

Q148N, a Novel Integrase Inhibitor Resistance Mutation Associated with Low-Level Reduction in Elvitegravir Susceptibility

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Abstract

The integrase strand transfer inhibitor (INSTI)-resistance mutations Q148H/K/R are arguably the most important INSTI-resistance mutations as they represented the first step to high-level dolutegravir cross-resistance. We describe an individual with transmitted four-class drug resistance whose virus sequence had the previously uncharacterized mutation Q148N. Infectious molecular HIV-1 clones containing Q148N alone and in combination with G140S demonstrated ~2.4–4.5 reduced elvitegravir susceptibility depending on the virus's genetic context but retained susceptibility to raltegravir and dolutegravir. This level of reduced elvitegravir susceptibility is lower than that observed with Q148H/K/R and in fact the infected individual responded to an initial treatment regimen containing tenofovir/emtricitabine/elvitegravir/cobicistat. Q148N was associated with a higher replication capacity than Q148H, suggesting that this mutation may be more fit in the absence of selective INSTI therapy.

A 50-YEAR-OLD HISPANIC homosexual man underwent HIV-1 seroconversion in August 2014. His CD4 count at the time of diagnosis was 405 cells/mm³ and his plasma HIV-1 RNA level was 3,980 copies/ml. A baseline genotypic resistance test revealed the nucleoside reverse transcriptase inhibitor-resistance mutations L210W and T215D that were interpreted as causing intermediate resistance to zidovudine and low-level resistance to tenofovir; the non-nucleoside reverse transcriptase inhibitor (NNRTI)-resistance mutations K101P and K103S that were interpreted as causing high-level resistance to each of the NNRTIs; and the protease inhibitor-resistance mutations D30N, L33F, I54V, N88D, and L90M that were interpreted as causing intermediate or high-level resistance to each of the PIs except darunavir. The patient was started on the once daily integrase strand transfer inhibitor (INSTI)-containing fixed-dose combination tenofovir/emtricitabine/elvitegravir/cobicistat.

In November 2014, the patient changed clinics and an INSTI resistance test was ordered on his August 2014 sample. The test revealed G140S, a nonpolymorphic accessory INSTI-resistance mutation, and Q148N, a previously uncharacterized mutation in the INSTI-binding pocket of integrase enzyme. At the same time, the plasma HIV-1 RNA

level was <50 copies/ml and CD4 count was 767 cells/mm³. Although the patient's genotypic resistance test was reported as being associated with intermediate resistance to raltegravir and elvitegravir based on the presence of G140S, no change to therapy was made because of the patient's virological and immunological response to therapy. The patient has since been monitored closely and multiple subsequent plasma HIV-1 RNA levels have been <50 copies/ml.

In 2015, we created a panel of infectious molecular clones to assess the effect of the novel mutation Q148N on INSTI susceptibility. The panel included site-directed HIV-1 mutants containing G140S+Q148N, Q148N alone, G140S+Q148H, and Q148H alone. It also contained a recombinant infectious molecular clone from the patient's August 2014 sample and clones in which G140S alone and G140S and Q148N were independently mutated back to the wild type residues at these positions. These clones were subjected for phenotypic susceptibility testing using the PhenoSense assay (Monogram).¹

Q148N ± G140S was associated with 2.4- to 4.5-fold reduced susceptibility to elvitegravir but not to raltegravir or dolutegravir (Table 1). This level of INSTI resistance was lower than that observed with Q148H alone (19-fold reduced

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TABLE 1. INTEGRASE STRAND TRANSFER INHIBITOR RESISTANCE MUTATIONS AND *IN VITRO* SUSCEPTIBILITY RESULTS OF VIRUS CLONES WITH AND WITHOUT Q148N

<i>IN</i> resistance mutations	Background ^a	RAL (fold change)	EVG (fold change)	DTG (fold change)	RC (%)
None	NL43	0.9	0.8	0.9	NA
Q148N	NL43	0.7	2.4	0.6	88
G140S, Q148N	NL43	0.6	3.1	0.6	NA
Q148H	NL43	19	5.5	0.6	27
G140S, Q148H	NL43	>150	>150	3.4	67
G140S, Q148N	Clinical sample	0.6	4.2	0.5	NA
Q148N	Clinical sample	1.0	4.5	0.7	NA
None	Clinical sample	1.0	1.3	1.1	NA

^aSite-directed mutants were created using the HIV-1 plasmid pNL43 and using a recombinant infectious clone created from the PCR-amplified cDNA extracted from plasma virus. All mutated clones were transfected and sequenced before susceptibility testing using previously published methods.¹⁴ Susceptibility testing and RC determination were determined using the PhenoSense assay (Monogram).[†] RC, replication capacity.

raltegravir susceptibility and 5.5-fold reduced elvitegravir susceptibility) or Q148H+G140S (>150-fold reduced susceptibility to raltegravir and elvitegravir and 3.4-fold reduced dolutegravir susceptibility). Among the site-directed mutants, Q148N had a higher replication capacity than Q148H (88% vs. 27%).

Comment

Q148 is conserved in all lentiviruses and plays an essential role in integrase strand transfer activity.^{2,3} Mutations at this position are arguably the most important INSTI-associated resistance mutations as they represent the first step in the pathway to high-level dolutegravir cross-resistance.^{4,5} Of 1,120 integrase sequences from patients receiving raltegravir in GenBank as of June 2015, 23%, 8%, and 1% had the previously characterized INSTI-resistance mutations Q148H, Q148R, and Q148K, respectively.⁶

Q148N was present in viruses from three raltegravir-experienced individuals and is the only other mutation at this position detected in GenBank sequences.^{6,7} Q148N is also the only mutation other than Q148H/R/K that has been shown to have strand transfer activity in biochemical assays.³ There have been too few published sequences obtained from elvitegravir-experienced individuals to determine whether Q148N occurs at a higher frequency after elvitegravir treatment than after raltegravir treatment. The rarity of Q148N and its weaker association with INSTI resistance suggest that it may be a revertant mutation arising from single nucleotide substitutions in viruses that once harbored Q148H (CAC or CAT) or Q148K (AAA or AAG) => Q148N (AAC or AAT).

The patient's response to therapy may reflect the relatively low-level reduction in elvitegravir susceptibility associated with Q148N and the relatively low plasma HIV-1 RNA level at the time of diagnosis. However, had a baseline INSTI genotypic resistance test been available and had the effect of Q148N been known, a different regimen would likely have been chosen.

Although transmitted INSTI-associated resistance has been practically absent in surveillance studies,^{8–10} there have been at least three case reports of INSTI-associated TDR before this one^{11–13}. This report therefore suggests that baseline INSTI genotypic resistance testing may play an in-

creasing role in the future, particularly in patients with transmitted resistance to other drug classes.

Sequence Data

The sequence of the clinical clone has been submitted to the GenBank under the accession number KU761565.

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Author Disclosure Statement

No competing financial interests exist.

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