A systematic review of the genetic mechanisms of dolutegravir resistance

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Background: Characterizing the mutations selected by the integrase strand transfer inhibitor (INSTI) dolutegravir and their effects on susceptibility is essential for identifying viruses less likely to respond to dolutegravir therapy and for monitoring persons with virological failure (VF) on dolutegravir therapy.

Methods: We systematically reviewed dolutegravir resistance studies to identify mutations emerging under dolutegravir selection pressure, the effect of INSTI resistance mutations on in vitro dolutegravir susceptibility, and the virological efficacy of dolutegravir in antiretroviral-experienced persons.

Results and conclusions: We analysed 14 studies describing 84 in vitro passage experiments, 26 studies describing 63 persons developing VF plus INSTI resistance mutations on a dolutegravir-containing regimen, 41 studies describing dolutegravir susceptibility results, and 22 clinical trials and 16 cohort studies of dolutegravir-containing regimens. The most common INSTI resistance mutations in persons with VF on a dolutegravir-containing regimen were R263K, G118R, N155H and Q148H/R, with R263K and G118R predominating in previously INSTI-naive persons. R263K reduced dolutegravir susceptibility 2-fold. G118R generally reduced dolutegravir susceptibility 5-fold. The highest levels of reduced susceptibility occurred in viruses containing Q148 mutations in combination with G140 and/or E138 mutations. Dolutegravir two-drug regimens were highly effective for first-line therapy and for virologically suppressed persons provided dolutegravir’s companion drug was fully active. Dolutegravir three-drug regimens were highly effective for salvage therapy in INSTI-naive persons provided one or more of dolutegravir’s companion drugs was fully active. However, dolutegravir monotherapy in virologically suppressed persons and functional dolutegravir monotherapy in persons with active viral replication were associated with a non-trivial risk of VF plus INSTI resistance mutations.

Introduction

The integrase strand transfer inhibitor (INSTI) dolutegravir has an improved safety profile, greater efficacy and lower cost compared with efavirenz.1–3 Dolutegravir will play a dominant role in first-line therapy in many countries, including those with and without high levels of pre-treatment drug resistance. Dolutegravir has also recently been recommended by the WHO as a preferred component for second-line therapy.4

Characterizing the mutations selected by dolutegravir and their effects on dolutegravir susceptibility are essential for identifying viruses less likely to respond to dolutegravir therapy and for monitoring persons with virological failure (VF) on a dolutegravir-containing regimen. The efficacy of dolutegravir in ART-experienced persons and in monotherapy and dual-therapy regimens informs the risk of VF and emergent dolutegravir resistance and the optimal antiretrovirals (ARVs) to be used in combination with dolutegravir.

Here, we systematically review published studies and meeting presentations on dolutegravir resistance. The review maps key dolutegravir resistance concepts and analyses: the mutations emerging in vitro and in vivo under dolutegravir selection pressure; the effect of INSTI resistance mutations on in vitro dolutegravir susceptibility; and the virological efficacy of dolutegravir in persons at increased risk of VF and drug resistance.

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Methods

Literature review

A systematic search of the NCBI PubMed database for all English language papers on dolutegravir resistance using the search string ‘Dolutegravir or GS1349572’ was last updated on 24 January 2019. A list of the titles and abstracts presented at scientific meetings during 2017 and 2018 that contained the drug name ‘Dolutegravir’ was also compiled. The scientific meetings included the Conference on Retroviruses and Opportunistic Infections (CROI), IAS Conference on HIV Science, International AIDS Conference, International Workshop on HIV Drug Resistance and Treatment Strategies (HIVDRW), IDWeek, InterScience Conference on Antimicrobial Agents and Chemotherapy, European AIDS Conference, European Meeting on HIV & Hepatitis, and Glasgow HIV Drug Therapy. Additional publications and meeting presentations were identified from the reference lists of identified papers.

Retrieved studies were reviewed in three stages. First, titles and/or abstracts were reviewed to identify studies relevant to dolutegravir resistance. Following the title/abstract review, complete publications or posters (in the case of meeting presentations) were reviewed to determine which studies contained data relevant to the three main areas of focus: (i) mutations emerging under dolutegravir selection pressure in vitro and in vivo; (ii) in vitro dolutegravir susceptibility data; and (iii) virological efficacy of dolutegravir. In (iii), we examined the virological efficacy of dolutegravir when used with a reduced number of companion ARVs or when used to treat persons with viruses containing either INSTI resistance mutations or mutations that reduce the activity of the ARVs used in combination with dolutegravir. In the third stage, studies were reviewed further to determine whether they merited inclusion in a table, a figure or the text. The complete list of studies meeting the first review stage are available in a publicly available companion Zotero reference database (https://www.zotero.org/groups/2262131/dtg_stanfordhivdb).

The following types of studies passing the first review stage were excluded: (i) review papers lacking primary data; (ii) studies containing drug resistance data for the INSTIs raltegravir or elvitegravir but not dolutegravir; (iii) in vitro experiments designed to understand the cellular, biochemical or biophysical (rather than genetic) mechanisms of dolutegravir resistance; (iv) studies of HIV-2 or non-group M viruses; and (v) studies containing replicating experiments designed to understand the cellular, biochemical or biophysical (rather than genetic) mechanisms of dolutegravir resistance.

Data extraction

The data in the sections that follow were extracted independently by three reviewers (R. W. S., P. M. G. and S. Y. R.) using standardized spreadsheets for in vitro selection data, in vivo selection data, drug susceptibility data and virological outcome data. The extracted data in these spreadsheets were then used to annotate the Zotero reference database. Discrepancies were handled by jointly reviewing the full text of studies with discrepancies.

Mutations emerging under dolutegravir selection pressure

For studies in which viruses were cultured in the presence of increasing dolutegravir concentrations (in vitro passage experiments), we recorded data about the pre-passage virus, including its subtype, whether it was a laboratory strain or a clinical isolate, and whether it contained pre-existing known or suspected INSTI resistance mutations; and the integrase mutations that developed during in vitro passage.

For studies of persons whose viruses developed established or suspected INSTI resistance mutations while receiving dolutegravir we recorded: (i) the extent of ART prior to dolutegravir therapy, including whether the person was ART naive, ART experienced but INSTI naive, or INSTI experienced but dolutegravir naive; (ii) whether the person was stably virologically suppressed, defined as having a plasma HIV-1 RNA virus level <50 copies/ml for ≥6 months on unchanged ART; (iii) the ARVs used in combination with dolutegravir, specifically, whether the person received dolutegravir monotherapy or dual therapy, dolutegravir plus two NRTIs, or dolutegravir plus an optimized background regimen; and (iv) the integrase mutations reported to develop during therapy.

INSTI resistance mutations were defined as mutations previously reported to be selected by raltegravir or elvitegravir and associated with reduced raltegravir or elvitegravir susceptibility. Non-polymeric INSTI resistance mutations were defined as those occurring in ≤1% of INSTI-naive persons and included H51Y, T66A/I/K, E92Q, G118R, F121Y, G140A/S/C, Y143C/G/H/K/R/S, S147G, Q148H/K/R, S153Y/F, N155H, S230R and R263K. Polymorphic accessory INSTI-associated mutations were defined as those occurring at a prevalence ≥1% of INSTI-naive persons in one or more subtypes and included L74I/M, Q95K, T97A, V151I, E157Q, G163/K/R and D232N.

In vitro susceptibility data

For studies containing in vitro susceptibility data, we recorded: (i) whether the virus was a clinical or laboratory isolate; (ii) the integrase mutations in the virus and, for laboratory isolates, whether the virus contained one or more mutations placed by site-directed mutagenesis; (iii) the virus subtype; (iv) the method of susceptibility testing; and (v) the fold reduction in susceptibility compared with WT. Duplicate results, defined as identical results on the same site-directed mutant performed by the same laboratory method, were excluded.

Virological outcome studies

In both clinical trials and cohort studies, study subjects were characterized according to: (i) their past ART history as either ART naive, ART experienced but INSTI naive, or INSTI experienced; (ii) whether they were stably virologically suppressed, defined as having a plasma HIV-1 RNA level <50 copies/ml on two or more occasions for a period of ≥6 months on unchanged ART; and (iii) the components of their dolutegravir-containing regimen: dolutegravir monotherapy or dual therapy, dolutegravir plus two NRTIs, or dolutegravir plus an optimized background regimen as defined by ARVs selected by a clinical trial subject’s care provider to be used in combination with dolutegravir. For clinical trial results reporting data at multiple timepoints, we extracted data from the 24 and 48 week timepoints.

Analysis

Mutations emerging under dolutegravir selection pressure

In vitro passage experiments included those performed in cell culture and those performed in humanized mice. The analysis of these experiments focused on established non-polymeric INSTI resistance mutations as well as novel non-polymeric mutations observed in multiple studies. The analysis of emergent INSTI resistance mutations in persons receiving dolutegravir compiled all reported established non-polymeric and polymorphic INSTI resistance mutations and additional novel non-
polymorphic integrase mutations. The analysis included reports of emergent INSTI resistance mutations from clinical trials, cohort studies and case reports to identify the full spectrum of dolutegravir-selected mutations in vivo.

In vitro susceptibility data

For site-directed mutants, the complete list of mutations was known. However, for most clinical isolates, neither the complete nucleotide sequence nor the complete list of integrase mutations was reported. Therefore, for clinical isolates, only those mutations provided by authors were reported. Of note, the INSTI-selected mutation V151I occurs in the NL43 laboratory isolate, the most common laboratory isolate used for creating site-directed mutants. Therefore, this mutation was excluded from our analyses. The Supplementary data (available at JAC Online) indicates which susceptibility tests were performed on NL43 site-directed mutants. The in vitro susceptibilities of site-directed mutants and clinical isolates containing the four most commonly selected INSTI resistance mutations among persons receiving dolutegravir—R263K, G118R, N155H and Q148H/R/K—were characterized.

Virological outcome studies

VF was defined across all studies using an ITT approach such that subjects discontinuing therapy for any reason, such as intolerance or non-adherence, were categorized as experiencing VF. This approach was adopted

Figure 1. Flow chart of study selection process. Of 742 studies identified through a PubMed search performed in January 2019 using the search string ‘Dolutegravir or GSK1349572’, 229 were read in their entirety following an initial review of titles and abstracts. Following a full-text review of these 229 studies and of 35 additional studies from meeting presentations, 95 studies met our inclusion criteria, containing data on mutations emerging under dolutegravir selection pressure in vitro and in vivo; in vitro dolutegravir susceptibility; and the risk of virological failure and drug resistance in clinical trials and cohorts.
both for studies that reported their results in this manner and for those that employed a narrower definition of protocol-defined VF. The 95% Clopper-Pearson CIs for proportions of VF and VF plus emergent INSTI resistance were estimated for each individual study. Pooled proportion and the $I^2$ statistic, a measure of heterogeneity among studies, were calculated using the random-effects model implemented in the R meta package for the proportions of VF and VF plus resistance at weeks 24 and 48.

## Results and discussion

### Dolutegravir-selected mutations

**In vitro passage experiments**

Fourteen studies described 86 in vitro passage experiments in which an HIV-1 group M virus was cultured with increasing dolutegravir concentrations (Table 1). In 62 experiments, mutations were selected after a median of 30 weeks (IQR 20–46 weeks). These 62 experiments used 37 clinically derived isolates and 25 laboratory isolates. Isolates lacking established INSTI-associated drug resistance mutations prior to dolutegravir passage were labelled as WT. Fifty-three of the viruses had a subtype B backbone; 4 had a subtype C backbone, 2 had a CRF02_AG backbone, 2 had a subtype D backbone and 1 had a CRF01_AE backbone.

R263K was the most commonly selected INSTI resistance mutation, developing in 33 experiments from six studies (Table 1). In 31 of these experiments, the baseline virus was WT (including five with the pre-existing polymorphic accessory resistance mutation, E157Q); the other two baseline viruses contained E92Q or N155H. Other commonly selected INSTI resistance mutations included S153Y/F (11 isolates, six studies), E138K (9 isolates, four studies), H51Y (7 isolates, four studies), T66A/I (3 isolates, two studies) and G118R (2 isolates, one study). In 21 experiments using 11 WT and 10 viruses containing INSTI resistance mutations, no mutations emerged, consistent with the difficulty in selecting dolutegravir resistance mutations in vitro. There were insufficient data to determine whether a particular subtype predisposed to the emergence of specific mutations.

There were two studies not shown in Table 1 of humanized mice infected with a laboratory HIV-1 strain and subsequently treated with dolutegravir monotherapy. In one study, one of five mice treated with dolutegravir monotherapy for 20 weeks developed the INSTI resistance mutations E138K, G140S, Q148H, R263K and E157Q. In another study, two of four mice treated with an injectable long-acting dolutegravir formulation developed R263K and E157Q.

In three additional studies, also not shown in Table 1, mutations outside of integrase were selected during dolutegravir in vitro passage and shown to reduce dolutegravir susceptibility, including (i) three nucleotide mutations and one nucleotide deletion (resulting in a downstream stop codon in nef) in the highly conserved six terminal nucleotides of the 3' polypurine tract (GGGGGG→GCATG) and one nucleotide mutation just upstream of the 3' polypurine tract; (ii) a nucleocapsid mutation, G19S, postulated to destabilize the integrase/viral DNA/dolutegravir complex; and (iii) gp41 envelope mutations A539V and A556T postulated to reduce susceptibility to multiple ARVs by enhancing cell-to-cell HIV-1 transmission and increasing intracellular HIV-1 inoculum size. In a fourth study, the second-generation INSTI cabotegravir was shown to select for HIV-1 LTR mutations, which were hypothesized to have a similar effect to 3' polypurine tract mutations. Only the first of these sets of mutations has so far been reported in persons receiving dolutegravir.

### Persons receiving dolutegravir

The studies in which persons receiving dolutegravir developed VF and one or more INSTI resistance mutations included: (i) 11 studies of INSTI-naive persons (Table 2); (ii) 8 studies of virologically suppressed persons receiving dolutegravir monotherapy for treatment simplification (Table 3); and (iii) 8 studies of persons who had a history of VF and INSTI resistance mutations on a raltegravir- or elvitegravir-containing regimen (Table 4). Emergent INSTI resistance mutations have not been reported in any of the trials of a first-line regimen comprising dolutegravir plus two NRTIs.

The 11 studies of INSTI-naive persons included 4 clinical trials, 1 cohort study and 6 case reports (Table 2). Three of the four clinical trials included 712 ART-experienced patients receiving dolutegravir plus an optimized backbone, which was required in the two largest trials to include at least one fully active ARV based on a pre-therapy genotypic resistance test. One of the clinical trials included 120 ART-naive persons receiving dolutegravir plus lamivudine. The cohort study included 310 ART-naive and ART-experienced INSTI-naive persons receiving dolutegravir plus two NRTIs. The six case reports included 3 ART-naive persons who received dolutegravir plus tenofovir plus emtricitabine and three ART-experienced persons who received dolutegravir plus an optimized background regimen or two NRTIs. Overall, 21 persons developed VF and an INSTI resistance mutation. The most common mutations were R263K in 13 persons and G118R in 6 persons. Other non-polymorphic resistance mutations were E138K/T (3 persons), N155H (2 persons), Q148K (1 person), S230R (1 person), T66I (1 person) and H51Y (1 person). The non-polymorphic mutation A49G developed in two persons.

The eight studies of persons receiving dolutegravir monotherapy for treatment simplification included three trials with 211 persons, three cohort studies with 92 persons, and two case reports (Table 3). Overall, 16 persons developed VF and an INSTI resistance mutation. The most common mutations were N155H (7 persons), Q148H/R (3 persons), R263K (2 persons), G118R (2 persons) and S147G (2 persons). Among the 16 persons with VF and emergent INSTI resistance mutations, 7 had previously received raltegravir or elvitegravir but had not previously experienced VF on a raltegravir- or elvitegravir-containing regimen.

In two studies, in which the 3' polypurine tract was sequenced, 2 of 17 persons had one or more nucleotide changes in this highly conserved region. One had virus containing two 3' polypurine tract nucleotide changes (GGGGGG→GGGAGC) compared with baseline and no reported integrase mutations, and another containing one polypurine tract nucleotide change also had the integrase mutation R263K.

The eight studies of persons with a history of VF and INSTI resistance mutations on a previous raltegravir- or elvitegravir-containing regimen included two of the VIKING trials (the Phase Ib VIKING and Phase III VIKING-4 trials), one cohort study and five case reports (Table 4). In these eight studies, 31 persons were reported to have developed VF on a dolutegravir-containing regimen. The spectrum of mutations selected by dolutegravir in this population differed from the spectrum in INSTI-naive persons.
| Author          | Yr     | Parent virus | Position (Cons) |
|-----------------|--------|--------------|-----------------|
|                 |        |              | 51 (H) | 66 (T) | 92 (E) | 118 (G) | 138 (E) | 140 (G) | 147 (S) | 148 (Q) | 153 (S) | 263 (R) |
| Kobayashi11     |        | NL43         | F/Y   | K      |        |        |        |        |        |        |        |
| Quashie12       |        | clinical (n = 3) | K      | K      |        |        |        |        |        |        |        |
|                 |        | clinical    |        |        |        |        |        |        |        |        |        |
|                 |        | clinical (C) |        |        |        |        |        |        |        |        |        |
|                 |        | clinical (02) |        |        |        |        |        |        |        |        |        |
| Oliveira14      |        | NL43-118R    | K      |        |        |        |        |        |        |        |        |
|                 |        | NL43-51Y/263K | K      |        |        |        |        |        |        |        |        |
| Anstett15       |        | NL43         | K      |        |        |        |        |        |        |        |        |
|                 |        | NL43-92Q     | K      |        |        |        |        |        |        |        |        |
|                 |        | NL43-140S    |        |        |        |        |        |        |        |        |        |
|                 |        | NL43-140S/148R |        |        |        |        |        |        |        |        |        |
|                 |        | NL43-155H    |        |        |        |        |        |        |        |        |        |
| Departureaux15  |        | NL43         | K      |        |        |        |        |        |        |        |        |
| Seki15          |        | NL43         | K      |        |        |        |        |        |        |        |        |
|                 |        | NL43-148H    |        |        |        |        |        |        |        |        |        |
|                 |        | NL43-148K    |        |        |        |        |        |        |        |        |        |
|                 |        | NL43-148R    |        |        |        |        |        |        |        |        |        |
| Brenner16       |        | NL43-118R    | K      |        |        |        |        |        |        |        |        |
|                 |        | clinical-118R (C) |        |        |        |        |        |        |        |        |        |
| Oliveira16      |        | NL43         | F      | K      |        |        |        |        |        |        |        |
| Brenner17       |        | clinical    |        |        |        |        |        |        |        |        |        |
|                 |        | clinical (n = 6) |        |        |        |        |        |        |        |        |        |
|                 |        | clinical (n = 2) |        |        |        |        |        |        |        |        |        |
| Andreatta18     |        | LAI (n = 2)  | Y      |        |        |        |        |        |        |        |        |
|                 |        | LAI-155H     |        |        |        |        |        |        |        |        |        |
|                 |        | LAI-148R     | K      |        |        |        |        |        |        |        |        |
| Oliveira18      |        | NL43         | K      |        |        |        |        |        |        |        |        |
|                 |        | NL43-157Q    | K      |        |        |        |        |        |        |        |        |
|                 |        | clinical    |        |        |        |        |        |        |        |        |        |
|                 |        | clinical (n = 6) |        |        |        |        |        |        |        |        |        |
|                 |        | clinical (C) |        |        |        |        |        |        |        |        |        |
|                 |        | clinical (01) |        |        |        |        |        |        |        |        |        |
|                 |        | clinical (02) |        |        |        |        |        |        |        |        |        |
|                 |        | clinical-157Q (n = 3) |        |        |        |        |        |        |        |        |        |
|                 |        | clinical-157Q (D) |        |        |        |        |        |        |        |        |        |
|                 |        | clinical-157Q (D) |        |        |        |        |        |        |        |        |        |

Cons, consensus subtype B amino acid.

*a* Columns contain established non-polymorphic INSTI resistance mutations. Mutations not shown include: M50I in two non-mutated viruses (Quashie12 and Oliveira14); E157Q in two viruses (Oliveira18); 101I/124A (Kobayashi11); 262K with NL43-51Y (Oliveira14); 75I/97A/154I in addition to 138K/140S in NL43-148H (Seki15); 193E with 51Y/153T in Clinical (C) (Quashie12) and with 92Q in NL43 (Seki15); 234F with 153Y in LAI (Andreatta18); 144D in LAI with the RT mutation 184V; 95K/146R in Clinical (Oliveira18).

*b* Clinical: virus isolates obtained from INSTI-naive individuals lacking known INSTI resistance mutations. NL43 and LAI are WT laboratory strains. Drug-resistance mutations in laboratory strains were placed by site-directed mutagenesis. Non-B subtypes are indicated in parentheses: C, D, CRF01_AE (01), and CRF02_AG (02). Replicate experiments yielding the same results are followed by the number of experiments (n) in parentheses. Notes: 22 subtype B are not shown including one developing polypurine tract mutations and 21 that did not develop mutations, including those with parent viruses 92Q (2), 92Q/155H (1), 51Y/118R (1), 143C (1), 143R (1), 148R (1), 155H (1), 263K (2), RT-65R (1), RT-184VI (2) and 8 without mutations.
No person developed R263K or G118R. Rather, the most common dolutegravir-selected mutations were T97A (22 persons), E138K/A/T (12 persons), N155H (7 persons), L74M/I (4 persons), Q148H+G140S (3 persons) and S147G (3 persons). The non-polymorphic mutations G149A and F139Y developed in two persons and one person, respectively.

R263K was the common mutation developing during in vitro passage and developing in INSTI-naive persons. G118R, E138K and H51Y also occurred both in vitro and in vivo, with E138K and H51Y occurring only in combination with other mutations. S153Y/F occurred commonly in vitro but has not yet been reported in patients receiving dolutegravir. N155H and Q148 mutations were not selected in vitro but developed in several INSTI-naive persons, particularly during dolutegravir monotherapy. Further studies are required to determine the frequency of dolutegravir-selected mutations outside of integrase in persons with VF on a dolutegravir-containing regimen.

G118R and R263K, which were previously reported in persons receiving raltegravir and elvitegravir, respectively, occurred in a much higher proportion of persons with VF on dolutegravir compared with the first-generation INSTIs. Indeed, no completely novel non-polymorphic INSTI-selected mutations were identified in persons receiving dolutegravir except for A49G, G149A and C0 polypurine tract mutations. However, complete integrase sequences were submitted to GenBank for just 2 of the 63 viruses from dolutegravir-treated persons with VF and INSTI resistance mutations, making it possible that many dolutegravir-selected mutations were not reported.

### Effect of INSTI resistance mutations on in vitro dolutegravir susceptibility

A total of 41 studies contained 572 dolutegravir in vitro susceptibility results (Table S1), including 395 results on site-directed mutants and 177 results on clinical isolates (Table S2). The site-directed mutants generally contained raltegravir- and elvitegravir-associated resistance mutations, mutations observed under dolutegravir selection pressure, and additional accessory INSTI-
Table 3. Emergent INSTI-associated DRMs in persons with sustained virological suppression receiving dolutegravir (DTG) monotherapy

| Author/Yr  | Population          | Past ART                          | DTG ART (q24h) | Subjects | Subjects with DRMs | Emergent INSTI DRMs<sup>a</sup> |
|------------|---------------------|-----------------------------------|----------------|----------|-------------------|---------------------------------|
| Wijting18  | RCT (DOMONO)        | 15% INSTI experienced; no h/o VF | DTG×48W (98 in immediate and delayed switch groups and 4 in a pilot study) | 102      | 5                 | (1) R263K                       |
|            |                     |                                   |                |          |                   | (2) N155H                       |
|            |                     |                                   |                |          |                   | (3) S230R                       |
|            |                     |                                   |                |          |                   | (4) E92Q, N155H                 |
|            |                     |                                   |                |          |                   | (5) 3’ polypurine tract mutations |
|            |                     |                                   |                |          |                   | (1) S147G, Q148R, N155H         |
|            |                     |                                   |                |          |                   | (2) E138K, G140S, N155H         |
| Blanco18   | RCT (DOLAM)         | 15% INSTI experienced; no h/o VF on an INSTI regimen | DTG×24W        | 31       | 2                 | (1) N155H, S147G                |
| Hocqueloux18| RCT (MONCAY) cohort | 17% INSTI experienced             | DTG×24W        | 78       | 2                 | (1) N155H, S147G                |
|            | (MONODOLU)          |                                   |                |          |                   | (2) R263K                       |
| Kattama16  | cohort (MONODOLU)   | 46% INSTI experienced             | DTG×24W        | 28       | 3                 | (1) E138K, G140A, Q148R         |
|            |                     |                                   |                |          |                   | (2) E92Q                        |
|            |                     |                                   |                |          |                   | (3) N155H                       |
| Rojas16    | cohort (DOLUMONO)   | 61% INSTI experienced             | DTG×24W        | 33       | 1                 | (1) G118R                       |
| Oldenbuettel17 | cohort (DOLUMONO) | 61% INSTI experienced; no h/o VF on an INSTI regimen | DTG×24W, DTG×24W | 31       | 1                 | (1) Q148H, G140S                |
| Brenner16  | case report         | h/o first-line ART with EVG-containing regimen | DTG×8W         | 1        | 1                 | (1) G118R                       |
| Malet18    | case report         | RAL experienced                   | DTG            | 1        | 1                 | (1) N155H                       |

RCT, randomized clinical trial; VF, virological failure; EVG, elvitegravir; RAL, raltegravir; W, weeks; h/o, history of.
<sup>a</sup>Underlined mutations indicate that the virus emerged in an INSTI-experienced person. In Wijting18, R263K, N155H and S230R were detected in persons in the main DOMONO study, which required a nadir CD4 count >200 cells/mm<sup>3</sup>, whereas E92Q/N155H and 3’ polypurine tract mutations occurred in four persons in the pilot DOMONO study, which included persons with a nadir CD4 count <200 cells/mm<sup>3</sup>. The virus with R263K reported by Hocqueloux18 also had a single G to A mutation in the 3’ polypurine tract, whereas two of the six nucleotides in the 3’ polypurine tract were mutated. The G118R mutation in Rojas16 was detected as a minority variant in 7% of viruses by next-generation sequencing. Complete sequences were unavailable for all sequences except Brenner16.

Table 3. Emergent INSTI-associated DRMs in persons with sustained virological suppression receiving dolutegravir (DTG) monotherapy

The next three plots in Figure 2 summarize the levels of reduced susceptibility for 17 isolates containing G118R without R263K. G118R alone or in combination with T66A, L74I and T97A reduced dolutegravir susceptibility between 5- and 15-fold. The two of the site-directed mutants contained a CRF02_AG integrase backbone. One G118R-containing clinical isolate lacking other reported INSTI resistance mutations had 30-fold reduced susceptibility; however, the complete list of mutations in this isolate was not available.48 Although both R263K and G118R were associated with reduced enzymatic activity and replication capacity, 49-51 the relative rarity of G118R may be due to its usual requirement for mutations at two nucleotides rather than one nucleotide, regardless of subtype.52

The next eight plots summarize the levels of reduced susceptibility for 64 isolates containing N155H (without R263K or G118R). N155H alone or in combination with one additional INSTI resistance mutation usually yielded <2-fold reduced dolutegravir susceptibility. However, in combination with Q148H/R or two or more additional resistance mutations, reduced susceptibility ranged from 2-fold to >15-fold. The highest levels of reduced susceptibility were found in a site-directed mutant containing the extremely rare mutation pair L74F+V75I52 and in clinical isolates containing N155H plus Q148H, G140S and T97A53 and N155H plus T97A, E138K and S147G.52

associated mutations. The complete integrase sequence was known for all of the site-directed mutants but was available for only 74 (41.8%) of the 177 clinical virus isolates.

The most commonly used assay was the recombinant virus reporter gene PhenoSense assay (Monogram Biosciences, South San Francisco; n=279). Most of the remaining assays were recombinant virus reporter gene assays developed by other laboratories, including ViVi, McGill University, the ANRS and the NCI. The site-directed mutants contained 127 distinct mutational patterns, and for 45 patterns susceptibility tests were performed with more than one assay. For these 45 patterns, the range/median ratio, a non-parametric measure of dispersion, was ≤1.0 for 21 patterns, 1.1–2.0 for 20 patterns and >2.0 for 4 patterns. The dispersion appeared to be lowest between the PhenoSense and ViVi assays (Table S3).

Figure 2 summarizes results on 281 site-directed mutants and clinical isolates containing patterns of INSTI resistance mutations characterized by four signature mutations: R263K, G118R, N155H and Q148H/R/R. The first eight plots in Figure 2 show the fold reductions in susceptibility for 42 isolates containing R263K. The median reduction in dolutegravir susceptibility was ~2-fold for isolates with R263K alone, ~5-fold for isolates with R263K plus two additional mutations, and 10- to 15-fold for isolates with R263K plus G118R, N155H or Q148R (usually in combination with one additional INSTI resistance mutation).
The final nine plots in Figure 2 summarize the levels of reduced susceptibility for 158 isolates containing Q148H/R/K without R263K, G118R or N155H. Q148H/R/K alone did not cause measurably reduced dolutegravir susceptibility. However, the median reduction in susceptibility was about 3-fold for the commonly occurring combination Q148H!G140S, 5- to 10-fold for Q148R!G140A/S and 10- to 20-fold for Q148K!E138K. In combination with an additional resistance mutation, including the polymorphic accessory resistance mutations, L74M and T97A, the reduction in susceptibility reached higher levels. The contribution of accessory mutations to reduced dolutegravir susceptibility was most striking in clinical isolates, possibly because these were likely to have additional background mutations that facilitate reduced susceptibility.39,44,53

Considering the above data, it is notable that of 251 isolates in the Stanford HIV Drug Resistance Database with Q148H/R/K, only 14 (5.6%) did not also include a G140 or E138 mutation: Q148H occurred alone in 3 (1.9%) of its 160 occurrences, Q148R in 11 (15.7%) of its 70 occurrences, and Q148K in 0 of its 11 occurrences. Several isolates lacking each of the four signature mutations in integrase were also reported to have 2-fold reduced dolutegravir susceptibility, including isolates with F121Y,54 S230R,55 E92Q!G140A56 and T66K!L74M, V151L and S153Y.57 Additionally, a site-directed mutant containing the five nucleotide changes selected in vitro in the 3' polypurine tract region displayed 23-fold reduced dolutegravir susceptibility.11

Table 4. Emergent INSTI-associated DRMs in INSTI-experienced persons receiving dolutegravir (DTG) in combination with an optimized background (OB) regimen

| Author/Yr | Population | DTG ART | Time to VF | Pre-DTG DRMs | Emergent INSTI DRMs |
|-----------|------------|---------|------------|--------------|---------------------|
| Eron13    | RCT (VIKING) | DTG 50 mg q24h + OB (Cohort I) | Day 11 | G140S, Y143H, Q148H | L74I/M, E138A |
|           |            |         | Day 11 | L74M, T97A, Y143R, G163R | E138K |
|           |            |         | W8     | none | L74I/M, T97A, G140S, Q148H |
|           |            |         | W24    | L74M, T97A, E138A, Y143R | N155H |
|           |            |         | W24    | L74M, T97A, Y143R | N155H |
|           |            | DTG 50 mg q12h + OB (Cohort II) | Day 11 | G140S, Q148H | N155H |
|           |            |         | Day 11 | G140S, Q148H | T97A |
|           |            |         | Day 11 | E138K, G140S, Q148H | T97A |
|           |            |         | Day 11 | G140S, Q148H | T97A |
|           |            |         | W8     | E138A, G140S, Q148H | T97A |
|           |            |         | W8     | G140S, Q148H | E138K, N155H |
|           |            |         | W16    | G140S, Q148H | T97A, E138K, N155H |
| Naeger16  | RCT (VIKING-4) | DTG 50 mg q12h + OB | W12    | G140S, Q148H | L74M, G149A, N155H |
|           |            |         | W24    | G140S, Q148H | T97A, E138K, G149A |
|           |            |         | W24    | E138A/K/T, G140S, Q148H | T97A |
|           |            |         | W24    | L74M, Q95K, T97A, Y143C | E138K, S147G |
|           |            |         | W4     | G140S, Q148H | T97A |
|           |            |         | W32    | G140S, Q148H | T97A |
| Castagna18 | cohort | DTG 50 mg q12h + OB | W84    | G140S, Q148H | E138K |
|           |            |         | W132   | T97A, G140S | E138K, Q148H |
|           |            |         | W132   | E138A, G140S, Q148H | T97A |
|           |            |         | W132   | L74I/M, G140S, Q148H | T66I, T97A |
|           |            |         | W172   | G140S, Q148H | L74I, T97A |
|           |            |         | W200   | E138A, G140S, Y143H/R/C, Q148H | T97A |
|           |            |         | W228   | G140S, Q148H | T97A, E138K |
|           |            |         | W276   | E138K, G140S, Q148H | T97A |
|           |            |         | W320   | Y143C | T97A, E138K, G140S, Q148H |
| Cargonico14 | case report | DTG 50 mg q12h + OB | W32    | N155H, E157Q | T97A, S147G |
| Hardy15   | case report | DTG 50 mg q12h + OB | W108   | S167G, V151L, N155H | T97A, E138K |
| Malet15   | case report | DTG 50 mg q12h + OB | W156   | G140S, Q148H | T97A, N155H |
| George18  | case report | DTG 50 mg q12h + OB | W24    | G140S, Q148H | T97A |
| Seatla18  | case report | DTG 50 mg q12h + OB | W44    | E138T, G140S, Q148H | T97A |
|           |            |         | W64    | E138K, G140A, Q148R | T97A, S147G |

Several additional baseline and follow-up mutations were noted for Naeger16. However, as sequences were not available for any of the studies, the lists of mutations do not show any mutations that are not known INSTI-associated DRMs or mutations at highly conserved positions. 31% of the persons in Castagna18 had previously been enrolled in one of the VIKING trials. In VIKING Cohorts I and II (Eron13) and in VIKING-4 (Naeger16), subjects had a period of functional dolutegravir monotherapy lead-in of 7 (VIKING-4) to 10 (VIKING Cohorts I and II) days before the ARVs accompanying dolutegravir were optimized. W, weeks.

The final nine plots in Figure 2 summarize the levels of reduced susceptibility for 158 isolates containing Q148H/R/K without R263K, G118R or N155H. Q148H/R/K alone did not cause measurably reduced dolutegravir susceptibility. However, the median reduction in susceptibility was about 3-fold for the commonly occurring combination Q148H+G140S, 5- to 10-fold for Q148R+G140A/S and 10- to 20-fold for Q148K+E138K. In combination with an additional resistance mutation, including the polymorphic accessory resistance mutations, L74M and T97A, the reduction in susceptibility reached higher levels. The contribution of accessory mutations to reduced dolutegravir susceptibility was most striking in clinical isolates, possibly because these were likely to have additional background mutations that facilitate reduced susceptibility.39,44,53

Considered the above data, it is notable that of 251 isolates in the Stanford HIV Drug Resistance Database with Q148H/R/K, only 14 (5.6%) did not also include a G140 or E138 mutation: Q148H occurred alone in 3 (1.9%) of its 160 occurrences, Q148R in 11 (15.7%) of its 70 occurrences, and Q148K in 0 of its 11 occurrences. Several isolates lacking each of the four signature mutations in integrase were also reported to have 2-fold reduced dolutegravir susceptibility, including isolates with F121Y,54 S230R,55 E92Q+G140A,56 and T66K+L74M, V151L and S153Y.57 Additionally, a site-directed mutant containing the five nucleotide changes selected in vitro in the 3' polypurine tract region displayed 23-fold reduced dolutegravir susceptibility.11

Studies in which dolutegravir plus an optimized background regimen was used for the treatment of INSTI-experienced persons...
showed that in viruses with Q148 mutations, a 3- to 4-fold reduction in dolutegravir susceptibility was associated with a measurably reduced response to therapy and that a 10-fold reduction in susceptibility was associated with a markedly reduced response to therapy.58,59

There is extensive cross-resistance between dolutegravir and bictegravir.53,60,61 The levels of reduced susceptibility are generally lower for bictegravir than dolutegravir for most patterns of INSTI resistance mutations. However, the clinical significance of these results is uncertain because bictegravir has not been used for salvage therapy in persons harbouring viruses with INSTI resistance mutations.

Our summary of published phenotypic data combined with data on the clinical significance of phenotypic thresholds provides partial insight into the concept of the genetic barrier to dolutegravir resistance. With few exceptions, two or more integrase mutations appear to be required to reduce dolutegravir susceptibility more than 3- to 4-fold and three or more mutations appear required to reduce dolutegravir susceptibility more than 10-fold.

However, the concept of the genetic barrier to resistance is complicated by several factors. First, it is not known whether the phenotypic thresholds for clinically significant reduced susceptibility cited above also apply to the R263K or G118R mutational pathways. Second, several INSTI resistance mutations, particularly G118R and R263K, reduce HIV-1 replication capacity,49–51 suggesting that the barrier to resistance is not simply a function of the number of mutations required for reducing susceptibility. Third, R263K, the most common mutation detected at the time of VF in INSTI-naive persons receiving dolutegravir, usually reduces susceptibility only about 2-fold, raising the question as to whether continued dolutegravir therapy with increased adherence and/or at the twice daily 50 mg dosage would lead to virus resuppression.

Finally, the published phenotypic data on clinical isolates do not account for possible unreported compensatory integrase mutations and for the potential effects of mutations outside of integrase.

Virological efficacy in populations at increased risk of drug resistance

There were 22 clinical trials and 19 cohort studies meeting one of the following inclusion criteria: (i) ART-naive persons receiving monotherapy or dual therapy (Table S4); (ii) ART-experienced virologically suppressed persons receiving monotherapy (Table S5) or dual therapy (Table S6); (iii) ART-experienced, INSTI-naive persons with active virus replication (Table 5); and (iv) INSTI-experienced persons with active virus replication (Table 6). Baseline genotypic resistance testing was performed in each of the clinical trials in
persons with active virus replication, to determine subject eligibility and/or to select the ARVs to be used in combination with dolutegravir (Tables 5, 6 and S4). We excluded 11 cohort studies containing highly heterogeneous populations with varying proportions of ART-naive and ART-experienced persons, varying proportions of ART-experienced persons with virological suppression, or varying numbers of ARVs used in combination with dolutegravir.62–72

ART-naive persons

There were two small studies of ART-naive persons treated with dolutegravir monotherapy. One was a cohort study of 20 ART-naive persons with baseline plasma HIV-1 RNA levels <100000 copies/mL, of whom 18 maintained virological suppression when receiving dolutegravir monotherapy for a median of 13 months.73 There was also one dose-finding 10 day dolutegravir monotherapy trial, which recruited 28 participants.74 Among those receiving 50 mg daily, the mean reduction in virus load was ~2.5 log copies/mL. No INSTI resistance mutations or reduction in INSTI susceptibility was observed.

Three clinical trials described 856 ART-naive persons treated with dolutegravir/lamivudine dual therapy.20,75,76 (Table S4). Each excluded persons with baseline plasma HIV-1 RNA levels ≥100000074 or ≥500000 copies/mL20 or genotypic evidence of lamivudine resistance. The largest trial was a randomized controlled trial (GEMINI) that reported a non-inferior 91% virological response rate at week 48 for 716 persons receiving dolutegravir/lamivudine compared with a similar number of persons receiving dolutegravir plus tenofovir/emtricitabine.75 The remaining two trials were pilot trials that enrolled 120 persons for 48 weeks (A5353)20,77 or 20 persons for 48 weeks.76 The VF rate was 10% in these two trials, except possibly for certain populations that appear to be at low risk of VF, including those who initiated ART shortly after HIV-1 infection or who had low PBMC HIV-1 DNA levels, factors associated with a smaller, less heterogeneous latent virus population.78,79

Table S6 lists the 13 studies of virologically suppressed persons on a stable ART regimen switched to dolutegravir dual therapy.
Table 6. Dolutegravir (DTG) plus optimized background (OB) in persons with virological failure and INSTI resistance on a raltegravir (RAL)- or elvitegravir (EVG)-containing regimen

| Author | Year | Population | Past ART | DTG ART | Subjects | Weeks | Percentage VF (95% CI)
|--------|------|------------|----------|----------|----------|-------|------------------------
| Eron | 2013 | RCT (VIKING – Cohort I) | heavily treated; h/o RAL VF and resistance | DTG (50 mg q12h) + OB | 27 | 24 | 59.3 (38.8–77.6)
| | | RCT (VIKING – Cohort II) | heavily treated; h/o RAL VF and resistance | DTG (50 mg q12h) + OB | 24 | 24 | 25 (9.8–46.7)
| Castagna | 2014 | RCT (VIKING 3) | heavily treated; h/o RAL VF and resistance | DTG (50 mg q12h) + OB | 183 | 24 | 31.1 (24.5–38.4)
| AkiI | 2015 | RCT (VIKING 4; with vs without OB x 7 days) | heavily treated; h/o INSTI VF and resistance | DTG (50 mg q12h) + OB | 30 | 24 | 53.3 (34.3–71.7)
| Naeger | 2016 | Cohort | heavily treated; h/o INSTI VF and resistance | DTG (50 mg q12h) + OB | 190 | 24 | 27.9 (21.6–34.8)
| | | ALL, 24 weeks<sup>a</sup> | | | 454 | 24 | 36.9 (26.4–47.0), I² = 75%
| | | ALL, 48 weeks<sup>b</sup> | | | 220 | 48 | 47.9 (27.5–68.3), I² = 79%

h/o, history of.
<sup>a</sup>VF, confirmed virus load ≥50 copies/mL or treatment discontinuation for any reason. For the cohort studies, the proportion of persons with VF after the median time of follow-up was provided.
<sup>b</sup>31% of the persons in Castagna18 had previously been enrolled in one of the VIKING trials.
<sup>c</sup>Pooled proportions and 95% CI of VF and VF with INST resistance estimated using a random-effects meta-regression. Note: In VIKING Cohorts I and II and in VIKING-4, subjects had a period of functional dolutegravir monotherapy lead-in of 7 (VIKING-4) to 10 (VIKING Cohorts I and II) days before the ARVs accompanying dolutegravir were optimized.

with lamivudine, rilpivirine, unboosted atazanavir or ritonavir-boosted darunavir.29,82–93 Dolutegravir/rilpivirine was used in one randomized clinical trial and in four cohort studies totalling 1067 persons. The randomized controlled SWORD trial demonstrated that dolutegravir/rilpivirine maintained 95% virological suppression at week 48 in 513 persons and was non-inferior to the control arm of continued unchanged ART. Dolutegravir/lamivudine was used in two randomized clinical trials, one single-arm pilot trial and three cohort studies totalling 504 persons. Dolutegravir/atazanavir and dolutegravir with boosted darunavir were each used in one small cohort study. In all but three studies, the proportion of persons with VF was ≤10%, and no person developed INSTI resistance mutations. The pooled proportion of VF at week 48 was 8.6% (95% CI 4.8%–12.3%). The extent of heterogeneity was high, with an I² of 82%, likely reflecting the inclusion of cohort studies and clinical trials, the heterogeneous ART histories of the study subjects, and the different dual drug combinations. Although persons in the larger studies were at low risk of VF (i.e. having no history of VF or of resistance to INSTIs, rilpivirine or lamivudine), several of the smaller studies included persons with multiple past VFs, including some harbouring viruses with reduced lamivudine susceptibility.

Our analyses of dolutegravir monotherapy and dual therapy in virologically suppressed persons included 20 studies that overlapped with 19 distinct studies in a recent systematic review of VF during dolutegravir-based monotherapy and dual therapy in virologically suppressed persons.94 We excluded three studies from this previous review, including two that had <10 persons95,96 and one that included persons with both virological suppression and active virus replication.64 We included four studies that were published following the completion date of this previous review.37,84,97

ART-experienced, INSTI-naive persons without virological suppression

There were two RCTs, one single-arm Phase I/II trial and one cohort study of dolutegravir-containing regimens in non-virologically suppressed, ART-experienced, INSTI-naive persons15,19,21,48 (Table 5). Pre-therapy genotypic resistance testing was performed on all persons in three clinical trials and on an unspecified proportion of persons in the cohort study.

In the DAWNING trial, dolutegravir + two NRTIs was superior to ritonavir-boosted lopinavir + two NRTIs in persons with VF on a first-line NNRTI-containing regimen, who were predicted to have one fully active NRTI—usually either zidovudine or tenofovir. The overall rate of VF at week 48 was 36.2%, with two persons (0.6% of subjects) developing one or more INSTI resistance mutations.

In the SAILING trial, dolutegravir plus an optimized background was superior to raltegravir plus an optimized background in persons with a history of resistance to two or more ARV classes, who nonetheless had a baseline genotypic resistance test, indicating that at least one ARV in addition to dolutegravir or raltegravir was fully active. In the dolutegravir arm, the proportion of persons with VF in this treatment-experienced population was 29.1%. Two persons (0.6% of subjects) developed one or more INSTI resistance mutations during the 48 week trial15 while another three (0.8% of subjects) developed such mutations after week 48.16

In the heavily treated adolescent population enrolled in the Phase I/II P1093 dose-finding trial, 39% of 23 subjects developed VF by week 48 and one developed emergent INSTI resistance.18 A
subsequent analysis of two additional treatment cohorts containing 38 additional adolescents later recruited to the study identified two additional cases of emergent INSTI resistance, one at week 52 and another at week 192. In the single cohort study, the proportion of persons with VF and emergent INSTI resistance mutations were 16.7% and 0.8%, respectively, after a median of 60 weeks.

The pooled proportion of VF at week 48 was 28.0% (95% CI 18.6%–37.5%), with a high degree of heterogeneity ($I^2$ 91%), reflecting the lower VF rate in the cohort study compared with the three clinical trials. The pooled proportion of VF plus INSTI resistance mutations at week 48 was 0.7% (95% CI 0.2%–1.2%; $I^2$ 0%).

Despite its high success rate in ART-experienced, INSTI-naive persons undergoing genotypic resistance testing, it is not known whether a regimen such as dolutegravir/tenofovir/lamivudine will be effective in persons with VF on a first-line NNRTI-containing regimen who harbour NRTI resistance mutations such as K65R and M184V. Both of these NRTI resistance mutations reduce HIV-1 replication fitness and NRTIs often appear to retain residual activity against NRTI-resistant viruses. Nonetheless, the proportion of persons with VF and emergent INSTI resistance during the first 48 weeks of therapy in low- and middle-income countries would likely be higher than the 0.7% observed in the studies of ART-experienced, INSTI-naive persons in which baseline genotypic resistance testing was performed in an attempt to avoid functional dolutegravir monotherapy.

INSTI-experienced persons without virological suppression

One multi-part clinical trial (VIKING Cohorts I and II, VIKING-3 and VIKING-4) and one cohort study investigated the response to dolutegravir plus an optimized background in persons with a history of INSTI resistance as a result of previous VF on a raltegravir or elvitegravir-containing regimen. (Table 6). Overall, the VIKING studies included 264 persons and the cohort study included 190 persons. Of the 190 persons in the cohort study, 31% had previously been enrolled in one of the VIKING studies. Dolutegravir at 50 mg twice daily was received in all persons except for a small number of persons in the dose-finding VIKING Cohort I trial. In VIKING Cohorts I and II and in VIKING-4, subjects had a period of functional dolutegravir monotherapy lead-in of 7 days (VIKING-4) to 10 days (VIKING Cohorts I and II) before the ARVs accompanying dolutegravir were optimized.

In nearly all persons in the VIKING trials and in the cohort study, the number of optimized background ARVs predicted to be fully active was low and the overall VF rate was high, ranging from 25% to 59% at week 24 and from 39% to 60% at week 48. The VIKING study defined genotypic dolutegravir resistance as Q148H/R/K in combination with one or more of the following accessory INSTI resistance mutations: L74M/I, T97A, E138A/K/T or G140S/A/C. In an analysis of the 183 person VIKING-3 study, the risk of VF at week 24 was 21% in the absence of a Q148 mutation, 42% in the presence of Q148H/R/K plus one accessory mutation, and 76% in the presence of Q148H/R/K and two accessory mutations.

Using in vitro susceptibility data obtained by the PhenoSense assay, the risk of VF was 24% for persons with viruses having ≤4-fold reduced dolutegravir susceptibility, 46% for persons with viruses having 4- to 10-fold reduced susceptibility, and 73% for persons with viruses having >10-fold reduced susceptibility. A similar analysis performed by the FDA found that a ≥3-fold (rather than 4-fold) reduction in dolutegravir susceptibility was associated with a reduced virological response.

Conclusions

The spectrum of dolutegravir-selected mutations and their effects on in vitro susceptibility is emerging but not yet complete because genotypic sequences have been published for a small proportion of cases of dolutegravir-associated VF, these cases have been reported primarily in subtype B viruses, and the effects of dolutegravir-selected mutations outside of integrase require further study.

The risk of VF and INSTI resistance on a dolutegravir-containing regimen depends on the ARVs used in combination with dolutegravir, a person’s prior ART experience, and whether a person is stably virologically suppressed. In INSTI-naive persons, several dolutegravir-containing two-drug combinations are likely to be highly effective for first-line therapy, treatment simplification, and even salvage therapy provided dolutegravir’s companion drug is fully active. However, actual and functional dolutegravir mono-therapy is associated with non-trivial risks of VF and emergent INSTI resistance.

The risk of functional monotherapy has implications for the use of dolutegravir plus two NRTIs in NRTI-experienced persons in low- and middle-income countries where genotypic resistance testing is not routinely available to guide therapy in persons with VF on a first-line NRTI/NNRTI-containing regimen or where viral load testing is not routinely available to confirm virological suppression in persons transitioning from a first-line NRTI/NNRTI-containing regimen. Studies designed to quantify this risk and to develop strategies to minimize it are urgently needed.

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Supplementary data

Tables S1 to S6 are available as Supplementary data at JAC Online.

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