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State of the Art in HIV Drug Resistance: Science and Technology Knowledge Gap

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Abstract

Resistance to antiretroviral therapy (ART) threatens the efficacy of human immunodeficiency virus type 1 (HIV-1) treatment. We present a review of knowledge gaps in the science and technologies of acquired HIV-1 drug resistance (HIVDR) in an effort to facilitate research, scientific exchange, and progress in clinical management. The expert authorship of this review convened to identify data gaps that exist in the field of HIVDR and discuss their clinical implications. A subsequent literature review of trials and current practices was carried out to provide supporting evidence. Several gaps were identified across HIVDR science and technology. A summary of the major gaps is presented, with an expert discussion of their implications within the context of the wider field. Crucial to optimizing the use of ART will be improved understanding of protease inhibitors and, in particular, integrase strand transfer inhibitors (INSTI) in the context of HIVDR. Limited experience with INSTI represents an important knowledge gap in HIV resistance science. Utilizing such knowledge in a clinical setting relies on accurate testing and analysis of resistance-associated mutations. As next-generation sequencing becomes more widely available, a gap in the interpretation of data is the lack of a defined, clinically relevant threshold of minority variants. Further research will provide evidence on where such thresholds lie and how they can be most effectively applied. Expert discussion identified a series of gaps in our knowledge of HIVDR. Addressing

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such gaps through further research and characterization will facilitate the optimal use of ART therapies and technologies. **(AIDS Rev. 2018;20:26-41)**

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Key words

Drug resistance, viral. Human immunodeficiency virus type 1. Genotyping techniques. Mutation. Sequence analysis.

Introduction

The evolving landscape of human immunodeficiency virus type 1 (HIV-1) treatment has led to continual improvements in patient outcomes. The expanding selection of antiretrovirals (ARVs) from six mechanistic classes provides a powerful armamentarium to the physician. However, the success of lifelong therapy relies on the continued efficacy of ARV regimens, whose barrier to genetic resistance is a crucial factor. Identifying and understanding resistance in relation to therapeutic options are critical to the appropriate selection, use, and sequencing of antiretroviral therapy (ART).

The objective of this review is to identify and discuss the key data gaps that exist with regard to acquired HIV-1 drug resistance (HIVDR) to focus and facilitate research, scientific exchange, and progress in clinical management of HIV disease. Emphasis will be placed on the gaps in our knowledge, and focus will be on the most commonly used treatment classes and specific ARVs. It is not intended to provide a comprehensive summary of resistance to each available treatment.

Resistance science

HIVDR is driven by the rapid rate and low fidelity of viral replication (approximately one nucleotide mutation per replicative cycle)[1-5]. The high mutation rate leads to a collection of many variants in each infected individual, often described as "quasispecies," and enables HIV to adapt very quickly to selection pressures, such as the presence of ART, leading to the selection and emergence of drug-resistant variants[2-5].

The genetic barrier to resistance of a regimen is broadly defined as the number of HIV mutations required for that drug regimen to fail (Fig. 1), and a low genetic barrier is a key factor contributing to treatment failure. This is because such drug regimen is strongly affected by drug exposure gaps, which are influenced by factors governing adherence. Resistance mechanisms can be complex, involving interactions between mutations and their associated pathways, and their impact on cross-resistance within ARV classes presents significant considerations for treatment decisions[5]. However, due to the complexity of genetic variants, their impact on therapeutic options is difficult to fully characterize. Numerous gaps exist in our knowledge of the mechanisms driving HIVDR (Table 1), which are important to address to reduce the impact of resistance in limiting therapeutic options.

Treatment classes

Gap 1: Full characterization of resistance mutations as they are identified

Knowledge gaps in resistance science differ significantly between treatment classes. The relative wealth of experience associated with nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) enables better-informed therapeutic decisions for these classes compared with those more recently introduced. Addressing this balance will be reliant on fully characterizing resistance mutations to better understand their actions and impacts within and across classes. In turn, such knowledge will facilitate the optimal use of all ARV classes in clinical settings.

Specific gaps within classes are outlined in more detail below.

NRTIs

Most NRTIs are chain terminators, i.e., they integrate at the terminus of a growing complementary deoxyribonucleic acid strand to block its extension. The primary mechanism conferring resistance to NRTIs is modification of the drug-binding site coded by the reverse transcriptase (RT) gene that allows HIV to preferentially bind analog deoxyribonucleotide triphosphate (dNTP) over phosphorylated NRTI (e.g., M184V,

Table 1. Knowledge gaps for the mechanisms of HIV drug resistance

Gaps: mechanisms of resistance
All classes
1. Full characterization of resistance mutations as they are identified: impact of mutations across ARV classes on treatment choice
Ritonavir-boosted protease inhibitors (PI/r)
2. Resistance barrier of PI/r in monotherapy and ARV-sparing regimens, and impact on subsequent therapy
3. Relationship of low-level viremia and resistance
4. Sequencing of the whole viral genome
Integrase strand transfer inhibitors (INSTI)
5. Activity in INSTI-naive patients, including those with an impaired NRTI backbone (e.g., M184V)
6. Genetic barrier to resistance with ongoing viremia/incomplete suppression
7. Activity in non-standard combinations (e.g., with a PI/r alone, with MVC, etc. [especially in the presence of (NNRTI) mutations])
Combination therapies
8. Combination therapy and the consequences of resistance
9. Appropriate dosing of MVC and use in combination therapy
10. Understanding the efficacy of recycled NRTIs in combination therapy and their use in second-line therapy

ARV: antiretroviral; MVC: maraviroc; NNRTI: non-nucleoside reverse transcriptase inhibitors; NRTI: nucleoside reverse transcriptase inhibitor; HIV: human immunodeficiency virus.

K65R, and Q151M)[6,7]. NRTI resistance may be alternatively driven by drug excision; so-called thymidine analog mutations (TAMs) facilitate the excision of non-extending NRTI, thereby unblocking RT (e.g., T215Y)[6,7].

TAMs occur only under selection pressure by thymidine analogs, such as zidovudine (ZDV) and stavudine, and can confer cross-resistance to impact subsequent use of tenofovir (TFV), abacavir, and didanosine[5,8]. Compared with wild-type virus, NRTI-resistant variants tend to exhibit higher fidelity in RT replication, sustaining the inhibition of NRTI incorporation. The antiviral effects observed within an NRTI combination may be modified by resistance mutations; as resistance mechanisms differ between different drugs and mutations to one NRTI may positively or negatively influence resistance to another NRTI[2,7,9].

NNRTIs

NNRTIs bind to a hydrophobic pocket within the RT that can tolerate relatively low conservation while not disrupting enzymatic activity, unlike the conserved active site or RT-dNTP binding site. Single mutations, occurring around the NNRTI pocket, can decrease binding of the drug[2,7]. Many first-generation NNRTIs are structurally rigid, and single mutations in the NNRTI pocket have a high impact, e.g., reducing binding and causing resistance. As resistance only requires a single mutation it can develop fast *in vivo,* e.g., following a single dose of nevirapine (NVP)[2,7]. The most common mutations observed under pressure of first-generation NNRTIs (NVP and efavirenz) are K103N (located on the pocket rim) and Y181C (within the pocket); these mutations modify molecular interactions through alteration of hydrophobic binding, loss of aromatic ring stacking, and increased steric hindrance[7].

Second-generation NNRTIs, rilpivirine (RPV) and etravirine, exhibit higher genetic barriers to resistance and retain activity against common NNRTI resistance mutations. RPV, for example, retains activity in the presence of K103N[7,10]. Combining RT-binding efficacy with enhanced flexibility in next-generation NNRTIs has the potential to further improve class resilience[2,7].

Protease inhibitors (PIs)

PI antiviral potency is primarily attributed to the inhibition of HIV aspartyl protease, but this leads to inhibition of multiple steps in the virus life cycle[11-13]. Inhibition of HIV protease blocks viral maturation resulting in the release of immature virions. These target new host cells, but fail to replicate because different parts of the replication cycle are severely disturbed (virus entry, RT, or post-RT). The greatest inhibitory potential of all PIs is seen at the entry step, with inhibition at subsequent steps varying moderately within the class[11].

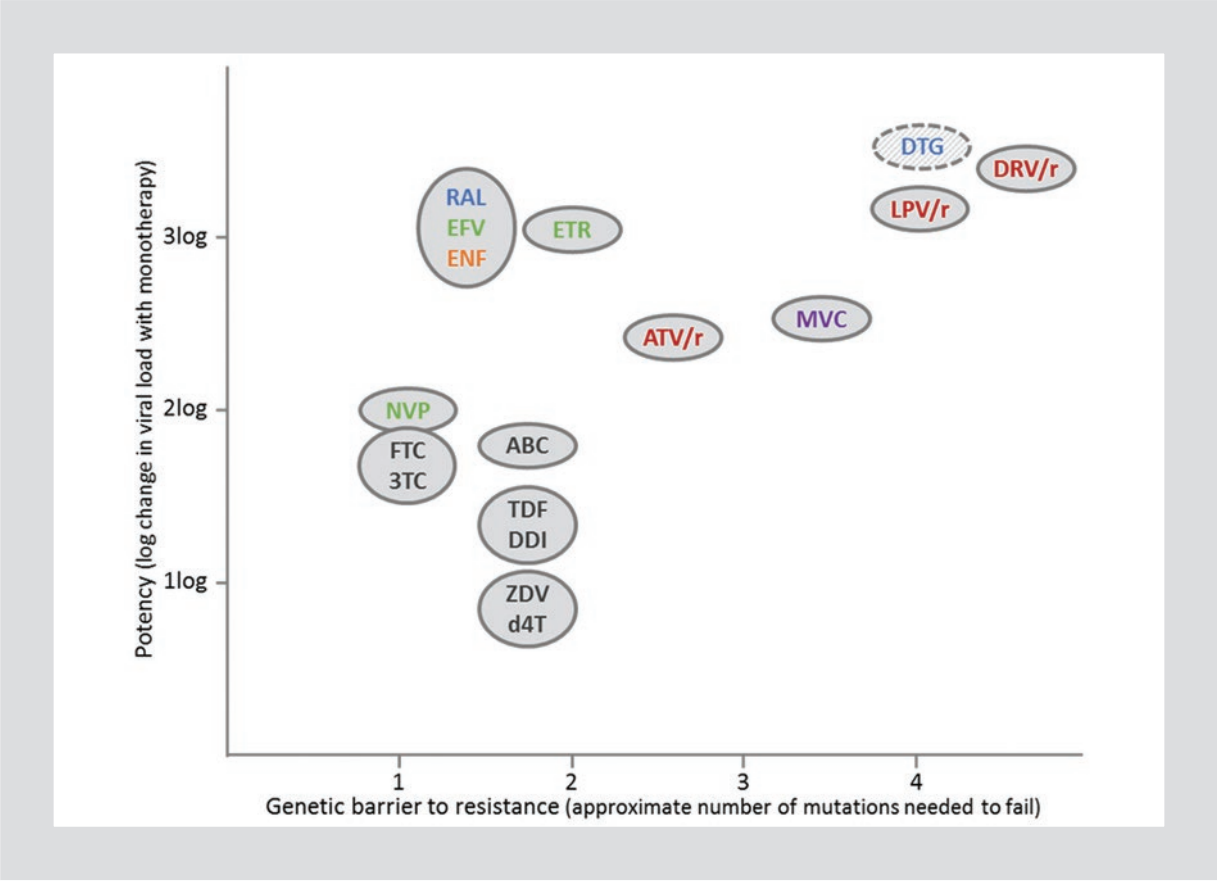

Figure 1. *Schematic of genetic barrier and potency of selected antiretrovirals. The genetic barrier and potency of an antiretroviral determine, in part, susceptibility to development of human immunodeficiency virus type 1 (HIV-1) resistance. This figure illustrates relative genetic barriers and potencies of commonly used antiretrovirals. Nucleoside reverse transcriptase inhibitors are depicted in black, non-nucleoside reverse transcriptase inhibitors in green, protease inhibitors in red, integrase inhibitors in blue, maraviroc in purple and enfuvirtide in orange. The appropriate position of dolutegravir represents a gap in our knowledge of this resistance profile* (adapted from Tang MW, Shafer RW[5]). *3TC: lamivudine; ABC: abacavir; ATV/r: atazanavir/ritonavir; ZDV: zidovudine; d4T: stavudine; DDI: didanosine; DRV/r: darunavir/ritonavir; DTG: dolutegravir; EFV: efavirenz; ENF: enfuvirtide; ETV: etravirine; FTC: emtricitabine; LPV/r: lopinavir/ritonavir; MVC: maraviroc; NVP: nevirapine; RAL: raltegravir; TDF: tenofovir disoproxil fumarate.*

These actions may explain one mechanism by which PI-based treatment failure sometimes occurs without protease mutations. PI resistance is a multi-step process in which initial mutations around the active drug binding site modify the overall protease structure and prevent PI binding. Structural modification can adversely influence the binding of HIV-Gag, thereby reducing viral fitness through lower viral replication. Mutations within the protease itself and Gag may further enhance resistance, and compensatory mutations may occur that overcome the loss of replicative ability[12,14]. In addition, mutations in the cytoplasmic tail of the envelope protein that is involved with viral entry (and that likely interacts with uncleaved Gag) have demonstrated PI resistance in the presence of wild-type *Gag* and *Pol* genes and may provide a further mechanism for PI failure in the absence of protease mutations[11,15].

High-level resistance to ritonavir-boosted PIs (PI/r) generally requires more than one resistance mutation in combination with at least one compensatory mutation in the protease and a Gag cleavage site mutation, which forms the basis of the high genetic barrier of PI/r[5,16]. Of the frequently used PIs, ritonavir-boosted lopinavir (LPV/r) and ritonavir-boosted darunavir (DRV/r) exhibit the highest genetic barriers within the class, requiring at least three to four mutations for treatment failure[5,16]. Most major PI resistance mutations confer broad class resistance (D30N and I50L confer high resistance to a single PI), but also reduce viral replication[5,16,17]. Although resistance to tipranavir/r (TPV/r) is not well understood, this PI retains activity against many LPV- and DRV-resistant viruses, awarding it a role in salvage therapy[5,18].

Despite the length of experience with PIs, their multimodal mechanisms of action are only recently being

uncovered. Combining this with their apparent versatility means that gaps in our knowledge of PI resistance remain. Until these are addressed, the optimal use of PIs, particularly with respect to apparent failure of other ARVs, remains to be defined.

Gap 2: Resistance barrier of PI/r in monotherapy and ARV-sparing regimens, and impact on subsequent therapy

The high genetic barrier of PI/r means that PI resistance is rare in patients experiencing the first-line failure with PI/r combination therapy[19-21], and PI/r monotherapy has shown efficacy at suppressing HIV replication as a maintenance/simplification strategy in ART-naive patients[22-28]. Although not recommended in guidelines, simplification with a PI/r monotherapy may present a feasible option for patients without a history of PI failure for whom NRTIs are no longer an option and for patients who wish to minimize their exposure to multiple classes of ARVs[22,23,25-29]. Recently, the large PIVOT trial demonstrated non-inferiority of long-term PI monotherapy compared with triple therapy following initial viral load (VL) suppression. This study met its primary objective of preserving future options; however, monotherapy was associated with significantly higher virologic failure[30].

When combined with appropriate VL monitoring, such an approach may preserve future treatment options and presents an attractive cost-saving option. Until further evidence addresses concerns regarding durable efficacy, low-level viremia (LLV) and propensity to resistance, understanding the apparent lack of resistance in the protease and the potential for PI/r monotherapy will remain a gap in our knowledge and therapeutic armoury.

Promising results have been obtained in dual-therapy trials, and these are discussed in more detail below (Section 1.2.2 ARV-sparing regimens).

Gap 3: Relationship of LLV and resistance

LLV, defined as persistent plasma HIV RNA levels in the range of 50-1000 copies/mL, is a common feature of PI/r therapy, and more frequent in PI/r monotherapy; but remarkably little resistance has been observed as associated with this scenario in the clinical trial setting[23,31,32], and there is limited guidance on the management of LLV[33]. LLV is associated with increased overall immune activation, risk of ART-failure and development of resistance[32,34]. Intensification strategies may be beneficial, but trials will be required to inform guidelines, and also to determine optimal timing and frequency of resistance testing in this setting. It was recently demonstrated that non-optimal drug levels and reduced susceptibility at LLV are independent predictors of virologic failure[35], and coupled with the choice of ART, points to the need for further investigation to understand potential implications for the management of LLV. Dual-sparing regimens may lend themselves to appropriate intensification strategies that have potential use in the instance of sustained LLV[36]. These are discussed in more detail below (Section 1.2.2 ARV-sparing regimens).

Gap 4: Sequencing of the whole viral genome

Recent studies provide increasing support for whole-genome sequencing, for which PI resistance is a prime candidate. The regions routinely sequenced when screening for PI resistance are the protease-RT and integrase (ART-experienced). Such practice does not explain PI failure in patients who do not have mutations in the protease. Although adherence may account for a portion of these patients, the accumulation of mutations beyond the major protease positions, which are ignored in current tests, may confer low phenotypic resistance and support the inclusion of novel areas in resistance analyses[11,12]. Thus, to find novel mutations ignored in current practices, it will be important to consider sequencing non-traditional areas, such as *Gag* (which may prove especially prudent in patients with LLV), and determine their clinical relevance.

Integrase strand transfer inhibitors (INSTIs)

The introduction of INSTIs provided additional treatment options for patients with drug-resistant virus[37]; however, they are gradually replacing PIs and NNRTIs within first-line regimens. Resistance to the first-generation INSTIs develops along distinct initial pathways, with the majority of mutations occurring in the active site of integrase, where they inhibit INSTI binding. Resistance predominantly involves independent changes at three positions: Q148, N155, and Y143. During prolonged failure, combinations of these three and additional mutations are observed[38-40]. These mutations impact viral fitness and are frequently observed with secondary mutations that enhance resistance or compensate for the negative effect on integrase activity[41-43].

Of the three currently approved INSTIs, first-generation compounds, raltegravir (RAL), and elvitegravir (EVG), exhibit a low genetic barrier to resistance and muta-

tions tend to confer cross-resistance, precluding the possibility of switching between RAL and EVG once resistance has developed[38,43-45]. Risk factors for resistance development include a high VL and low activity of the background regimen[45]. Dolutegravir (DTG), a second-generation INSTI, exhibits different resistance patterns and a higher genetic barrier, with resistance being identified only in a limited number of pathways[40,46]. Any relationship between the levels of INSTI, overall risk of resistance and pathways of resistance remains to be established. The superiority of DTG over other INSTIs may result from higher potency, prolonged binding time to integrase and reduced replicative capacity for virions with DTG resistance mutations[46,47].

INSTIs represent an important knowledge gap in HIV drug resistance; and clinical trials investigating INSTIs have used varied definitions of virologic failure and different types of resistance testing and analyses, limiting inter-trial comparisons[48]. Consensus on these terms and the publication of associated data will facilitate more valuable analysis in future research.

Gap 5: Activity in INSTI-naive patients, including those with an impaired NRTI backbone

DTG is unique in the fact that, to date, *de novo* mutations conferring resistance to DTG, or NRTIs used in DTG regimens have not been identified in ART-naive patients[49]. However, data are limited, with very few failures, analysis of only the first samples at failure and only known INSTI resistance mutations are being reported. These reports and should, therefore, be interpreted with caution. INSTI-containing regimen activity in INSTI-naive patients with an impaired NRTI backbone has yet to be characterized. Addressing this gap will facilitate our understanding of appropriate treatment options for such patients.

Gap 6: Genetic barrier to resistance with ongoing viremia/incomplete suppression

Despite the low genetic barrier of RAL, resistance mutations have been identified in relatively few patients experiencing virologic failure on RAL, although this number varies according to the definition of virologic failure and remains to be fully explained[45]. Patients with RAL-resistant virus require careful management to avoid the evolution to DTG resistance with the appearance of double mutants carrying Q148. The limitations of available DTG studies leave a gap for analyses that use consistent criteria applicable to real-life data.

Gap 7: Activity in non-standard combinations

There are currently no data to indicate any benefit of DTG in specific instances that currently prompt the continued use of PI/r such as LLV, a scenario commonly associated with new mutations[34]. In addition, the benefit of PI/r versus DTG in scenarios such as isolated NNRTI resistance remains to be determined. Pooled data may provide evidence to shape such guidance, but these analyses have yet to be conducted and remain a gap in current knowledge.

There is an additional lack of data surrounding the recent shift from PI to RAL in post-exposure prophylaxis. Although data support the shift, the limited availability of high-quality evidence to fully characterize how this regimen may impact resistance has the potential to emerge as a future gap[50].

Chemokine (C-C Motif) Receptor 5 (CCR5) (entry) inhibitors

Failure on maraviroc (MVC), currently the only approved Chemokine (C-C Motif) Receptor 5 (CCR5) inhibitor (used when CCR5-tropic virus is confirmed), tends to occur in the presence of previously undetected chemokine receptor type 4-tropic (or dual-tropic) viruses as minority species that pre-existed the use of MVC[51,52]. However, resistance mutations that also enable HIV to enter a cell through CCR5 in the presence of inhibitor do occur, either through adaptation to reduced levels of CCR5 or through inhibitor-bound CCR5[53-56].

Tropism can be identified genotypically (relatively easy and logistically more manageable) or phenotypically and validated testing is recommended before initiation of MVC[5,57]. Both assays profile tropism through the ***env*** genes, with suitability of MVC selection, determined accordingly. Validation of a tropism assay is critical, and concordance between laboratories has been successfully demonstrated by the European Coreceptor Proficiency Panel Test[58-60].

Gaps in our knowledge of MVC resistance occur in its use within combination therapies. These are discussed in more detail below (Section 1.2.2 ARV-sparing regimens).

Influence of combination therapy profiles on resistance

Triple therapy

ART regimens typically combine two or more active drugs[33]. NRTIs are currently regarded as the best

backbone in first-line therapy, but different criteria among studies prevent reliable comparisons, and the paucity of data means that investigators have yet to determine on which characteristic this efficacy is based and if this will remain true with newer regimens.

Dual NRTIs are selected based on their *in vivo* activity and genetic barrier to resistance: mutations that impact viral fitness by significantly decreasing RT activity or enhancing susceptibility to another NRTI. Combining NRTIs with an NNRTI have demonstrated high efficacy, and the synergistic effects of such combinations may act to reduce resistance, for example, EFV inhibits the excision of TDF[6,61]. In the reverse scenario, E138K confers low-level resistance to RVP, but this is enhanced by the presence of a M184V/I background, an example of mutation synergy across NNRTI and NRTI classes[62]. As such, the efficacy of these dual combinations may be influenced even by minority variants that diminish NNRTI susceptibility, and which may also impact future use of NNRTI-based regimens[63,64].

Gap 8: Combination therapy and the consequences of resistance

As new ART regimens are trialed, gaps remain in our knowledge of the consequences of resistance in combination therapies. Resistance to NNRTIs accounts for 25-50% of first-line failure. The first-line failure due to PI resistance is rare, and failure due to INSTI resistance varies among the class. Understanding the mechanisms of action that can increase the barrier to resistance with combination therapy, and the consequences of failure in this setting, will better inform physicians and ultimately enhance treatment options.

ARV-sparing regimens

Triple combination therapy is the current standard of care, but suboptimal virologic suppression has the potential to lead to multiple class resistance, which can significantly impact future regimens[65]. In an effort to enhance tolerability, preserve future options and reduce costs, simplified approaches are under investigation. This review discusses studies in the context of current knowledge gaps, for a comprehensive overview of ARV-sparing studies, see the recent review by Baril et al.[66].

Recent ARV-sparing trials have combined 1NRTI + PI/r[67,68]. Initial studies suggest good efficacy and safety of these dual regimens, coupled with low rates of resistance. In the GARDEL trial, LPV/r + lamivudine (3TC) demonstrated non-inferiority to triple therapy at 48 weeks with no primary PI mutations in either arm (it is notable that most patients in the triple therapy arm received ZDV/3TC, which may not be an ideal comparator). Although the low emergence of M184V in the dual therapy arm raised queries over suitability as first-line therapy[68], studies for this regimen as simplification from triple therapy have been very encouraging. The open-label extension study has extended findings from GARDEL and supports the non-inferiority of dual LPV/r + 3TC to triple therapy at 48 weeks. A single emergence of M184V RT resistance mutation in a patient receiving dual therapy was found to be present after cessation of earlier treatment with TFV + 3TC + EFV[69]. These data are further supported by similar findings in the SALT and ATLAS studies that examined switching to atazanavir (ATV)/r + 3TC in virologically suppressed patients[70,71].

NRTI-sparing regimens combining PI/r + INSTI have demonstrated comparable efficacy to 2NRTIs + PI/r[29,72-74]. In the PROGRESS study, 8 patients receiving LPV/r + RAL versus 5 patients receiving TFV/FTC + LPV/r were tested for resistance, and 3 in the LPV/r + RAL group were found to have INSTI resistance-associated mutations (RAM), with the earliest detection at week 16. One patient also had LPV/r RAMs at week 72[74]. Comparable virologic suppression was found between ATV + RAL and triple therapy in the SPARTAN trial. No PI resistance developed, although INSTI resistance was detected in 4 patients[73]. Similar efficacy was noted for DRV/r + RAL versus triple therapy in NEAT001. However, of patients qualifying for resistance analysis who received DRV/r + RAL, 29.5% had INSTI (15/55) or PI (1/57) RAMs versus none in patients who received triple therapy; and the frequency of INSTI mutations at virologic failure was associated with baseline VL ($p = 0.007$)[75,76].

In terms of PI-sparing regimens, 48 weeks results from PADDLE, a pilot study combining DTG + 3TC in ART-naive patients, demonstrated rapid virologic suppression, followed by maintained suppression and tolerability. No mutations were identified in the only patient who experienced virologic failure (integrase and protease regions did not amplify) who later resuppressed without changes in regimen[77]. These promising results will be developed in further trials (NCT02491242, NCT02582684, NCT02527096, and NCT02263326).

In the second-line setting, both the SECOND LINE study and EARNEST study demonstrated the non-inferiority of LPV/r + NRTIs to LPV/r + RAL with no or few emergent PI-associated mutations in either study, respectively[29,72]. Approximately 3-14% of patients who received LPV/r + RAL developed resis-

tance mutations to RAL in both trials[29,72]. The low incidence of resistance with this regimen is further evidenced by the SELECT trial, which demonstrated non-inferiority of LPV/r + RAL to LPV/r + NRTIs and supports the alternative option of second-line dual therapy in resource-limited settings[78].

Management following failure on PI/r + RAL regimens has yet to be defined but must be carefully managed to fully preserve future options within the classes. Failure on therapy with a high genetic barrier may result from non-adherence or minority drug-resistant variants, additional considerations for clinical decisions that may be further confounded by availability of ARVs and incomplete virologic suppression[33,79].

The apparent protection to resistance offered by the combination of PIs with NRTIS remains to be elucidated. Understanding whether PIs protect NRTIs or vice versa, or if the long half-life of TFV/FTC protects itself and, in combination with PIs, leads to a low rate of resistance, will enhance our knowledge of mechanisms of actions and class synergy. Developing this knowledge may expand the rationale for recycling NRTIs and provide confidence for the use of such regimens[29].

Gap 9: Appropriate dosing of MVC and use in combination therapy

MVC is rarely used as the third agent of triple therapy and has been investigated in NRTI-sparing studies with PI/r[80]. The recent, large MODERN study designed to assess MVC + DRV/r versus TFV/FTC + DRV/r was terminated following inferior efficacy of DRV/r + MVC (NCT01345630), which was similar in overall outcome to dual treatment studies with ATV-RTV[81]. It should be noted that MVC was used at 150 mg once daily and more encouraging results were seen with LPV/r + MVC 150 mg once-daily, possibly due to increased MVC exposure[80,82]. In contrast, the MITOX study showed a decreased drug level in patients with DRV/r + MCV[83]. Until the relationship between MVC exposure and resistance is understood, the appropriate dosing of MVC and its optimal place in combination therapy remain to be determined; and this relatively well-tolerated ARV with potential anti-inflammatory activity remains to be fully utilized.

Gap 10: Understanding the efficacy of recycled NRTIs in combination therapy and their use in second-line therapy

A surprising outcome from the EARNEST study was the efficacy demonstrated by LPV/r + recycled NRTIs. This outcome may be explained, at least in part, by the multi-step activity of PIs and their possible synergy with NRTIs[11,29]. Furthermore, in the SELECT trial, the presence of three or more NRTI mutations at entry was associated with reduced virologic failure in the dual- and triple-therapy arms[78]. These confounding results present another gap in our knowledge of the mechanisms of resistance in combination therapies and how these can be overcome. Recycling previously failed NRTIs with another active drug may provide previously unexplored therapeutic pathways for patients with limited treatment options and warrants further investigation.

Resistance technology

Drug resistance can be investigated using genotypic and/or phenotypic assays. Genotyping identifies specific resistance mutations, and phenotyping determines drug susceptibility. European guidelines recommend monitoring by genotyping, which is generally more widely used due to its relative cost, availability, high level of standardization, ease of use, and short time scale compared with phenotypic testing[33,84-86]. Despite the advantages of phenotyping, these challenges make it an impractical option and, as such, this review will focus on gaps associated with genotyping. Although genotyping is recommended in most clinical situations, phenotypic assays can prove valuable in heavily pre-treated patients with complex resistance[86,87]. Moreover, the results from phenotypic tests still provide highly valuable data for the improvement of genotypic interpretation systems.

As technology develops, its appropriate use often remains to be defined. Closing such technological gaps in our knowledge will enhance the identification of resistance variants and promote consistency in analysis (Table 2).

Sequencing

Gap 1: Optimization of source material for resistance testing

The short half-life of HIV in plasma means plasma-isolated virus represents the most recently selected variant[3]. The lag time between the detection of resistance mutations in the plasma and in peripheral blood mononuclear cells (PBMCs)[3] has led to plasma as the standard source for resistance analysis to investigate recent therapy failures. However, inter-compartment heterogeneity has been reported between resistance

Table 2. Knowledge gaps in the technology of HIV drug resistance

Gaps: technology
Current technologies
1. Optimization of source material for resistance testing
2. Recommendations to provide consistency and standardization of definitions and practice for genotyping resistance mutations
3. Consistent bioinformatics and data analyses
Next-generation sequencing
4. Establishing a clinically relevant interpretation threshold for sequencing analysis
5. Simple, affordable assays with consistent bioinformatics for global use
Genotyping recommendations and practices
6. Evidence to establish consistent recommendations for genotyping: update to the relevance and rationale for recommending genotyping before commencement of ART
7. Use of whole-genome next-generation sequencing in clinical practice
8. Value of sequencing novel regions
9. Necessity for genotyping ahead of initiating an INSTI or entry inhibitor

HIV: human immunodeficiency virus; INSTI: integrase strand transfer inhibitor; ART: antiretroviral therapy.

mutations harbored in the PBMC reservoir and in the plasma[88-91]. The utility of proviral DNA PBMC resistance testing in place of, or in addition to, ribonucleic acid (RNA) plasma samples look promising but remain to be determined, but two-compartment testing may provide a more complete picture of viral resistance in patients with complex treatment history[89,91].

Gap 2: Recommendations to provide consistency and standardization of definitions and practice for genotyping resistance mutations

Current guidelines inadequately address resistance mutations and lack consistency in their definitions[33,86]. Virologic failure is a prime example: definitions include "VL > 50 copies/mL 6 months after starting therapy[33]," "VL > 1000 copies/mL based on two consecutive measurements in 3 months[92]," and "inability to achieve or maintain suppression of viral replication to an RNA level of < 200 copies/mL[93]." Misconceptions of resistance terminology may have implications on the clinical interpretations of resistance testing. Addressing this gap through clear definitions of terms relied on in resistance technologies will facilitate consistent, comparable data collection and analyses.

Population sequencing

Sanger (population) sequencing is the current standard of care for clinical use. Viral genes are amplified with multiple primers using polymerase chain reaction (PCR) to generate DNA for sequencing that is compiled into a consensus sequence by analysis software.

Gap 3: Consistent bioinformatics and data analyses

The reliability of electropherogram analysis varies extensively between individuals, assays, and laboratories[94-96]; appropriate quality control is critical to ensure the validity and comparability of resistance testing. Sequence interpretation is dependent on an individual's ability to recognize low-frequency mutations and quality assurance programs are in place to minimize erroneous reporting, but subjective analyses can hinder consistent evaluation[94,96,97]. The bioinformatics tool recall has been developed with external validation to help overcome this problem (and provide much faster turnover) and may go some way to addressing this gap[95,97] (http://pssm.cfenet.ubc.ca/).

Next-generation sequencing (NGS)

Next-generation deep sequencing represents a powerful approach to sequence multiple individual template molecules by physical separation, providing enhanced sensitivity for the detection of low-frequency variants and tropism prediction[84,98,99]. NGS can sequence the multiple variants present in a single specimen and detects minority variants more reliably than Sanger sequencing[98-100].

NGS is prone to its own inherent sources of systematic error, including insertions/deletions associated with homopolymer stretches and sampling errors, which further confound the already challenging bioinformatic processing of this technology[84,98,101,102]. Furthermore, the same errors as made by HIV RT which

contribute to resistance development are also commonly made by enzymes used in NGS. For example, K65R is based on an RT error in a homopolymer stretch, an area also prone to errors by NGS[99].

Gap 4: Establishing a clinically relevant interpretation threshold for sequencing analysis

The limited ability of Sanger sequencing to detect minority variants can lead to an underestimation of the resistance burden. This technique does not have the sensitivity to detect minority variants that form < 20% of the population; and the lower the VL, the less sensitive it is for minority variants[96,103]. In contrast, NGS detects minority variants of 1% provided the VL is high enough, but the clinical significance of such sensitivity has yet to be agreed by experts. For example, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC)-induced mutations contribute to the generation of inactivated HIV variants, the majority of such mutations are unlikely to enhance viral adaptation[104]. This suggests variants with signs of APOBEC editing can be excluded from analysis[105].

A convenient interpretation cutoff of 10% is often used, similar to the output from Sanger sequencing. This cutoff allows collection of data, while not over-interpreting minority variants. As such, minority variants below 10% are detected but not taken into account, despite a lack of evidence to determine their clinical importance. However, an increased risk of virologic failure on NNRTI-based ART (particularly EFV or NVP) has been observed when minority variants are present[63,100,106,107].

The dose-dependent relationship between resistance and virologic failure risk implies that the proportion and quantity of resistant variants impacts ART outcome[63,106]. Although it is clear that a lower threshold identifies more mutations, a sensitivity balance must be reached for each drug class that optimizes clinical benefit while reducing the potential for misinterpretation[108,109]. The cutoff is likely to be mutation- and drug-specific, e.g., a 2% interpretation cutoff has been proposed for K103N in patients starting predominantly 2NRTIs + EFV, whereas others may require more sensitive detection[110]. A further confounding factor to selecting an interpretation threshold is the clinical relevance of absolute numbers of a mutant versus the percentage of a variant within a population, an approach adopted by some studies but which has yet to be fully characterized[63]. For example, K103N may reach clinical significance only at a presence of 2000 copies/mL, whereas others may require more sensitive detection (Fig. 2)[110].

Furthermore, NGS has demonstrated enhanced detection of low-frequency PI resistance mutations. Although the limited available data suggest this has a low clinical impact[111], the full implications of such screening have yet to be characterized[100,112]. As resistance to established drug classes continues to be characterized, further data will also be required to guide the appropriate interpretation of INSTI resistance analysis.

Gap 5: Simple, affordable assays with consistent bioinformatics for global use

The future of current technologies will depend on those in development. As the benefit of genotyping is increasingly evidenced but the discrepancy of use remains, there is need for a simple and affordable assay for global implementation, ideally with alternative technologies that eliminate the variability introduced by PCR to ensure greater accuracy[113].

As NGS develops and the ability for multiplexing increases, this technology has the potential to become a more cost-effective and efficient option than Sanger sequencing, particularly in centralized institutes[102]. Centralizing also removes the need for a bioinformatic pipeline at the clinic. NGS whole genome sequencing (WGS) can be relatively cheap if Ultra Deep sequencing is not required. However, NGS technology has yet to be widely implemented beyond the research setting and currently relies on in-house protocols that may not allow for reliable comparison between sites. Intuitive, standardized bioinformatics methodology, and even interpretation algorithms, will circumvent limitations introduced by operator error and enhance the validity of inter-center comparison[102]. Further development and investment into research are required to fully optimize and standardize this technique and realize its place in the future of HIV management.

Recently introduced technologies and those under development will continue to enhance the relevance and accessibility of resistance testing. Mutation-specific assays demonstrate high sensitivity and specificity; their associated cost-effectiveness and the widespread expertize of such assays mean they can be readily implemented in all types of settings. Furthermore, methods of entire genome sequencing, although more costly, may widen the application and outputs of resistance testing[102]. As the use of such technologies become more widespread, there will be a par-

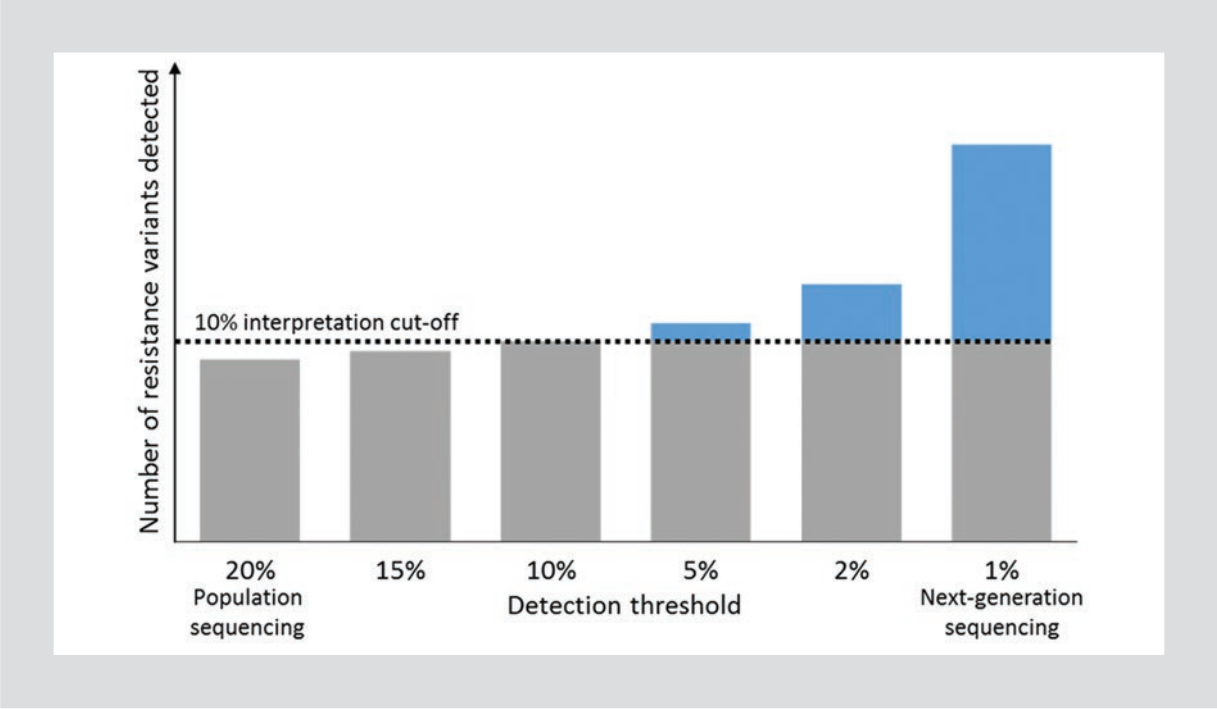

Figure 2. *In a patient with minority resistance variants, lowering the detection threshold increases the number of resistance variants detected. Blue areas indicate variants lost at the common interpretation cutoff of 10%* (adapted from an original figure by Martin Däumer[109]).

ticular need for guidance on the clinical interpretation of deep sequencing and a clear indication of the implications of each result. This will help avoid a tendency to exclude drugs unnecessarily, thereby preserving options.

Genotyping recommendations and practices

Understanding baseline mutations at treatment failure informs clinical decisions, thereby decreasing the likelihood of subsequent treatment failure. Baseline resistance testing is the current standard of care but its regional implementation varies according to resources[86,114].

Gap 6: Evidence to establish consistent recommendations for genotyping

Current guidance recommends genotyping before the selection of ART, but despite the impact and cost-effectiveness of this approach[115-117], it remains to be fully implemented in regions without appropriate resources[114]. In some instances there may be a perceived advantage of selective genotyping, particularly for NNRTI-resistant mutations[118]. Guidelines that take into account specific ARV classes may be of greatest clinical benefit but further investigation is required to determine the impact of baseline resistance testing across the drug classes. Current, limited evidence suggests this may have less impact before use of PIs or INSTIs than NNRTIs[114,119].

Recommendations for genotyping in the presence of LLV also remain to be defined. While persistent LLV is a risk factor for resistance, immune activation and virologic failure[34], the significance of associated resistance testing and management remains a source of debate and is complicated by the issue of adherence[120,121]. The lack of evidence to define appropriate thresholds for LLV testing in the era of NGS has led to recommended cutoffs ranging from 50 to 200 copies/mL, but the potential for artifacts may mask the relevance of testing at very low levels of viremia[121]. The high prevalence of LLV makes this a significant knowledge gap in guiding appropriate testing[120,121].

There is a need for consensus guidelines on the timing and degree of resistance testing, and on subsequent treatment choice in the resistance environment. Reversion dynamics differ between viral variants in the absence of drug selection pressure, often according to the fitness cost of a mutation[122]. Therefore, the timing

of resistance testing may influence the success of future ART. Furthermore, informed consensus guidance will rely on a greater knowledge of the impact of mutations on treatment choice, supporting the need for full characterization of resistance mutations as they are identified.

Gap 7: Use of whole-genome next-generation sequencing in clinical practice

As the availability and cost-effectiveness of NGS technologies improve, WGS will become a more accessible and viable option, with the potential to transform the field of resistance testing[123,124]. As technologies evolve to make WGS a possibility, it will be important to understand if and how it can be optimally utilized. While the relevance and clinical application of WGS remains to be determined, there are multiple avenues of preliminary support for WGS. The ability of WGS to accurately determine cell tropism before the use of MVC makes it an attractive alternative to phenotypic assays and conventional sequencing[125-127]. The technique also allows the routine sequencing of larger sections of Gag, which are known to influence PI resistance, including in the absence of protease mutations[128]. WGS is also of scientific interest. For example, the technique has identified low-frequency variants, not detected by conventional sequencing, that impact the early immune response. Such research presents potential pathways for better understanding of how the body responds to early viremia[129]. Finally, although not covered in the scope of this review, it is worth also noting that the ability to rapidly sequence whole genomes may have particular application in the surveillance of HIVDR through accurate profiling of viral diversity[129,130].

Gap 8: Value of sequencing novel regions

The knowledge gap associated with WGS also includes arguments for extending the current practice to sequence beyond traditional regions of the genome. This is discussed above in relation to PI resistance. The recent characterization of the multi-step activity of PIs serves as an example of how our evolving knowledge of ARV classes must be used to appropriately update resistance testing practices. As PI resistance testing continues to be optimized, there is a need to establish which regions may be important to genotype, but are not currently recommended, across each class of ARV. This need may prove particularly relevant as we gain further understanding of INSTIs.

Gap 9: Necessity for genotyping ahead of initiating an INSTI or entry inhibitor

While the impact of baseline resistance testing has yet to be fully characterized across the ARV classes, the significance of low-frequency NNRTI resistance mutations has received extensive attention. In contrast, the impact of low-frequency mutations on NRTIs + INSTI combinations remains to be determined. Where baseline screening has been carried out, there has generally been an absence of INSTI resistance in ART-naive patients[119], but low frequency of mutations have been identified in some studies, adding to the debate over INSTI screening in ART-naive patients[131]. It remains to be determined if such patients are suitable to receive INSTIs and, if so, which[49]. Studies such as SAILING and VIKING are paving the way, with evidence for the use of INSTIs in the management of ART-experienced, INSTI-naive patients as well as those with extensive multi-class resistance, including INSTI mutations[46,132,133].

Conclusion

Appropriate selection and sequencing of ART is the most efficacious and cost-effective method of managing lifelong HIV therapy at a population level. As trials investigate alternatives to NNRTI-based first-line therapy, simplified regimens show promise as a method of preserving future options[29,30,68,71,72,76,77]. Such strategies may also overcome problems associated with ART availability. Our current understanding of resistance mechanisms and their subsequent impact on ART selection is incomplete, including the optimal application of the coreceptor blocker, MVC. Defining the genetic barrier to resistance of combination therapies and the consequences of failure will help optimize first-line therapy and also inform decision-making in the event of treatment failure. This understanding will, in turn, lead to optimized genetic resistance testing to facilitate improved clinical management at an individualized level. In particular, INSTIs represent an important knowledge gap in resistance science. The gaps identified in this review highlight a particular need for a more complete understanding of INSTIs and the impact of resistance on the use of this class. Such knowledge will allow for more informed decision-making and provide support for optimizing future guidelines.

Applying this knowledge relies on the accurate identification and appropriate interpretation of resis-

tance mutations. Baseline genotyping provides important information for clinical decisions, but the relevance and application of testing need continual review and updating. The adoption of routine resistance testing within the clinical setting may have dramatic implications for the management of HIV, but gaps remain in the use and interpretations of these technologies. The ability of NGS to detect minority variants down to 1% and the ease of sequencing whole genomes provide significant advantages over other assays[98,100]. Deep sequencing has demonstrated its potential as an all-inclusive genotypic and coreceptor tropism assay, detecting multiple minority variants from samples with ≥1000 copies/mL[109,134]. Its applications are multiple and may extend to improving the prediction of virologic outcomes on ART, including salvage therapy, which will facilitate the problematic management of ART-experienced patients[135]. The primary gap identified in resistance technologies is the appropriate interpretation of these assays. Establishing a clinically relevant threshold will not only guide therapeutic decisions but also promote comparable data analyses.

In summary, the identification of data gaps within HIV resistance science and technology represent an opportunity to guide future research, facilitate scientific exchange and, ultimately, lead to progress in the clinical management of HIVDR.

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Conflicts of interest

Dr. Marina R. Bobkova reports grants from the Russian Science Foundation and personal fees from AbbVie, outside the submitted work; Dr. Charles A. Boucher has received research grants from Merck and personal fees from AbbVie and ViiV Healthcare; Dr. Dorr is an employee of AbbVie and may hold stock and has a patent issued to AbbVie; Prof. Anna Maria Geretti reports personal fees from AbbVie, grants and personal fees from BMS, grants, personal fees and non-financial support from Gilead, grants and personal fees from Janssen, personal fees from Pfizer, other from Roche and grants and personal fees from ViiV Healthcare, outside the submitted work; Dr. Chien-Ching Hung reports grants from Janssen and ViiV Healthcare, advisory board fees from AbbVie, Gilead and Janssen and personal fees from AbbVie, Gilead and BMS, outside the submitted work; Dr. Rolf Kaiser reports personal fees from Gilead, AbbVie, Janssen, Roche, Siemens, ViiV Healthcare and Alere, outside the submitted work; Dr. Anne-Geneviève Marcelin has nothing to disclose; Dr. Adrian Streinu-Cercel reports a grant from AbbVie, personal fees from AbbVie, BMS and J&J and participation as principal investigator in trials from Gilead, BMS and MSD, outside the submitted work; Dr. Jean van Wyk was an employee of AbbVie when this work was carried out, and is currently an employee of ViiV Healthcare; Dr. Anne-Mieke Vandamme reports personal fees from AbbVie and Gilead, outside the submitted work; and spouse is receiving consultancy fees from AbbVie, Gilead and ViiV Healthcare.

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