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# HIV-1 Isolates with the RT Mutation Q145M Retain Nucleoside and Non-Nucleoside RT Inhibitor Susceptibility

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**ABSTRACT**

Q145M, a mutation in a conserved HIV-1 reverse transcriptase (RT) region, was reported to decrease susceptibility to multiple RT inhibitors. We report that Q145M and other Q145 mutations do not emerge with RT inhibitors nor decrease RT inhibitor susceptibility. Q145M should therefore not be considered an RT inhibitor resistance mutation.

Genotypic resistance testing is part of the routine management of patients with HIV-1 infection. To optimize genotypic resistance test interpretation, it is essential to track virus mutations that cause or contribute to HIV-1 drug resistance. Although the RT mutations responsible for most nucleoside RT inhibitor (NRTI) and non-nucleoside RT inhibitor (NNRTI) resistant viruses are known, there have been recent reports that several less widely recognized RT mutations may also decrease RT inhibitor susceptibility usually in combination with one or more of the known drug resistance mutations (reviewed in (10)).

However, two rare novel mutations, Q145M/L, have been reported to cause high-level resistance to multiple NRTIs and NNRTIs even in the absence of other known RT inhibitor resistance mutations. When placed in an HXB2 clone, pHXB2delta2-261RT, these mutations were reported to cause more than 10- to 100-fold resistance to the NRTIs zidovudine, lamivudine, stavudine, didanosine, tenofovir, and abacavir, and to the NNRTIs nevirapine and efavirenz in both cell culture and enzymatic assays (5, 6). Despite the potential importance of this report, no subsequent studies have confirmed nor contradicted these findings either in the above cited HXB2 backbone, in another HIV-1 clone, or in clinical isolates.

We undertook several analyses and experiments, to determine whether Q145M/L should be considered drug resistance mutations and be included in genotypic resistance tests reports. Specifically, we determined whether mutations at RT position 145 were selected by RT inhibitors, contributed to decreased RT inhibitor susceptibility, or interfered with a virological response to RT inhibitors.

Table 1 shows that six mutations at position 145 occur in about 0.1% to 0.2% of HIV-1-infected patients. Columns 2 through 5 of Table 1 show that Q145M and other mutations at this position are not associated with NRTI or NNRTI therapy in the HIV Drug Resistance Database. Columns 6 through 8 show that in a large database of HIV-1 RT sequences from a commercial reference laboratory, Q145 mutations were as likely to occur in viruses without RT mutations as they were to occur in viruses with RT inhibitor resistance mutations. This lack of association with RT inhibitor therapy and RT inhibitor resistance mutations demonstrates that Q145 mutations are not selected by RT inhibitor therapy.

To assess the phenotypic impact of Q145M, we performed *in vitro* susceptibility testing on three infectious molecular clones containing Q145M and one containing Q145V (PhenoSense; Monogram, South San Francisco, CA) (7). One of the three infectious molecular clones with Q145M was a site-directed mutant created on a pNL4-3 backbone using QuickChange XL Site Directed Mutagenesis kit (Stratagene, La Jolla, CA) to change the RT codon 145 of pNL4-3 from CAG to ATG. The remaining three infectious molecular clones were created by ligating patient-derived RT amplicons into a vector lacking RT codons 24 to 311 as previously described (3). Each of the four recombinant infectious molecular clones were transfected into C8166 cells and expanded in SupT1 cells to create multiple aliquots of cell-free virus stocks that were tested for RT inhibitor susceptibility (Phenosense Assay, Monogram, South San Francisco) (7). Table 2 shows that each of the three infectious molecular clones with Q145M and the clone with Q145V were fully susceptible to each of the FDA-licensed NRTIs and the first three licensed NNRTIs.

Among patients undergoing HIV-1 genotypic resistance testing at Stanford University Hospital Virology Laboratory for whom antiretroviral treatment history and clinical follow-up were available, mutations at position 145 did not interfere with the response to standard first line treatment regimens. Among 2 of 2 patients with Q145M, 2 of 2 with Q145L, 8 of 9 with Q145V, 1 of 1 with Q145H, and 3 of 3 with Q145E, treatment with a standard first line treatment regimen led to sustained virological suppression (<75 plasma HIV-1 RNA copies/ml; Siemans bDNA assy).

Examination of amino acids 143 through 157 in the three-dimensional structure of HIV-1 RT shows that amino acids 142 through 147 are part of beta-sheet 8; positions 155 to 157 are part of alpha-helix E; and positions 148 to 154 form a connecting loop (2). However, in contrast to Q151, which is within 8 to 11 angstroms from the template, primer, and incoming dNTP, Q145 is more than 20 angstroms from each of these structural entities (2).

RT amino acids 143 through 157 are conserved in group M HIV-1 viruses with the rare mutations at position 145 being the only mutations occurring in untreated individuals and the multi-nucleoside resistance mutation Q151M occurring in about 2% of NRTI-treated persons. In HIV-1 Group O and HIV-$1_{CPZ}$ isolates, Q145C/H have been rarely reported. In HIV-2, Q145I is the consensus variant and Q145V/M/T are other common variants at this position. In other primate lentiviruses, positions 145 to 148 are highly variable whereas positions 149 to 157 are nearly completely conserved (Table 3).

Although the previously published site-directed mutagenesis experiment and in vitro susceptibility results were performed in a pHXB2 backbone and ours were performed in a pNL4-3 backbone, this is not likely to explain the differences in results that we obtained because there is no example for such marked difference in in vitro drug susceptibility results obtained using pHXB2 or pNL4-3 vectors. Indeed, the Antivirogram assay (Vircolab, Mechelen Belgium) uses an HXB2-derived vector (1) whereas the PhenoSense assay uses a pNL43-derived vector (7). Although differences in reproducibility between the Antivirogram and PhenoSense assays have been reported, the results of these two assays are generally concordant (8, 11).

In conclusion, multiple lines of evidence suggest that the RT mutation Q145M and other mutations at this position do not confer RT inhibitor resistance and should not be reported as RT inhibitor resistance mutations on current genotypic resistance tests reports. Novel drug-resistance mutations should ideally be confirmed using a standardized phenotypic assay to validate their biological and potential clinical significance.

## REFERENCES


1. **Hertogs, K., M. P. de Bethune, V. Miller, T. Ivens, P. Schel, A. Van Cauwenberge, C. Van Den Eynde, V. Van Gerwen, H. Azijn, M. Van Houtte, F. Peeters, S. Staszewski, M. Conant, S. Bloor, S. Kemp, B. Larder, and R. Pauwels.** 1998. A rapid method for simultaneous detection of phenotypic resistance to inhibitors of protease and reverse transcriptase in recombinant human immunodeficiency virus type 1 isolates from patients treated with antiretroviral drugs. Antimicrob.Agents Chemother. **42:**269-276.
2. **Huang, H., R. Chopra, G. L. Verdine, and S. C. Harrison.** 1998. Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: implications for drug resistance. Science **282:**1669-1675.
3. **Johnston, E., K. M. Dupnik, M. J. Gonzales, M. A. Winters, S. Y. Rhee, T. Imamichi, and R. W. Shafer.** 2005. Panel of prototypical infectious molecular HIV-1 clones containing multiple nucleoside reverse transcriptase inhibitor resistance mutations. AIDS **19:**731-3.
4. **Kuiken, C., T. Leitner, B. Folery, B. Hahn, P. Marx, F. McCutchan, S. Wolinsky, and B. Korber.** 2008. HIV Sequence Compendium 2008. Los Alamos National Laboratory, Theoretical Biology and Biophysics, Los Alamos, NM.
5. **Paolucci, S., F. Baldanti, G. Maga, R. Cancio, M. Zazzi, M. Zavattoni, A. Chiesa, S. Spadari, and G. Gerna.** 2004. Gln145Met/Leu changes in human immunodeficiency virus type 1 reverse transcriptase confer resistance to nucleoside and nonnucleoside analogs and impair virus replication. Antimicrob Agents Chemother **48:**4611-7.
6. **Paolucci, S., F. Baldanti, M. Tinelli, G. Maga, and G. Gerna.** 2003. Detection of a new HIV-1 reverse transcriptase mutation (Q145M) conferring resistance to nucleoside and non-nucleoside inhibitors in a patient failing highly active antiretroviral therapy. AIDS **17:**924-7.
7. **Petropoulos, C. J., N. T. Parkin, K. L. Limoli, Y. S. Lie, T. Wrin, W. Huang, H. Tian, D. Smith, G. A. Winslow, D. J. Capon, and J. M. Whitcomb.** 2000. A novel phenotypic drug susceptibility assay for human immunodeficiency virus type 1. Antimicrob Agents Chemother **44:**920-8.

8. **Qari, S. H., R. Respess, H. Weinstock, E. M. Beltrami, K. Hertogs, B. A. Larder, C. J. Petropoulos, N. Hellmann, and W. Heneine.** 2002. Comparative analysis of two commercial phenotypic assays for drug susceptibility testing of HIV-1. J Clin Microbiol **40:**31-5.

9. **Shafer, R. W., S. Y. Rhee, D. Pillay, V. Miller, P. Sandstrom, J. M. Schapiro, D. R. Kuritzkes, and D. Bennett.** 2007. HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. AIDS **21:**215-223.

10. **Shafer, R. W., and J. M. Schapiro.** 2008. HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. AIDS Rev **10:**67-84.

11. **Zhang, J., S. Y. Rhee, J. Taylor, and R. W. Shafer.** 2005. Comparison of the precision and sensitivity of the Antivirogram and PhenoSense HIV drug susceptibility assays. J Acquir Immune Defic Syndr **38:**439-444.

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**Table 1. Prevalence of Q145 Mutations in HIV-1-Infected Persons By RT Inhibitor History (HIV Drug Resistance Database) and Co-occurrence With Other RT Inhibitor Resistance Mutations (Quest Laboratory Database)**

| | HIV Drug Resistance Database* | | | | Quest Laboratory Database† | | |
|---|---|---|---|---|---|---|---|
| | Naïve (n=11,458) | NRTI (n=4,110) | NRTI + NNRTI (n=13,684) | Δ Naïve vs Rx | No RTI Resistance Mutations (n=106,906) | RTI Resistance Mutations (n=128,286) | Δ WT vs Mut |
| Q145M | 0.03% | 0.05% | 0.02% | None | 0.04% | 0.07% | +0.03% |
| Q145L | 0.03% | 0.05% | 0% | -0.1% | 0.01% | 0.01% | None |
| Q145V | 0.15% | 0.05% | 0.06% | -0.9% | 0.07% | 0.07% | None |
| Q145E | 0.09% | 0.02% | 0.02% | -0.7% | 0.2% | 0.2% | None |
| Q145C | 0.21% | 0% | 0.02% | -1.9% | 0.07% | 0.03% | -0.04% |
| Q145H | 0.15% | 0.05% | 0.02% | -0.12% | 0.05% | 0.11% | +0.06% |

*The HIV Drug Resistance Database contains treatment histories of the persons from whom the viruses with Q145M were obtained: Naïve – persons receiving no antiretrovirals; NRTI – persons receiving nucleoside RT inhibitors but no non-nucleoside RT inhibitors; NRTI+NNRTI – persons had received nucleoside + nonnucleoside RT inhibitors. Sequences from the same patient having the same Q145 mutation were counted as only one sequence. †The Quest Laboratory Database contains larger numbers of sequences than the HIV Drug Resistance Database. However, the treatment histories associated with those sequences is not known. Therefore, the presence or absence of known nonpolymorphic RT inhibitor resistance mutations (9) was used as an imperfect but logical surrogate for past RT inhibitor selective pressure.

**Table 2.** ***In vitro*** **RT Inhibitor Susceptibilities of Infectious Molecular Clones With RT Codon 145 Mutations***

| Clone | Mutations | NRTIs | | | | | | | NNRTIs | | | RC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AZT | d4T | TDF | ABC | ddI | 3TC | FTC | DLV | EFV | NVP | (%) |
| pNL43-Q145M | K102Q, **Q145M**, S162C, K277R, I293V | 0.7 | 0.9 | 1.1 | 0.8 | 0.9 | 1.1 | 1.1 | 0.9 | 0.9 | 0.9 | 12 |
| 38086 | K49R, V60I, I135V, **Q145M**, Q174H, G196E, Q207E, R211K, V245K | 1.5 | 0.9 | 1.1 | 0.9 | 1.2 | 1.1 | 1.1 | 1.5 | 0.9 | 1.0 | 12 |
| Q9016 | K122E, **Q145M**, I202V, F214L | 0.4 | 0.9 | 0.7 | 0.8 | 0.9 | 1.2 | 1.1 | 0.5 | 0.6 | 0.4 | 54 |
| 14682 | 122E, 135V, **Q145V**, 200A | 0.7 | 0.9 | 0.9 | 1.0 | 1.2 | 1.3 | 1.3 | 1.1 | 0.7 | 0.5 | 33 |

*_In vitro_ susceptibility testing was performed using the PhenoSense assay (Monogram; South San Francisco). The numbers indicate the fold change in susceptibility compared to the pNL43 wildtype control. Abbreviations: NRTIs – nucleoside RT inhibitors, NNRTIs – Nonnucleoside RT inhibitors, AZT - zidovudine, d4T - stavudine, TDF - tenofovir, ABC - abacavir, ddI - didanosine, 3TC - lamivudine, FTC - emtricitabine, DLV - delavirdine, EFV - efavirenz, NVP - nevirapine, RC - replication capacity

**Table 3. Amino Acid Variability of RT Positions 143 to 157 Among Primate Lentiviruses***

| | | | 143 | 144 | 145 | 146 | 147 | 148 | 149 | 150 | 151 | 152 | 153 | 154 | 155 | 156 | 157 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HIV-1 | Group M Consensus | | R | Y | Q | Y | N | V | L | P | Q | G | W | K | G | S | P |
| $SIV_{CPZ}$ | CPZ.TZ.01.TAN1 | AF447763 | – | F | – | – | – | – | – | – | – | – | – | – | – | – | – |
| $SIV_{CPZ}$ | CPZ.CD.90.ANT | U42720 | – | – | C | – | – | – | – | – | – | – | – | – | – | – | – |
| HIV2 | H2A.DE.x.BEN | M30502 | – | – | I | – | K | – | – | – | – | – | – | – | – | – | – |
| $SIV_{AGM}$ | VER.KE.x.AGM155 | M29975 | – | – | – | F | – | C | – | – | – | – | – | – | – | – | – |
| $SIV_{SYK}$ | SYK.KE.x.KE51 | AY523867 | – | – | – | F | K | – | – | – | – | – | – | – | – | – | – |
| $SIV_{GSN}$ | GSN.CM.99.CN166 | AF468659 | – | – | – | F | K | – | – | – | – | – | – | – | A | – | – |
| $SIV_{COL}$ | COL.CM.x.CGU1 | AF301156 | – | – | V | – | K | – | – | – | – | – | – | – | – | – | – |
| $SIV_{SUN}$ | SUN.GA.98.L14 | AF131870 | – | – | V | – | – | – | – | – | – | – | – | – | – | – | – |
| $SIV_{MON}$ | MON.NG.x.NG1 | AJ549283 | – | – | – | F | R | – | – | – | – | – | – | – | – | – | – |
| $SIV_{SAB}$ | SAB.SN.x.SAB1c | U04005 | – | – | – | – | K | – | – | – | – | – | – | – | – | – | – |
| $SIV_{DEB}$ | DEB.CM.99.CM40 | AY523865 | – | – | E | F | R | – | – | – | – | – | – | S | A | – | – |

*The table contains each of the unique patterns of differences from the HIV-1 group M consensus among full-genome primate lentivirus sequences reported in the 2008 Los Alamos Sequence Compendium (4). Only those patterns for which two or more isolates containing the same difference from the HIV-1 Group M consensus is shown.