopy by Shaik et al 2018; PDB ID: 6MEO(Shaik et al., 2019).

Tables

Drug Class	Action	Drug and Associated Mutations	Resistance Mechanism
Nucleotide/side reverse transcription inhibitor	Competitive inhibitor of viral nucleic acid. Drugs lack a 3'-OH and act as chain terminators when they are incorporated into elongating viral DNA by RT	3TC and FTC -M184V TDF and ddI- K65R	<i>Exclusion</i> - Causes a conformational change in the nucleic acid binding cleft that allows better discrimination of incoming dNTPs over drug.
		AZT, d4T, ddI - Q151M complex ddI and ABC- L74V	<i>Exclusion</i> - Alters the hydrogen bonding networks between RT and incoming deoxyribose, allowing better discrimination of dNTP over drug
		AZT- TAMs; M41L, D67N, K70R, L210W, T215F/Y, K219E/Q T69 insertion	<i>Excision</i> - Pyrophosphorolysis; RT enzyme mediated reaction that removes the chain-terminating residue and allow reverse transcription to continue.
		AZT- T369V/I AZT- N348I	<i>Connection domain mutations</i> - Decreases RNase H activity through poorly defined mechanism.
		AZT and D4T-D549N, H539N AZT, 3TC and ABC- Q509L	<i>RNase H mutations</i> - Decreases RNase H activity and hence reduces RNA template degradation, allowing more efficient excision of AZT. Q509L alters the RNase primer grip which affects the proximity of the RNA/DNA complex to the RNase H and hence degradation efficiency.
Non-nucleotide reverse transcription inhibitors	Allosteric inhibition of RT. The binding pocket is located ~10	All- Y181C EFV, NVP, RPV- Y188L RPV- F227L	<i>Loss/change of key hydrophobic interactions</i> -Causes loss of loss of the aromatic ring interactions with NNRTIs
		All- K100I EFV and NVP- G190A	<i>Steric Hindrance</i> - Changes the shape of the binding pocket

	Å from the polymerase active site.	EFV - K103N ETR and RPV- K101E ETR and RPV- E138K	<i>Pocket Entrance Mutations</i> - Affect the binding pocket and interferes with the entry/exit of NNRTIs in and out of the pocket
Protease Inhibitors	Competitive inhibitors of the protease enzyme. Bind at the catalytic site of the protease enzyme with high affinity thus blocking activity	ATV- I50L, I84V, N88S DRV- I47V, I50V, I54M/L, I84V, LPV- V32I, I47V/A, L76V, V82A/F/T/S	<i>Major mutations</i> - Amino acid mutations that arise within or proximal to the catalytic binding site to the drug. Enlarge the binding cleft, thus reducing the affinity of the PI. The drugs also affect the protease flap dynamics
		ATV- L10I/F/V/C, G16E, K20R/M/I/T/V, L24I, L33I/F/V, E34Q, M36I/L/V, M46I/L, F53L/Y, D60E, I62V, I64L/M/V, A71V/I/T/L, G73C/S/T/A, V82A/T/F/I, I85V, L90M, I93L/M DRV- V11I, L33F, T74P, L89V LPV- L10F/I/R/V, K20M/R, L24I, L33F, M46I/L, I50V, F53L, I54V/L/A/M/T/S, L63P, A71V/T, G73S, I84V, L90M	<i>Minor mutations</i> - Affect PI susceptibility through complementary mechanisms. Refer to main text.
		NC/p1 site- A431V, K436E, I437T/V P1/p6 site- L449F	<i>Gag cleavage site mutations</i> - Enhance efficiency of gag cleavage.
		Envelope gp41 mutations	Unknown mechanism
Integrase strand transfer inhibitors	Selectively inhibit strand transfer that integrates the proviral DNA into the host's genome	RAL and EVG E92QV/N155H	<i>Mutations in the CCD</i> - Around the catalytic triad that coordinate two Mg ²⁺ required for IN catalyses. N155 is not in direct contact with INSTIs but leads to conformational change of the catalytic pocket

		RAL, EVG, DTG and BIC- G140CS/Q148HKR	Confers cross-resistance to all INSTIs in clinical use. Q148 mutations affect conformational change of the catalytic pocket
		RAL- T97A/Y143CHR,	Y143 is in direct contact with RAL in the catalytic pocket. Mutation affects binding of RAL to the catalytic pocket.
		RAL-T66IR/S153/F121	Mutations in the CCD, around the catalytic triad that coordinate the two Mg ²⁺ required for IN catalyses.
		DTG and BIC- R263K/S153/F121	<i>Mutations in the CTD</i> - Involved in acetylation regulation, which enhances DNA binding. The mechanism is incompletely understood.
		3'-polypurine tract mutations	Prevents plus strand syntheses by interfering with RNA primer binding
		four 5' terminal bases of the LTR	Decreases binding of INSTIs to integrase
Entry Inhibitors			
<ul style="list-style-type: none"> Fusion inhibitors 	Peptide mimetics of HR2. Selectively inhibit the function of gp41	ENF- HR1 mutations: G36D/S, V38A, Q40H, N42T/E/S, N43D/S/K, L44M, L45M	Mechanism not fully understood. DRMs disrupts HR1- HR2 interaction which may increase discrimination between the fusion inhibitors and the native HR2
<ul style="list-style-type: none"> CCR5 co-receptor antagonist 	Allosteric inhibitors of CCR5	MVC- Tropism shift MVC- Gp120 mutations: P13S/G15GA, I20F/A25D/I26V, T2I/G11S/I26V/V34A and N13H/I2T/R18K/T22A	From CCR5 to CXCR4 or dual tropic. Mechanism not fully understood. Virus gp120 is able to bind to the drug-bound form of CCR5

Table 1: mechanism of HIV drug resistance to ARTs in clinical use

RT; reverse transcriptase, dNTP; deoxynucleotide triphosphate, CCD; catalytic core domain, CTD; C-terminal domain, NRTI; nucleotide/side reverse transcription inhibitor, NNRTI; non-nucleotide reverse transcription inhibitor, PI; protease inhibitor, INSTI; integrase strand transfer inhibitor, 3TC; lamivudine, FTC; emtricitabine, TDF; tenofovir, ddl; didanosine, AZT; zidovudine, D4T; stavudine, TAMs; thymidine analogue mutations, EFV; efavirenz, NVP; nevirapine, ETR; etravirine, RPV; rilpivirine, RAL; raltegravir, EVG; elvitegravir, DTG; dolutegravir, HR1; heptad repeat 1, HR2; heptad repeat 2,

	First-line	Second-line
Adults and adolescents	TDF+ 3TC (or FTC) + DTG	2 NRTIs ^b + ATV/r (or LPV/r) 2 NRTIs ^b + DTG ^c
Women and adolescent girls of childbearing age who decline use of consistent contraception	TDF+ 3TC (or FTC) + EFV	2 NRTIs ^b + ATV/r (or LPV/r)
Children > 6 years and >15 Kg ^a	ABC+3TC+DTG	2 NRTIs + ATV/r (or LPV/r) ^d 2 NRTIs ^b + DTG ^e
Neonates	AZT+3TC+RAL	

Table 2: Preferred regimens from the World Health Organisation updated guidelines on ART treatment 2018.

TDF; tenofovir, 3TC; lamivudine, FTC; emtricitabine, DTG; dolutegravir, EFV; efavirenz, ABC; abacavir, AZT; zidovudine, RAL; raltegravir, NRTI; nucleoside (tide) reverse transcription inhibitor, ATV/r; ritonavir boosted atazanavir, LPV/r; ritonavir boosted lopinavir.

^a Paediatric weight-based dosing is currently being decided

^b AZT following TDF or ABC failure and vice versa.

^c If failing NNRTI-based first-line ART

^d In children failing first-line DTG based ART

^e In children failing first-line LPV/r based ART