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Review

Deep sequencing for HIV-1 clinical management

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ABSTRACT

The emerging HIV-1 resistance epidemic is threatening the impressive global advances in HIV-1 infection treatment and prevention achieved in the last decade. Next-generation sequencing is improving our ability to understand, diagnose and prevent HIV-1 resistance, being increasingly cost-effective and more accessible. However, NGS still faces a number of limitations that need to be addressed to enable its widespread use. Here, we will review the main NGS platforms available for HIV-1 diagnosis, the factors affecting the clinical utility of NGS testing and the evidence supporting –or not- ultrasensitive genotyping over Sanger sequencing for routine HIV-1 diagnosis. Now that global HIV-1 eradication might be within our reach, making NGS accessible also to LMICs has become a priority. Reductions in sequencing costs, particularly in library preparation, and accessibility to low-cost, robust but simplified automated bioinformatic analyses of NGS data will remain essential to end the HIV-1 pandemic.

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1. Introduction

Human immunodeficiency virus type 1 (HIV-1) infection affects more than 35 million people globally and causes nearly 1.5 million deaths every year (UNAIDS, 2016; World Health Organization, 2015). More than half of the people in need worldwide are already receiving antiretroviral treatment (ART). If the other half could be treated, the HIV-1 pandemic could potentially be ended. The emerging HIV-1 resistance epidemic (WHO, 2015), however, poses a major threat to achieving that goal.

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Although HIV-1 infection cannot be cured (Chun et al., 2015), antiretroviral therapy (ART) is able to persistently block HIV-1 replication, limiting HIV-1's damage on the immune system (Vigano et al., 2015). This prevents and/or reverts immune deterioration in most subjects, prolonging life expectancy and increasing quality of life (Egger et al., 2002; Günthard et al., 2014; Rodger et al., 2013; Strategies for Management of Antiretroviral Therapy (SMART) Study Group et al., 2008; Thompson et al., 2012). Furthermore, ART is the most effective tool available to prevent onward HIV-1 transmission (Cohen et al., 2011) and one of the most cost-effective interventions in medicine (Sax et al., 2005; Yazdanpanah et al., 2007).

However, ART pressure might not be sufficient to block HIV-1 replication in a number of situations; i.e., if suboptimal ART is prescribed, there is pre-existing HIV-1 drug-resistance, drug penetration in target cells is insufficient, patient's treatment adherence is incomplete, or when drug-drug interactions decrease ART levels or increase toxicity (Iniesta-Navalón et al., 2015). Lack of suppression of viral replication in the presence of ART, even at cryptic levels, allows HIV-1 resistance to evolve with further accumulation of drug resistance mutations and ultimately leads to overt virological failure (Paredes and Clotet, 2010) (Fig. 1).

In each individual, HIV-1 is structured following a quasispecies distribution (Domingo et al., 2012, 1997; Lauring and Andino, 2010), i.e., a swarm of highly-related but genotypically different viral variants. Such distribution is consequence of a high virus replication rate in the absence of ART (10^{9-14} new virions created per day) combined with high mutation and recombination rates (1 mutation and 2–3 recombination events, respectively, per each new virion)(Abram et al., 2010; Coffin, 1995; Onafuwa-Nuga and Telesnitsky, 2009; Schlub et al., 2014; Smyth et al., 2012) due to the lack of proofreading activity of HIV-1's polymerase (Ji and Loeb, 1992).

The quasispecies distribution allows HIV-1 to rapidly adapt and escape from adverse drug or immune pressure. It also implies that potentially relevant low-frequency mutants exist, which might not be detected by less sensitive HIV-1 genotypic assays. In other words, clinicians might miss important information to optimize ART choices. Novel point-mutation assays and next-generation sequencing (NGS) techniques increase the sensitivity of genotyping from 20% to approximately 1% of mutants in the quasispecies (Codoñer et al., 2011; Simen et al., 2009a). Similarly, NGS approaches provide improved sensitivity to detect CXCR4-using or X4 HIV, which has implications for immune recovery, clinical progression and virological response to CCR5 antagonists (Casadellà et al., 2015a; Swenson et al., 2012a).

In an era when ART is indicated for anyone living with HIV-1 anywhere in the World, HIV-1 genotyping remains key for both clinical management (Asboe et al., 2012; Department of Health and Human Services, 2016a, 2016b; European AIDS Clinical Society, 2015) and public health surveillance, and will play an essential role to ending the HIV-1 pandemic in the coming decades (Gupta et al., 2009).

2. Sequencing strategies

2.1. Sanger sequencing

For many years, virus population sequencing using Sanger's technique (Sanger and Coulson, 1975) has been the gold standard for HIV-1 drug resistance testing, both for research and clinical routine. Sanger sequencing is feasible for most laboratories with basic molecular biology equipment, is straightforward to perform and generally affordable, particularly with home-brew methods. It is easily scalable to a few dozen tests per week using a single PCR instrument and a single technician with part-time dedication, which fits many small or mid-scale HIV-1 laboratories. Sanger-based genotyping has been extensively validated in clinical trials and is supported by equally validated and often publicly available laboratory protocols and interpretation algorithms and rules that can be retrieved automatically (Gifford et al., 2009; Tang et al., 2012). This allows standardized reporting of resistance testing results to clinicians, researchers and public health officials, which has been instrumental in the past to ensure its acceptability among HIV-1 caregivers and policymakers.

However, due to its intrinsic sequencing chemistry, Sanger sequencing can only provide a consensus sequence of the whole quasispecies in each HIV-1-infected individual, being able to detect only those nucleotides present in at least 10–20% of the virus population. There is solid evidence that, at least in some cases, low-frequency genotypic information missed by Sanger sequencing might impact ART efficacy and could be important to improve HIV-1 resistance surveillance (Chabria et al., 2014; Gianella and Richman, 2010). The advent and rapid technical evolution of NGS platforms, coupled with rapid reductions in costs, simplification of laboratory procedures, improvements in turnaround time to results and testing scalability, as well as the development of automated bioinformatic pipelines are gradually increasing NGS use in HIV-1 diagnostics.

2.2. Next generation sequencing

All NGS techniques perform parallel sequencing of hundreds of thousands to millions of individual DNA molecules, enabling the quantification of different viral variants from the same sample at clonal level with higher sensitivity than Sanger sequencing and at cheaper cost per base (Chabria et al., 2014; Eisenstein, 2015; Goodwin et al., 2016; Metzker, 2010). Technically, the field is rapidly evolving and different NGS platforms have become available. Their main characteristics are summarized in Table 1.

All NGS platforms available to date require reverse transcription and PCR amplification before HIV-1 sequencing. This limits the lower sensitivity threshold to the intrinsic error rate of the reverse transcriptase, i.e., 10^{-4} or 1 error per every 10^4 nucleotides copied. Therefore, even in the presence of high HIV-1 RNA levels, it is unrealistic to expect any reliable detection of HIV-1 variants below 0.5 to 1% in the virus population. Also, true assay sensitivity of any ultrasensitive genotyping method depends on the number of RNA molecules in the original sample. The RNA copy in the assay depends on the plasma HIV-1 RNA concentration, the volume of plasma used and the efficiency of the RNA extraction process (discussed in (Paredes et al., 2007)). The efficiency of the reverse transcriptase step also determines the starting copy number, since NGS platforms sequence DNA and not RNA molecules. As a rule of thumb, reliable detection of variants at 1% frequency will require HIV-1 RNA levels of at least 1000 copies/mL.

Although mutant detection above 1% frequency is generally robust and reliable, linear quantification of mutants in the 1% to 100% range is often affected by biases during library preparation due to the presence of resistance mutations or polymorphisms in primer binding sites, PCR-founding effects or random resampling of input DNA molecules. Primer ID partially avoids PCR resampling bias by including a random sequence tag in the first primer so that every template receives a unique ID (Brodin et al., 2015; Jabara et al., 2011; Keys et al., 2015; Seifert et al., 2016; Zhou et al., 2015). Sequences obtained with this strategy can then be identified, the initial copy number can be quantified and the error and bias can be corrected to a great extent. However, this approach requires an amplicon-based strategy and high coverage to obtain enough reads with identical primer IDs, what makes it an expensive procedure.

As NGS is approaching the clinic, a number of challenges must be overcome before it becomes generally available for routine