Drug Resistance Updates 37 (2018) 17–39

Contents lists available at ScienceDirect

Drug Resistance Updates

journal homepage: www.elsevier.com/locate/drup

Hepatitis C virus drug resistance associated substitutions and their clinical relevance: Update 2018

Maria C. Sorbo[a], Valeria Cento[d,*], Velia C. Di Maio[a], Anita Y.M. Howe[b], Federico Garcia[c], Carlo F. Perno[e], Francesca Ceccherini-Silberstein[a]

[a] Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome, Italy
[b] Centre for Excellence in HIV/AIDS, St Paul's Hospital, Vancouver, BC, Canada
[c] Clinical Microbiology Service, Hospital Universitario San Cecilio, Granada, Spain
[d] Residency program in Microbiology and Virology, Università degli Studi di Milano. Milan, Italy
[e] Department of Oncology and Oncohematology, Università degli Studi di Milano. Milan, Italy

ARTICLE INFO

Keywords:
Direct acting antiviral agents
Treatment failure
Fold-change
HCV genotypic resistance testing
HCV sequencing

ABSTRACT

Nowadays, due to the development of potent Direct-Acting Antiviral Agents (DAAs) that specifically target NS3, NS5A and NS5B viral proteins, several new and highly efficacious options to treat chronic Hepatitis C virus (HCV) infection are available. The natural presence of resistance associated substitutions (RASs), as well as their rapid emergence during incomplete drug-pressure, are intrinsic characteristics of HCV that greatly affect treatment outcome and the chances to achieve a virolgical cure. To date, a high number of RASs in NS3, NS5A, and NS5B have been associated *in vivo* and/or *in vitro* with reduced susceptibility to DAAs, but no comprehensive RASs list is available. This review thus provides an updated, systematic overview of the role of RASs to currently approved DAAs or in phase II/III of clinical development against HCV-infection, discriminating their impact in different HCV-genotypes and DAAs, providing assistance for a fruitful use of HCV resistance testing in clinical practice.

Introduction

Hepatitis C infection is a leading cause of liver diseases, liver cirrhosis, and hepatocellular carcinoma. Globally, over 170 million people are infected with hepatitis C virus (HCV), and about 71 million of individuals have viraemic infections in 2015 (Polaris Observatory HCVC, 2017).

Because of the high replication rate and the lack of proofreading activity of viral NS5B polymerase, HCV shows an intrinsic predisposition to mutate and exists within the same host as a population of slightly different viral-variants, known as "quasispecies" (Ogata et al., 1991). Genetic variability among viral strains circulating worldwide is very high, and the 7 HCV genotypes (GTs) and the > 80 confirmed subtypes (Smith et al., 2018; Smith et al., 2014) show different geographical distribution, pathogenesis and response to anti-HCV treatment. The major HCV GT-1, GT-2, and GT-3 account ~85% of all HCV infections (Messina et al., 2015). The other GTs are less prevalent globally, yet can reach high prevalence in certain geographic regions (Messina et al., 2015). GT-1 has long been the most difficult to cure, due to its high prevalence (it is the far most common worldwide) and showed poor responsivity to interferon, when compared to more "easy" GT-2 and GT-3. The development of direct acting antiviral agents (DAAs) has incredibly improved GT-1 (and GT-4) treatment options, while GT-3 has emerged as a "difficult" to eradicate virus. GT-3 is spread worldwide, and reaches high prevalence in South Asia, Russia, and Australia, as well as in "special populations" (i.e. people who inject drugs) in Western Countries. Among HCV GTs, GT-3 presents unique predisposition in promoting progression of fibrosis, cirrhosis and HCC (Kanwal et al., 2014; McCombs et al., 2014), and it is currently associated with lowest sustained virological response (SVR) rates with DAAs, particularly in presence of cirrhosis.

DAA failure is an unfortunate event that can happen with all HCV GTs, and in a variety of clinical situations, and is frequently associated with the presence of HCV resistance-associated variants (RAVs) (EASL, 2017; AASLD-IDSA, 2018; Pawlotsky, 2016; Sarrazin, 2016). The development of drug resistance is indeed an intrinsic, and to some extent unavoidable, characteristic of antiviral therapies. RAVs found at failure are generally developed during treatment, but in some patients they may pre-exist as naturally occurring variants before treatment, impairing DAA efficacy especially in GT-1a and GT-3 (Komatsu et al.,

* Corresponding author.
E-mail addresses: valeria.cento@unimi.it (V. Cento), carlo.perno@unimi.it (C.F. Perno), ceccherini@med.uniroma2.it (F. Ceccherini-Silberstein).

https://doi.org/10.1016/j.drup.2018.01.004
Received 25 September 2017; Received in revised form 29 November 2017; Accepted 11 January 2018

2017; Zeuzem et al., 2017; Harrington et al., 2017).

A RAV is defined by the presence of one or more resistance-associated substitutions (RASs), amino acid substitutions able to adversely impact the activity of DAAs *in vitro* and/or *in vivo* in treated patients.

As with HIV, the classification of a specific amino acid substitution as a RAS is possible through genotypic and phenotypic analyses. Genotypic analyses include population sequencing and next-generation sequencing (NGS); each of them differs in the ability of detecting single specific substitutions within the infecting viral quasispecies: as population sequencing can detect only variants representing > 15-20% of the quasispecies, NGS can reach those with a 0.1-1% prevalence. In clinical trials only RASs present > 15% have shown to be clinically relevant, so HCV genotypic resistance testing in daily clinical practice can be optimally carried out by population sequencing. If NGS is used, a 15% cut-off for reporting RASs is recommended (Pawlotsky, 2016). Drug-specific RASs that are found at a lower frequency may not convey sufficient resistance to reduce SVR rates with currently available DAA regimens (AASLD-IDSA, 2018).

HCV resistance started to gain clinical relevance in 2011, when DAAs were first approved for clinical use. Since then, the role of either natural or acquired RAVs in clinical management and optimization of HCV-treatment has been a matter of discussion. Until recently, the emergence of RAVs (often with RAS on multiple DAA-targets) at failure had a significant impact on the efficacy of a second-, or third-line regimen (AASLD-IDSA, 2018; Pawlotsky, 2016; Sarrazin, 2016; Di Maio et al., 2017; Li and De Clercq, 2017). The utility of RAS testing depends upon both patient characteristics and DAA regimen (AASLD-IDSA, 2018). However, the recent approval of new pangenotypic drug combinations, with a very high genetic barrier to resistance and antiviral potency may change the debate. Nevertheless, until newer DAAs become extensively available in all countries, and the issue of resistance will not be overcome, the HCV genotypic resistance testing is, and will be, an essential diagnostic tool for tailoring personalized treatments, particularly after a DAA-failure (AASLD-IDSA, 2018). Outside the US, the universal use of HCV resistance testing is limited by the lack of a validated, widely available, and easy to acess assay. In addition, since many RASs may have a different prevalence and impact according to viral genotype and even subtype, the interpretation of the resistance profile is very complex, and is only partially supported by the currently available algorithms/databases (such as Geno2pheno) (Kalaghatgi et al., 2016), reviews (Pawlotsky, 2016; Sarrazin, 2016; Lontok et al., 2015), or international guidelines (AASLD-IDSA, 2018), that may not be as satisfactory updated fo the current clinical need.

In the “changeable and dynamic” scenario of HCV-treatment, resistance-interpretation tools need to be continuously updated as new information emerge. To support clinicians and virologists in their daily clinical practice, this review provides an extended update of both single and multiple RASs, reported as a graphic summary of clinically relevant substitutions found in failing patients, along with their *in vitro* fold-change reduction in drug-susceptibility.

Methodology

This systematic review analyze all DAAs approved in Europe, USA and Japan as of September 2017 (Table 1) (AASLD-IDSA, 2018; JSH, 2014). Experimental drugs in phase II and III in clinical development were also analyzed, and results are reported in Supplementary Material. All papers published in peer-reviewed journals reporting results of approved drugs and experimental phase II and III drugs from clinical trials and/or real-life studies were included, along with abstracts presented at the most important international congresses in the last few years (such as EASL, AASLD, CROI), and not yet published.

Wild-type amino acids can largely differ according to HCV-genotype/subtype and geographic origin (Bukh, 2016). Reference amino acid sequence for each HCV genotype was defined as reported by Geno2Pheno (Table 2) (Kalaghatgi et al., 2016). In the context of DAA treatment, any amino acid change from the reference sequence at an amino acid position that has been associated *in vitro* with reduced susceptibility of a virus to one or more antiviral drugs is commonly defined as RAS (AASLD-IDSA, 2018; Pawlotsky, 2016). This definition suits well *de novo* developed substitutions found at DAA failure when comparing with the baseline HCV sequence from the same patient. However, it should be noted that in the context of viruses with natural resistance or emergent resistance, it would be more appropriate to use the term “variants” instead of substitutions. Moreover, for pre-existing RAVs, as we do not know if a real substitution occurred in the patient or that this viral variant was passed on during a transmission event, the term “polymorphism” is preferred.

As indicated by the latest international guidelines, before define a RAS, it is necessary to specify: the HCV genotype (eg, genotype 1, 2, 3, etc) and subtype (eg, 1a vs 1b); the HCV protein (eg, NS5A); and the amino acid position (eg, 93) (AASLD-IDSA, 2018). Each RAS (such as Y93H) is indicated by a first letter for the reference amino acid (eg. Y), a number for the amino acid position in the wild-type protein (eg. 93), and a second letter representing the amino acid actually found in the sequence analyzed (eg. H). Due to the quasispecies nature of HCV, some patients may be simultaneously infected by multiple variants harbouring different amino acids at positions associated with drug resistance (i.e. Y93Y/H/M in NS5A). This means that such patients would have variants with either the amino acids tyrosin (Y), histidine (H) or methionine (M) at position 93 of the NS5A protein.

A specific substitution may, or may not, confer a phenotypic reduction of susceptibility to one or multiple antiviral agents. For this reason, a drug-specific RAS is an amino acid substitution that reduces the susceptibility of a virus to a specific drug, while drug-class RASs are amino acid substitutions that reduce the susceptibility of a virus to at least one (but possibly more) member of a drug-class (AASLD-IDSA, 2018).

In the following paragraphs, both RASs observed during *in vitro* studies and those observed at virological-failure in DAA-treated patients are reported. Since the number of patients who failed treatment with most recent DAAs is extremely low, no specific cut-off for RASs prevalence at failure was used. The criteria to be included in the present analysis as *in vivo* RASs were thus: a) to be found as a *de novo* developed variant in failing patients; or b) to have a demonstrated impact on virological response even if found as natural RAS/RAV.

For a complete overview of both *in vitro* and *in vivo* RASs, please refer to Fig. 1, panel A (NS3-RASs), panel B (NS5A-RASs), and panel C (NS5B-RASs). Potential clinical relevance of NS5A-RASs is further analyzed in Fig. 2.

Individual resistance profiles for approved DAAs

NS3 protease inhibitors

NS3-protease inhibitors (PIs) are peptidomimetics inhibitors able to prevent viral polyprotein cleavage by competing with the natural NS3 serine-protease substrates (Bartenschlager et al., 2013). With the exeption of the catalytic triad, only 47% of NS3-residues are fully-conserved across HCV-GTs (Cento et al., 2012), making it difficult to design pangenotypic inhibitors.

First-generation PIs, such as asunaprevir, paritaprevir, simeprevir, vaniprevir, showed high antiviral potency, though with poor GT-coverage, low genetic barrier to resistance and considerable cross-resistance at amino acid positions V36, T54, R155, A156 and D168 (Fig. 1, panel A; Table 3). Second-generation approved PIs, such as grazoprevir, glecaprevir and voxilaprevir exhibit improved genetic barrier to resistance, and enhanced antiviral activity against multiple HCV-GTs (Table 1). Yet, GT-3 is still “difficult-to-treat” with PIs, probably because of some active site polymorphisms (R123T-I132L-D168Q) (Soumana et al., 2016). Among second-generation PIs, grazoprevir show high activity with 0.2 nM EC_{50} values against GT-1, yet