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Hepatitis C Virus Resistance to Direct-Acting Antiviral Drugs in Interferon-Free Regimens

Jean-Michel Pawlotsky[1,2]

[1]*National Reference Center for Viral Hepatitis B, C and D, Department of Virology, Hôpital Henri Mondor, Université Paris-Est, Créteil; and* [2]*INSERM Unité 955, Créteil, France*

Treatment of hepatitis C virus (HCV) infection has progressed considerably with the approval of interferon-free, direct-acting antiviral (DAA)-based combination therapies. Although most treated patients achieve virological cure, HCV resistance to DAAs has an important role in the failure of interferon-free treatment regimens. The presence of viral variants resistant to NS5A inhibitors at baseline is associated with lower rates of virological cure in certain groups of patients, such as those with genotype 1a or 3 HCV, those with cirrhosis, and/or prior nonresponders to pegylated interferon–based regimens. DAA-resistant HCV is generally dominant at virological failure (most often relapse). Viruses resistant to NS3-4A protease inhibitors disappear from peripheral blood in a few weeks to months, whereas NS5A inhibitor–resistant viruses persist for years. Re-treatment options are available, but first-line treatment strategies should be optimized to efficiently prevent treatment failure due to HCV resistance.

Keywords: Resistance; Resistance-Associated Substitutions; Treatment Failure; Retreatment.

Treatment of hepatitis C virus (HCV) infection has progressed considerably since the approval in 2014 of direct-acting antivirals (DAAs) and the subsequent availability of interferon (IFN)-free, DAA-based combination therapies. Despite the high rates of virological cure achieved with these treatments, the infection is not eliminated from a substantial number of patients (1%–15%, depending on the patient group and regimen).[1,2] Factors that influence the ability of infected patients to be cured include the patients' metabolism of the DAA agents, their genetic background (eg, polymorphisms in the *IL28B* gene), whether they have extensive fibrosis or cirrhosis, their adherence to therapy, and resistance of the HCV to DAAs, which is an important factor in the failure of IFN-free regimens.

We review the principles of HCV resistance to DAAs, the role of HCV resistance in IFN-free treatment virological failures, the dynamics of resistant viruses after treatment failure, ways to prevent failure due to resistance, re-treatment options, and the utility of HCV resistance testing at different time points of therapy.

Principles of HCV Resistance to DAAs

Definition of Viral Resistance and Resistance-Associated Substitutions

HCV has a quasispecies distribution. Patients are infected by complex mixtures of genetically distinct but closely related viral populations of different sizes. Their respective proportions depend on their replication capacities in their environment (defined as fitness). HCV populations coexist in equilibrium at any time point, but any change in the environment tips the equilibrium and alters the quasispecies distribution.[3]

The viral populations that constitute the quasispecies differ by amino acid polymorphisms that emerge by mutation during replication and are subsequently selected based on their effects on viral fitness. Natural polymorphisms that lie in a viral protein region important for the antiviral effect of a DAA may confer reduced susceptibility to the DAA or DAA class. Such polymorphisms can be present in major, highly fit viral populations. However, they are more often present in minor viral populations because they generally reduce fitness compared with wild-type viruses (ie, viruses without these polymorphisms).

When a DAA is administered, positive selection of viral variants with reduced susceptibility to this drug defines viral resistance. Complete inhibition of DAA-sensitive wild-type viruses opens the replication space, allowing variants with reduced susceptibility to rapidly outgrow them. Additional, so-called compensatory or secondary amino acid substitutions, or fitness-associated substitutions—either naturally present or acquired by mutation during replication of the resistant virus on drug administration—may increase the fitness of resistant variants, leading to their rapid outgrowth on treatment (breakthrough) or after treatment (relapse) and influencing their posttreatment persistence.

Abbreviations used in this paper: **DAA, direct-acting antiviral; EC_{50}, 50% effective concentration; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IFN, interferon; RAS, resistance-associated substitution; RdRp, RNA-dependent RNA polymerase; SVR, sustained virological response.**

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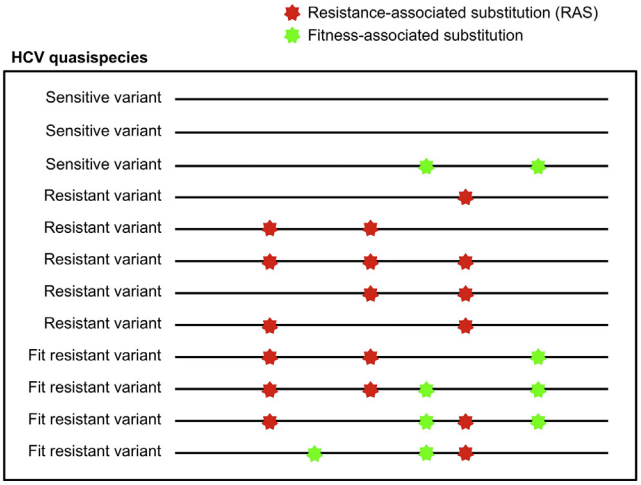

Figure 1. Definitions in HCV resistance. Viral variants are individual full-length viruses that constitute the HCV quasispecies in a patient. They are organized as viral populations, made of identical variants that are different from the variants in other populations. The sequence of sensitive variant genomes does not contain amino acids that confer reduced susceptibility to the antiviral action of an HCV DAA. Compared with the sequence of sensitive variants, the sequence of resistant variants contains one or several RASs, which are single amino acid changes that reduce susceptibility to a DAA or a class of DAAs. The sequence of resistant variants sometimes also contains one or several fitness-associated substitution(s), which are single amino acid changes that do not alter DAA susceptibility but increase the fitness of the resistant variants, giving them a replication advantage over other resistant variants. Fitness-associated substitution(s) can also be present in the sequence of sensitive variants, improving their replication capacity or having no effect in the absence of the RAS(s). The populations of viral variants coexist within each patient's viral quasispecies, under the pressure of Darwinian selection forces. In the absence of DAA treatment, sensitive viruses are generally (but not always) the fittest. When DAAs are administered, resistant variants (variants carrying RASs) are selected. Their outgrowth depends on their fitness in the presence of the drug more than on the level of resistance conferred by the RASs. When treatment is stopped, the outcome of competition between the variants also depends on their respective fitness.

The term "resistance-associated variant" is often used to indifferently describe the amino acid substitutions that reduce the susceptibility of a virus to a drug or drug class or, alternatively, the viral variants with reduced susceptibility that carry these substitutions. This term is inaccurate and should no longer be used. Instead, the amino acid substitutions that confer resistance must be called "resistance-associated substitutions" (RASs), and the viral variants that carry these RASs (and thereby have reduced susceptibility to the DAA) must be called "resistant variants" (see Figure 1). This terminology will be used throughout this review article.

Factors That Affect HCV Resistance to DAAs

Resistance of HCV to DAAs is determined by 3 major factors.[4] One is the genetic barrier to resistance, related to the number and type of nucleotide substitutions required for emergence of RASs during replication and to the number and type of RASs required for a viral variant to acquire full resistance to the drug. The genetic barrier to resistance varies with drug class, specific drug, and HCV genotype or subtype. It determines the likelihood that resistant viruses are generated during replication. Resistance is also determined by the fitness of resistant virus populations, which is independent of the level of resistance conferred by the RASs (the most resistant variants are not necessarily the fittest and vice versa). Fitness determines the likelihood that generated resistant viruses persist in minor or major populations. Resistance is finally determined by level of drug exposure compared with the drug's 50% and 90% inhibitory concentrations in vitro. In vivo exposure affects the ability of a drug to inhibit replication of resistant variants.

Characteristics of HCV Resistance to DAAs

In patients receiving antiviral treatment, HCV kinetics are typically biphasic.[5] The first-phase HCV RNA decline is rapid and results from the direct inhibitory effect of the drug(s) on viral replication. This phase depends on drug potency, exposure, and virus susceptibility. The second, slower-phase HCV RNA decline results from the progressive loss of HCV from cells due to degradation of nonreplicating viral RNAs by effectors of the innate immune system. This second phase is influenced by drug potency, genetic features of the host, and the severity of liver disease and can be accelerated by ribavirin through unclear mechanisms.[6]

In patients treated with a combination of DAAs, the absolute amount of each viral population present in the quasispecies at baseline evolves following individual kinetics that depend on the starting amount of the viral population, its susceptibility to the antiviral action of the drugs, and its fitness in the presence of the drugs (see Figure 2). Sensitive viral populations are rapidly eliminated following a typical biphasic decline if treatment duration is sufficient. In contrast, resistant variants, which are only partly or not at all inhibited, slowly decrease, remain at the same level, or expand. Some are still present in the liver when treatment is stopped, even though they were undetectable in peripheral blood during treatment. After treatment is withdrawn, these resistant variants start replicating again, eventually acquire mutations that increase their fitness, and propagate in the liver, ultimately causing virological relapse (Figure 2).

At treatment failure (breakthrough or relapse), if adherence and treatment duration have been appropriate, most if not all of the viral variants in the quasispecies are resistant to one or several of the drugs administered. After treatment, some variants (such as those resistant to NS3-4A protease inhibitors) disappear within a few weeks to months. They are replaced by wild-type, DAA-sensitive virus. The wild-type virus either persists in the liver—if therapy is not long enough to clear it—and outgrows them after treatment withdrawal, or it is generated by spontaneous mutation of resistant viruses (reversion to wild-type) if the original wild-type virus is cleared during therapy.