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FULL PAPERS

Clinical relevance of genotypic resistance testing today

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For many years, the major clinical goals of anti-HIV therapy were, at best, to control viral replication, prevent progression of the disease, and decrease the death toll of advanced disease caused by resistant viruses (DHHS, 2015). Since the beginning of therapeutic era, resistance has been a major contributor to the failure of anti-HIV therapy and disease progression. Today, many advances have been made in HIV management, thanks to the ongoing improvement of antiretroviral therapy and the extensive and careful use of resistance testing from the time when treatment starts, and beyond (DHHS, 2015; EACS, guidelines 2015; HIV/AIDS Italian Expert Panel, 2015). Information about drug resistance helps in selecting more effective antiretroviral regimens, and, in turn, in ensuring high rates of virologic success (Zaccarelli *et al.* AIDS 2005). For these reasons, antiretroviral drug resistance testing on plasma samples is currently recommended for HIV-1-infected individuals both at entry into care (regardless of whether antiretroviral therapy will be initiated immediately) and at virological failure (DHHS, 2015; EACS, 2015; HIV/AIDS Italian Expert Panel, 2015; Vandamme *et al.,* 2011).

Nowadays, genotypic resistance testing (GRT) is also crucial in patients with low or undetectable plasma viral load. Indeed, during antiretroviral therapy, many patients have episodes of low-level viremia (50-1000 copies/mL) that lead to uncertainty regarding what clinical intervention is appropriate (HIV/AIDS Italian Expert Panel, 2015). Studies have shown that low-level viremia can be predictive of progressive viral rebound (Laprise *et al.,* 2013; Ryscavage *et al.,* 2014), and that HIV drug resistance detected during low-level viraemia is associated with subsequent virologic failure (Santoro *et al.,* 2014; Swenson *et al.,* 2014). As such, in-house genotypic resistance assays have recently been developed by a number of laboratories to improve the performance of commercial assays at low-level viremia (Santoro *et al.,* 2014; Gonzales-Serna *et al.,* 2014). With these in-house assays, the success rate of amplification/sequencing is about 70% for viremia levels of 50-200 copies/mL, reaching >90% for viremia levels of 500-1000 copies/mL (Santoro *et al.,* 2014). Therefore, in principle, and in practice, there are no major reasons today not to perform the resistance testing whenever the viral load goes above 50 copies/ml.

The evaluation of drug resistance can be also helpful in clinical practice to plan drug switches for intolerance, toxicity or simplification in suppressed HIV-1 infected patients with good virologic control. In this context, a GRT in peripheral blood mononuclear cells (PBMCs) may be a valid alternative tool when there is inadequate information about prior plasma resistance or antiretroviral resistance, according to data obtained in several studies (HIV/AIDS Italian Expert Panel, 2015).

In addition, GRT from peripheral blood mononuclear cells (PBMCs) may be a valid alternative in patients on antiretroviral therapy with low or undetectable virus load to plan drug switches, as suggested by Italian guidelines for the management of HIV-1 infected patients, according to data obtained in several studies (Parisi *et al.,* 2007; Palmisano *et al.,* 2009; Delaugerre *et al.,* 2012; HIV/AIDS Italian Expert Panel, 2015; Armenia *et al.,* 2016).

In light of the development of a new wave of integrase inhibitors (INIs), it is crucial that resistance monitoring guidelines be revised (DHHS 2015; EACS 2015; HIV/AIDS Italian Expert Panel, 2015). Work recently presented in Boston at the annual Conference on Retroviruses and Opportunistic Infections (CROI) 2016 by Katherine Lepik and colleagues showed that although the prevalence of resistance to INIs remains low compared with resistance to other antiretrovirals, it is increasing with expanded INI use (Lepik *et al.,* 2016). In particular, prevalence of INI resistance /1000 antiretroviral treated persons significantly increased from 1.07 in 2009 to 6.8 in 2015. Until 2013, most new cases of INI resistance were associated with raltegravir use. In 2014 and 2015, 8/19 cases (42%) of new resistance to integrase inhibitors followed elvitegravir or dolutegravir use (Lepik *et al.,* 2016). These data highlight the importance of resistance testing for INI not only in patients for whom this antiretroviral class has failed, but also in INI-naïve patients who start these drugs as a first-line or in subsequent regimens (DHHS, 2015; HIV/AIDS Italian Expert Panel, 2015). Moreover, the evaluation of INI resistance in INI-naïve persons is useful for proper monitoring of viral evolution during INI treatment.

INI-resistance testing in drug-naïve patients is also useful to assess potential transmission of INI resistance (HIV/AIDS Italian Expert Panel, 2015). Recent data have also highlighted the importance of INI-resistance testing in patients who failed at low viremia levels, because it provides useful information about emerging resistance at early failures (Armenia *et al.,* 2015). For these low viremia levels, the test is still reliable, reproducible; indeed the amplification and interpretation efficiency is 82% at viremia levels of 51-500 copies/mL, while it is 94% with viremia levels of 500-1000 copies/mL (Armenia *et al.,* 2015). So, the same feasibility in clinical practice applies to the resistance test for integrase, as it is for reverse transcriptase and protease (see above). In light of the extensive use of this class, laboratories performing resistance tests should be able to provide the integrase test without restrictions.

Despite genetic variability between different HIV-1 subtypes (up to 12% of the nucleotide sequence), to date no clinical data clearly indicate the need of a particular diagnostic and therapeutic carefulness against non-B subtypes (Santoro *et al.,* 2013). However, recent findings suggest

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that therapeutic efficacy can differ according to HIV-1 subtype. Armenia and colleagues have shown that even though a high proportion of HIV-1 patients starting a first-line regimen containing a boosted-ritonavir protease inhibitor achieved and maintained virological success, those infected with CRF02_AG might have a poorer response (Armenia *et al.,* 2016). The presence of specific mutations such as K20I, K70R and L89M (tightly related to CRF02_AG) may correlate with this phenomenon (Armenia e*t al.,* 2016). More studies are needed to better characterize the impact of HIV-1 subtype on therapeutic efficacy.

In conclusion, assessment of drug resistance remains an important factor in determining the effectiveness of antiretroviral therapy for HIV-1 infection (DDHS, 2015; EACS, 2015; HIV/AIDS Italian Expert Panel, 2015; Vandamme *et al.,* 2011). Preventing resistance is easier and far more productive than treating it, and drug resistance data inform the selection of antiretroviral regimens that lead to high rates of virologic success.

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