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Genotypic correlates of resistance to the HIV-1 strand transfer integrase inhibitor cabotegravir

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ABSTRACT

Cabotegravir (CAB) is an integrase strand transfer inhibitor (INSTI) formulated as a long-acting injectable drug approved for pre-exposure prophylaxis and use with a long acting rilpivirine formulation for therapy in patients with virological suppression. However, there has been no comprehensive review of the genetic mechanisms of CAB resistance. Studies reporting the selection of drug resistance mutations (DRMs) by CAB and the results of *in vitro* CAB susceptibility testing were reviewed. The impact of integrase mutations on CAB susceptibility was assessed using regularized regression analysis. The most commonly selected mutations in the 24 persons developing virological failure while receiving CAB included Q148R (n = 15), N155H (n = 7), and E138K (n = 5). T97A, G118R, G140 A/R/S, and R263K each developed in 1–2 persons. With the exception of T97A, G118R, and G140 A/R, these DRMs were also selected *in vitro* while G140R was selected in the SIV macaque model. Although these DRMs are similar to those occurring in persons receiving the related INSTI dolutegravir, Q148R was more likely to occur with CAB while G118R and R263K were more likely to occur with dolutegravir. Regularized regression analysis identified 14 DRMs significantly associated with reduced CAB susceptibility including six primary DRMs which reduced susceptibility on their own including G118R, Q148 H/K/R, N155H, and R263K, and eight accessory DRMs including M50I, L74 F/M, T97A, E138K, and G140 A/C/S. Isolates with Q148 H/K/R in combination with L74M, E138 A/K, G140 A/S, and N155H often had >10-fold reduced CAB susceptibility. M50I, L74M, and T97A are polymorphic mutations that alone did not appear to increase the risk of virological failure in persons receiving a CAB-containing regimen. Careful patient screening is required to prevent CAB from being used during active virus replication. Close virological monitoring is required to minimize CAB exposure to active replication to prevent the emergence of DRMs associated with cross-resistance to other INSTIs.

1. Introduction

Cabotegravir (CAB) is an integrase strand transfer inhibitor (INSTI) developed as a long-acting (LA) injectable antiretroviral drug with a long half-life, permitting infrequent dosing. CAB-LA has been approved for pre-exposure prophylaxis (PrEP) and, in combination with a long-acting formulation of the nonnucleoside RT inhibitor (NNRTI) rilpivirine (RPV-LA), it has been approved for maintenance therapy in patients with virological suppression and no history of virological failure or known or suspected resistance to CAB or RPV. CAB/RPV is administered as two separate extended-release injectable suspensions either monthly or bimonthly. CAB-LA is approved bimonthly for PrEP.

CAB is a highly potent analog of dolutegravir. As a result of its reduced water solubility and high protein binding it has a prolonged plasma half-life. Moreover, it can be formulated into a nanosuspension which can maintain prolonged suppressive protein-adjusted 90% inhibitory concentrations in plasma (Spreen et al., 2013). However, in contrast to other antiretroviral inhibitors it is not approved for use in persons with ongoing viral replication. As with all antiretrovirals, the INSTIs can select for amino acid mutations that confer reduced susceptibility and efficacy. INSTI resistance can be selected during sub-optimal ART with an INSTI-containing regimens, and in rare cases can be transmitted to antiretroviral-naïve individuals (McClung et al., 2021). Information related to drug resistance to new antiretrovirals can be

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generated from several sources, including *in vitro* selection in cell culture, *in vitro* susceptibility tests, and the genetic changes in viruses from patients with virological failure while receiving the antiretroviral. Here we review published data on CAB resistance to generate a framework to better understand the optimal use of this new treatment and prevention modality.

2. Material and methods

2.1. Search strategy

We searched PubMed and conference proceedings using the search terms "cabotegravir" and "GSK1265744". Relevant publications and conference proceedings were reviewed to characterize the genetic determinants of CAB resistance and the incidence of INSTI drug-resistance mutations (DRMs) in patients receiving CAB (Fig. 1).

2.2. Mutation definitions

Mutations were defined as amino acid differences from the consensus subtype B sequence. INSTI DRMs were defined as the 51 mutations on the IAS-USA list of resistance-associated mutations (Wensing et al., 2019) or assigned a mutation penalty score for any INSTI in the Stanford HIV Drug Resistance Database (HIVDB) genotypic resistance interpretation program (Paredes et al., 2017). Additional potential INSTI-associated DRMs included those reported to emerge either *in vitro* or *in vivo* in patients receiving CAB (M50I, T122N, and Q146 L/R).

2.3. Phenotypic data

Phenotypic susceptibility data were reported as fold-change in IC_{50} compared to a standard reference virus lacking known INSTI DRMs. Fold-change results that were greater than 128 or that could not be calculated because the IC_{50} exceeded the highest concentration of drug tested were reported as 128-fold. Published phenotypic data were characterized according to laboratory performing the assay and whether the viruses were laboratory-generated site-directed mutants or recombinant viruses containing the integrase coding sequence from clinical samples.

2.4. Regularized regression analysis

To evaluate the impact of INSTI DRMs on CAB susceptibility, we performed Least Absolute Shrinkage and Selection Operator (LASSO) regression analysis, which is useful for selecting a subset of predictors when the set of possible predictors is large (Taylor and Tibshirani, 2015). For this analysis, we included each of the INSTI and potential INSTI DRMs that occurred two or more times in the phenotypic dataset. The dependent variable was the $\log_{10}$ fold change in CAB susceptibility. We calculated the mean and 95% confidence intervals of the LASSO importance values for each mutation by running 10-fold cross-validation without replacement 1000 times using the R package glmnet (https://cran.r-project.org/web/packages/glmnet/index.html). The complete list of phenotypic susceptibility results and the supporting R code are available as Supplementary Material.

3. Results

3.1. Search results

A search conducted August 11, 2022, identified 272 PubMed publications. After reviewing all titles and abstracts, 62 were selected for full-text review. Four studies presented at scientific meetings between 2019 and 2022 containing data on CAB resistance that was not subsequently published and one additional study published only as a pre-print on bioRxiv were also reviewed. Of these 67 publications, four contained data from *in vitro* passage experiments (Oliveira et al., 2018; Smith et al., 2020; Yoshinaga et al., 2015, 2018), one contained data from experiments in non-human primates (Radzio-Basu et al., 2019), 16 contained *in vitro* susceptibility data (Cheung et al., 2022; Hachiya et al., 2017a; Hassounah et al., 2017; Jeffrey et al., 2022; Ndashimye et al., 2021; Oliveira et al., 2018; Overton et al., 2021; Pham et al., 2018; Rizzardini et al., 2020; Saladini et al., 2019; Shahid et al., 2019; Smith et al., 2018, 2020; Yoshinaga et al., 2015, 2018; Zhang et al., 2018), ten contained data from randomized clinical trials (Eshleman et al., 2017, 2022; Marzinke et al., 2021; Overton et al., 2021; Sutton et al., 2022; Swindells et al., 2020), and four contained data from open-label trials or clinical cohorts (Fig. 1).

3.2. In vitro passage experiments

Yoshinaga et al. performed four passage experiments using a subtype B variant (Yoshinaga et al., 2015). Two non-polymorphic mutations were observed in CAB-resistant viruses: Q146L or S153Y (Table 1). In a second publication from the same laboratory, viruses with the pre-existing INSTI DRMs E92Q, N155H, Q148H, Q148R, and Q148K were cultured in the presence of increasing CAB concentrations (Yoshinaga et al., 2018). No additional mutations were selected in the viruses

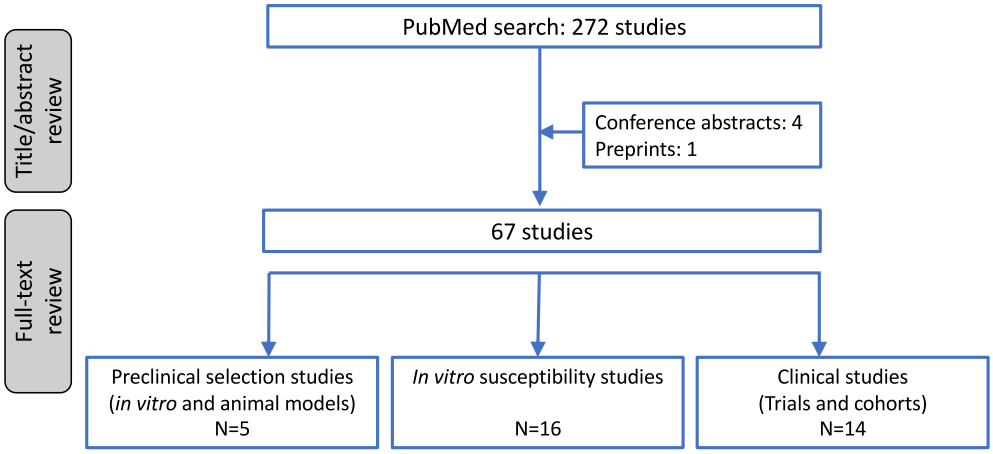

Fig. 1. Flow chart of study selection process. 272 studies were identified through a search of PubMed using the search terms "Cabotegravir" or "GSK1265744". Four additional studies were presented at scientific conferences and one was published as a bioRxiv preprint. Sixty-seven of these studies were selected for full-text review to determine whether they contained CAB resistance data from *in vitro* passage experiments or animal model studies, *in vitro* susceptibility data, or emergent resistance in patients receiving cabotegravir. Overall, data was extracted from 35 studies. The scientific abstracts reviewed included those presented between 2019 and 2022 at the following conferences: Conference on Retroviruses and Opportunistic Infections, International AIDS Conference, International AIDS Society Conference on HIV Science, International Workshop on HIV Drug Resistance, HIV Glasgow Drug Therapy, and European AIDS Conference.

Table 1
Integrase mutations selected during *in vitro* passage in the presence of cabotegravir.

Reference	Initial isolate	Mutations selected during passage
Yoshinaga 2015 (Yoshinaga et al., 2015)	Subtype B	Q146L S153Y
Yoshinaga 2018 (Yoshinaga et al., 2018)	Q148K Q148R Q148H	E138K L74M, E138K G140S C56S, L74M, V75A, T122N, E138K, G140S, G149A, M154I
Oliveira 2018 (Oliveira et al., 2018)	Subtype B	L74M, G140S, S147G, Q148K[a] H51HY R263K (8)[b] R263K, S153A L74I, E138K, G140GS, Q148R[c] N155H Q146L
	Subtype C	R263K S147G
	CRF01_AE	S153Y, G163R
	CRF02_AG	L74LM, E138K, Q148R, R263K[d] S153F
Smith 2020 (Smith et al., 2020)	Subtype B	L74M, Q148R V75A, G140S, Q148H T122N, G140S, Q148H L74M, V75A, G140S, Q148H

[a] The same isolate developed H51HY during *in vitro* passage with dolutegravir and S153Y with bictegravir.
[b] R263K occurred with M50I in 4 of 8 *in vitro* passage experiments.
[c] The same isolate developed Q95QK + Q146R with dolutegravir and bictegravir.
[d] The same isolate developed 263 K during *in vitro* passage with dolutegravir and S153SF, E157EK with bictegravir.

containing E92Q or N155H. G140S, C56S, L74M, V75A, T122N, E138K, M154I, and G149A were selected in the virus containing Q148H; L74M and E138K were selected in the virus containing Q148R; and E138K was selected in the virus containing Q148K (Table 1). T122N and M154I were observed transiently.

Oliveira et al. performed 19 passage experiments using clinical isolates belonging to different subtypes in the presence of increasing concentrations of CAB (Oliveira et al., 2018). R263K was selected in eight passage experiments, often in combination with M50I. Q148R/K was selected in three experiments in combination with three other DRMs. Miscellaneous additional mutations were selected in eight other experiments including H51Y, S153A plus R263K, N155H, Q146L, S147G, S153F, and S153Y plus G163R. Fewer mutations were selected by dolutegravir and bictegravir compared with CAB in the same passage experiments. Q148R or Q148K did not develop during passage experiments with dolutegravir and bictegravir.

3.3. Animal model studies

During CAB monotherapy in macaques acutely infected with a chimeric SIVmac239 virus that contains a chimeric SIVmac239 containing an HIV-1 RT gene, three mutations were selected in different experiments including G118R, G140R, and E92Q/G (Radzio-Basu et al., 2019). In the context of the SIV integrase, G118R and G140R were each independently associated >345-fold reduced susceptibility to all five INSTIs including CAB in the Monogram BioSciences PhenoSense assay (Petropoulos et al., 2000).

3.4. Selection of INSTI-associated DRMs in patients receiving CAB

HIV-1 integrase was sequenced in patient samples from seven randomized clinical trials of CAB efficacy and safety, including five trials in which CAB/RPV was used for maintenance ART in patients with virological suppression (Margolis et al., 2017, 2015; Orkin et al., 2020; Overton et al., 2021; Swindells et al., 2020) and two trials in which CAB was used for PrEP (Delany-Moretlwe et al., 2022; Landovitz et al., 2021) (Table 2).

LATTE was a phase IIb 96-week dose ranging trial in which 181 ART-naïve patients were initially treated with 10 mg–60 mg oral CAB in combination with two NRTIs for 24 weeks (Margolis et al., 2017). Patients who were virologically suppressed continued their original CAB dose in combination with 25 mg oral RPV for 72 weeks. One patient in the 10 mg arm developed virological failure and the INSTI-resistance mutation Q148R. During the open label follow-up period for this study, in which all patients received daily 30 mg oral CAB plus 25 mg RPV 25, two patients developed virological failure and INSTI resistance-mutations at weeks 132 and 264 (Sutton et al., 2022): E138K, G140A, and Q148R in one patient and G140S and Q148R in another.

LATTE-2 was a phase IIb non-inferiority 96-week trial in which ART-naïve patients were initially treated with 30 mg oral CAB + abacavir + lamivudine. Following virological suppression, 230 patients were randomized 1:1 to receive 400 mg CAB-LA plus 600 mg RPV-LA every 4 weeks or 600 mg CAB-LA plus 900 mg RPV-LA mg every 8 weeks. One patient receiving the q8 week regimen developed virological failure and the INSTI-resistance mutation Q148R.

FLAIR was a phase III 96 week trial in which ART-naïve patients were initially treated with dolutegravir, abacavir, and lamivudine for 20 weeks (Orkin et al., 2020). Patients were then randomized to continue their current regimen or switch to 400 mg CAB-LA plus 600 mg RPV-LA every 4 weeks following a 4 week induction period with daily 30 mg oral CAB plus 25 mg oral RPV for 4 weeks. Among the 283 patients switching to CAB-LA plus RPV-LA, three patients developed INSTI DRMs by week 48 including Q148R in two patients and G140R in one patient. No additional patients developed virological failure or emergent resistance by week 96 (Orkin et al., 2021b) but by week 124, one patient developed N155H + R263K (Orkin et al., 2021a).

ATLAS was a phase III 48 week trial in which patients with virological suppression for ≥6 months while taking standard oral ART were randomized to receive 400 mg CAB-LA plus 600 mg RPV-LA every 4 weeks or to continue their suppressive ART regimen (Swindells et al., 2020). Among the 308 patients receiving CAB-LA/RPV-LA, one patient developed virological failure and an INSTI DRM (N155H) by week 48. Among 52 patients in an extension phase, none developed virological failure and emergent resistance by week 96 (Swindells et al., 2022).

ATLAS-2M was a phase III 48 week trial in which 1045 patients with virological suppression for ≥6 months while taking standard oral ART were randomized to receive 400 mg CAB-LA 400 mg plus 600 mg RPV-LA every 4 weeks or 600 mg CAB-LA plus 900 mg RPV-LA every 8 weeks (Overton et al., 2021). Among the eight confirmed cases of virological failure in the 8 weekly regimen, five developed INSTI DRMs while both of the confirmed cases of virological failure in the 4 weekly regimen developed DRMs (Table 2).

HPTN083 was a trial which randomized 2283 persons to receive 600 mg CAB-LA every 8 weeks for PrEP following a 5-week lead-in with oral CAB versus tenofovir and emtricitabine (Landovitz et al., 2021). There were four unrecognized infections at the start of the trial and 12 incident infections during the trial. The incident infections occurred in three persons receiving oral CAB, in four persons while receiving appropriately timed CAB injections, and in five persons who discontinued CAB. INSTI DRMs developed in one person with a baseline infection, two persons developing virological failure on oral CAB, and two persons receiving appropriately timed injections (Landovitz et al., 2021; Marzinke et al., 2021). HPTN084 was a similarly designed study but incident infection with INSTI DRMs did not develop in any of the 1614 trial participants (Delany-Moretlwe et al., 2022; Eshleman et al., 2022).

Overall, among patients receiving CAB/RPV all but one patient developed one or more RPV DRMs. Risk factors for virological failure and HIVDR in patients receiving CAB/RPV were proviral RPV DRMs, a higher BMI (which has been associated with lower week 8 CAB

Table 2
INSTI drug-resistance mutations (DRMs) in trial participants with virological failure (VF) while receiving cabotegravir (CAB).

Study name and description	Number of patients on CAB	Weeks of treatment	INSTI DRMs[a]
Latte (Margolis et al., 2017) (CAB/RPV)[b] Dose-ranging trial. Oral CAB+2NRTIs x 24 weeks followed by oral CAB/RPV in patients with VS	10 mg (n = 60) 30 mg (n = 60) 60 mg (n = 61)	96	Q148R (10 mg)
Latte-2 (Margolis et al., 2017) (CAB/RPV) CAB/ABC/3 TC x 20 weeks followed by CAB-LA 400 mg/RPV 600 mg q4 weeks vs. CAB-LA 600 mg/RPV 900 mg q8 weeks in patients with VS without a history of VF	q4 weeks (n = 115) q8 weeks (n = 115)	96	Q148R (q8 weeks)
ATLAS (Swindells et al., 2020) (CAB/RPV) VS x ≥ 6 months after standard oral ART followed by CAB/RPV oral lead-in x 4 weeks followed by CAB-LA 400 mg/RPV 600 mg q4 weeks vs continued oral ART	308	48	N155H
FLAIR (Orkin et al., 2020) (CAB/RPV)[c] VS after DTG/ABC/3 TC x 20 weeks followed by CAB/RPV oral lead-in x 4 weeks followed by CAB-LA 400 mg/RPV 600 mg q4 weeks vs. continued DTG/ABC/3 TC	283	48	G140R Q148R (in 2 participants)
ATLAS 2 M (Overton et al., 2021) (CAB/RPV)[d] CAB-LA 400 mg/RPV 600 mg q4 weeks vs. CAB-LA 600 mg/RPV 900 mg q8 weeks in patients with VS ≥ 6 months without a history of VF	q8 weeks (n = 523) q4 weeks (n = 522)	48	<u>q4 weeks</u> N155 N/H E138 E/K, Q148R <u>Q8 weeks</u> Q148R Q148Q/R, N155 N/H T97A, N155H N155H Q148Q/R, N155 N/H
HPTN083 (Landovitz et al., 2021; Marzinke et al., 2021) (CAB; PrEP) Oral CAB x 5 weeks followed by CAB-LA 600 mg IM at weeks 5, 9 and then q8 weeks vs TDF/FTC daily	n = 2283 4 baseline infections 12 incident infections	N/A	<u>Baseline infections</u> E138K, Q148K <u>Incident infections</u> E138 E/K, G140 G/S, Q148R E138A, Q148R R263K G140A, Q148R
HPTN084 (Delany-Moretlwe et al., 2022; Eshleman et al., 2022) (CAB; PrEP) Oral CAB x 5 weeks followed by CAB-LA 600 mg IM at weeks 5, 9 and then q8 weeks vs TDF/FTC daily	n = 1614 4 incident infections	N/A	None

Abbreviations: VS – virological suppression; RPV – rilpivirine; DTG – dolutegravir; ABC – abacavir; 3 TC – lamivudine; TDF – tenofovir; FTC – emtricitabine; PrEP - pre-exposure prophylaxis; LA – long-acting; q4 weeks – every 4 weeks; q8 weeks – every 8 weeks; IM – intramuscular.

Footnote.

[a] List of DRMs reported. The complete sequences were not published.

[b] During a maintenance phase in which all participants received 30 mg, DRMs developed in two persons including E138K + G140A + Q148R at week 132 and G140S + Q148 R at week 264 (Sutton et al., 2022).

[c] During long-term follow-up, one patient developed N155H + R263K (Orkin et al., 2021a).

[d] Six of seven participants with INSTI-DRMs also developed RPV-DRMs. All six participants with RPV-DRMs also developed CAB-DRMs.

concentrations), and HIV-1 subtype A6 (Cutrell et al., 2021; Overton et al., 2021).

In addition to the randomized trials described above, four additional studies described resistance in persons receiving CAB/RPV including an open-label single arm implementation trial enrolling 430 patients (Johnson-Oldenbuttel et al., 2022), two clinical cohorts enrolling 236 (Wyen et al., 2022) and 51 (Christopoulos et al., 2022) persons, respectively, and 35 patients receiving compassionate use of CAB/RPV because they were in need of parenteral ART (D'Amico et al., n.d.). Three of the patients receiving compassionate CAB/RPV developed virological failure and emergent INSTI-resistance mutations including one with G118R, one with Q148R and N155H, and one with E138K and Q148R. One of the 717 persons in the other three studies was reported to have suspected virological failure with the emergence of N155 N/S (Johnson-Oldenbuttel et al., 2022).

In total, 24 CAB-experienced persons developed emergent INSTI-resistance mutations in clinical trials and cohort studies including 15 who developed Q148R, 7 who developed N155H, 2 who developed R263K, and 1 who developed G118R. In contrast, among 16 NRTI-experienced, INSTI-naïve persons receiving a dolutegravir -containing regimen in three phase III trials (Cahn et al., 2013; Paton et al., 2022; Underwood et al., 2022), 8 developed R263K, 6 developed G118R, 2 developed N155H, and 1 developed a Q148 mutation (Q148R). Q148R was more likely to develop in persons receiving CAB ($p < 0.001$; Fishers Exact Test) while R263K ($p = 0.007$) and G118R ($p = 0.01$) were more likely to develop in persons receiving dolutegravir.

3.5. *In vitro susceptibility*

Sixteen studies reported *in vitro* susceptibility data including three published by ViiV Healthcare (Jeffrey et al., 2022; Yoshinaga et al., 2015, 2018), three by the British Columbia Centre for Excellence in HIV/AIDS (BCE) (Cheung et al., 2022; Shahid et al., 2019; Zhang et al., 2018), three by McGill University (Hassounah et al., 2017; Oliveira et al., 2018; Pham et al., 2018), two by the National Cancer Institute (NCI) (Smith et al., 2018, 2020), one by Sienna University (Saladini et al., 2019), one by Nagoya University (Hachiya et al., 2017b), one by the Ugandan JCRC (Ndashimye et al., 2021), and two containing data from clinical trials using the PhenoSense assay (Overton et al., 2021; Rizzardini et al., 2020). The studies by ViiV, the NCI, and the Nagoya University analyzed site-directed mutants. The studies by Sienna University, the Ugandan JCRC, and the isolates tested by the PhenoSense assay were clinical isolates. The BCE and McGill University laboratories tested both clinical and laboratory isolates.

Susceptibility results were available for 425 isolates including 368 containing one (n = 116), two (n = 149), or ≥ three (n = 103) INSTI DRMs. Twenty mutations were present in 10 or more isolates including: M50I, T66I, L74M/I, E92Q, Q95K, T97A, E138 A/K, G140 A/C, Y143R, S147G, Q148 H/K/R, N155H, E157Q, G163R, and R263K. Fig. 2 shows phenotypic susceptibility for the 57 patterns of mutations having two or more susceptibility results: 24 patterns with one DRM, 21 with two DRMs, and 12 with three DRMs. Seven mutations were present as the only INSTI DRM had a median fold reduction in susceptibility ≥2.0-fold including G118R (8-fold, 3 isolates), Q148R (4.1-fold, 11 isolates), G140R (3.8-fold, 2 isolates), Q148K (3.1 fold, 3 isolates), R263K (2.5-fold, 8 isolates), S153Y (2.2-fold, 4 isolates), and N155H (2.1-fold, 14 isolates). Q148H, which appeared in many highly resistant isolates containing 2 or 3 DRMs had a median fold reduced susceptibility of 1.9 in the absence of other DRMs. G140R alone had a fold-reduction in susceptibility of 6.7 fold in a clinical isolate from a patient with virological failure while receiving CAB but just a 1.1 fold reduction in susceptibility in a site-directed mutant. Neither the complete sequence nor list of accompanying mutations in the clinical isolate were published.

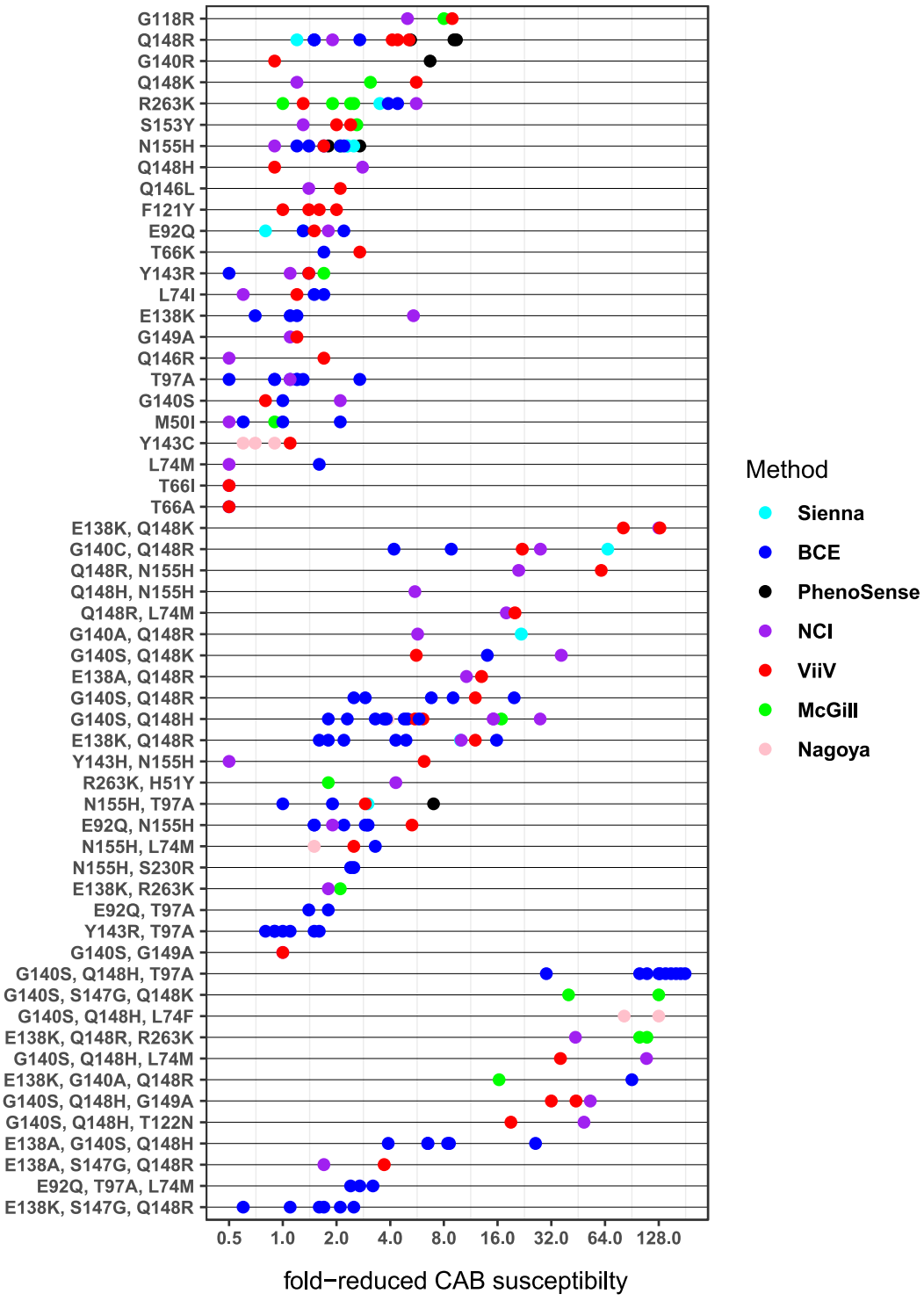

Fig. 2. Fold reduction in susceptibility for HIV-1 isolates containing one, two, or three INSTI drug-resistance mutations (DRMs) for which at least two results were available. The X-axis indicates the fold-reduction in susceptibility on a base 2 log scale. Overlapping fold-reductions were minimally adjusted so that all results would be displayed. HIV-1 isolates tested by ViiV and NCI were site-directed mutants whereas those tested by the remaining laboratories were clinical isolates. Four results that were more than 10 times higher than the median of at least five other results were included in our analysis but are not shown in the figure. Five clinical isolates tested by the Joint Clinical Research Centre in Uganda (Ndashimye et al., 2021) that contained either N155H or Y143R alone are not shown.

Regularized regression analysis of the impact of INSTI DRMs on CAB susceptibility demonstrated that the following 14 mutations had a mean importance >0.1 and a p value < 0.001: M50I, L74 F/M, T97A, G118R, E138K, G140 A/C/S, Q148 H/K/R, N155H, and R263K (Fig. 3). Each of these mutations occurred in ten or more isolates with the exception of G140C, L74F, and G118R which occurred in seven, six, and four isolates, respectively. M50I, L74 F/M, T97A, E138K, and G140 A/C/S appeared to reduce susceptibility only when they occurred with other mutations, particularly at position 148 (Fig. 2). Although six site-directed mutants containing L74F underwent susceptibility testing, this mutation has been reported in only a single person. F121C, which was present in just a single clinical isolate, in combination with another rare mutation T97M,

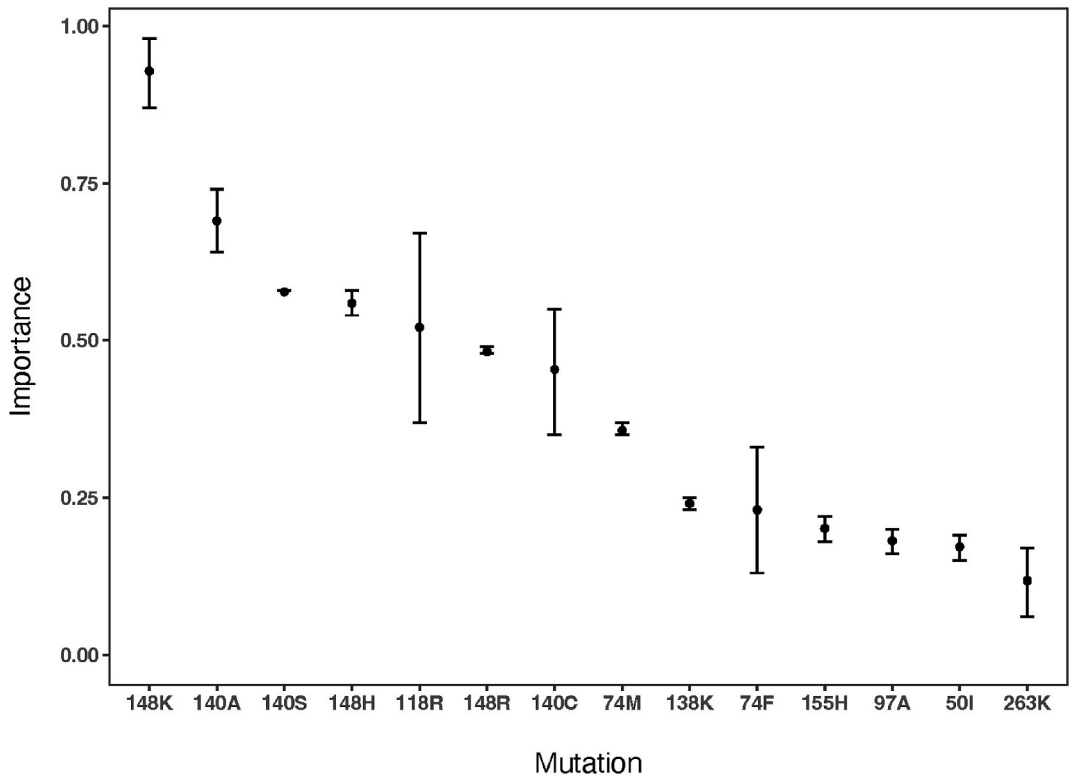

Fig. 3. Regularized regression analysis of the integrase mutations significantly associated with reduced CAB susceptibility by LASSO for predicting $\log_{10}$ fold reduced susceptibility. Ten-fold cross-validation was performed 1000 times without replacement using the glmnet R package. The mean importance (points) and 95% confidence intervals (error bars) for each mutation are shown.

was associated with a 77-fold reduction in CAB susceptibility.

Most isolates tested for CAB susceptibility was also tested for dolutegravir susceptibility by the same assay. The overall regression coefficient between CAB and dolutegravir was 0.73 ($p < 1e\text{-}16$). The correlation increased with the number of INSTI-resistance mutations. Isolates with one DRM had a median 1.2-times greater fold reduced CAB susceptibility (paired *t*-test; $p < 0.001$) while those with two DRMs had a median 1.9-times greater fold reduced CAB susceptibility (paired *t*-test; $p < 0.001$) compared with dolutegravir. Although 46 isolates had reductions in susceptibility that were four fold higher for CAB as compared to dolutegravir (indicated by blue points in Fig. 4), we were not able to discern a specific pattern of mutations that influenced the relative fold resistance to these two INSTIs.

4. Discussion

We reviewed three types of studies to characterize the genotypic changes associated with CAB resistance: studies reporting mutations emerging during *in vitro* passage experiments, studies reporting mutations developing in HIV-infected persons receiving CAB, and studies reporting the effect of integrase mutations on *in vitro* CAB susceptibility. The most commonly selected mutations in the 24 persons with

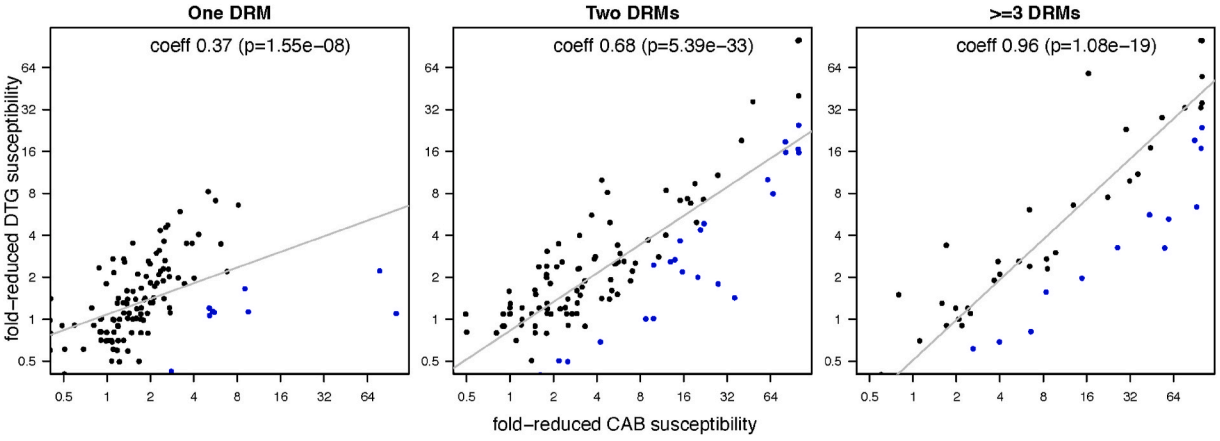

Fig. 4. Correlation between reduced CAB and dolutegravir susceptibility. Susceptibility results on both axes are shown on a $\log_2$ scale according to whether the tested isolates contained one, two, or three or more INSTI-resistance mutations. Blue points indicate those for which the fold-reduced susceptibility for CAB was four or more times higher than the fold-reduced susceptibility for dolutegravir. Overall, 293 isolates containing 30 INSTI-resistance mutations were compared. The following 15 mutations occurred in 10 or more viruses and accounted for 91% of all mutations: T66I, L74M, E92Q, T97A, E138 A/K, G140 A/S, Y143R, S147G, Q148 H/K/R, N155H, and R263K.

virological failure and emergent resistance while receiving CAB included E138K, Q148R, and N155H which occurred in 5–15 persons while T97A, G118R, G140 A/R/S, and R263K occurred in 1–2 persons. Each of these mutations with the exception of T97A, G118R, and G140 A/R were also reported to emerge *in vitro*, while G140R was reported to emerge in a non-human primate receiving CAB. None of the 24 sequences with emergent resistance were publicly available, thus it is not possible to know whether additional mutations were also present in these viruses. Although CAB and dolutegravir have similar molecular structures, they select for different but overlapping DRMs with Q148R occurring more commonly in patients receiving CAB but G118R and R263K occurring more commonly in patients receiving dolutegravir. The predilection for developing Q148 mutations may have a structural basis as mutations at this position appear to be selected more often for CAB than for dolutegravir *in vitro* as well as *in vivo* (Oliveira et al., 2018; Rhee et al., 2019; Smith et al., 2018).

Three of the mutations associated with reduced CAB susceptibility were polymorphic. M50I has a prevalence of 3%–34% in INSTI-naïve persons depending on subtype. L74M and T97A have a prevalence of 1%–5% depending on subtype. Considering the frequency of these mutations in INSTI-naïve persons and the infrequency with which CAB resistance emerges in persons receiving PrEP or CAB/RPV, it is unlikely that these mutations are important risk factors for virological failure on a CAB-containing regimen.

Two novel mutations, L74I and G140R, gained attention because of the ATLAS and FLAIR trials. L74I is a highly polymorphic mutation with a prevalence of 3%–35% in the eight most common subtypes. It is the consensus amino acid in subtype A viruses belonging to the A6 clade which is common in several countries of the former Soviet Union. Subtype A6 viruses with L74I were present at baseline in five of the six persons in the ATLAS and FLAIR trials with virological failure and emergent INSTI or RPV resistance. However, In contrast to L74M, L74I is not more common in persons receiving INSTIs compared with in INSTI-naïve persons nor has it been shown to reduce susceptibility to any of the INSTIs either alone or in combination with other mutations (Hu et al., n. d.; Jeffrey et al., 2022).

G140R is an extremely rare mutation with reduced replication capacity that has been reported in one person receiving CAB and not in any of the more than 2500 persons receiving an INSTI (Tzou et al., 2020). It has also been selected in a non-human primate receiving CAB. Although a site-directed mutant containing G140R did not reduce CAB susceptibility, the one clinical isolate with this mutation had seven-fold reduced CAB susceptibility suggesting that it requires a specific mutational background to reduce CAB susceptibility.

Two mechanisms of INSTI resistance involving mutations outside of integrase have been reported including one caused by mutations in and near the 3′ polypurine tract (3′PPT) and one caused by mutations in the envelope glycoproteins. 3′PPT mutations have been selected during *in vitro* passage experiments with dolutegravir (Malet et al., 2017), however, they are rare in persons receiving INSTIs (Malet et al., 2019) and there are conflicting data on whether these mutations contribute to reduced susceptibility in all cell lines (Dekker et al., 2022; Smith et al., 2021; Wei and Sluis-Cremer, 2021). Envelope mutations that increase the efficiency of cell-to-cell viral spread have been shown to reduce HIV-1 susceptibility to multiple antiretroviral drug classes in multi-cycle replication assays (Hikichi et al., 2021; Van Duyne et al., 2019). These mutations have not been reported in clinical isolates from INSTI-treated persons.

Our analysis of *in vitro* susceptibility data had several limitations. First, the findings were likely biased by the distribution of mutations in the viruses selected for susceptibility testing. Second, there was a large spread in the susceptibility for viruses containing the same DRMs likely explained by the different assays used to determine CAB susceptibility and because many of the isolates were obtained from patient samples that almost certainly had additional polymorphic mutations that can influence susceptibility. Finally, considering the large number of potential INSTI resistance mutations relative to the number of phenotypic results, it is possible that mutations other than those that we identified may also contribute to reduced susceptibility.

5. Conclusions

CAB occupies a unique niche in HIV prevention and therapy. Because it is either used alone (for PrEP) or in combination with RPV (which has a low genetic barrier to resistance), CAB-containing regimens are expected to have a low-genetic barrier to resistance. Although CAB and dolutegravir have similar molecular structures, they select for different but overlapping DRMs with Q148R occurring more commonly in patients receiving CAB but G118R and R263K occurring more commonly in patients receiving dolutegravir. The extent to which these differences reflect intrinsic properties of the two drugs as opposed to the clinical scenarios in which they are used require further study. Careful pretreatment screening is required to prevent CAB from being used in persons with active HIV-1 replication or in persons harboring viruses with reduced RPV susceptibility. Close virological monitoring is required to identify patients with unsuppressed viral load as quickly as possible, to minimize virus exposure to CAB and the generation of HIV-1 with cross-resistance to other INSTIs.

Declaration of competing interest

PRH has served as a consultant to ViiV in the past 5 years outside the submitted work. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data and code are available as supplementary material.

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Appendix A. Supplementary data

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