CONCISE COMMUNICATION

High-level dolutegravir resistance can emerge rapidly from few variants and spread by recombination: implications for integrase strand transfer inhibitor salvage therapy

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The integrase strand transfer inhibitor (INSTI) dolutegravir is commonly used in combination antiretroviral therapy regimens and retains strong potency even with primary resistance mutations to some other INSTIs. Acquisition of accessory mutations to primary mutations results in significant increases in dolutegravir resistance. Previously, we reported that addition of the secondary mutation T97A can result in rapid treatment failure in individuals with INSTI mutations at positions 140 and 148. Here, we conducted a detailed case study of one of these individuals and find that T97A-containing HIV emerged from a large replicating population from only a few ($\leq$4) viral lineages. When combined with primary INSTI resistance mutations, T97A provides a strong selective advantage; the finding that T97A-containing variants spread by replication and recombination, and persisted for months after discontinuing dolutegravir, has important implications as dolutegravir is rolled out worldwide.

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Introduction

Effective combination antiretroviral therapy (cART) reduces mortality and morbidity of people with HIV (PWH), and is a key component of treatment as prevention strategies to eliminate HIV in populations [1,2]. Approximately 60% of infected individuals worldwide now have access to cART, and integrase strand transfer inhibitors (INSTIs), including dolutegravir (DTG), are recommended in guidelines for treatment of treatment-naïve, and -experienced individuals, including in resource limited countries (https://clinicalinfo.hiv.

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gov/en/guidelines/adult-and-adolescent-arv/whats-new-guidelines; https://apps.who.int/iris/bitstream/handle/10665/325892/WHO-CDS-HIV-19.15-eng.pdf). Development of drug resistance, which emerges in a substantial proportion of treated individuals, threatens to compromise treatment and eradication strategies. Understanding the emergence of resistance, especially to INSTIs, is critical to public health efforts to preserve effective cART. Several distinct patterns conferring resistance to INSTIs have been described, typically including primary mutations at amino acid positions Y143, Q148 or N155 [3]. The role of accessory mutations, which by themselves do not confer high level resistance, but contribute to resistance, remains uncertain. T97A is an accessory IN mutation present in approximately 4–5% of INSTI-naïve individuals, including individuals with non-B subtypes [4] where it has little effect on INSTI resistance; T97A is present in increased frequency in INSTI-experienced individuals (11–29%) and increases INSTI resistance when combined with primary mutations [5,6]. We previously reported that the addition of T97A to a resistance profile already containing Q148H and G140S mutations rapidly resulted in >10-fold increase dolutegravir (DTG) resistance [7], likely due to drug-resistant variants present at low levels prior DTG initiation [8]. To investigate the emergence of T97A-containing variants, we used detailed sequencing analyses to analyze HIV populations present prior to and following initiation of DTG in one of these cases. Surprisingly, the T97A-containing population emerged from only a few distinct variants that were present in a large replicating population of HIV, exceeding 1×10^5 cells in this individual. Subsequent spread of the T97A mutant during DTG therapy was facilitated by recombination. These data indicate that T97A has a strong selective advantage, and that rapid emergence of resistance can spread in large viral populations from only a few resistant variants.

Materials and methods

Study participant

The study participant was enrolled in an IRB-approved clinical study of resistance and provided written informed consent for study procedures and sample analysis (NCT 01976715, NIAID IRB FWA 0005897; [7]).

Sample collection and integrase and envelope amplification

Viral RNA was extracted from plasma samples and single genome sequencing (SGS) and next generation sequencing (NGS) were carried out (see Supplemental Digital Content, http://links.lww.com/QAD/C542) using primers, probes, and conditions as described (see Tables 1 and 2, Supplemental Digital Content, http://links.lww.com/QAD/C542).

Data analysis

Single genome sequences of HIV-1 integrase and *env* region, respectively, were aligned and subjected to neighbor-joining phylogenetic analysis, population genetics and recombination analyses (see Supplemental Digital Content, http://links.lww.com/QAD/C542). Sequences are available at GenBank (OP160627-OP160877).

Results

Participant clinical course

The study participant was a 53-year-old man with longstanding (approximately 20 years) subtype B HIV infection, extensive treatment experience, including with raltegravir (RAL), viral RNA levels of 136 476 copies/ml, $CD4^+$ cell count of 259 cells/μl and a history of 4-class drug resistance (Fig. 1, [7]). He was undergoing therapy with tenofovir DF + emtricitabine + lopinavir/ritonavir + atazanavir, but he continued to have elevated viral RNA >100 000 copies/ml on multiple occasions. He did not have co-morbid infections or neoplastic diseases, and reported strong adherence with his antiretroviral regimen. To design a new and optimized regimen, he underwent genotyping which noted extensive resistance including integrase mutations G140S and Q148H (Fig. 1); T97A was not detected by routine NGS sequencing [7]. Subsequent therapy (Fig. 1) with DTG-containing regimen (tenofovir DF + emtricitabine + darunavir/ritonavir + dolutegravir + maraviroc). After initiating DTG therapy, viral RNA levels declined to <50 copies/ml by 12 weeks and remained <50 copies/ml through 21 weeks of the new regimen. At week 24, viral RNA level had risen to 1638 copies/ml plasma despite adequate plasma levels of DTG and darunavir [7]. Genotypic analysis of protease, reverse transcriptase, and integrase region in rebound viremia revealed that T97A in integrase was the only new mutation present (Fig. 1). Phenotypic analysis revealed a >10-fold increase in DTG IC50, compared with pre-DTG HIV [7].

Virologic and phylogenetic analysis

To investigate the dynamics of emergence of T97A-containing HIV, we used single genome sequencing to analyze HIV variants present in plasma at multiple points prior to, during, and after DTG-containing therapy (Fig. 1). Prior to DTG-containing therapy, HIV populations were genetically diverse, (average pairwise distance (APD) of 1.1%) (Table 1). INSTI mutations G140S and Q148H were present in all single genomes, but T97A was not present in any of the SGS recovered pre-DTG therapy (Fig. 1, Table 1). Upon rebound, SGS analysis of HIV populations revealed the viral diversity of this rebound population (0.2%) was substantially lower than that of the pre-DTG therapy virus, indicating that HIV variants in the

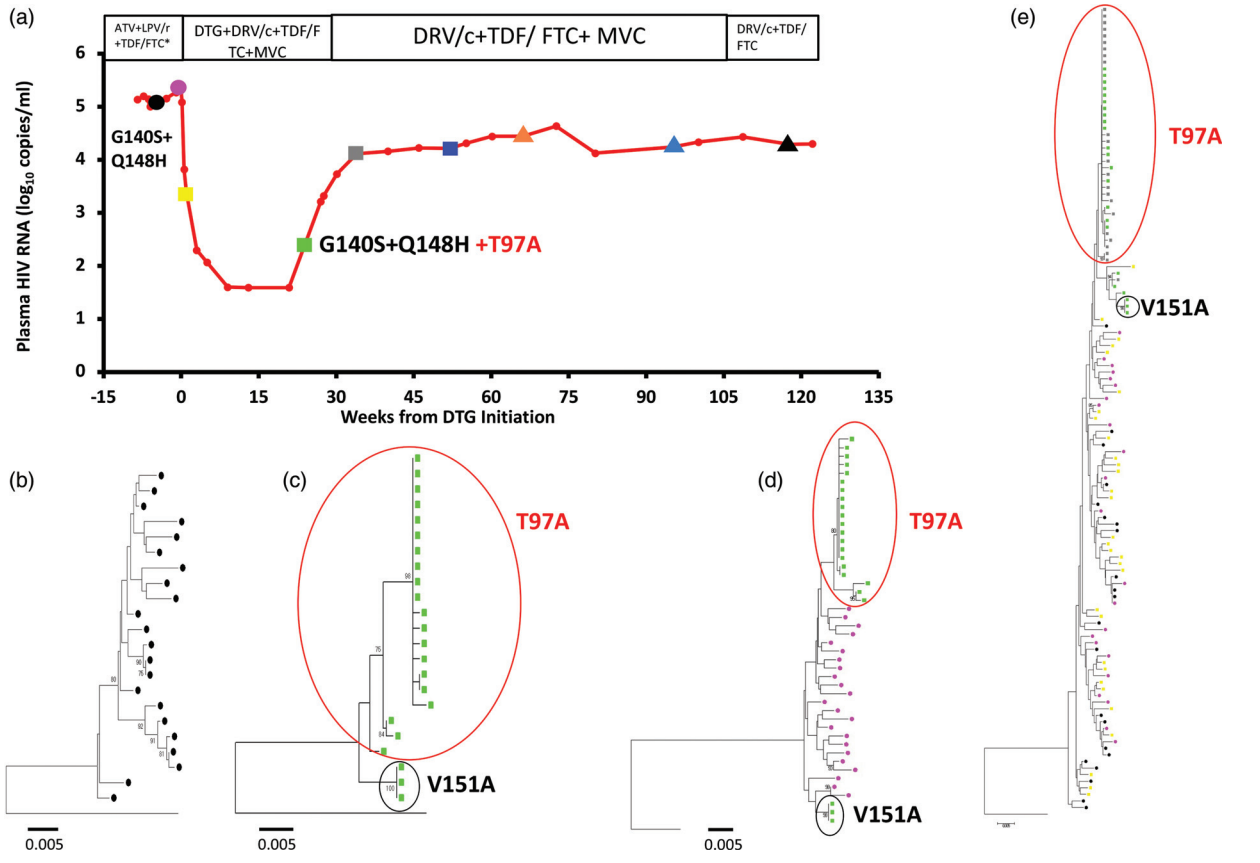

Fig. 1. The clinical course of study participation and the emergence of T97A. Time of sampling and emergence of INSTI DRMs are indicated in various colors and positions with amino acid change, respectively (a). Circles represent time-points prior to DTG initiation, rectangles time on DTG-containing regimen and triangles time after DTG discontinuation. Neighbor joining trees at prior to DTG initiation (b), at time of T97A emergence (c), at the time of DTG initiation and the emergence of T97A (d), and the tree containing pre-DTG sequences and sequences from two time points after viral rebound are shown. *The study participant had been receiving RAL-containing therapy prior to study participation. DTG, dolutegravir; INSTI, integrase strand transfer inhibitor; RAL, raltegravir.

rebound virus population were highly related. The T97A mutation was present on the majority (20/23 SGS) of the early rebound variants (Fig. 1). A second, phylogenetically distinct lineage not containing T97A but having the integrase resistance mutation V151A comprised the remaining variants in the rebound (Fig. 1). Analysis of subsequent samples obtained 7 weeks later revealed T97A in all SGS, V151A was not detected in any SGS.

Table 1. Emergence and persistence of INSTI mutations with population shift analysis prior to, during, and after DTG-containing therapy.

	Pre-DTG (weeks from DTG initiation)		On-DTG (weeks from DTG initiation)				Post-DTG (weeks from DTG initiation)		
	−7	−1	1	27	34	52	66	95	118
Single genomes in pol (*n*)	22	23	31	23	23	22	21	20	21
APD (%)	1.1	1.2	1.2	0.5	0.2	0.4	0.4	0.9	1.1
INSTI mutations (% of SGS with mutation)									
G140S + Q148H	100	100	100	100	100	100	100	100	100
H51Y	0	0	0	0	0	0	9	0	0
T97A	0	0	0	87	100	100	100	80	5
V151A	0	0	0	13	0	0	0	0	0
G163R	0	0	3.4	0	0	0	0	0	0
Population shift analyses*	Pop1			Pop2					Pop3

DTG, dolutegravir; INSTI, integrase strand transfer inhibitor; SGS, single genome sequencing.
*Populations are defined using panmixia analyses of single genome sequences obtained at indicated time points; probability that Pop1, Pop2, and Pop3 represent a single population (are panmictic) is 1×10^{-9}; all panmixia analyses were performed with sequence sets with and without T97A.

In the setting of high phenotypic resistance to DTG and concern for emergence of additional INSTI resistance mutations, DTG therapy was discontinued 28 weeks after rebound. We investigated subsequent samples obtained over the course of 14 months; T97A persisted, but the frequency of the mutation declined, and 15 months after DTG was discontinued, only 1/21 SGS contained T97A (Table 1).

We performed additional phylogenetic and population genetic analyses to investigate the origin of the T97A-resistant HIV. Phylogenetic analysis of all SGS at the rebound time point revealed that all emerged T97A variants were highly related (APD = 0.2%), but phylogenetic analysis indicated that the T97A variants were distinct (Fig. 1, bootstrap values of the T97A branches >70), suggesting the T97A rebound could have emerged from a limited number of independent T97A variants, or alternatively, from a single variant that had diversified after limited cycles of replication. To investigate whether these variants had a single recent origin, we analyzed all the single nucleotide polymorphisms (SNPs) in the SGS in these rebound variants. As shown in Figure 1, Supplemental Digital Content, http://links.lww.com/QAD/C543, four different SNP patterns were present in T97A variants in rebound viremia (Figure 1, Supplemental Digital Content, http://links.lww.com/QAD/C543), suggesting there were four different T97A-containing variants that were responsible for rebound viremia.

Subsequently, each of these four initial variants persisted, but several additional T97A variants were also present with mosaic SNP patterns consistent with recombination events among the initial T97A variants (Figure 1A, Supplemental Digital Content, http://links.lww.com/QAD/C543); as previously demonstrated, the SGS amplification strategy eliminates artifactual recombination events [9]. Boot-scanning phylogenetic analysis indicated recombination had taken place among the initial variants to generate these new chimeric T97A-containing variants (Figure 1B, Supplemental Digital Content, http://links.lww.com/QAD/C543). The recombinants accounted for approximately 13% of all the SGS recovered during the rebound. Taken together, these data demonstrate that ongoing HIV replication during suboptimal therapy with DTG, resistant variants spread by replication of the initial rebound as well as by recombination among variants.

Rapid emergence of T97A variants may be the result of a number of factors; including the size of the replicating HIV population and the overall replication capacity of the virus. It is possible that the size of the replicating population was relatively small, permitting the new variants to emerge quickly. Alternatively, the replicating population could be large, but the T97A mutation conferred substantial selective advantage, permitting rapid emergence even in a large replicating population. Replicating population sizes of drug resistant HIV has not been extensively investigated, and to distinguish between these two possibilities, we measured the size of the replicating HIV population as previously described [10] (Fig. 1). We determined that the replicating population size at the DTG was initiated was large, exceeding 150 000 cells transmitting replication-competent HIV per day in this individual. As such, the rapid emergence of T97A variants was not due to a small replicating population, but more likely the result of selective advantage over the variants containing G140S and Q148H alone.

We obtained additional insights on the dynamics of HIV resistance by studying the HIV populations present prior to and following DTG using sensitive geographic subdivision methods to detect the presence of population shift [8]. In the absence of cART, HIV populations remain genetically indistinguishable on a population level ('panmictic') for prolonged periods, and undergo population shifts to become non-panmictic slowly (>2–3 years) [10]. As shown in Table 1, the rebound population that emerged approximately 4 months after DTG initiation was not panmictic with the pretherapy virus. This new population that emerged on DTG persisted for >1 year (>300 HIV generations), including after DTG was discontinued, and was gradually replaced as virus replication continued. These data indicate that the T97A-containing population persisted in the absence of DTG selection pressure, consistent with the replication capacity (79%, range 50–125%) measured in the Phenosense assay [7], but was gradually out-competed by variants without T97A.

We did not detect variants with any additional known resistance mutations in integrase in addition to the T97A, and no additional mutations were detected in RT or protease [5,11]. We also investigated whether changes in HIV *env* may contribute to emergence of resistance. Recently, Van Duyne *et al.* [12] reported that mutations in *env* may facilitate cell-to-cell spread of drug resistant variants *in vitro* even in the presence of adequate levels of DTG and darunavir [13]; such a mechanism might explain rebound. Analysis of envelope SGS from samples obtained pre-DTG, and post rebound revealed no substitutions at positions (Y61H, P81S, and A556T) associated with resistance in cell culture [12].

Discussion

DTG has rapidly become one of the most widely prescribed antiretrovirals in the world. *De novo* DTG resistance in treatment-naïve individuals is infrequent, but increased frequency of DTG resistance is reported in individuals with prior INSTI resistance. T97A has been identified in a number of variants resistant to DTG, bictegravir, and cabotegravir [14,15], and may play an important role in INSTI resistance by influencing the viral fitness in viruses possessing primary INSTI resistance

mutations [16,17]. How T97A increases drug resistance in viruses possessing primary drug resistance mutations remains uncertain. In X-ray crystallography studies of dimers of the catalytic domain of HIV integrase, the T97 residue is present in an alpha helix that is relatively close to the dimer interface[18]; it is possible the T97A affects catalytic activity, multimerization, or another structural element of this region. A better understanding of the mechanism of resistance is necessary to develop useful strategies to treat individuals with resistance. In this case study, we found that T97A variants [4] emerged quickly from a large (approximately 150 000) replicating population; this frequency of variants (4/150 000) approximates what might be produced given the mutation rate of the HIV RT (3.4×10^{-5}/site/replication cycle [19]) and is consistent with the long-standing prediction that single nucleotide changes conferring drug resistance preexist prior to antiretroviral drug selection pressure [8]. Early studies identified multiple variants present in rebound viremia during drug therapy and suggested roles for genetic drift and selection in emergence of resistance [20–23]. INSTI resistance is likely to be complex; previously Pham and coworkers described emergence of resistance mutations S153Y and R263K in PBMC without the high rebound in blood [24]. Here, T97A emerged from a limited number of variants, and spread through replication and recombination. After discontinuing DTG, T97A-containing variants persisted for a prolonged period (>400 viral generations), suggesting robust replication of these variants.

In this case study, we also detected a minor population of variants with V151A, which is a rare mutation selected *in vitro* but rarely reported in vivo patients [5,11]. In our participant, V151A was not present in subsequent time points after the initial rebound, strongly suggesting T97A-containing variants outcompeted V151A.

These data demonstrate that clinically significant DTG resistance from T97A-containing variants with primary resistance mutations have a substantial selective advantage, emerge quickly in large replicating populations, and may persist for prolonged periods in the absence of drug selection pressure. As T97A is present in numerous subtypes, in geographic areas where DTG is undergoing rollout, it may be already be present when primary mutations such as mutations at IN positions 140 or 148 emerge. The persistence of T97A for prolonged periods in the absence of INSTI supports the explanation by Bailey *et al.* [25] that the frequency of T97A is increasing over time in drug-naïve individuals worldwide due to its fitness in the absence of drug selection pressure after transmission with other resistance mutations. Judicious DTG stewardship and more intensive monitoring in INSTI-experienced individuals is indicated to preserve this drug class and prevent transmission of drug resistance.

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Conflicts of interest

There are no conflicts of interest.

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