**TITLE**

Integrase Strand Transfer Inhibitor Resistance Mutations for the Surveillance of Transmitted HIV-1 Drug Resistance

**RUNNING TITLE**

INSTI Resistance Mutations for HIV-1 TDR Surveillance

**AUTHORS**

Philip L. TZOU\*1, Soo-Yon RHEE1, Diane DESCAMPS2, Dana S. CLUTTER3, Bradley HARE4, Orna MOR5, Maxime GRUDE6, Neil PARKIN7, Michael R. JORDAN8, Silvia BERTAGNOLIO9, Jonathan M. SCHAPIRO10, P. Richard HARRIGAN11, Anna Maria GERETTI12, Anne-Geneviève MARCELIN6, Robert W. SHAFER1 for the WHO HIVResNet working groups.

**AFFILIATIONS**

1Division of Infectious Diseases, Department of Medicine, Stanford University, Stanford CA, U.S.; 2Université de Paris; IAME, INSERM, F-75018 Paris, France; AP-HP, Hôpital Bichat, Laboratoire de Virologie, F-75018 Paris, France; 3Kaiser-Permanente Medical Care Program – Northern California, South San Francisco, CA; 4Kaiser-Permanente Medical Care Program – Northern California, San Francisco, CA; 5Central Virology Laboratory, Sheba Medical Center, Ministry of Health, Ramat-Gan, Israel and Sackler School of Medicine, Tel-Aviv University, Ramat-Aviv Israel; 6Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique (IPLESP), AP-HP, Hôpital Pitié-Salpêtrière, Department of Virology, F-75013 Paris, France; 7Data First Consulting Inc., Belmont CA, U.S; 8Tufts University School of Medicine, Boston MA, U.S.; 9Department of HIV and Global Hepatitis Programme, WHO, Geneva, Switzerland; 10National Hemophilia Center, Shaba Medical Center, Ramat Gan, Israel; 11Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada, 12Department of Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, Liverpool, UK

**\*CORRESPONDING AUTHOR**

P.L. Tzou

1000 Welch Road

Suite 202

Palo Alto, CA, 94304, USA

Tel: 650-736-8108

Email: philiptz@stanford.edu

Abstract: 250 words

Main text: 3682 words

**ABSTRACT**

Introduction: Integrase (IN) strand transfer inhibitors (INSTIs) are expected to be widely adopted globally, requiring surveillance of resistance emergence and transmission. We therefore sought to develop a standardized list of INSTI resistance mutations suitable for the surveillance of transmitted INSTI resistance.

Methods: To characterize the suitability of the INSTI-resistance mutations for transmitted HIV drug resistance surveillance, we classified them according to their presence on published expert lists, conservation in INSTI-naïve persons, frequency in INSTI-treated persons, and contribution to reduced *in vitro* susceptibility. Mutation prevalences were determined using IN sequences from 17,302 INSTI-naïve and 2,450 INSTI-treated persons. 53.3% of the INSTI-naïve sequences and 20.0% of INSTI-treated sequences were from non-B subtypes. Approximately 10% of sequences were from persons who received dolutegravir alone or a first-generation INSTI followed by dolutegravir.

Results: 59 established INSTI-resistance mutations were present on one or more of four published expert lists. They were classified into three main non-overlapping groups: 29 relatively common nonpolymorphic mutations, occurring in five or more individuals and significantly selected by INSTI treatment; eight polymorphic mutations; and 22 rare mutations. Among the 29 relatively common INSTI-selected mutations, 24 emerged as candidates for inclusion on a list of INSTI surveillance drug resistance mutations: T66A/I/K, E92G/Q, G118R, F121Y, E138A/K/T, G140A/C/S, Y143C/H/R/S, S147G, Q148H/R/K, N155H, S230R, and R263K.

Conclusion: A set of 24 nonpolymorphic INSTI-selected mutations is likely to be useful for quantifying INSTI-associated TDR. This list may require updating as more sequences become available from INSTI-experienced persons infected with HIV-1 non-subtype B viruses and/or receiving dolutegravir.

**INTRODUCTION**

Monitoring transmitted HIV drug resistance (TDR) is performed genotypically by testing for drug-resistance mutations (DRMs) highly specific for antiretroviral (ARV) drug selective pressure. A consensus list of surveillance DRMs (SDRMs) makes it possible to compare the prevalence of TDR over time and across different regions. However, developing such a consensus list of DRMs is challenging because many mutations contribute to reduced drug susceptibility including some that occur as natural variants or polymorphisms in ARV drug-naïve individuals.

We previously proposed a set of SDRMs for HIV-1 protease and reverse transcriptase (RT) based on the following criteria: (i) recognized as DRMs by HIV drug-resistance experts; (ii) being non-polymorphic regardless of subtype; and (iii) being not exceedingly rare (i.e. mutations resulting exceedingly rarely from drug pressure were excluded).1, 2 This set of SDRMs has been widely used to facilitate meta-analyses of TDR surveillance data generated by different public health and research groups particularly in low and middle income countries.3, 4

Dolutegravir, one of the drugs within the integrase strand transfer inhibitors (INSTI) class, has been recommended by the 2018 World Health Organization (WHO) ARV guidelines as the preferred first-line ART.5, 6 Several low and middle-income countries (LMIC) have already transitioned to dolutegravir and many more are in the planning phase; it is therefore important to report the emergence or transmission of resistance to this class of drugs using standardized approaches that generate comparable findings.7 Although INSTI resistance has been rare in population-based studies,8-14 there have now been multiple case reports of INSTI-associated TDR.15-19 However, because of the many mutations reported to be associated with reduced INSTI susceptibility, there is no standardized SDRM list to use when quantifying INSTI-associated TDR. We analyzed published data on INSTI-associated DRMs to develop a consensus list of candidate INSTI-associated surveillance DRMs using an approach similar to that used for an earlier list of HIV-1 protease and RT SDRMs.

**METHODS**

**Established INSTI-resistance mutations**

Established INSTI-resistance mutations were defined as mutations present on one or more of the following four mutation lists: Stanford HIV Drug Resistance Database (HIVDB), National Agency for AIDS Research (ANRS), REGA Institute, International Antiviral Society (IAS)-USA. INSTI-resistance positions were also ranked according the number of mutations at each position on these mutation lists, the extent of conservation of the position, and the literature on the mechanistic importance of the mutation to reduced INSTI susceptibility.

**Nonpolymorphic INSTI-resistance mutations**

The prevalence of each mutation in INSTI-naïve and INSTI-treated persons was determined using HIV-1 group M integrase sequences in HIVDB generated by direct PCR dideoxynucleotide sequencing. Sequences containing two or more signature APOBEC mutations were excluded. Mutations occurring in more than one sequence from the same persons were counted just once.

Mutation prevalences were calculated using two approaches. In the primary approach, amino acids were counted regardless of whether they occurred alone or as part of an electrophoretic mixture (i.e., the amino acid was present in combination with another amino acid – usually the wild type consensus). In a secondary analysis, mutation prevalence was determined counting only those amino acids present in pure unmixed form. Mutations which had large differences in prevalence among INSTI-naïve or INSTI-treated individuals between the two approaches were noted.

HIV-1 subtype was determined using the HIVDB subtyping program. The prevalence of each amino acid at each integrase position was determined among all INSTI-naïve and INSTI-treated persons and for each HIV-1 subtype and circulating recombinant form (CRF). Nonpolymorphic mutations were defined as mutations occurring at a prevalence <0.2% in all INSTI-naive persons, <0.5% in all subtypes for which more than 1,000 sequences were available, and <1% in all subtypes for which more than 200 sequences were available.

**INSTI-selected mutations**

 Each IN amino acid variant was examined for its association with INSTI-selection pressure by comparing its proportion in INSTI-treated individuals with its proportion in INSTI-naïve individuals using a Fisher’s Exact test. The Holm’s method was used to control the family-wise error rate for multiple-hypothesis testing at an adjusted p value ≤0.05.20 This analysis was performed separately for established INSTI-resistance mutations and for all remaining IN amino acids that occurred in five or more INSTI-treated persons and were at least three as common among INSTI-treated compared with INSTI-naïve persons.

**Phenotypic susceptibility**

To characterize the effects of established INSTI-resistance mutations on raltegravir, elvitegravir, and dolutegravir, we analyzed HIV-1 isolates in HIVDB for which *in vitro* phenotypic susceptibility data were available for each of these INSTIs. Phenotypic results were expressed as the fold-change in susceptibility defined as the 50% effective concentration (EC50) of the tested isolate divided by the EC50 of the standard wild-type control isolate used for the assay. Viruses from the same individual that contained the same pattern of INSTI-resistance mutations were excluded.

The effects of the established INSTI-resistance mutations on susceptibility were quantified using least squares regression, an approach previously published for each of the other ARV drug classes.21-23 The presence or absence of each mutation was an explanatory variable and the log10-fold change in susceptibility was the response variable. In each INSTI regression model, mutation coefficients were proportional to the contribution of the mutation to reduced susceptibility. Five-fold cross-validation was performed on randomly chosen subdivisions of the complete dataset. The complete dataset used for the regression analyses is downloadable (HIVDB Genotype-Phenotype Dataset, <https://hivdb.stanford.edu/pages/genopheno.dataset.html>).

**Principal components analysis**

We performed a principal components analysis to jointly analyze the INSTI-associated DRMs, according to six often overlapping characteristics:24 (i) recognizability: DRMs on four lists were assigned a 2 and those on two-to-three lists were assigned a 1; (ii) positional importance: DRMs at positions 66, 92, 118, 121, 138, 140, 143, 148, 155, and 263 were assigned a 2 and those at positions 145, 146, 147, 149, 151, and 153 were assigned a 1; (iii) non-polymorphism: DRMs with a naïve prevalence <0.2 were assigned a 2 and those with a naïve prevalence between 0.2 and 1.0 were assigned a 1; (iv) frequency: DRMs occurring in ≥10 INSTI-treated persons were assigned a 2 and those in five to nine persons were assigned a 1; (v) statistical association with INSTI therapy: DRMs with a corrected p ≤0.000001 (1.0E-6) were assigned a 2 and those with a corrected p between 1.0E-6 and 0.05 were assigned a 1; and (vi) statistical association with reduced susceptibility: DRMs with a linear regression coefficient ≥1.0 for one or more INSTIs were assigned a 2 and those with a coefficient between 0.5 and 1.0 were assigned a 1. If not otherwise applicable a 0 was assigned for each characteristic.

**Correlation network analysis**

We selected all IN sequences containing one or more of the 59 INSTI-associated DRMs. For persons with more than one IN sequence, we included only those sequences with a nonredundant pattern of INSTI-associated DRMs. Additionally, positions containing mixtures of a DRM and another amino acid were ignored. The first step in the network analysis was to create a list of positively correlated substitution pairs having a nonparametric Spearman correlation coefficient (*rho*) of (>0.075), at least three occurrences of each mutation, and a p value ≤0.00001. We used the R package igraph to create an undirected weighted network graph from the adjacency matrix of positively correlated amino acid substitution pairs.25 In this network, an edge was created between all correlated amino acid substitution pairs meeting the above criteria. Edge widths were positively correlated with *rho*.

**RESULTS**

**Established INSTI-resistance mutations**

Table 1 shows that 59 mutations at 26 positions that were on one or more mutation lists including 21 on four lists, 11 on three lists, 12 on two lists, and 15 on one list. Positions 66, 92, 118, 121, 138, 140, 143, 148, 155, and 263 were classified as important positions because they either had multiple DRMs (positions 66, 92, 138, 140, 143, and 155) or had a single DRM at a conserved position associated with a published mechanism of INSTI resistance (positions 118, 121, and 263).26, 27 Positions 145-147, 149, 151, and 153 were considered to be in important regions of IN by virtue of being in the flexible conserved “catalytic loop” extending between positions 140 to 149 or of being adjacent to the catalytic glutamate at position 152.26

**Mutation prevalence**

**Available sequences:** The prevalences of the 59 established INSTI-resistance mutations were determined using 17,425 sequences from 17,302 INSTI-naïve persons and 2,783 sequences from 2,450 INSTI-treated persons. The sequences from INSTI-treated persons were from 1,741 (71.1%) raltegravir-treated persons, 285 (11.6%) elvitegravir-treated persons, 152 (6.2%) dolutegravir-treated persons, 196 (8.0%) persons who received more than one INSTI, and 76 (3.1%) from persons with uncertain INSTI treatment histories.

 The sequences from INSTI-naïve individuals included 8,136 (46.7%) subtype B, 3,194 (18.3%) subtype C, 2,019 (11.6%) CRF01\_AE, 1,456 (8.4%) subtype A, 978 (5.6%) CRF02\_AG, 486 (2.8%) subtype D, 268 (1.5%) subtype F, 244 (1.4%) subtype G, and 644 (3.7%) sequences belonging to other CRFs and unique recombinant forms (URFs). The sequences from INSTI-treated persons included 2,226 (79.7%) subtype B sequences; 557 (20.3%) sequences belonged to other subtypes, CRFs, and URFs.

**Nonpolymorphic INSTI-selected DRMs:** Table 2 shows the prevalence of each of the 59 established INSTI-resistance mutations among INSTI-naïve and INSTI-treated persons overall and among INSTI-naïve persons according to subtype. The mutations are divided into (i) 29 nonpolymorphic mutations present in five or more INSTI-treated persons; (ii) 8 polymorphic mutations present in five or more INSTI-treated persons; and (iii) 22 rare mutations present in fewer than five INSTI-treated persons.

Of the 29 nonpolymorphic mutations present in five or more INSTI-treated persons, each was significantly selected by INSTI treatment with corrected p values <0.05: H51Y, T66A/I/K, V75I, E92G/Q, Q95K, H114Y, G118R, F121Y, E138A/K/T, G140A/C/S, Y143C/H/R/S, Q146R, S147G, Q148H/K/R, N155H, S230R, and R263K (Table 2). However, H51Y, Q95K, and H114Y were often present as part of an electrophoretic mixture in combination with the consensus amino acid and would not have met statistical significance had mutations been restricted to those occurring in an unmixed form (Supplementary Table 1). All of the nonpolymorphic INSTI-selected mutations were at highly conserved positions with the exception of S230R because S230N was present in 5.0% of INSTI-naïve sequences.

Among the 29 nonpolymorphic INSTI-selected mutations, Q95K, E138K and R263K were the only mutations with a prevalence in naïve persons above 0.1% (0.14% for Q95K, 0.18% for E138K and 0.13% for R263K). Nineteen of the 22 R263K mutations and ten of the 32 E138K mutations occurred as part of a mixture with wildtype (Supplementary Table 2). Although E138K and R263K could arise as a result of APOBEC-mediated DNA editing, none of the sequences with either of these mutations contained a stop codon or had other evidence for G-to-A hypermutation.

**Polymorphic established INSTI-resistance DRMs:** The eight polymorphic established INSTI-resistance mutations were A49P, L74I/M, T97A, E157Q, G163K/R, and D232N. With the exception of L74I, which was the most polymorphic, each was significantly associated with INSTI-treatment. E157Q occurred in 2.3% of all naïve sequences with prevalences of 3.2% in naïve subtype B and 6.4% in naïve CRF02\_AG sequences. L74M had T97A each had overall naïve prevalences of slightly more than 1%. However, L74M occurred in 8.3% of naïve CRF02\_AG sequences and T97A occurred in more than 5% of naïve subtype A and CRF02\_AG sequences. G163K/R had prevalences just below 0.5% overall but had prevalences above 5% in subtype F sequences. A49P and D232N had overall prevalences of 0.2% and 0.4%, respectively.

**Rare established INSTI-resistance DRMs:** Twenty-two of the established INSTI-resistance mutations were rare occurring in fewer than five INSTI-treated persons. Two of these 22 mutations were significantly selected by INSTI therapy (Y143G, and N155S). These rare mutations included 13 of the 44 mutations on two or more lists and nine of the 15 mutations on just one list.

***In vitro* susceptibility**

Phenotypic susceptibility results were available on 1,717 virus isolates, of which 988 (57.6%) were tested using the PhenoSense assay. Raltegravir susceptibility results were available on 1,555 isolates, of which 970 were tested using the PhenoSense assay. Elvitegravir susceptibility results were available on 1,443 isolates, of which 896 were tested using the PhenoSense assay. Dolutegravir susceptibility results were available on 754 isolates, of which 280 were tested using the PhenoSense assay.

Of the 29 nonpolymorphic INSTI-associated mutations that occurred in five or more persons, 21 had a regression coefficient ≥0.5 log10 to one or more INSTIs consistent with a ≥3.2-fold contribution to reduced susceptibility: T66A/I/K, E92G/Q, G118R, F121Y, E138T, G140A/C/S, Y143C/R/S, S147G, Q148H/K/R, N155H, S230R, and R263K (Table 3). The remaining eight nonpolymorphic INSTI-associated mutations, H51Y, V75I, Q95K, H114Y, E138A/K, Y143H, and Q146R, had regression coefficients <0.5 log10.

Two of the eight polymorphic mutations (A49P and G163K) and 14 of the 23 very rare mutations (L74F, E92V, Y143A/G/K, P145S, Q146I/L/P, V151A/L, S153Y, and N155S/T) also had a regression coefficient ≥0.5 log10.

**Non-established INSTI-selected mutations**

 Thirty-two additional nonpolymorphic mutations occurred in five or more INSTI-treated persons were significantly selected by INSTI therapy (corrected p<0.05) including four mutations at established INSTI-resistance positions (H51D, E92A, Q95R, and N155D); and one mutation (N142T) in the highly conserved catalytic loop extending between positions 140 and 149 (Table 4).

**Principal components analysis**

We jointly analyzed each of the 59 established INSTI-resistance mutations according to six often overlapping characteristics – recognizability, positional importance, non-polymorphism, frequency, statistical association with INSTI therapy, and statistical association with reduced INSTI susceptibility – as outlined in the Methods. For each feature, each DRM was scaled to have a high (2), intermediate (1), or low (0) association (Table 5). As the six mutational features were often correlated with one another, we performed a principal components analysis to cluster the mutations in two dimensions. This analysis yielded three main groupings of mutations (Figure 1). One contained 24 of the 29 nonpolymorphic INSTI-selected mutations, another contained the 22 rare mutations, and a third contained the eight polymorphic mutations.

Of the 29 nonpolymorphic INSTI-selected mutations, 20 were tightly clustered. Three mutations, T66K, F121Y, and Y143S were somewhat less tightly clustered because they occurred in just six to eight treated persons. S230R was also less tightly clustered in part because it was at a position containing the common polymorphism S230N. Five additional nonpolymorphic INSTI-selected mutations (H51Y, V75I, Q95K, H114Y, and Q146R) did not cluster with the remaining 24 mutations. None of these five mutations were significantly associated with reduced INSTI susceptibility and each was relatively uncommon or owed its association with therapy to usually being present as part of a mixture with wildtype.

**Correlation network analysis**

Figure 2 shows that of the 59 INSTI-resistance mutations, 25 frequently co-occurred with one or more other mutations including 20 nonpolymorphic INSTI-selected mutations (H51Y, E92Q, H114Y, E138A/K/T, G140A/C/S, Y143C/H/R/S, S147G, Q148H/K/R, N155H, S230R, and R263K), three polymorphic INSTI-selected mutations (L74M, T97A, and D232N), and two rare mutations (A49G and Y143G). Among the signature raltegravir-resistance mutation at position 148, Q148H was strongly linked with G140S and E138A/T; Q148R with E138K, G140A/C, and S147G; and Q148K with E138K and G140A. Y143C/G/H/R were each significantly linked with T97A. Y143C was also strongly linked to S230R.

**DISCUSSION**

We analyzed 59 INSTI-resistance mutations on four expert panel mutation lists according to six often overlapping characteristics. Despite their large number, the mutations could be classified into three main groups: 29 nonpolymorphic mutations significantly selected by INSTI treatment; eight polymorphic mutations of which seven were significantly selected by INSTI treatment, and 22 rare mutations of which nearly all were too uncommon to be significantly selected by INSTI treatment. The 29 nonpolymorphic INSTI-selected mutations were subjected to more scrutiny and 24 emerged as strong candidates for inclusion on a list of INSTI surveillance DRMs: T66A/I/K, E92G/Q, G118R, F121Y, E138A/K/T, G140A/C/S, Y143C/H/R/S, S147G, Q148H/R/K, N155H, S230R, and R263K.

HIV-1 DRMs can be classified according to whether they are nonpolymorphic in the absence of ARV therapy, whether they are selected *in vitro* during ARV passage experiments or *in vivo* in persons receiving ARV therapy, their effects on *in vitro* susceptibility, and virological response to ARV therapy. Of the 24 suggested INSTI surveillance DRMs, 18 were on all four of the published expert DRM lists, five were on three lists, and one was on two lists. Therefore, the presence of one of these mutations in a person initiating therapy is evidence for pre-treatment resistance, regardless of whether it arose from previous therapy or from transmitted resistance.

We used a strict threshold for defining polymorphisms by excluding mutations with an overall prevalence ≥0.25%, a prevalence ≥0.5% in any subtype or CRF with 1000 or more persons, or a prevalence ≥1.0% in any subtype or CRF with 200 or more persons. This threshold is twice as high as the threshold we used to define nonpolymorphic mutations in a paper published in 2009 to select DRMs for RT inhibitor and protease inhibitor-associated TDR surveillance.2 It was possible to use a lower threshold for INSTI-resistance mutations without sacrificing sensitivity because the rarity of transmitted INSTI resistance may have made it unlikely that nonpolymorphic INSTI-resistance DRMs were detected in INSTI-naïve persons.2 It was possible to use a lower threshold for INSTI-resistance mutations without sacrificing sensitivity because the rarity of transmitted INSTI resistance may have made it unlikely that nonpolymorphic INSTI-resistance DRMs were detected in INSTI-naïve persons. Therefore, the presence of an INSTI surveillance DRM in an untreated person strongly suggests that the transmitted virus had previously been exposed to INSTI treatment.

The WHO drug resistance surveillance program initially classified TDR in recently infected persons using the list of protease and RT SDRMs. In 2014, surveillance of TDR was deprioritized in favor of estimating the prevalence of drug resistance in all patients initiating first-line ARV therapy – referred to as pretreatment drug resistance (PDR). PDR includes those with TDR as well as those in whom ARV therapy had been interrupted or who had received ARV therapy to prevent mother-to-child transmission.28, 29

As WHO-recommended surveys of PDR aim to predict susceptibility of virus to ARV drugs used in first- and second-line regimens, in order to inform global and national ART selection, the Stanford HIVdb drug resistance interpretation system has been used to interpret the data, because it takes into consideration the contribution of polymorphisms to predicted drug resistance.29 In contrast, the SDRM list is designed for epidemiological purposes – i.e., documentation of transmission of drug-resistant virus – and therefore is not intended for use when analyzing data from surveys of PDR. Nonetheless, for many investigators, the list will be useful for distinguishing polymorphic from nonpolymorphic INSTI DRMs because polymorphic DRMs that occur in the absence of therapy are often subtype dependent and may influence regional and temporal estimates of TDR.

A set of DRMs designed for public health surveillance as opposed to individual patient management need not contain rare mutations, which are less likely to be recognized as DRMs by experts, public health officials, and laboratory personnel. Rare DRMs are also less likely to be significantly associated with ARV drug selection pressure. The 22 rare mutations in this study included nine variants at the well-recognized INSTI-resistance positions 92, 143, 148, and 155 and eight variants at the highly conserved positions 145, 146, 149, and 153. Of these, E92V, V151A/L, and N155S/T have been observed during *in vitro* passage with investigational INSTIs and are associated with variably reduced susceptibility to current INSTIs.30, 31 Y143A/K are raltegravir-selected variants associated with reduced raltegravir susceptibility.32, 33 P145S and Q146P are elvitegravir-selected mutations associated with reduced elvitegravir susceptibility.34, 35 S153Y/F have been selected *in vitr*o by multiple INSTIs and cause minimal reductions in INSTI susceptibility.30, 36

Approximately 80% of the sequences from treated persons were subtype B viruses and these were usually from persons treated with raltegravir and elvitegravir. This is not surprising because INSTIs have been primarily used in upper-income countries that have a lower prevalence of non-subtype B viruses37-41 with relatively small numbers of reports of INSTI resistance emanating from low- and middle-income countries.42, 43

WHO’s 2018 ARV guidelines recommend the use of dolutegravir as preferred first-line ART.5, 6 It is expected that the new regimen will be adopted widely in many regions of the word, including settings with non-B subtype and where programmatic challenges, such as ARV drug stock outs, might result in the emergence of INSTI resistance and eventually its transmission.7 As INSTIs become more widely used in areas with a high prevalence of non-B variants this list may need to be updated. In addition, virological failure with emergent resistance has been uncommon in persons receiving the second-generation INSTIs, dolutegravir and bictegravir. Several of the rare mutations might eventually be considered SDRM candidates should they prove to be common after more sequences become available, particularly sequences from persons with virological failure on a second-generation INSTI such as dolutegravir, bictegravir, or cabotegravir.

**FUNDING**

This work was supported by the National Institute of Allergy and Infectious Diseases at the National Institutes of Health (R24 AI136618) and the Agence Nationale de Recherches sur le SIDA et les hépatites virales.

**TRANSPARENCY DECLARATIONS**

PLT, SYR, and RWS were supported by a grant from the National Institute of Allergy and Infectious Diseases at the National Institutes of Health (R24 AI136618). DD, MG, and AGM were supported by grants from the Agence Nationale de Recherches sur le SIDA et les hépatites virales. Funders had no decision-making role in manuscript. The remaining authors have none to declare.

**FIGURE LEGENDS**

Figure 1

Principal components analysis of the 59 INSTI-resistance mutations on one or more expert lists. The original characteristics or variables were related to the number of expert lists containing the mutation, the positional importance of the mutation, the frequency of the mutation in INSTI-naïve and INSTI-experienced persons, and the statistical association of the mutation with reduced susceptibility to one or more INSTIs. The mutations at the upper right are nonpolymorphic mutations significantly associated with INSTI treatment. Those at the lower right are polymorphic mutations significantly associated with therapy. These on the left are rare nonpolymorphic mutations which nearly always occurred too infrequently to be associated with therapy.

Figure 2

Correlation network analysis of the 24 INSTI-resistance that most frequently co-occurred with one or more other INSTI-resistance mutations. INSTI-resistance mutations having a nonparametric Spearman correlation coefficient (*rho*) of >0.075 and a p value ≤0.00001 are linked with an edge. Edge thickness is proportional to rho with the greatest thicknesses for the edge between G140S and Q148H (rho = 0.93), Y143C and S230R (rho = 0.65), and G140A and Q148R (*rho* = 0.38).

**REFERENCES**

1. Shafer RW, Rhee SY, Pillay D, *et al*. HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. *AIDS* 2007; **21**:215-23.

2. Bennett DE, Camacho RJ, Otelea D, *et al*. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. *PLoS One* 2009; **4**:e4724.

3. Gupta RK, Gregson J, Parkin N, *et al*. HIV-1 drug resistance before initiation or re-initiation of first-line antiretroviral therapy in low-income and middle-income countries: a systematic review and meta-regression analysis. *Lancet Infect Dis* 2018; **18**:346-55.

4. Rhee SY, Blanco JL, Jordan MR, *et al*. Geographic and temporal trends in the molecular epidemiology and genetic mechanisms of transmitted HIV-1 drug resistance: an individual-patient- and sequence-level meta-analysis. *PLoS Med* 2015; **12**:e1001810.

5. World Health Organization. Updated recommendations on first-line and second-line antiretroviral regimens and post-exposure and recommendations on early infant diagnosis. Interim guidance. <https://www.who.int/hiv/pub/guidelines/ARV2018update/en/> 2018.

6. Vitoria M, Hill A, Ford N, *et al*. The transition to dolutegravir and other new antiretrovirals in low-income and middle-income countries: what are the issues? *AIDS* 2018; **32**:1551-61.

7. World Health Organization. Global Action Plan on HIV Drug Resistance 2017-2021: 2018 Progress Report. <https://www.who.int/hiv/pub/drugresistance/gap-hivdr-progress2018/en/> 2018.

8. Chen I, Zhang Y, Cummings V, *et al*. Analysis of HIV Integrase Resistance in Black Men Who Have Sex with Men in the United States. *AIDS Res Hum Retroviruses* 2017; **33**:745-8.

9. Ji H, Patterson A, Taylor T, *et al*. Prevalence of Primary Drug Resistance Against HIV-1 Integrase Inhibitors in Canada. *J Acquir Immune Defic Syndr* 2018; **78**:e1-e3.

10. Tostevin A, White E, Dunn D, *et al*. Recent trends and patterns in HIV-1 transmitted drug resistance in the United Kingdom. *HIV Med* 2017; **18**:204-13.

11. Stekler JD, McKernan J, Milne R, *et al*. Lack of resistance to integrase inhibitors among antiretroviral-naive subjects with primary HIV-1 infection, 2007-2013. *Antivir Ther* 2015; **20**:77-80.

12. Banaez Ocfemia MC, Saduvala N, Oster AM, *et al*. Transmitted HIV-1 drug resistance among men who have sex with men - 11 U.S. jurisdictions, 2008 -2011. *21st Conference on Retroviruses and Opportunistic Infections (CROI)*; March 3-6, 2014; Boston, MA Abstract 579 2015.

13. Acosta RK, Willkom M, Martin R, *et al*. Resistance Analysis of Bictegravir/Emtricitabine/Tenofovir Alafenamide in HIV-1 Treatment-Naive Patients Through 48 Weeks. *Antimicrobial agents and chemotherapy* 2019; **63**:e02533-18.

14. Jeong W, Jung IY, Choi H, *et al*. Integrase Strand Transfer Inhibitor Resistance Mutations in Antiretroviral Therapy-Naive and Treatment-Experienced HIV Patients in South Korea. *AIDS Res Hum Retroviruses* 2019; **35**:213-6.

15. Varghese V, Pinsky BA, Smith DS, Klein D, Shafer RW. Q148N, a Novel Integrase Inhibitor Resistance Mutation Associated with Low-Level Reduction in Elvitegravir Susceptibility. *AIDS Res Hum Retroviruses* 2016; **32**:702-4.

16. Young B, Fransen S, Greenberg KS, *et al*. Transmission of integrase strand-transfer inhibitor multidrug-resistant HIV-1: case report and response to raltegravir-containing antiretroviral therapy. *Antivir Ther* 2011; **16**:253-6.

17. McGee KS, Okeke NL, Hurt CB, McKellar MS. Canary in the Coal Mine? Transmitted Mutations Conferring Resistance to All Integrase Strand Transfer Inhibitors in a Treatment-Naive Patient. *Open Forum Infect Dis* 2018; **5**:ofy294.

18. Volpe JM, Ward DJ, Napolitano L, *et al*. Five Antiretroviral Drug Class-Resistant HIV-1 in a Treatment-Naive Patient Successfully Suppressed with Optimized Antiretroviral Drug Selection. *J Int Assoc Provid AIDS Care* 2015; **14**:398-401.

19. Boyd SD, Maldarelli F, Sereti I, *et al*. Transmitted raltegravir resistance in an HIV-1 CRF\_AG-infected patient. *Antivir Ther* 2011; **16**:257-61.

20. Aickin M, Gensler H. Adjusting for multiple testing when reporting research results: the Bonferroni vs Holm methods. *Am J Public Health* 1996; **86**:726-8.

21. Rhee SY, Taylor J, Fessel WJ, *et al*. HIV-1 protease mutations and protease inhibitor cross-resistance. *Antimicrobial agents and chemotherapy* 2010; **54**:4253-61.

22. Melikian GL, Rhee SY, Taylor J, *et al*. Standardized comparison of the relative impacts of HIV-1 reverse transcriptase (RT) mutations on nucleoside RT inhibitor susceptibility. *Antimicrobial agents and chemotherapy* 2012; **56**:2305-13.

23. Melikian GL, Rhee SY, Varghese V, *et al*. Non-nucleoside reverse transcriptase inhibitor (NNRTI) cross-resistance: implications for preclinical evaluation of novel NNRTIs and clinical genotypic resistance testing. *J Antimicrob Chemother* 2014; **69**:12-20.

24. Pedregeosa F, Varoquaux G, Gramfort A, *et al*. Scikit-learn: machine learning in python. *J Machine Learning Res* 2011; **12**:2825-30.

25. Csardi G, T N. igraph - The network analysis package. <https://igraph.org/> 2018.

26. Mouscadet JF, Delelis O, Marcelin AG, Tchertanov L. Resistance to HIV-1 integrase inhibitors: A structural perspective. *Drug Resist Updat* 2010; **13**:139-50.

27. Hare S, Smith SJ, Metifiot M, *et al*. Structural and functional analyses of the second-generation integrase strand transfer inhibitor dolutegravir (S/GSK1349572). *Mol Pharmacol* 2011; **80**:565-72.

28. World Health Organization. Guidelines on the Public Health Response to Pretreatment HIV Drug Reistance. <http://www.who.int/hiv/pub/guidelines/hivdr-guidelines-2017/en/> 2017.

29. World Health Organization. Surveillance of HIV drug resistance in adults initiating antiretroviral therapy (pre-treament HIV drug resistance). <https://apps.who.int/iris/bitstream/handle/10665/112802/9789241507196_engpdf?sequence=1> 2014.

30. Kobayashi M, Yoshinaga T, Seki T, *et al*. In Vitro antiretroviral properties of S/GSK1349572, a next-generation HIV integrase inhibitor. *Antimicrobial agents and chemotherapy* 2011; **55**:813-21.

31. Jones GS, Yu F, Zeynalzadegan A, *et al*. Preclinical evaluation of GS-9160, a novel inhibitor of human immunodeficiency virus type 1 integrase. *Antimicrobial agents and chemotherapy* 2009; **53**:1194-203.

32. Huang W, Frantzell A, Fransen S, *et al*. Multiple genetic pathways involving amino acid position 143 of HIV-1 integrase are preferentially associated with specific secondary amino acid substitutions and confer resistance to raltegravir and cross-resistance to elvitegravir. *Antimicrobial agents and chemotherapy* 2013; **57**:4105-13.

33. Canducci F, Ceresola ER, Boeri E, *et al*. Cross-resistance profile of the novel integrase inhibitor Dolutegravir (S/GSK1349572) using clonal viral variants selected in patients failing raltegravir. *J Infect Dis* 2011; **204**:1811-5.

34. Shimura K, Kodama E, Sakagami Y, *et al*. Broad antiretroviral activity and resistance profile of the novel human immunodeficiency virus integrase inhibitor elvitegravir (JTK-303/GS-9137). *J Virol* 2008; **82**:764-74.

35. Smith SJ, Zhao XZ, Burke TR Jr., *et al*. Efficacies of Cabotegravir and Bictegravir against drug-resistant HIV-1 integrase mutants. *Retrovirology* 2018; **15**:37.

36. Margot NA, Hluhanich RM, Jones GS, *et al*. In vitro resistance selections using elvitegravir, raltegravir, and two metabolites of elvitegravir M1 and M4. *Antiviral Res* 2012; **93**:288-96.

37. Nguyen T, Fofana DB, Le MP, *et al*. Prevalence and clinical impact of minority resistant variants in patients failing an integrase inhibitor-based regimen by ultra-deep sequencing. *J Antimicrob Chemother* 2018; **73**:2485-92.

38. Cavalcanti Jde S, Ferreira JL, Guimaraes PM, *et al*. High frequency of dolutegravir resistance in patients failing a raltegravir-containing salvage regimen. *J Antimicrob Chemother* 2015; **70**:926-9.

39. Molina JM, Lamarca A, Andrade-Villanueva J, *et al*. Efficacy and safety of once daily elvitegravir versus twice daily raltegravir in treatment-experienced patients with HIV-1 receiving a ritonavir-boosted protease inhibitor: randomised, double-blind, phase 3, non-inferiority study. *Lancet Infect Dis* 2012; **12**:27-35.

40. Hurt CB, Sebastian J, Hicks CB, *et al*. Resistance to HIV integrase strand transfer inhibitors among clinical specimens in the United States, 2009-2012. Clin Infect Dis 2014; 58:423-31.

41. Marcelin AG, Grude M, Charpentier C, *et al*. Resistance to integrase inhibitors: a national study in HIV-1-infected treatment-naive and -experienced patients. *J Antimicrob Chemother* 2019; **74** issue 5: 1368-1375.

42. Pujari SN, Gaikwad S, Joshi K, *et al*. Integrase Resistance-Associated Mutations on Raltegravir Failure in Western India: A Preliminary Analysis. *J Acquir Immune Defic Syndr* 2018; **77**:e42-e5.

43. Ndashimye E, Avino M, Kyeyune F, *et al*. Absence of HIV-1 Drug Resistance Mutations Supports the Use of Dolutegravir in Uganda. *AIDS Res Hum Retroviruses* 2018; **34**:404-14.

|  |
| --- |
| **Table 1. HIV-1 Integrase Mutations Reported on Four Lists of INSTI-Resistance Mutations** |
| Position | Cons1 | AA | HIVDB2 | IAS-USA2 | ANRS2 | REGA2 | # Algs3 |  | Position | Cons1 | AA | HIVDB2 | IAS-USA2 | ANRS2 | REGA2 | # Algs3 |
| **66** | T | A | Y | Y | Y | Y | 4 |  | **155** | N | T | Y |  | Y | Y | 3 |
| **66** | T | I | Y | Y | Y | Y | 4 |  | **230** | S | R | Y |  | Y | Y | 3 |
| **66** | T | K | Y | Y | Y | Y | 4 |  | **51** | H | Y | Y |  |  | Y | 2 |
| **74** | L | M | Y | Y | Y | Y | 4 |  | **74** | L | F | Y |  | Y |  | 2 |
| **92** | E | Q | Y | Y | Y | Y | 4 |  | **75** | V | I | Y |  | Y |  | 2 |
| **97** | T | A | Y | Y | Y | Y | 4 |  | **92** | E | V | Y |  |  | Y | 2 |
| **121** | F | Y | Y | Y | Y | Y | 4 |  | **138** | E | T | Y |  | Y |  | 2 |
| **138** | E | A | Y | Y | Y | Y | 4 |  | **143** | Y | A | Y |  | Y |  | 2 |
| **140** | G | A | Y | Y | Y | Y | 4 |  | **143** | Y | G | Y |  | Y |  | 2 |
| **140** | G | S | Y | Y | Y | Y | 4 |  | **143** | Y | K | Y |  |  | Y | 2 |
| **143** | Y | C | Y | Y | Y | Y | 4 |  | **146** | Q | P | Y |  |  | Y | 2 |
| **143** | Y | H | Y | Y | Y | Y | 4 |  | **149** | G | A | Y |  |  |  | 1 |
| **143** | Y | R | Y | Y | Y | Y | 4 |  | **151** | V | A | Y |  |  | Y | 2 |
| **147** | S | G | Y | Y | Y | Y | 4 |  | **157** | E | Q | Y |  | Y |  | 2 |
| **148** | Q | H | Y | Y | Y | Y | 4 |  | **163** | G | K | Y |  |  | Y | 2 |
| **148** | Q | K | Y | Y | Y | Y | 4 |  | **49** | A | G |  |  |  | Y | 1 |
| **148** | Q | R | Y | Y | Y | Y | 4 |  | **49** | A | P |  |  |  | Y | 1 |
| **155** | N | H | Y | Y | Y | Y | 4 |  | **95** | Q | K | Y |  |  |  | 1 |
| **263** | R | K | Y | Y | Y | Y | 4 |  | **114** | H | Y |  |  |  | Y | 1 |
| **138** | E | K | Y | Y | Y | Y | 4 |  | **146** | Q | I |  |  |  | Y | 1 |
| **118** | G | R | Y | Y | Y | Y | 4 |  | **146** | Q | K |  |  |  | Y | 1 |
| **74** | L | I | Y |  | Y | Y | 3 |  | **146** | Q | L |  |  |  | Y | 1 |
| **92** | E | G | Y | Y |  | Y | 3 |  | **146** | Q | R |  |  |  | Y | 1 |
| **140** | G | C | Y |  | Y | Y | 3 |  | **148** | Q | E |  |  | Y |  | 1 |
| **143** | Y | S | Y |  | Y | Y | 3 |  | **148** | Q | G |  |  | Y |  | 1 |
| **145** | P | S | Y |  | Y | Y | 3 |  | **148** | Q | N | Y |  |  |  | 1 |
| **151** | V | L | Y |  | Y | Y | 3 |  | **163** | G | R | Y |  |  |  | 1 |
| **153** | S | F | Y |  | Y | Y | 3 |  | **230** | S | G |  |  |  | Y | 1 |
| **153** | S | Y | Y |  | Y | Y | 3 |  | **232** | D | N | Y |  |  |  | 1 |
| **155** | N | S | Y |  | Y | Y | 3 |  |  |  |  |  |  |  |  |  |
| 1Cons: Consensus amino acid (AA). 2Lists of INSTI Resistance Mutations: HIVDB: Stanford HIV Drug Resistance Database interpretation system; IAS-USA: International AIDS Society – USA Antiviral Resistance Mutations; ANRS – National Agency for AIDS Research interpretation system; Rega – Rega Institute drug resistance interpretation system. 3#Algs: # algorithms containing the mutation. |

|  |
| --- |
| **Table 2. Prevalence of INSTI-Resistance Mutations In INSTI-Naïve and Treated Persons** |
| Pos | AA | Prevalence in INSTI-Naïve Persons | Treated Prevalence – All Subtypes(n=2,450)# persons (%) | Corrected P |
| All Subtypesn=17,302# persons (%) | A(n=1447) | B(n=8040) | C(n=3194) | D(n=484) | F(n=268) | G(n=244) | 01(n=2019) | 02(n=978) | Highest |
| ***Nonpolymorphic*** |
| **51** | Y | 4 (0.0%) | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.1% | 12 (0.5%) | <1.0E-6 |
| **66** | A | 7 (0.0%) | 0.1% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 17 (0.7%) | <1.0E-6 |
| **66** | I | 8 (0.0%) | 0.1% | 0.0% | 0.1% | 0.2% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% | 36 (1.5%) | <1.0E-6 |
| **66** | K | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 6 (0.2%) | 0.0001 |
| **75** | I | 14 (0.1%) | 0.1% | 0.1% | 0.1% | 0.4% | 0.0% | 0.0% | 0.0% | 0.0% | 0.4% | 22 (0.9%) | <1.0E-6 |
| **92** | G | 4 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 10 (0.4%) | 0.00001 |
| **92** | Q | 1 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 137 (5.6%) | <1.0E-6 |
| **95** | K | 24 (0.1%) | 0.1% | 0.0% | 0.5% | 0.0% | 0.4% | 0.0% | 0.0% | 0.3% | 0.5% | 27 (1.1%) | <1.0E-6 |
| **114** | Y | 4 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 5 (0.2%) | 0.05 |
| **118** | R | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 9 (0.4%) | <1.0E-6 |
| **121** | Y | 1 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 6 (0.2%) | 0.0006 |
| **138** | A | 1 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 47 (1.9%) | <1.0E-6 |
| **138** | K | 32 (0.2%) | 0.1% | 0.3% | 0.1% | 0.4% | 0.0% | 0.0% | 0.0% | 0.0% | 0.4% | 127 (5.2%) | <1.0E-6 |
| **138** | T | 2 (0.0%) | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 15 (0.6%) | <1.0E-6 |
| **140** | A | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 40 (1.6%) | <1.0E-6 |
| **140** | C | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 9 (0.4%) | <1.0E-6 |
| **140** | S | 5 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 379 (15.5%) | <1.0E-6 |
| **143** | C | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 81 (3.3%) | <1.0E-6 |
| **143** | H | 3 (0.0%) | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 44 (1.8%) | <1.0E-6 |
| **143** | R | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 134 (5.5%) | <1.0E-6 |
| **143** | S | 2 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 9 (0.4%) | 0.000009 |
| **146** | R | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 8 (0.3%) | 0.000002 |
| **147** | G | 5 (0.0%) | 0.0% | 0.0% | 0.0% | 0.2% | 0.0% | 0.0% | 0.1% | 0.0% | 0.2% | 45 (1.8%) | <1.0E-6 |
| **148** | H | 4 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.4% | 0.0% | 0.0% | 0.4% | 344 (14.0%) | <1.0E-6 |
| **148** | K | 1 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 15 (0.6%) | <1.0E-6 |
| **148** | R | 7 (0.0%) | 0.0% | 0.0% | 0.0% | 0.2% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% | 159 (6.5%) | <1.0E-6 |
| **155** | H | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 575 (23.5%) | <1.0E-6 |
| **230** | R | 7 (0.0%) | 0.1% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 50 (2.1%) | <1.0E-6 |
| **263** | K | 22 (0.1%) | 0.2% | 0.2% | 0.0% | 0.0% | 0.8% | 0.0% | 0.0% | 0.2% | 0.8% | 24 (1.0%) | <1.0E-6 |
| ***Polymorphic*** |
| **49** | P | 42 (0.2%) | 0.1% | 0.4% | 0.0% | 0.8% | 0.4% | 0.0% | 0.1% | 0.0% | 0.8% | 16 (0.7%) | 0.01 |
| **74** | I | 1259 (7.3%) | 24.1% | 4.1% | 5.6% | 3.9% | 4.5% | 11.9% | 2.2% | 17.0% | 24.1% | 176 (7.2%) | 1 |
| **74** | M | 262 (1.5%) | 2.0% | 0.8% | 0.8% | 1.4% | 1.5% | 4.5% | 1.3% | 8.3% | 8.3% | 133 (5.4%) | <1.0E-6 |
| **97** | A | 305 (1.8%) | 6.5% | 0.6% | 0.8% | 5.2% | 6.0% | 2.9% | 0.6% | 6.3% | 6.5% | 292 (11.9%) | <1.0E-6 |
| **157** | Q | 400 (2.3%) | 1.2% | 3.2% | 0.7% | 3.9% | 0.4% | 1.6% | 0.7% | 6.4% | 6.4% | 132 (5.4%) | <1.0E-6 |
| **163** | K | 40 (0.2%) | 0.1% | 0.2% | 0.1% | 0.2% | 5.6% | 0.0% | 0.1% | 0.3% | 5.6% | 32 (1.3%) | <1.0E-6 |
| **163** | R | 81 (0.5%) | 0.2% | 0.3% | 0.6% | 0.2% | 7.8% | 0.4% | 0.2% | 0.0% | 7.8% | 116 (4.7%) | <1.0E-6 |
| **232** | N | 72 (0.4%) | 1.1% | 0.5% | 0.2% | 0.2% | 0.0% | 0.8% | 0.0% | 0.2% | 1.1% | 119 (5.0%) | <1.0E-6 |
| ***Rare*** |
| **49** | G | 4 (0.0%) | 0.2% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% | 4 (0.2%) | 0.1 |
| **74** | F | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 1 (0.0%) | 1 |
| **92** | V | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 1 (0.0%) | 1 |
| **143** | A | 1 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 2 (0.1%) | 0.6 |
| **143** | G | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 4 (0.2%) | 0.006 |
| **143** | K | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 1 (0.0%) | 1 |
| **145** | S | 4 (0.0%) | 0.0% | 0.0% | 0.0% | 0.2% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% | 4 (0.2%) | 0.2 |
| **146** | I | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0 (0.0%) | 1 |
| **146** | K | 1 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 4 (0.2%) | 0.02 |
| **146** | L | 1 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 1 (0.0%) | 1 |
| **146** | P | 2 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 1 (0.0%) | 1 |
| **148** | E | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0 (0.0%) | 1 |
| **148** | G | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0 (0.0%) | 1 |
| **148** | N | 2 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 3 (0.1%) | 0.3 |
| **149** | A | 8 (0.0%) | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 2 (0.1%) | 1 |
| **151** | A | 2 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 4 (0.2%) | 0.06 |
| **151** | L | 2 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 1 (0.0%) | 1 |
| **153** | F | 11 (0.1%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% | 0.0% | 0.2% | 2 (0.1%) | 1 |
| **153** | Y | 1 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.4% | 0.0% | 0.0% | 0.0% | 0.4% | 3 (0.1%) | 0.1 |
| **155** | S | 0 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 3 (0.1%) | 0.04 |
| **155** | T | 1 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 2 (0.1%) | 0.6 |
| **230** | G | 28 (0.2%) | 0.2% | 0.3% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.3% | 3 (0.1%) | 1 |

|  |
| --- |
| **Table 3. Linear Regression Coefficients for each INSTI-Resistance Mutation’s Contribution to the Log10-Fold Reduction in INSTI Susceptibility** |
| Pos | AA | #  IsolatesTested1 | Linear regression coefficient2 | Coefficient ≥0.5 |
| RAL3 | EVG3 | DTG3 | Maximum4 |
| ***Nonpolymorphic*** |
| **66** | A | 14 | -0.12 14 | 0.57 14 | -0.29 6 | 0.57 | ✓ |
| **66** | I | 58 | 0.06 50 | 0.72 55 | -0.30 25 | 0.72 | ✓ |
| **66** | K | 7 | 1.10 5 | 1.56 5 | 0.37 6 | 1.56 | ✓ |
| **92** | G | 8 | 0.14 8 | 0.85 8 | 0.16 2 | 0.85 | ✓ |
| **92** | Q | 96 | 0.55 78 | 0.98 75 | 0.16 42 | 0.98 | ✓ |
| **118** | R | 23 | 0.79 19 | 0.39 18 | 0.97 21 | 0.97 | ✓ |
| **121** | Y | 26 | 1.03 21 | 1.30 23 | 0.25 11 | 1.30 | ✓ |
| **138** | T | 3 | 0.01 2 | -0.34 2 | 0.74 3 | 0.74 | ✓ |
| **140** | A | 30 | 0.45 27 | 0.47 23 | 0.62 19 | 0.62 | ✓ |
| **140** | C | 10 | 0.41 9 | 0.58 9 | 0.27 9 | 0.58 | ✓ |
| **140** | S | 159 | 0.37 143 | 0.54 106 | 0.60 115 | 0.60 | ✓ |
| **143** | C | 45 | 0.62 44 | 0.06 36 | 0.11 24 | 0.62 | ✓ |
| **143** | R | 62 | 1.20 56 | 0.22 43 | 0.07 37 | 1.20 | ✓ |
| **143** | S | 14 | 0.77 14 | 0.14 12 | 0.07 2 | 0.77 | ✓ |
| **147** | G | 49 | 0.12 33 | 0.51 41 | 0.02 21 | 0.51 | ✓ |
| **148** | H | 139 | 1.37 126 | 1.03 92 | 0.25 102 | 1.37 | ✓ |
| **148** | K | 36 | 1.41 34 | 1.45 27 | 0.68 24 | 1.45 | ✓ |
| **148** | R | 118 | 1.29 100 | 1.22 85 | 0.26 83 | 1.29 | ✓ |
| **155** | H | 159 | 0.97 140 | 1.02 112 | 0.28 98 | 1.02 | ✓ |
| **230** | R | 39 | 0.43 36 | 0.51 30 | -0.12 10 | 0.51 | ✓ |
| **263** | K | 55 | 0.05 45 | 0.72 38 | 0.57 48 | 0.72 | ✓ |
| **51** | Y | 24 | 0.09 16 | 0.32 18 | 0.21 13 | 0.32 |  |
| **75** | I | 16 | 0.12 16 | 0.29 16 | 0.09 14 | 0.29 |  |
| **95** | K | 16 | -0.08 11 | 0.24 14 | 0.04 10 | 0.24 |  |
| **114** | Y | 2 | -0.10 2 | 0.13 2 | NA | 0.13 |  |
| **138** | A | 20 | 0.22 15 | 0.29 9 | -0.06 19 | 0.29 |  |
| **138** | K | 93 | 0.18 78 | 0.05 70 | 0.20 71 | 0.20 |  |
| **143** | H | 23 | 0.21 23 | -0.11 21 | 0.20 6 | 0.21 |  |
| **146** | R | 2 | 0.16 2 | 0.42 2 | 0.34 2 | 0.42 |  |
| ***Polymorphic*** |
| **49** | P | 3 | 0.15 3 | -0.11 3 | 1.10 2 | 1.10 | ✓ |
| **163** | K | 6 | 0.61 4 | 0.61 4 | 0.03 6 | 0.61 | ✓ |
| **74** | I | 69 | 0.18 57 | 0.09 55 | 0.06 32 | 0.18 |  |
| **74** | M | 101 | 0.24 83 | 0.19 78 | 0.16 61 | 0.24 |  |
| **97** | A | 177 | 0.36 154 | 0.46 131 | 0.17 105 | 0.46 |  |
| **157** | Q | 80 | 0.11 70 | 0.06 62 | 0.17 48 | 0.17 |  |
| **163** | R | 42 | 0.18 32 | 0.11 29 | 0.12 22 | 0.18 |  |
| **232** | N | 140 | 0.07 135 | 0.09 133 | 0.01 13 | 0.09 |  |
| ***Rare*** |
| **74** | F | 13 | 0.66 13 | 0.64 13 | 0.11 13 | 0.66 | ✓ |
| **92** | V | 5 | 0.69 5 | 1.17 5 | 0.28 1 | 1.17 | ✓ |
| **143** | A | 12 | 1.09 12 | 0.38 12 | NA | 1.09 | ✓ |
| **143** | G | 15 | 1.02 15 | 0.25 13 | -0.09 3 | 1.02 | ✓ |
| **143** | K | 2 | 1.10 2 | NA | 0.56 2 | 1.10 | ✓ |
| **145** | S | 2 | -0.09 2 | 1.58 2 | -0.25 2 | 1.58 | ✓ |
| **146** | I | 1 | 0.52 1 | 1.86 1 | 0.51 1 | 1.86 | ✓ |
| **146** | L | 3 | 0.25 3 | 0.89 3 | 0.28 2 | 0.89 | ✓ |
| **146** | P | 7 | 0.05 2 | 0.53 7 | -0.25 2 | 0.53 | ✓ |
| **151** | A | 5 | 0.56 4 | 0.45 4 | 0.01 2 | 0.56 | ✓ |
| **151** | L | 2 | 1.06 2 | 1.24 2 | 0.54 2 | 1.24 | ✓ |
| **153** | Y | 18 | 0.11 15 | 0.37 16 | 0.51 9 | 0.51 | ✓ |
| **155** | S | 5 | 0.87 5 | 1.28 4 | 0.31 1 | 1.28 | ✓ |
| **155** | T | 1 | 0.73 1 | 1.45 1 | 0.44 1 | 1.45 | ✓ |
| **49** | G | 3 | 0.15 3 | -0.47 3 | -0.19 3 | 0.15 |  |
| **146** | K | 1 | -0.08 1 | 0.20 1 | 0.07 1 | 0.20 |  |
| **148** | E | 0 | NA | NA | NA | NA |  |
| **148** | G | 0 | NA | NA | NA | NA |  |
| **148** | N | 4 | -0.33 4 | 0.12 4 | -0.36 4 | 0.12 |  |
| **149** | A | 4 | 0.10 4 | 0.07 4 | 0.21 4 | 0.21 |  |
| **153** | F | 9 | -0.02 8 | 0.34 9 | 0.20 4 | 0.34 |  |
| **230** | G | 6 | 0.00 6 | -0.10 6 | 0.22 3 | 0.22 |  |
| 1The number of HIV-1 isolates undergoing susceptibility testing to RAL, EVG, or DTG. The specific number of tests per INSTI is the subscript in columns 4, 5, and 6. 2The effect of INSTI-resistance mutations on susceptibility was quantified using least squares regression. 3RAL: raltegravir, EVG: elvitegravir, DTG: dolutegravir. 4The highest of the three coefficients for RAL, EVG, and DTG. |

|  |
| --- |
| **Table 4. Previously Unrecognized Nonpolymorphic Mutations with Significantly Higher Prevalence in INSTI-Treated Compared with INSTI-Naïve Persons** |
| Pos | AA | Prevalence in INSTI-Naïve Persons | Treated Prevalence – All Subtypes(n=2,450)# persons (%) | Corrected P |
| All Subtypesn=17,302# persons (%) | A(n=1447) | B(n=8040) | C(n=3194) | D(n=484) | F(n=268) | G(n=244) | 01(n=2019) | 02(n=978) | Highest |
| **170** | A | 2 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 23 (0.9%) | <1.0E-6 |
| **79** | I | 31 (0.2%) | 0.0% | 0.3% | 0.1% | 0.2% | 0.0% | 0.0% | 0.0% | 0.1% | 0.3% | 40 (1.6%) | <1.0E-6 |
| **51** | D | 3 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 20 (0.9%) | <1.0E-6 |
| **70** | R | 11 (0.1%) | 0.1% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 17 (0.7%) | <1.0E-6 |
| **112** | S | 9 (0.1%) | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 14 (0.6%) | 0.000002 |
| **92** | A | 1 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.4% | 0.0% | 0.0% | 0.4% | 9 (0.4%) | 0.000002 |
| **39** | R | 30 (0.2%) | 0.2% | 0.3% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.3% | 20 (1.0%) | 0.000003 |
| **95** | R | 10 (0.1%) | 0.0% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 14 (0.6%) | 0.000004 |
| **253** | N | 8 (0.0%) | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.1% | 12 (0.5%) | 0.00001 |
| **96** | N | 1 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 8 (0.3%) | 0.00001 |
| **142** | T | 3 (0.0%) | 0.0% | 0.0% | 0.0% | 0.2% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% | 8 (0.3%) | 0.0002 |
| **171** | R | 5 (0.0%) | 0.1% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 9 (0.4%) | 0.0002 |
| **212** | L | 17 (0.1%) | 0.4% | 0.1% | 0.0% | 0.2% | 0.8% | 0.0% | 0.0% | 0.1% | 0.8% | 13 (0.5%) | 0.0006 |
| **264** | R | 15 (0.1%) | 0.0% | 0.1% | 0.2% | 0.4% | 0.0% | 0.0% | 0.0% | 0.1% | 0.4% | 12 (0.5%) | 0.0007 |
| **195** | H | 7 (0.0%) | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 9 (0.4%) | 0.0008 |
| **196** | T | 13 (0.1%) | 0.0% | 0.1% | 0.0% | 0.2% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% | 11 (0.5%) | 0.001 |
| **253** | Y | 21 (0.1%) | 0.3% | 0.2% | 0.0% | 0.2% | 0.0% | 0.0% | 0.0% | 0.2% | 0.3% | 13 (0.6%) | 0.002 |
| **155** | D | 3 (0.0%) | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 6 (0.2%) | 0.005 |
| **76** | V | 2 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.4% | 0.4% | 0.0% | 0.0% | 0.4% | 5 (0.2%) | 0.01 |
| **160** | S | 3 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 5 (0.2%) | 0.02 |
| **91** | V | 8 (0.0%) | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 7 (0.3%) | 0.02 |
| **177** | R | 3 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.4% | 0.0% | 0.0% | 0.1% | 0.4% | 5 (0.2%) | 0.02 |
| **195** | N | 11 (0.1%) | 0.0% | 0.1% | 0.0% | 0.2% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% | 8 (0.3%) | 0.02 |
| **229** | N | 5 (0.0%) | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 6 (0.3%) | 0.02 |
| **195** | R | 15 (0.1%) | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.1% | 9 (0.4%) | 0.02 |
| **272** | E | 3 (0.0%) | 0.0% | 0.0% | 0.0% | 0.2% | 0.0% | 0.0% | 0.1% | 0.0% | 0.2% | 5 (0.2%) | 0.02 |
| **277** | R | 3 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.1% | 5 (0.2%) | 0.02 |
| **241** | F | 8 (0.0%) | 0.0% | 0.1% | 0.0% | 0.2% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% | 7 (0.3%) | 0.02 |
| **136** | E | 6 (0.0%) | 0.1% | 0.0% | 0.0% | 0.0% | 0.4% | 0.0% | 0.1% | 0.0% | 0.4% | 6 (0.2%) | 0.02 |
| **56** | Y | 19 (0.1%) | 0.0% | 0.1% | 0.2% | 0.0% | 0.0% | 0.0% | 0.0% | 0.2% | 0.2% | 10 (0.4%) | 0.02 |
| **53** | K | 11 (0.1%) | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.4% | 0.0% | 0.2% | 0.4% | 5 (0.2%) | 0.04 |
| **68** | F | 5 (0.0%) | 0.0% | 0.0% | 0.0% | 0.0% | 0.4% | 0.0% | 0.0% | 0.0% | 0.4% | 5 (0.2%) | 0.05 |

|  |
| --- |
| **Table 5. Established INSTI-Resistance Mutations Scaled According to Six Often Overlapping Mutation Characteristics** |
| DRM | Recognizability | Positional Importance | Non-Polymorphism | Treatment Frequency | Association with INSTI Rx | Association with Reduced Susceptibility | Sum |
| **143R** | 2 | 2 | 2 | 2 | 2 | 2 | 12 |
| **148H** | 2 | 2 | 2 | 2 | 2 | 2 | 12 |
| **148K** | 2 | 2 | 2 | 2 | 2 | 2 | 12 |
| **148R** | 2 | 2 | 2 | 2 | 2 | 2 | 12 |
| **155H** | 2 | 2 | 2 | 2 | 2 | 2 | 12 |
| **66A** | 2 | 2 | 2 | 2 | 2 | 1 | 11 |
| **66I** | 2 | 2 | 2 | 2 | 2 | 1 | 11 |
| **92Q** | 2 | 2 | 2 | 2 | 2 | 1 | 11 |
| **140A** | 2 | 2 | 2 | 2 | 2 | 1 | 11 |
| **140S** | 2 | 2 | 2 | 2 | 2 | 1 | 11 |
| **143C** | 2 | 2 | 2 | 2 | 2 | 1 | 11 |
| **147G** | 2 | 2 | 2 | 2 | 2 | 1 | 11 |
| **263K** | 2 | 2 | 2 | 2 | 2 | 1 | 11 |
| **66K** | 2 | 2 | 2 | 1 | 1 | 2 | 10 |
| **92G** | 2 | 2 | 2 | 2 | 1 | 1 | 10 |
| **118R** | 2 | 2 | 2 | 1 | 2 | 1 | 10 |
| **121Y** | 2 | 2 | 2 | 1 | 1 | 2 | 10 |
| **138A** | 2 | 2 | 2 | 2 | 2 | 0 | 10 |
| **138K** | 2 | 2 | 2 | 2 | 2 | 0 | 10 |
| **138T** | 1 | 2 | 2 | 2 | 2 | 1 | 10 |
| **140C** | 2 | 2 | 2 | 1 | 2 | 1 | 10 |
| **143H** | 2 | 2 | 2 | 2 | 2 | 0 | 10 |
| **143S** | 2 | 2 | 2 | 1 | 1 | 1 | 9 |
| **155S** | 2 | 2 | 2 | 0 | 1 | 2 | 9 |
| **230R** | 2 | 0 | 2 | 2 | 2 | 1 | 9 |
| **143G** | 1 | 2 | 2 | 0 | 1 | 2 | 8 |
| **155T** | 2 | 2 | 2 | 0 | 0 | 2 | 8 |
| **51Y** | 1 | 0 | 2 | 2 | 2 | 0 | 7 |
| **75I** | 1 | 0 | 2 | 2 | 2 | 0 | 7 |
| **92V** | 1 | 2 | 2 | 0 | 0 | 2 | 7 |
| **143A** | 1 | 2 | 2 | 0 | 0 | 2 | 7 |
| **143K** | 1 | 2 | 2 | 0 | 0 | 2 | 7 |
| **145S** | 2 | 1 | 2 | 0 | 0 | 2 | 7 |
| **151L** | 2 | 1 | 2 | 0 | 0 | 2 | 7 |
| **163K** | 1 | 0 | 1 | 2 | 2 | 1 | 7 |
| **49P** | 0 | 0 | 1 | 2 | 1 | 2 | 6 |
| **74M** | 2 | 0 | 0 | 2 | 2 | 0 | 6 |
| **95K** | 0 | 0 | 2 | 2 | 2 | 0 | 6 |
| **97A** | 2 | 0 | 0 | 2 | 2 | 0 | 6 |
| **146R** | 0 | 1 | 2 | 1 | 2 | 0 | 6 |
| **153Y** | 2 | 1 | 2 | 0 | 0 | 1 | 6 |
| **146I** | 0 | 1 | 2 | 0 | 0 | 2 | 5 |
| **146P** | 1 | 1 | 2 | 0 | 0 | 1 | 5 |
| **151A** | 1 | 1 | 2 | 0 | 0 | 1 | 5 |
| **153F** | 2 | 1 | 2 | 0 | 0 | 0 | 5 |
| **157Q** | 1 | 0 | 0 | 2 | 2 | 0 | 5 |
| **163R** | 0 | 0 | 1 | 2 | 2 | 0 | 5 |
| **232N** | 0 | 0 | 1 | 2 | 2 | 0 | 5 |
| **74F** | 1 | 0 | 2 | 0 | 0 | 1 | 4 |
| **74I** | 2 | 0 | 0 | 2 | 0 | 0 | 4 |
| **146L** | 0 | 1 | 2 | 0 | 0 | 1 | 4 |
| **148E** | 0 | 2 | 2 | 0 | 0 | 0 | 4 |
| **148G** | 0 | 2 | 2 | 0 | 0 | 0 | 4 |
| **148N** | 0 | 2 | 2 | 0 | 0 | 0 | 4 |
| **114Y** | 0 | 0 | 2 | 1 | 1 | 0 | 4 |
| **146K** | 0 | 1 | 2 | 0 | 0 | 0 | 3 |
| **149A** | 0 | 1 | 2 | 0 | 0 | 0 | 3 |
| **49G** | 0 | 0 | 2 | 0 | 0 | 0 | 2 |
| **230G** | 0 | 0 | 2 | 0 | 0 | 0 | 2 |