Revising the concept of genetic barrier according to combination therapy

Introduction
Potent antiretroviral therapy is the most effective intervention to reduce HIV-related morbidity and mortality. Despite this success, the emergence of drug-resistance still remains one of the major causes of virological failure. Drug-resistance can compromise future treatment options, and has been associated with an increased risk of death among patients failing HAART (Hogg et al., 2006; Zaccarelli et al., 2007; Kozal et al., 2007). Intrinsic HIV-1 properties lead to the emergence of drug resistance. Their knowledge is critical to set up an antiretroviral regimen aimed at achieving maximal virological suppression. They are:

1. Genetic diversity. Due to the lack of proof-reading mechanism, HIV-1 reverse transcription is a highly error-prone process. It has been estimated that at least 1 mutation is introduced in HIV-1 genome at each replication cycle. The rate of recombination has been estimated to be even higher (Batorsky et al., 2011). As a consequence, HIV exists in a single individual as a mixture of genetically different variants, whose distribution reflects the relative fitness of each single virus.

2. Replication Rate. HIV-1 is characterized by high speed of replication. In blood of drug-naive patients, HIV may reach a titer ranging 10⁻³ to 10⁶ copies/ml, in lymph nodes this concentration may be 2 to 3 orders of magnitude higher. Moreover, HIV is characterized by a short generation time (1-3 days). Thus, this high and erroneous turn over represents the driving force of viral evolution and variation within a single patient.

These characteristics make HIV prone to drug-resistance. It has been estimated that every possible variant containing one resistance mutation and many variants containing two resistance mutations can be spontaneously generated and persist as minority species in HIV-1 infected individuals naive to antiretroviral drugs (Clotet et al., 2010). In presence of antiretroviral drugs, drug resistant variants may replicate better than the wild-type. Thus, if therapy failed to completely suppress ongoing viral replication, drug resistant variants become dominant in the viral population, thus leading to virological rebound and failure.

Several factors contribute the emergence of drug-resistance. Among them, the genetic barrier to drug resistance plays a critical role. This review will specifically address the clinical meaning and new concepts regarding the topic of genetic barrier to resistance, whose knowledge is critical to set up a highly effective antiretroviral therapy.

Defining genetic barrier to resistance
Genetic barrier to resistance denotes the ability of the virus to acquire a sufficient number of critical drug-resistance mutations to overcome the activity of the drug. Stated differently, the genetic barrier refers to the threshold above which clinically meaningful resistance develops to a drug or a drug class. Traditionally, HIV-1 requires the accumulation of multiple mutations to overcome the activity of boosted protease inhibitors and the thymydine analogues. These drugs are thus characterized by a high genetic barrier to resistance (Hsu et al., 2004; Kempf et al., 2004; Eron et al., 1996). The use of drugs with high genetic barrier to resistance (such as ritonavir boosted protease inhibitors) has been generally associated with a lower emergence of drug resistance at virological failure (Eron et al., 2006; Gallant et al., 2004; Gathe et al., 2004; Ortiz et al., 2008; Riddler et al., 2008; Walmsley et al., 2002 and 2009). Conversely, a single drug resistance mutation is generally sufficient to compromise the use of the nucleoside reverse transcriptase inhibitor (NRTI) lamivudine and emtricitabine, the first generation NNRTI nevirapine and efavirenz, and the fusion inhibitor enfuvirtide. These drugs are thus characterized by low genetic barrier to resistance. Drugs with intermediate barrier to resistance are the integrase inhibitors raltegravir and elvitegravir, the second generation NNRTI etravirine, and the NRTI tenofovir, abacavir and didanosine. The genetic barrier to the class of CCR5 antagonists has not yet clinically defined.

Applying the concept of genetic barrier to resistance

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to clinic
The use of drugs with low genetic barrier to resistance does not unequivocally mean decreased rate of virological success. This has been highlighted by different studies. Indeed, a recent study has compared the efficacy of abacavir/lamivudine and tenofovir/emtricitabine when combined with ritonavir boosted atazanavir (traditionally considered as a high genetic barrier drug) or with efavirenz (traditionally considered as a low genetic barrier drug). The authors found that, in patients with plasma HIV-RNA < 10^4 copies/ml at the time of starting therapy, time to virological failure was similar for abacavir/lamivudine versus tenofovir/emtricitabine with ritonavir boosted atazanavir (hazard ratio [HR] 1.25, 95% CI 0.77, 1.96) (Sax et al., 2011). Differently, in patients with plasma HIV-RNA > 10^4 copies/ml, virological failure rate was significantly higher for abacavir/lamivudine than for tenofovir/emtricitabine when given with either efavirenz or ritonavir boosted atazanavir (Sax et al., 2011). Recently, Rockstroh and co-workers compared 3 years of antiretroviral therapy with raltegravir (with intermediate genetic barrier) or efavirenz (with low genetic barrier) as part of a combination regimen in the ongoing STARTMRK study of treatment-naive patients infected with HIV. The study shows that raltegravir produced durable viral suppression and immune restoration that was at least equivalent to efavirenz through 156 weeks of therapy (Rockstroh et al., 2011).

Overall findings support that the combined use of highly potent drugs even if with low genetic barrier to resistance (as in the case of emtricitabine, tenofovir, and efavirenz) allows the achievement of virological success at a rate comparable to that obtained when drugs with high genetic barrier to resistance are used. These results can be explained by two factors:
1. In the setting of full adherence, the combined use of potent drugs, even if with low genetic barrier to resistance, allows a rapid and long-term suppression of ongoing viral replication. This is crucial to avoid the emergence drug-resistance. Consistent with this concept, different studies have shown that the combined use of tenofovir, emtricitabine and efavirenz correlates with a lower rate of drug resistance emergence compared to the combined use of lamivudine, zidovudine and efavirenz (Gallant JE et al., 2006, Margot NA et al., 2009). Several studies have also shown a reduced selection of the mutation M184V/I in patients treated with emtricitabine than in those treated with lamivudine (Svicher et al., 2009; Margot et al., 2009; McColl et al., 2011). This result can be explained by the prolonged intracellular half-life of emtricitabine compared to lamivudine (Feng et al., 1999), and by the consequent higher potency of emtricitabine than lamivudine (Schinazi et al., 1993; Saag et al., 2006).
2. As mentioned in the introduction, every possible variant containing one resistance mutation and many variants containing two resistance mutations can be spontaneously generated and persist as minority species in HIV-1 infected individuals naïve to antiretroviral drugs (Fig 1A, B) (Closter et al., 2010). This explains why mono- or dual-therapy rapidly and inexorably leads to virological failure. Conversely, the probability that 3 drug resistance mutations (conferring resistance to 3 different drugs) can be spontaneously generated in the same viral genome is extremely low (Closter et al., 2010). This means that (excluding events of broad transmitted drug resistance) no drug-naïve patients harbor viral strains resistant to 3 antiretroviral drugs (Fig 1A, B). This explains why the combined use of potent drugs (even if with low genetic barrier to resistance) can keep under full control the virus, thus allowing a prolonged virological suppression.

In this light, the classical concept of genetic barrier should be revised, and applied not to the single drug but to the entire regimen. Thus, in the setting of full adherence, potent drugs with low/medium genetic barrier to resistance can be combined together, allowing a rapid achievement and long-term maintenance of virological success. This is the reason why antiretroviral regimens including first-generation NNRTIs, with a NRTI backbone of emtricitabine and tenofovir, today considered by all guidelines as optimal or preferred option, achieve high rate of virological success, and are frequently preferred as first line antiretroviral regimen.

Factors affecting genetic barrier to resistance.
Genetic barrier to resistance can be influenced by different factors as reported below:
1. Impact of HIV-1 subtypes and recombinants on genetic barrier to resistance. HIV-1 has spread into different subtypes and recombinant forms that differ in around 10% of their nucleotide sequence. This genetic diversity among HIV-1 subtypes and recombinants can modulate genetic barrier to resistance. At this regard, a paradigmatic example is represented by the K65R mutation known to confer resistance to tenofovir. This mutation has been found to occur at high frequency in patients infected with HIV-1 subtype C failing tenofovir, stavudine and didanosine, and rarely in patients infected with HIV-1 subtype B (Martinez-Cajias et al., 2009). This finding can be explained by the fact that in subtype C, the generation of K65R requires only a single nucleotide substitution, while in subtype B, K65R requires 2 nucleotide substitutions to appear. In addition, recent studies have shown that in HIV-1 subtype C (and not in subtype B), the nucleotide sequence in the genomic region including the RT codon 65 increases the ability of HIV-1 RT to introduce mutations in the viral genome and in particular at codon 65 (Brenner et al., 2006). Thus, overall findings support why in subtype B the K65R mutation is rarely detected at virological failure, and by this light, tenofovir can be considered a drug with genetic barrier to resistance lower in subtype B, but with intermediate barrier in subtype C. Due to the similar evolution process, the same concept applies to abacavir (that in subtype C selects for K65R with a frequency higher than subtype B), as well as d4T and d4T (drugs with limited use today).
Figure 1. Fig. 1. A) The probability that a single (or at most double) drug resistance mutations pre-exist as minority species in drug-naïve patients is high. This means that (virtually) all drug-naïve patients harbor viral strains resistant to 1 (at most 2) antiretroviral drugs. Conversely, the probability that 3 drug resistance mutations (conferring resistance to 3 different drugs) co-exist in the same viral genome is extremely low. This means that (virtually) no drug-naïve patient harbors viral strains resistant to 3 antiretroviral drugs.

B) In a drug-naïve patient, the natural HIV-1 variability allows only the circulation of viral strains resistant to a single drug. The replication of these strains can be suppressed by the other active drugs included in the combination therapy. This allows a full suppression of viral replication even when drugs with low genetic barrier to resistance are used. Dotted lines indicate the loss of drug activity, while colored lines indicate when the drug is active.
2. Genetic barrier and the role of minority drug resistance strains

As mentioned previously, minority drug resistant species can pre-exist even before the onset of therapy. Their presence can be due to natural HIV evolution and/or to the transmission of HIV resistant strains from a treated individual. The advent of ultra-deep sequencing methodologies has allowed their detection in both drug-naive and drug-treated patients, and the evaluation of their impact on virological response to antiretroviral therapy.

A systematic review of the literature and a pooled analysis has recently examined the relationship between the presence of baseline low-frequency HIV-1 drug resistance mutations and the risk of virologic failure with NNRTI-based regimens in treatment-naive adults (Li et al., 2011). First, this study has shown that the presence of minority variants correlates with higher rate of virological failure. This rate is further increased in the setting of poor adherence. Again, this highlights the importance to full adherence (in particular when drugs with low genetic barrier to resistance are used) as critical factor to limit drug resistance emergence. Secondly, this study has shown a dose-dependent increased risk of virologic failure in patients with a higher proportion of drug-resistant variants. In line with this result, Goodman and his group have recently defined the threshold quantity of K103N minority resistant variants above that in which patients fail the NNRTI containing therapy (Goodman et al., 2011). By multiplying the proportion of virus with K103N in RT and the viral load, a threshold of >2,000 copies/ml of HIV carrying such NNRTI-resistance mutation has been correlated with an increased risk of viral failure to an efavirenz-containing regimen. Thus, this finding highlights that the quantity, rather than just presence, of minority resistant quasispecies, plays a game in regulating the efficacy of NNRTI-based first line therapies, and highlights the importance to establish a cut-off below which the risk of failure declines.

So far, the ultra-deep sequencing methodologies have been used to evaluate the role of single drug-resistance mutation in minority species on virological response to antiretroviral regimen. Further studies are needed to evaluate the linkage of drug resistance mutations (especially in the settings of broad transmitted drug resistance) and their impact on virological failure.

2. Role of polymorphisms

Recent studies have highlighted a potential role of RT polymorphisms in modulating virological response to NNRTI-containing first line HAART. An example is represented by polymorphisms at position 135 that have been shown to correlate with higher rate of virological failure to efavirenz containing regimen. Structural analysis has shown the ability of the polymorphisms at position 135 to stabilize the emergence of the K103N conferring full resistance to first generation NNRTI.

Conclusions

Drug resistance still represents a major cause of virological failure. The knowledge of factors affecting its emergence is critical to set up antiretroviral regimens aimed at achieving maximal virological suppression. The use of drugs with low genetic barrier to resistance has always represented matter of debate and discussion for their higher tendency to develop drug resistance. However, due to intrinsic HIV-1 properties, in the absence of drug-pressure, there are no chance for HIV to spontaneously develop 3 or more than 3 drug resistance mutations in the same viral genome, and thus to become completely resistant to the combination regimen. This can explain why the combined use of 3 potent drugs, even if characterized by low/medium genetic barrier to resistance, allows the rapid achievement of virological success and the long-term maintenance of virological suppression in a quite high proportion of patients starting their first line antiretroviral regimen. This suggests to revise the concept of genetic barrier that should be applied to the entire regimen and not to the single drug. The advent of highly advanced methodologies, such as ultra-deep pyrosequencing, will let us better clarify this new concept.
References

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