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HCV DAA genotypic drug resistance testing

Approximately 80% of individuals infected with Hepatitis C Virus (HCV) go on to have a chronic infection that can lead to hepatocellular carcinoma. Traditionally classed as a chronic infection that could occasionally be treated with interferon and ribavirin, the advent of Directly-Acting Antivirals (DAAs) in 2011 has allowed healthcare professionals to cure HCV infected individuals. The advent of DAAs has allowed for interferon-free treatments with greater tolerability and reduced side-effects, particularly for HCV infected individuals with renal disease, or immunosuppression. In addition, cure rates (determined by the sustained virological response (SVR) 6 months after stopping treatment) have been improved to over 90% across all genotypes with DAAs, as compared to 55% average treatment success with pegylated interferon alpha and ribavirin.

Development of new DAAs and treatment strategies continues to improve cure rates, even for difficult-to-treat HCV genotypes. Currently, each of the licenced anti-HCV DAAs target one of three gene-products of the virus: NS3 (Serine protease/RNA helicase), NS5A (phosphoprotein that is also an interferon resisting protein) and NS5B (RNA dependent RNA polymerase). EASL and NICE no longer produce guidance documents on the recommended treatment combinations, as the development and understanding of anti-HCV DAAs is rapidly evolving.

NS3 inhibitors

Early anti-HCV DAAs targeted the NS3A protease (boceprevir and telaprevir) and are effective against genotype 1 viruses. These agents are all licenced in the UK and can significantly boost response to treatment although they were poorly tolerated and treatment discontinuation rates were high.

Simeprevir is a licenced NS3/4A protease inhibitor for genotypes 1 or 4 HCV infection. It can be given in combination with or without interferon, depending on the ability of the individual to tolerate the treatment. Treatment with simeprevir and PEG-IFN/RBV, was found to induce significantly improved SVR when compared with PEG-IFN/RBV alone (79.2% vs 45.6%) and has an improved side effect profile when compared to the first generation PIs boceprevir and telaprevir.

Since 2011, many more anti-HCV DAAs have been developed that target the NS3 product: Asunaprevir, grazoprevir, paritaprevir, voxilaprevir. Currently, only grazoprevir and paritaprevir are recommebded by NICE as UK HCV treatment options, as part of combination therapies with an NS5A inhibitor.

Micropathology Ltd can offer a genotype 1a NS3A genotypic resistance test covering codons 1-181 of the gene and all reported DAA resistance associated mutations that are currently known, including the Q80K polymorphism (associated with reduced response to simeprevir). As HCV DAAs have advanced, and the use of NS3A inhibitors has decreased in many clinics, Micropathology Ltd can offer this test on a research-only basis and it is not UKAS accredited.

NS5A inhibitors

In 2016, a new DAA was licenced in the UK as an alternative treatment option for HCV infection in individuals with genotype 1 or 4. Zepatier is a fixed dose combination drug (elbasvir-grazoprevir). Elbasvir inhibits non-structural viral protein, NS5A, and grazoprevir inhibits NS3/4A protease. Zepatier can be taken as a stand-alone treatment whereas earlier NS3A/4A DAAs are used in combination with peginterferon alpha and/or ribavirin.

However, there are certain NS5A polymorphisms that mean combination therapy may be necessary. For example, in genotype 1a infected individuals the presence of one or more polymorphisms at positions M28, Q30, L31, or Y93 of the NS5A coding region are associated with a reduced response to treatment. Sustained virological response was achieved in 98% of subjects without NS5A resistance associated polymorphisms following 12 weeks therapy compared with 70% of subjects with NS5A resistance associated polymorphisms.

Additional NS5A inhibitors have been developed and licenced in the UK, including ledipasvir, daclatasvir and ombitasvir. Pibrentasvir is available in combination with glevaprevir (AbbVie), but it is not used as much as other options in the UK.

It is recommended that individuals infected with HCV are offered viral load, genotyping and resistance testing prior to treatment initiation. These tests allow clinicians to take the most appropriate “tailored” treatment option for the patient, as some DAAs are not appropriate for non-genotype 1 infections.

Micropathology Ltd have developed an NS5A testing protocol for patients known to be genotype 1a positive. Our assay is based on RT-PCR amplification and Sanger sequencing of codons 14-106 of the NS5A region in order to ascertain the polymorphism genotype.

NS5B inhibitors

The NS5B gene product of HCV is an RNA-dependent RNA polymerase and theoretical development of resistance to this target for novel DAAs is limited, because of the critical nature of the polymerase in viral replication. There are currently 2 NS5B inhibitors licenced in the UK: sofosbuvir and dasabuvir.

The prodrug sofosbuvir can be used in combination with peginterferon alpha and ribavirin for the treatment of chronic HCV infection of genotypes 1-6, with compensated liver disease. Monotherapy is not advised. Dasabuvir is recommended

in combination with ombitasvir-paritaprevir-ritonavir (+/- ribavirin) for genotype 1 infections with or without cirrhosis.

Currently, the Micropathology Ltd assay for NS5B is predominantly for genotyping purposes, but it can be used for limited DAA resistance information. It covers codons 222-345 of the NS5B gene of any genotype, allowing resistance information to be provided for all known mutations associated with sofosbuvir (codons 282, 289, 320 and 321). However, as this assay can only provide information regarding dasabuvir codon 316 and does not detect codons 348-556, dasabuvir resistance cannot currently be reported.

In all instances, HCV RNA levels should be monitored throughout therapy and treatment discontinued if viral load rises above 25 IU/ml at 4, 12 or 24 weeks. For appropriate levels of information regarding HCV DAA resistance, a minimum viral load of 500 IU/mL is required to be able to report minority variant resistance at around the 10% virus population level. Although it is possible to test samples with viral loads <500 IU/mL, there is a risk that sequence analysis will only detect wild-type (non-resistant) sequence data, even if resistance mutations are present at low level.

References

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