HIV-1 drug resistance in people on dolutegravir-based antiretroviral therapy: a collaborative cohort analysis

Tom Loosli, Stefanie Hossmann, Suzanne M Ingle, Hajra Okhai, Katharina Kusejko, Johannes Mouton, Pantxika Bellecave, Ard van Sighem, Melanie Stecher, Antonella d'Arminio Monforte, M John Gill, Caroline A Sabin, Gary Maartens, Huldrych F Günthard, Jonathan A C Sterne, Richard Lessells, Matthias Egger*, Roger D Kouyos**

Summary

Background The widespread use of the integrase strand transfer inhibitor (INSTI) dolutegravir in first-line and second-line antiretroviral therapy (ART) might facilitate emerging resistance. The DTG RESIST study combined data from HIV cohorts to examine patterns of drug resistance mutations (DRMs) and identify risk factors for dolutegravir resistance.

Methods We included cohorts with INSTI resistance data from two collaborations (ART Cohort Collaboration, International epidemiology Databases to Evaluate AIDS in Southern Africa), and the UK Collaborative HIV Cohort. Eight cohorts from Canada, France, Germany, Italy, the Netherlands, Switzerland, South Africa, and the UK contributed data on individuals who were viraemic on dolutegravir-based ART and underwent genotypic resistance testing. Individuals with unknown dolutegravir initiation date were excluded. Resistance levels were categorised using the Stanford algorithm. We identified risk factors for resistance using mixed-effects ordinal logistic regression models.

Findings We included 599 people with genotypic resistance testing on dolutegravir-based ART between May 22, 2013, and Dec 20, 2021. Most had HIV-1 subtype B (n=351, 59%), a third had been exposed to first-generation INSTIs (n=193, 32%), 70 (12%) were on dolutegravir dual therapy, and 18 (3%) were on dolutegravir monotherapy. INSTI DRMs were detected in 86 (14%) individuals; 20 (3%) had more than one mutation. Most (n=563, 94%) were susceptible to dolutegravir, seven (1%) had potential low, six (1%) low, 17 (3%) intermediate, and six (1%) high-level dolutegravir resistance. The risk of dolutegravir resistance was higher on dolutegravir monotherapy (adjusted odds ratio [aOR] 34·1, 95% CI 9·93–117) and dolutegravir plus lamivudine dual therapy (aOR 9·21, 2·20–38·6) compared with combination ART, and in the presence of potential low or low (aOR 5·23, 1·32–20·7) or intermediate or high-level (aOR 13·4, 4·55–39·7) nucleoside reverse transcriptase inhibitor (NRTI) resistance.

Interpretation Among people with viraemia on dolutegravir-based ART, INSTI DRMs and dolutegravir resistance were rare. NRTI resistance substantially increased the risk of dolutegravir resistance, which is of concern, notably in resource-limited settings. Monitoring is important to prevent resistance at the individual and population level and ensure the long-term sustainability of ART.

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Introduction

The integrase strand transfer inhibitor (INSTI) dolutegravir was approved in 2013 in the USA and shortly afterwards in the EU to treat HIV-1. In 2019, WHO recommended dolutegravir as the preferred drug for first-line and second-line antiretroviral therapy (ART) in all populations, including pregnant women and those of childbearing age. Since then, dolutegravir-based ART has been rolled out globally,[1] with about 100 countries including dolutegravir in their treatment guidelines by mid-2020.[2]

Dolutegravir has a high genetic barrier to resistance,[3,4] and relatively few people living with HIV are so far known to have developed resistance.[5–7] The mutations leading to dolutegravir resistance can differ between HIV-1 subtypes. In individuals without previous exposure to INSTI-based ART, dolutegravir resistance is mainly associated with the Arg263Lys mutation,[8,9] which was observed in three cases of dolutegravir resistance in the NADIA trial.[10] The Asn155His mutation was present in two individuals with subtype A and C in the SAILING trial,[11] while the Gly118Arg mutation appears to be facilitated by a natural polymorphism in subtype C.[12] In a recent study in Ethiopia, the Gln148His/Lys/Arg mutation was found to be less prevalent in subtype C.[13] Pre-existing mutations, such as those acquired during a first-generation INSTI regimen, might directly confer resistance to dolutegravir or facilitate the accumulation of additional mutations.[14,15]

The risk factors and the mutational patterns that confer resistance to dolutegravir in vivo are less well established than for older antiretroviral drugs.[16] The widespread use

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See Comment page e696

*Contributed equally

Department of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland (T Loosli MSc, K Kusejko PhD, Prof H F Günthard MD, Prof R D Kouyos PhD); **Institute of Medical Virology, University of Zurich, Zurich, Switzerland** (T Loosli, K Kusejko, Prof H F Günthard, Prof R D Kouyos); **Institute of Social and Preventive Medicine (ISPM), University of Bern, Bern, Switzerland** (S Hossmann MSc, Prof M Egger MD); **Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK** (S M Ingle PhD, Prof J A C Sterne PhD, Prof M Egger); **Institute for Global Health, University College London, London, UK** (H Okhai PhD, Prof C A Sabin PhD); **Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa** (J Mouton MD, Prof G Maartens MD); **Virology Laboratory, University Hospital Bordeaux, Bordeaux, France** (P Bellecave PhD); **Stichting HIV Monitoring, Amsterdam, Netherlands** (A van Sighem PhD); **German Center for Infection Research (DZIF), Partner-Site Cologne-Bonn, Cologne, Germany** (M Stecher PhD); **Department I of Internal Medicine, University Hospital Cologne, University of Cologne, Cologne, Germany** (M Stecher); **Italian Cohort Naive Antiretrovirals (ICONA), University of Milan, Milan, Italy** (Prof A d'Arminio Monforte MD); **Southern Alberta Clinic,**

Calgary, AB, Canada (Prof M J Gill MB)**; Department of Medicine, University of Calgary, Calgary, AB, Canada** (Prof M J Gill)**; KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP), University of KwaZulu-Natal, Durban, South Africa** (R Lessells PhD)**; Centre for the AIDS Programme of Research in South Africa (CAPRISA), Durban, South Africa** (R Lessells)**; Centre for Infectious Disease Epidemiology and Research, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa** (Prof M Egger)

Correspondence to: Prof Matthias Egger, Institute of Social and Preventive Medicine, University of Bern, CH-3012 Bern, Switzerland **matthias.egger@unibe.ch**

Research in context

Evidence before this study

We searched Scopus on March 20, 2023, for all publications from inception using the terms "dolutegravir" or "DTG", "resistant" or "resistance", and "HIV". The available evidence on resistance evolution in people living with HIV with virological failure on dolutegravir-based antiretroviral therapy (ART) is scarce. Most studies assessed the efficacy of dolutegravir-based regimens in clinical studies. They reported drug resistance in individuals with virological failure as a secondary objective or reported single or multiple cases of individuals developing resistance on dolutegravir-based ART. Clinical trials such as the NADIA trial showed high viral suppression even in people with nucleoside reverse transcriptase inhibitor (NRTI) resistance. Previous analyses included only a few people experiencing failure on dolutegravir; the SINGLE trial with 39 people with virological failure on dolutegravir was the largest. The highest number of individuals with dolutegravir resistance was nine study participants in the NADIA trial. There is evidence that dolutegravir resistance in individuals on a dolutegravir monotherapy might be more likely, and studies suggest that HIV-1 subtype and mutations acquired during a first-generation integrase strand transfer inhibitor (INSTI)-based regimen might affect the risk of dolutegravir resistance.

Added value of this study

To our knowledge, DTG RESIST is the first study systematically investigating resistance in people living with HIV experiencing viraemia on dolutegravir-based ART using a multicohort collaboration design reflecting real-world routine care. We collected genotypic resistance tests and clinical data from eight observational HIV cohorts. This resulted in a large dataset of individuals experiencing viraemia on a dolutegravir regimen (599 individuals). It allowed a robust assessment of drug resistance mutations and risk factors for dolutegravir resistance. Cross-resistance of first-generation INSTIs does not appear to explain the mutation patterns in individuals with HIV who experience virological failure on dolutegravir-based ART regimens. Those who received dolutegravir monotherapy or dolutegravir plus lamivudine dual therapy and those infected with non-B subtypes were more likely to develop resistance. Resistance to NRTIs was a major risk factor for dolutegravir resistance, indicating that individuals receiving functional monotherapy are more likely to develop dolutegravir resistance.

Implications of all the available evidence

HIV-1 drug resistance is a significant threat to the sustainability of current and future ART for combating the ongoing HIV-1 pandemic. Our collaborative analysis shows that cases of dolutegravir resistance are rare at present but not negligible. Given the global dolutegravir roll-out, this might lead to increased frequencies and transmission of dolutegravir resistance, particularly in individuals with resistance to NRTIs. Although the evidence regarding subtype differences is tentative, it indicates that non-B subtypes, which are most relevant for the global roll-out of dolutegravir, might be associated with an increased risk of resistance.

of dolutegravir in resource-limited settings, where ART regimens are highly standardised, drugs are recycled, access to adherence support and viral load and resistance testing is limited, and the risk for drug stock-outs is higher, might facilitate the emergence of resistance. In the DTG RESIST study, we combined data from European, North American, and South African cohorts to identify risk factors for dolutegravir resistance and examine the patterns of resistance mutations across different HIV-1 subtypes.

Methods

Study design and population

The DTG RESIST project was discussed in two HIV cohort collaborations: the ART Cohort Collaboration (ART-CC)[17] and the International epidemiology Databases to Evaluate AIDS (IeDEA)[18] in Southern Africa. Six of the 21 ART-CC cohorts participated: the Agence Nationale de la Recherche sur le SIDA et les hépatites virales (ANRS CO3) Aquitaine Cohort, France; the AIDS Therapy Evaluation in the Netherlands cohort (ATHENA); the Köln/Bonn Cohort (CBC), Germany; the Italian Cohort of Antiretroviral-Naïve Patients (ICONA); the Southern Alberta Clinic Cohort (SAC), Canada; and the Swiss HIV Cohort Study (SHCS). The main reason for the non-participation of the other cohorts was the lack of access to resistance data. The UK Collaborative HIV Cohort (UK CHIC) Study and linked UK HIV Drug Resistance Database (UKHDRD), although not formally part of the ART-CC collaboration, also joined. In IeDEA Southern Africa, the South African Aid for AIDS (AfA) cohort was the only cohort with access to INSTI resistance data. The clinical data were provided by the data centres of the two cohort collaborations, ART-CC and IeDEA, and the genotypic data by the cohorts. Genotypic data were the consensus nucleotide sequences identified through genotypic resistance testing. There were two exceptions: AfA provided a list of mutations, and Aquitaine provided the Stanford resistance algorithm output. UK CHIC provided all data directly to the DTG RESIST study team. The appendix (p 2) provides further details.

See Online for appendix

We included participants who underwent genotypic resistance testing from plasma HIV-1 RNA covering the integrase gene between 2 weeks after starting and up to 2 months after stopping any dolutegravir-based regimen. The latest test was considered in the case of multiple genotypic resistance tests. Participants with unknown dates of initiation of dolutegravir-based ART were excluded. The analysis of risk factors for dolutegravir

resistance was restricted to individuals with at least 1 year of clinical data before the genotypic resistance test, ensuring the availability of viral load data and assessment of viral load testing frequency.

The Human Research Ethics Committee of the University of Cape Town and the Cantonal Ethics Committee of the Canton of Bern granted permission to analyse these data.

Procedures

We determined HIV-1 subtypes from the integrase gene using COMET (Context-based Modeling for Expeditious Typing)[19] and REGA.[20] If REGA and COMET output differed, the subtype with higher support was assigned. As nucleotide sequences were not available for AfA, we used subtype information from the cohort based on the reverse transcriptase and protease. For Aquitaine, information on subtype was used where available and otherwise considered unknown. The Aquitaine subtypes were characterised locally using Blast analysis on the SmartGene HIV module on at least two genes. In the analysis, we grouped HIV-1 subtypes other than the four most common subtypes in the study population (B, C, A, G) as "other" (F, AE, D, 06_CPX, AG, 18_CPX, AD, H, unknown; appendix p 3).

Individuals prescribed raltegravir or elvitegravir before starting the dolutegravir-based regimen were considered exposed to first-generation INSTIs. Viral load testing frequency was calculated for individuals with more than 1 year of follow-up before the genotypic resistance test. We quantified HIV-1 viral load as the area under the curve (AUC) of the $\log_{10}$-transformed viral load measurements from dolutegravir initiation to the genotypic resistance test sample date. To account for differences in detection limits, we set any viral load measurement below 50 copies per mL to 0 copies per mL. For individuals who initiated ART with the dolutegravir-based regimen, we excluded viral loads at ART initiation by setting measurements within the first 180 days from the first HIV-1 RNA measurement to 0. Time on dolutegravir-based ART was calculated in years from dolutegravir initiation to genotypic resistance testing. The ART regimen at genotypic resistance testing was the regimen an individual took 14 days before the test. If available, genotypic resistance test results from earlier timepoints were used to assess previous nucleoside reverse transcriptase inhibitor (NRTI) resistance. We defined monotherapy as ART consisting of dolutegravir only. Dolutegravir dual therapy was defined as dolutegravir combined with a second antiretroviral drug. Dolutegravir-based regimens comprising dolutegravir and two or more antiretroviral drugs were defined as combination therapy with three or more drugs.

Outcomes

We defined two HIV drug resistance outcomes: the level of resistance to dolutegravir and the presence of known drug resistance mutations (DRMs). The Stanford HIV Database version 9.0 and the Stanford HIVdb algorithm[21] were used to categorise drug resistance levels as susceptible (score below 10), potential low (10–14), low (15–29), intermediate (30–59), or high (≥60). We defined INSTI DRMs[22] as all mutations associated with INSTIs by the Stanford HIVdb algorithm, including major and accessory mutations. We used the same approach to assess resistance to all other antiretroviral drugs, whereby drug resistance to tenofovir alafenamide (not covered by the Stanford algorithm) was considered equal to tenofovir disoproxil fumarate resistance. Resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) was calculated as the median of the

	Analysis of resistance-conferring mutations (n=599)	Analysis of risk factors for dolutegravir resistance (n=540)
Sex		
Female	187 (31%)	175 (32%)
Male	412 (69%)	365 (68%)
Age at dolutegravir initiation, years	44 (36–52)	45 (37–52)
HIV subtype		
B	351 (59%)	316 (59%)
C	69 (12%)	63 (12%)
A	54 (9%)	51 (9%)
G	42 (7%)	39 (7%)
Other*	83 (14%)	71 (13%)
ART regimen at dolutegravir initiation		
Combination therapy with ≥3 antiretrovirals	511 (85%)	455 (84%)
Dual therapy (dolutegravir and other)	51 (9%)	50 (9%)
Dual therapy (dolutegravir and lamivudine)	19 (3%)	17 (3%)
Monotherapy	18 (3%)	18 (3%)
ART duration at dolutegravir initiation, years	6·7 (0·95–14)	7·9 (2·4–15)
Missing	4 (<1%)	4 (<1%)
Year of dolutegravir initiation	2016 (2015–2017)	2016 (2015–2017)
Year of genotypic resistance testing	2018 (2017–2019)	2018 (2017–2019)
Availability of additional (previous) genotypic resistance tests		
Yes	395 (66%)	356 (66%)
No	204 (34%)	184 (34%)
Dolutegravir regimen initiation		
Switch to dolutegravir-based ART	486 (81%)	470 (87%)
Initiation on dolutegravir-based ART	113 (19%)	70 (13%)
Duration on dolutegravir-based ART at genotypic resistance testing, years	1·4 (0·58–2·7)	1·6 (0·67–2·8)
Exposure to first-generation INSTI		
Yes	193 (32%)	184 (34%)
No	406 (68%)	356 (66%)
CD4 count at genotypic resistance testing, cells per μL	412 (213–674)	433 (218–681)
Missing	129 (22%)	115 (21%)
Viral load AUC of $\log_{10}$ copies per mL during dolutegravir-based ART	3·6 (2·2–5·0)	3·6 (2·3–4·9)
Missing	10 (2%)	0
Number of HIV tests per year	3·0 (2·0–4·3)	3·3 (2·3–4·3)
Missing	20 (3%)	0

(Table 1 continues on next page)

	Analysis of resistance-conferring mutations (n=599)	Analysis of risk factors for dolutegravir resistance (n=540)
(Continued from previous page)		
Cohort		
AfA	9 (2%)	9 (2%)
Aquitaine	64 (11%)	59 (11%)
ATHENA	66 (11%)	64 (12%)
CBC	89 (14%)	76 (14%)
ICONA	8 (1%)	5 (<1%)
SAC	92 (15%)	87 (16%)
SHCS	118 (20%)	108 (20%)
UK CHIC/UKHDRD	153 (26%)	132 (24%)

Data are n (%) or median (IQR). ART=antiretroviral therapy. INSTI=integrase strand transfer inhibitor. AUC=area under the curve. AfA=Aid for AIDS. Aquitaine=Agence Nationale de la Recherche sur le SIDA et les hépatites virales (ANRS) CO3 Aquitaine Cohort. ATHENA=AIDS Therapy Evaluation in the Netherlands. CBC=Cologne/Bonn Cohort. ICONA=Italian Cohort of Antiretroviral-Naïve Patients. SAC=Southern Alberta Clinic Cohort. SHCS=Swiss HIV Cohort Study. UK CHIC/UKHDRD=UK Collaborative HIV Cohort/UK HIV Drug Resistance Database. *Other subtypes are as follows: for the analysis of resistance conferring mutations—Unknown, n=30 (5%); F, n=19 (3%); AE, n=10 (2%); D, n=10 (2%); 06_CPX, n=6 (1%); AG, n=4 (<1%); 18_CPX, n=2 (<1%); AD, n=1 (<1%); and H, n=1 (<1%). For the analysis of risk factors for dolutegravir resistance—Unknown, n=27 (5%); F, n=15 (3%); D, n=10 (2%); AE, n=7 (1%); 06_CPX, n=5 (<1%); AG, n=4 (<1%); 18_CPX, n=1 (<1%); AD, n=1 (<1%); and H, n=1 (<1%).

Table 1: **Demographics and clinical characteristics in the study population**

scores for efavirenz, etravirine, nevirapine, and rilpivirine. Finally, we calculated resistance to NRTIs as the median of abacavir, zidovudine, emtricitabine or lamivudine, and tenofovir disoproxil fumarate scores. We used alternative definitions in sensitivity analyses.

Statistical analysis

We used descriptive statistics to present the characteristics of the study population and the different INSTI DRMs. A negative binomial generalised linear model, adjusting for HIV-1 subtype, exposure to first-generation INSTIs, and sex, was used to analyse the number of major and accessory INSTI DRMs. We used ordinal logistic regression to identify risk factors for developing resistance, including cohort as a random effect. We considered variables based on availability and clinical relevance. We included sex, age at initiation, time on the dolutegravir-based regimen, HIV-1 subtype, type of ART (combination ART based on three drugs or more, dolutegravir plus lamivudine dual therapy, other dolutegravir dual therapy, or monotherapy), exposure to first-generation INSTIs, HIV-1 viral load, viral load testing frequency, and resistance to NRTIs. If the sequencing did not cover the reverse transcriptase gene, the missing data were included as a separate category. All analyses were performed in R, version 4.0.5.

We did several sensitivity analyses. First, we replaced the NRTI resistance variable with the presence or absence of the Met184Val/Ile mutation (sensitivity analysis S1). Further, we did logistic regression with the same covariables as in the main risk factor analysis, using susceptible versus any dolutegravir resistance as the outcome (S2). We also considered dolutegravir resistance according to the WHO definition, whereby potential low is considered susceptible (S3). We repeated the risk factor analyses excluding study participants where reverse transcriptase was not sequenced (S4) and excluding participants on dolutegravir monotherapy (S5). Given the widespread use of combined tenofovir disoproxil fumarate, lamivudine, and dolutegravir, we restricted the analysis of NRTIs to tenofovir disoproxil fumarate and lamivudine, using the higher resistance level of the two to quantify NRTI resistance (S6). In the subset of people on a dolutegravir plus two NRTIs regimen, we calculated NRTI resistance specific to the two NRTIs used in each participant (S7). The main analysis could not assess whether NRTI and NNRTI resistance mutations pre-existed or were acquired on dolutegravir-based therapy. Sensitivity analysis S8 restricted the study population to participants with available genotypic resistance tests before the genotypic resistance test used in the main analysis.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

A total of 599 people met the eligibility criteria and were included in the analysis of mutations conferring resistance to dolutegravir; 540 (90%) had more than 1 year of follow-up since starting the dolutegravir-based regimen and were included in the analysis of risk factors for dolutegravir resistance. The appendix shows the number of participants included in the risk factor analysis by presence or absence of INSTI DRMs (appendix p 4), and by dolutegravir resistance levels (appendix p 5).

The study participants included in the two analyses (mutations conferring dolutegravir resistance and risk factors for dolutegravir resistance) were similar (table 1): most participants were men living with HIV-1 subtype B who were on combination ART with three or more antiretroviral drugs (see appendix p 3 for details on ART regimens). The median year of starting dolutegravir was 2016. Participants had been on dolutegravir for a median of 1·4 years at the time of genotypic resistance testing, and the median AUC of $\log_{10}$ viral load (copies per mL) accumulated during this period was 3·6. The first genotypic resistance test was done on May 22, 2013, and the last on Dec 20, 2021. About a third of participants had previously been exposed to first-generation INSTIs; most were exposed to raltegravir (142/193), followed by elvitegravir (38/193), and both elvitegravir and raltegravir (13/193). A total of 129 participants did not have a CD4 measurement within a year of the genotypic resistance test, ten did not have any recorded HIV-1 RNA measurements before the genotypic resistance test,

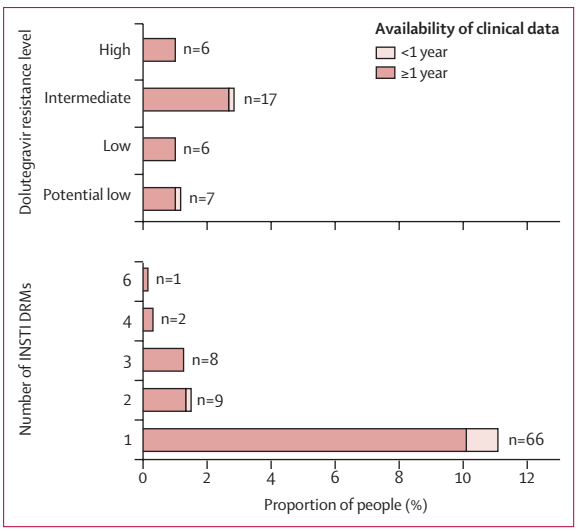

Figure 1: **Prevalence of dolutegravir resistance and INSTI DRMs**
Genotypic resistance tests of 599 people with genotypic resistance testing on dolutegravir-based antiretroviral therapy were analysed using the Stanford resistance algorithm to determine INSTI DRMs and resistance level to dolutegravir. Both major and accessory INSTI DRMs were considered for the number of INSTI DRMs. People with no INSTI DRMs (n=513, 86%) and those who are susceptible to dolutegravir (n=563, 94%) are not displayed. INSTI=integrase strand transfer inhibitor. DRM=drug resistance mutation.

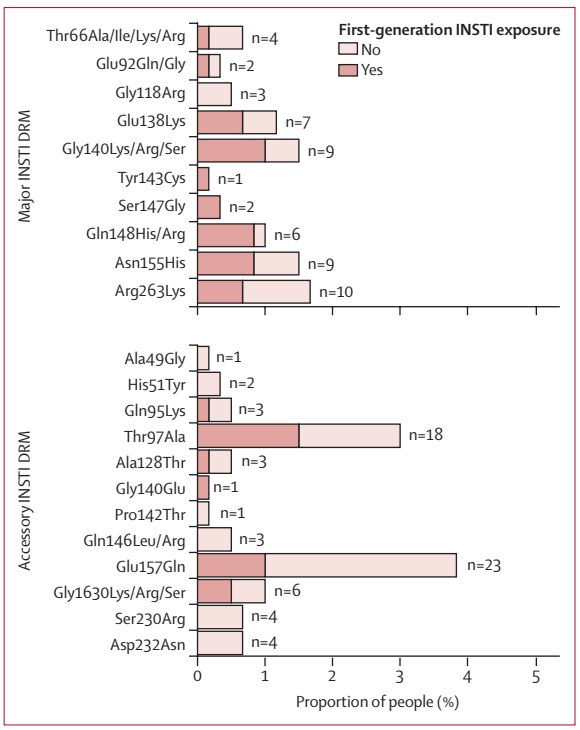

Figure 2: **INSTI DRMs found in 599 people experiencing viraemia on a dolutegravir-based regimen**
DRMs were classified as major and accessory according to the Stanford resistance database.[22] Bars are coloured by previous history of first-generation INSTIs (raltegravir, elvitegravir). INSTI=integrase strand transfer inhibitor. DRM=drug resistance mutation.

and in 62 people sequencing did not cover reverse transcriptase.

At least one major or accessory INSTI DRM was found in 86 (14%) of the 599 study participants; 20 (3%) had more than one mutation (appendix pp 6–7). The proportion of first-generation INSTI exposure was similar among participants with and without INSTI DRMs (28 [33%] of 86 and 165 [32%] of 513, respectively). 563 study participants (94%) were fully susceptible to dolutegravir, with potential low, low, intermediate, and high levels of dolutegravir resistance being observed in seven (1%), six (1%), 17 (3%), and six (1%) participants, respectively (figure 1).

The most common major INSTI DRM was Arg263Lys (n=10), which only once occurred with another major INSTI DRM (appendix p 6). Other common major mutations included Gly140Lys/Arg/Ser (n=9), Asn155His (n=9), Gln148His/Arg (n=6), and Glu138Lys (n=7). The Gly118Arg, which has the strongest impact on susceptibility to dolutegravir, was only observed three times. Among accessory DRMs, Glu157Gln (n=23) and Thr97Ala (n=18) were the most common. The distribution of INSTI resistance mutations was similar in people previously exposed to first-generation INSTIs and those not exposed (figure 2). There was no statistically significant association of specific DRMs with first-generation INSTI experience. For HIV-1 subtype, we found a significant association after adjusting for multiple testing for the accessory INSTI DRM Thr97Ala (adjusted p value=0·015, see appendix p 8). This DRM occurred in six (11%) of 54 people with HIV-1 subtype A, four (10%) of 42 people with subtype G, six (2%) of 351 people with subtype B, and none of 69 people with HIV-1 subtype C.

The results from the negative binomial model of the number of mutations showed little evidence of a difference between HIV-1 subtypes. The total INSTI DRM count (including both accessory and major DRMs) was higher in first-generation INSTI-exposed individuals (adjusted rate ratio [aRR] 1·59, 95% CI 0·98–2·59; figure 3). This association became stronger when considering only the number of major INSTI DRMs (aRR 2·67, 1·25–5·87; see appendix p 9 for further details).

The prevalence of predicted resistance (low, intermediate, or high) to NRTIs and NNRTIs was substantially higher in the presence of dolutegravir resistance (table 2). Among genotypic resistance tests with coverage of the reverse transcriptase, the prevalence of at least low-level NRTI resistance was 7% overall (39 of 530), but 32% (seven of 22) among those with dolutegravir resistance. The corresponding figures for NNRTI resistance were 15% (82 of 530) and 50% (11 of 22).

The risk of dolutegravir resistance was higher on dolutegravir monotherapy compared with combination ART with three or more drugs (adjusted odds ratio [aOR] 34·09, 95% CI 9·93–117·01) and for the dolutegravir plus lamivudine dual regimen (aOR 9·21, 2·20–38·55; figure 4). The risk of resistance was also increased in the

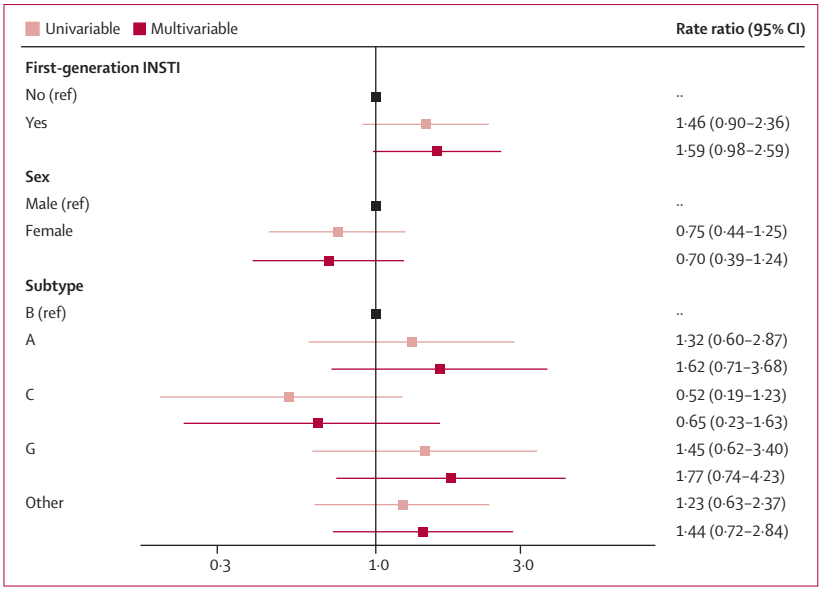

Figure 3: **Rate ratios for number of INSTI DRMs**
A negative binomial generalised linear model was fit to the number of major and accessory INSTI DRMs in 599 people with viraemia on dolutegravir-based antiretroviral therapy. The plot shows univariable and multivariable point estimates and 95% CIs of rate ratios. INSTI=integrase strand transfer inhibitor. DRM=drug resistance mutation.

	Susceptible and potential low dolutegravir resistance (n=570)	Low, intermediate, and high dolutegravir resistance (n=29)
NRTI resistance level		
Susceptible	467 (82%)	13 (45%)
Potential low	9 (2%)	2 (7%)
Low	10 (2%)	1 (3%)
Intermediate	9 (2%)	2 (7%)
High	13 (2%)	4 (14%)
Reverse transcriptase not covered in genotypic resistance test	62 (11%)	7 (24%)
NNRTI resistance level		
Susceptible	414 (73%)	11 (38%)
Potential low	23 (4%)	0
Low	18 (3%)	0
Intermediate	34 (6%)	4 (14%)
High	19 (3%)	7 (24%)
Reverse transcriptase not covered in genotypic resistance test	62 (11%)	7 (24%)

Data are n (%). Data are given for the entire study population. NRTI resistance level is based on median resistance score to abacavir, zidovudine, emtricitabine or lamivudine, and tenofovir disoproxil fumarate or tenofovir alafenamide. NNRTI resistance level is based on median resistance score to efavirenz, etravirine, nevirapine, and rilpivirine. NRTI=nucleoside reverse transcriptase inhibitor. NNRTI=non-nucleoside reverse transcriptase inhibitor.

Table 2: **Resistance levels to NRTIs and NNRTIs by dolutegravir susceptibility**

presence of potential low or low-level NRTI resistance (aOR 5·23, 1·32–20·71) or intermediate or high level (aOR 13·44, 4·55–39·68), compared with no NRTI resistance. Non-B HIV-1 subtypes were also associated with increased resistance, particularly subtype A (aOR 3·12, 0·84–11·61 compared with subtype B), but associations were not statistically significant (appendix p 10). Similarly, there was weak, statistically non-significant evidence for an association of viral load with dolutegravir resistance (aOR 1·19, 0·76–1·85 per standard deviation of the $\log_{10}$ virus load AUC).

The results of the risk factor analyses were similar when replacing the NRTI resistance variable with the Met184Val/Ile mutation (sensitivity analysis S1, n=540/540, appendix p 12), when analysing susceptible versus any dolutegravir resistance as the outcome in a logistic regression (S2, n=540/540, appendix p 13), or when considering dolutegravir resistance levels following the WHO definition, where potential low-level resistance is considered susceptible (S3, n=540/540, appendix p 14). The exclusion of 58 individuals with missing reverse transcriptase sequences allowed the inclusion of both NRTI and NNRTI resistance in the model. The results for NRTI resistance were similar, and intermediate or high-level NNRTI resistance was also associated with dolutegravir resistance (aOR 2·72, 95% CI 0·94–7·86; S4, n=482/540, appendix p 15). Results were similar when excluding individuals on dolutegravir monotherapy (S5, n=474/540, appendix p 16). The analysis restricted to lamivudine and tenofovir disoproxil fumarate (S6, n=540/540, appendix p 17) confirmed that dolutegravir resistance was associated with both potential low or low and intermediate or high-level resistance to these NRTI drugs. Similarly, when restricting the analysis to people on a dolutegravir regimen with two NRTIs, we found similar results for the specific NRTIs (S7, n=309/540, appendix p 18). Finally, in sensitivity analysis S8, we used data on pre-existing NRTI resistance and found that dolutegravir resistance was associated with previous intermediate or high-level NRTI resistance (n=356/540, appendix p 19).

Discussion

In this collaborative analysis of eight large cohort studies, we identified INSTI DRMs in 86 (14%) of 599 people living with HIV with a genotypic resistance test while viraemic on dolutegravir-based ART. Resistance to dolutegravir according to the Stanford algorithm was present in 36 (6%) individuals. Dolutegravir resistance was associated with dolutegravir monotherapy, lamivudine–dolutegravir dual therapy, and resistance to NRTIs. Exposure to first-generation INSTI was associated both with more resistance mutations and higher levels of dolutegravir resistance, but the association with dolutegravir resistance was not statistically significant. A wide range of INSTI DRMs was present. The polymorphic accessory INSTI DRM Thr97Ala was detected more frequently in subtypes A and G (compared with subtypes B and C), consistent with previously reported data.[23] The major INSTI DRMs

Gly140Lys/Arg/Ser and Gln148His/Arg were detected in five out of six people with high-level dolutegravir resistance.

Dolutegravir monotherapy, dolutegravir plus lamivudine dual therapy, and resistance to the NRTI backbone were most strongly associated with dolutegravir resistance in our study. The complete sequence analysis, which allowed us to distinguish between NRTI and NNRTI resistance, suggests that the association might be mediated via NRTI resistance. It was robust when considering only lamivudine and tenofovir disoproxil fumarate resistance in a sensitivity analysis. As the main analysis was cross-sectional, it did not allow the timing of NRTI resistance relative to dolutegravir resistance to be determined. However, an additional analysis in people with previous resistance tests suggests that NRTI resistance might often have pre-dated dolutegravir resistance. These results suggest that resistance to NRTI backbone drugs from previous regimens might have promoted the emergence of dolutegravir resistance. However, it is also possible that previous NRTI resistance reflects adherence issues, which might facilitate the emergence of dolutegravir resistance.

The results from our study align with previous studies[10,24,25] that showed associations of dolutegravir resistance with dolutegravir monotherapy or NRTI resistance. The NADIA trial found no evidence that resistance to NRTIs affects the effectiveness of dolutegravir-based ART.[10] The NADIA trial does not, however, contradict the results of our study because outcomes differed: NADIA examined the risk of virological failure, whereas our study focused on the risk of dolutegravir resistance among individuals tested for drug resistance while on dolutegravir-based ART. Resistance to NRTIs might not affect treatment failure risk but still increase the chances of acquiring resistance in case of failure. Research on other drug classes[26] indicates that drug regimens with high and low genetic barriers can have similar failure rates but different probabilities of acquiring resistance.

Our study contributes important new information on dolutegravir resistance in individuals receiving different dolutegravir-based ART regimens by examining risk factors for dolutegravir resistance in real-world cohort data from different settings. The cohort collaboration resulted in a large dataset of genotypic resistance test results in people who experienced viraemia on dolutegravir. Our results are central to informing HIV treatment and monitoring policies in the context of the continued expansion of dolutegravir-based treatment regimens. The pooling of data from diverse routine clinical cohorts also has limitations. The participating cohorts include individuals in routine care but practices regarding when and for whom genotypic resistance testing is done will differ between cohorts. Further, the personalised approach to ART and HIV care in the European settings will not be generalisable to other settings, particularly to low-income and middle-income countries. In our regression models, we accounted for this heterogeneity between cohorts by including cohort as a random effect, but confounding by cohort might still have affected our results. Furthermore, genotypic resistance testing before starting or switching ART might have prevented some individuals from receiving dolutegravir, thus introducing selection bias. However, pretreatment resistance to dolutegravir was unlikely during the study period.[27]

A further limitation of our study is the dominance of HIV-1 subtype B, which was expected considering that our study population comprised mainly individuals from European countries, where subtype B predominates. More data from people with non-B subtypes are needed. The prospective arm of DTG RESIST is ongoing within the framework of IeDEA:[18] individuals with virological

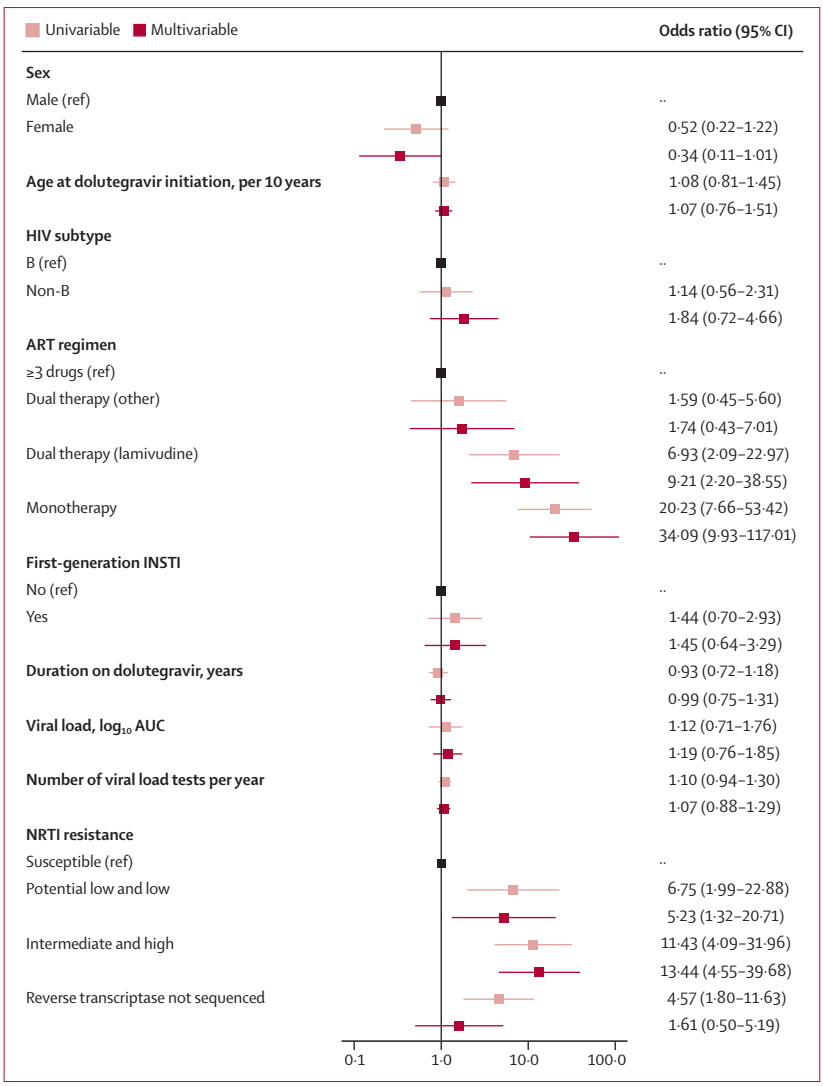

Figure 4: **Odds ratios for dolutegravir resistance levels with 95% CIs from univariable and multivariable ordinal logistic models for genotypic dolutegravir resistance**
Cohorts were included as random effect. Dolutegravir resistance levels in people with viraemia on dolutegravir-based ART were assessed using the Stanford resistance algorithm. ART=antiretroviral therapy. AUC=area under the curve. NRTI=nucleoside reverse transcriptase inhibitor.

failure on dolutegravir-based ART are prospectively enrolled in around 20 sites across sub-Saharan Africa, South America, and Asia. Furthermore, WHO plans to launch sentinel surveys of acquired HIV resistance to dolutegravir among people receiving dolutegravir-based ART.[28] We could not assess adherence or drug interactions with rifampicin, which might influence the emergence of dolutegravir resistance.[29] Adherence and rifampicin use were not recorded consistently and comparably in the participating cohorts. In our study population, the dolutegravir-based regimens were too heterogeneous to investigate dolutegravir resistance outcomes of specific regimens and treatment histories. Lastly, there is growing evidence that mutations outside integrase can confer dolutegravir resistance.[30–32] Our study was based on pol sequences, which did not allow us to investigate the effects of these mutations.

The associations we found with dolutegravir resistance, resistance to NRTI backbone drugs, and trends for HIV-1 subtype and unsuppressed virus load have important implications for ensuring the long-term sustainability of ART. Although overall INSTI resistance was rare in our population, and although the low risk of virological failure will further reduce the incidence of resistance among people treated with dolutegravir, dolutegravir resistance is still a concern. Firstly, the duration of dolutegravir therapy and the duration of viraemia while receiving dolutegravir were relatively short in our population: the median time on dolutegravir was less than 2 years, and drug resistance might emerge more frequently in settings where individuals remain viraemic for a longer time on dolutegravir regimens. This could happen in resource-limited settings where guidelines recommend not switching from dolutegravir-based therapy unless multiple viral loads above 1000 copies per mL have been documented and where delays in regimen switching are common.[33] Secondly, the strong association of dolutegravir resistance with NRTI resistance suggests that the risk of resistance might be higher in people with previous failure on NNRTI-based first-line therapy, among whom the prevalence of NRTI resistance is much higher than in our study population. The WHO guidelines recommend dolutegravir in first-line, second-line, and third-line ART. This multiplicity of roles combined with the recycling of drugs and limited access to viral load and drug resistance testing will facilitate the emergence of dolutegravir resistance. Finally, even a relatively low level of acquired dolutegravir resistance in the millions of people receiving dolutegravir-based ART could lead to rising levels of transmitted INSTI resistance, which could negatively affect both treatment and prevention.

In conclusion, our study underlines the importance of resistance testing, especially in treatment-experienced people. Although rare, dolutegravir resistance can develop in people who experience viraemia on a dolutegravir-containing ART regimen. Monitoring the emergence of such resistance is important to prevent resistance at the individual and the population level and to ensure the long-term sustainability of ART.

Contributors

RDK, ME, HFG, RL, and JACS conceptualised the study. TL, SH, SMI, and HO curated the data. TL, CAS, RDK, ME, and JACS devised the methodology. TL and RDK conducted the formal analysis and validation. TL, SH, and SMI managed the data. SH and SMI provided project administration. HO, KK, JM, AvS, MS, Ad'AM, MJG, CAS, PB, and GM contributed resources. TL and KK applied and adapted the software. HFG, JACS, RL, ME, and RDK provided supervision. TL created the figures. TL, RL, ME, and RDK wrote the original draft of the manuscript. All authors reviewed the manuscript. TL and RDK directly accessed and verified the underlying data reported in the manuscript. ME, TL, and RDK had full access to all the data in the study; other authors had access to the data from their cohort, but not to the data from the other cohorts. All authors had the final responsibility for the decision to submit for publication.

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Data sharing

Study data will not be publicly available. Data can be made available to interested researchers. De-identified participant data and a data dictionary can be made available and shared under a data transfer agreement. Requests for access to DTG RESIST data should be sent to matthias.egger@unibe.ch. Nucleotide sequences are available on GenBank for the cohorts where local regulations allowed data sharing (see appendix p 2).

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