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Dynamic escape of pre-existing raltegravir-resistant HIV-1 from raltegravir selection pressure

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ABSTRACT

Using quantitative deep HIV-1 sequencing in a subject who developed virological failure to deep salvage therapy with raltegravir, we found that most Q148R and N155H mutants detected at the time of virological failure originated from pre-existing minority Q148R and N155H variants through independent evolutionary clusters. Double 148R + N155H mutants were also detected in 1.7% of viruses at virological failure in association with E138K and/or G163R. Our findings illustrate the ability of HIV-1 to escape from suboptimal antiretroviral drug pressure through selection of pre-existing drug-resistant mutants, underscoring the importance of using fully active antiretroviral regimens to treat all HIV-1-infected subjects.

1. Introduction

Integrase strand transfer inhibitors are a new family of antiretrovirals that reached HIV clinics in 2007 (McColl and Chen, 2010; Steigbigel et al., 2008). Resistance to raltegravir, the first integrase inhibitor available for HIV treatment, evolves through three seemingly exclusive pathways (Fransen et al., 2009) characterized by a signature mutation in the integrase catalytic centre (Y143R/C, Q148R/H/K or N155H) plus several accessory mutations that increase resistance or improve viral replication (Cooper et al., 2008; Paredes and Clotet, 2010). Clonal analyses suggested that Q148R/H/K and N155H mutations did not coexist in individual genomes, which is consistent with the low replication capacity of double Q148R/H/K + N155H mutants *in vitro* (Fransen et al., 2009; McColl and Chen, 2010). The fitness impact of raltegravir resistance mutations, however, may be modulated *in vivo* by accessory mutations in integrase, as well as by epistatic effects of other HIV-1 genes (Buzon et al., 2010).

2. Clinical case

We report the case of a 42 year-old male diagnosed with HIV in 1988 (Fig. 1) with history of alcoholism and illicit drug use until 1993. He was treated for pulmonary tuberculosis and *Pneumocystiis jirovecii* pneumonia in 1993 and 2004, respectively. Although he had good adherence to antiretrovirals in the recent years, he never achieved HIV-1 RNA suppression <50 copies/mL and his CD4+ T cell counts remained extremely low (the nadir and zenith CD4+ counts were, respectively, 1 and 169 cells/mm^3). After receiving 13 different antiretrovirals during 15 years, he began salvage therapy with tenofovir disoproxil fumarate (245 mg QD), raltegravir (200 mg BID), darunavir (600 mg BID) and ritonavir (100 mg BID) on July 2007. HIV-1 RNA levels decreased 2 $\log_{10}$ four weeks after

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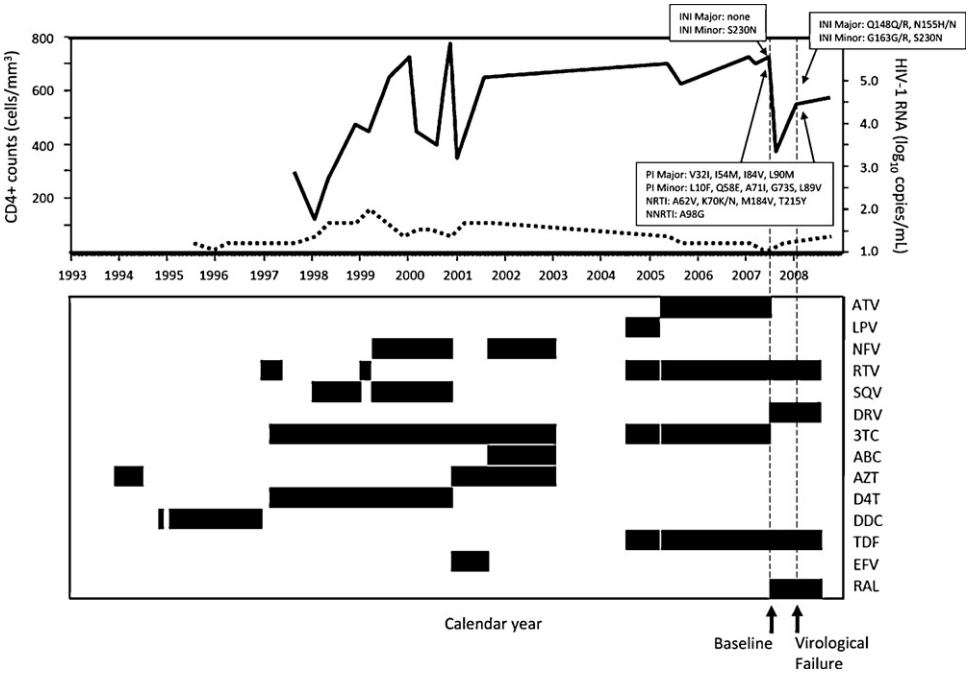

Fig. 1. Antiretroviral treatment history and virological and immunological evolution. The continuous line in the upper graph represents HIV-1 RNA levels in copies/mL transformed in a logarithmic scale; the dashed line in the upper graph represents CD4+ T-cell counts (CD4 counts) in cells/mm^3. Horizontal bars in the lower graph represent the time period during which a given antiretroviral drug was prescribed. Vertical dashed lines represent the timepoints when population and quantitative deep sequencing was performed; i.e., baseline and virological failure. Boxes include resistance mutations detected by population sequencing at baseline and virological failure. PI: protease inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; IN: integrase; ATV: atazanavir; LPV: lopinavir; NFV: nelfinavir; RTV: ritonavir; SQV: saquinavir; DRV: darunavir; 3TC: lamivudine; ABC: abacavir; AZT: zidovudine; D4T: stavudine; DDC: zalcitabine; TDF: tenofovir diprivoxil fumarate; EFV: efavirenz; RAL: raltegravir; None: antiretroviral treatment interruption. Graph developed with and adapted from the ART-AiDE (Antiretroviral Therapy – Acquisition & Display Engine) Program, Stanford HIV Drug Resistance Database.

therapy initiation, but rebounded 1 $\log_{10}$ twenty weeks later. A few weeks after virological failure, the patient was admitted to the intensive care unit and died of acute respiratory distress.

3. Methods

3.1. HIV-1 population and quantitative deep sequencing

HIV-1 RNA was extracted from 1 mL of plasma 3 weeks before initiation of salvage antiretroviral therapy (baseline) and at virological failure (VF), 24 weeks after treatment initiation. Plasma was centrifuged at 35,000 rpm (9000 × *g*) during 90 min at 4 °C. Viral pellets were resuspended and viral RNA was extracted (QIAamp UltraSens Virus Kit™, QIAGEN, Valencia, CA). Three One-Step RT-PCRs (SuperScript® III One-Step RT-PCR System with Platinum® Taq High Fidelity, Invitrogen, Carlsbad, CA) were performed in parallel per each sample. Primers used were 1571-L23 (HXB2: 1417 → 1440) 5′-ATT TCT CCT ACT GGG ATA GGT GG-3′ and 5464-L27 (HXB2: 5464 → 5438) 5′-CCT TGT TAT GTC CTG CTT GAT ATT CAC-3′. Cycling conditions were: 30 min at 52 °C, 2 min at 94 °C; 30 s at 94 °C, 30 s at 56 °C, 4 min at 68 °C, for 30 cycles; followed by 5 min at 68 °C. Triplicate RT-PCR products were pooled, column-purified (QIAquick PCR Purification Kit, QIAGEN, Valencia, CA) and used for both viral population and quantitative deep HIV-1 sequencing (QDS).

3.1.1. Viral population genotyping

For population sequencing, triplicate nested PCRs were performed in parallel (Platinum® Taq DNA Polymerase High Fidelity, Invitrogen, Carlsbad, CA) with primers 2084-U26 (HXB2: 2084 → 2109) 5′-ATT TTT TAG GGA AGA TCT GGC CTT CC-3′ and 5456-L26 (HXB2: 5456 → 5431) 5′-TGT CCT GCT TGA TAT TCA CAC CTA GG-3′. Cycling conditions were: 2 min at 94 °C; 30 s at 94 °C, 30 s at 56 °C, 4 min at 68 °C, for 30 cycles; followed by 5 min at 68 °C. Again, nested PCR products were pooled and column-purified (QIAquick PCR Purification Kit, QIAGEN, Valencia, CA). Protease, reverse transcriptase and integrase genes were sequenced in-house (BigDye v3.1, Applied Biosystems, Foster City, CA, USA) and resolved by capillary electrophoresis (ABI 7000, Foster City, CA, USA). Genotypes were interpreted automatically with the HIVdb program implemented in the Stanford drug resistance database (Liu and Shafer, 2006; Rhee et al., 2003).

3.1.2. Quantitative deep sequencing

Pooled purified RT-PCR products were used as template to generate two overlapping amplicons. Excluding primer sites, amplicon 1 encompassed the integrase codons 91 to 178 (HXB2: 4499–4763), whereas amplicon 2 included the integrase codons 132 to 224 (HXB2: 4621–4900). The overlapping region between the two amplicons thus encompassed codons 132–178 in the Integrase catalytic center (HXB2: 4621–4763). Each amplicon was generated in triplicate during 30 cycles of PCR amplification (Platinum® Taq DNA Polymerase High Fidelity, Invitrogen, Carlsbad, CA). Primers for amplicon 1 were Beta-F (HXB2: 4481 → 4499) 5′- AGA AGC AGA AGT TAT TCC A -3′ and Beta R (HXB2 4763 → 4780) 5′-TTG TGG ATG AAT ACT GCC-3′; primers for amplicon 2 were Gamma-F (HXB2: 4606 → 4621) 5′- TTA AGG CCG CCT GTT G -3′ and Gamma R (HXB2: 4900 → 4917) 5′-TGT CCC TGT AAT AAA CCC-3′. In addition, primers contained 454 sequencing adapters A or B and 8-nucleotide sample identifier tags in the 5′-end. Triplicate PCR products were pooled and purified (QIAquick PCR Purification Kit, QIAGEN, Valencia, CA). Quantitative Deep Sequencing was performed at 454 Life Sciences, Branford, CT, USA.

Raw output sequences were filtered to ensure high quality (Table 1). The overlapping region between the two amplicons (integrase codons 132–178) was extracted. Sequences resulting