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Advances in Treatment of Chronic Hepatitis C Virus (HCV) Infection

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Abstract

Hepatitis C virus (HCV) infection is a prominent cause of chronic liver disease and may lead to serious complications such as liver failure and need for a transplant. The virus is transmitted via exposure to blood and is classified into various genotypes based on genetic mutations in the virus. Current treatment options for HCV infection are not effective in all patients, and there are limited options for patients infected with a genotype other than genotype 1. Two new medications have been approved recently for treatment of HCV infection. Simeprevir (Olysio®) gained U.S. Food and Drug Administration (FDA) approval in November 2013, and sofosbuvir (Sovaldi®) was approved in December 2013. Information from clinical trials with each of the medications supports their safety and efficacy in appropriate patient populations. The adverse effects are generally tolerable; however, for some patients, the adverse effects, drug interactions and cost can be limiting factors.

Introduction

Hepatitis C virus is a blood-borne pathogen leading to long-term hepatic complications if not properly treated.[1] Current therapy for HCV includes ribavirin (RBV), peg-interferon alfa (PegIFN) and two options for protease inhibitors (boceprevir and telaprevir), which can only be used in one genotype of HCV infection.[2-4] Two new medications were recently approved for treatment of chronic HCV infection: simeprevir (Olysio®) in November 2013 and sofosbuvir (Sovaldi®) in December 2013.[5,6] These new medications can provide more treatment options to patients with different genotypes or for whom prior treatment with RBV and PegIFN was unsuccessful.[7,8] Safety and efficacy have been demonstrated in several clinical trials.[9-12] The adverse reactions and cost of these medications are important factors to consider when determining treatment for HCV infection in a patient.

Disease State Overview

Hepatitis C infects approximately 170 million individuals worldwide, which has become both a public health and an economic issue.[13] Being the most common blood-borne pathogen, the HCV is one of the leading causes of chronic liver disease and the third leading cause of death in patients with end stage renal disease.[1,2] All individuals who are infected with HCV are at a greater risk for developing long-term complications such as cirrhosis, liver failure and hepatocellular carcinoma.[14]

Hepatitis C virus is a single-stranded, positive RNA virus, which has a high rate of mutation, leading to therapy resistance and immune system evasion.[15] Presentation varies from patient to patient in that some patients may present with mild hepatitis or inflammation of the liver, while others experience scarring or even liver cancer.[16] The first phase begins with HCV exposure which results in high viral titers being expressed in the blood. In this early stage, which typically lasts six to eight weeks, a T-cell mediated immune response is apparent in the absence of liver damage. The second phase of HCV infection begins with the generation of HCV specific antibodies and T-cells. This phase is also characterized by an increase in liver enzyme levels, which is evidence of hepatocyte destruction. Phase three, which takes place 12 to 24 weeks after infection, shows variations in HCV viremia as well as decreasing capabilities of functional T-cells. Phase four, or 24 weeks post-infection, marks the end of the acute phase of the HCV infection as cellular immunity continues to evolve.

The virus itself does not cause damage to the liver, but rather the response it elicits from the immune system causes healthy liver tissue to be replaced with scar tissue.[18] Typically, the preferred treatment for HCV is PegIFN along with RBV, a nucleoside analogue, to which approximately 55 percent of patients respond. For those patients who are non-responders, few treatment options exist, which has led to further research and development of HCV medications.

Treatment for HCV has evolved over the past years owing to advances in research and drug therapy.[2] Treatment in the mid-1990s consisted of interferon monotherapy, which was injected three times a week for a total duration of six to 12 months. In the late 1990s, RBV was added to interferon, which dramatically increased a patient's sustained virologic response (SVR). A sustained virologic response is defined as a lack of HCV in the blood after 24 weeks of treatment and remains the best indication of how successful therapy is in a patient.[18] Therapy evolved once more to the current regimen of PegIFN in addition to RBV.[2] The ultimate goal of treatment in patients with HCV is to reach an SVR, as defined above. Currently, there is no vaccine to prevent HCV.[19]

Genotyping plays an important role in determining treatment for HCV.[20] Currently, there are six clinically significant genotypes (1-6) identified for HCV, of which some are found to be more prevalent in different parts of the world. For example, genotypes 1 through 3 are more common in the United States and Europe, genotype 4 is found most often in Egypt, and genotype 6 is more common in South Asia. Based on what genotype a person has, treatment and also response to treatment will differ. HCV genotyping is done to provide

the most effective therapy and to get the maximum response out of the medication prescribed.

Overview of New Medications

Two new medications have recently been approved for the treatment of chronic HCV infection.[6] The first is simeprevir (Olysio®), approved Nov. 22, 2013.[5] The second is sofosbuvir (Sovaldi®), approved Dec. 6, 2013.

Simeprevir is a HCV NS3/4A protease inhibitor to be used in combination with PegIFN and RBV to treat chronic HCV infection in patients with genotype 1 HCV infection.[7] The HCV NS3/4A protein is a serine protease that is essential in cleaving the single HCV polyprotein precursor into the 10 individual proteins needed for HCV maturation and replication.[21,22] Inhibition of NS3/4A prevents the viral replication of HCV.[21] Simeprevir is the third HCV NS3/4A protease inhibitor to receive approval, with the previous two being boceprevir (Victrelis®) and telaprevir (Incivek®), each approved in 2011.[5] Simeprevir differs from the other two protease inhibitors in that it is administered only once daily; however, all three protease inhibitors are only effective in genotype 1 HCV infection and must be used with both PegIFN and RBV.[3,4,7] Simeprevir is a 150 mg capsule taken once daily with food.[7] The treatment schedule is 12 weeks of simeprevir with PegIFN and RBV for 24 or 48 weeks, depending on prior treatment status.

Sofosbuvir is a NS5B polymerase inhibitor.[8] The HCV protein NS5B is an RNA polymerase that transcribes viral RNA in order to produce HCV proteins.[8,23] Sofosbuvir is a nucleotide prodrug that is converted in the liver to a uridine analog, which then inhibits the work of NS5B by incorporating into HCV RNA and acting as a chain terminator, thereby preventing the completion of viral replication. Sofosbuvir has shown efficacy in treating genotypes 1, 2, 3 and 4 HCV infection.[8] Sofosbuvir is a 400 mg tablet taken once daily without regard to food. Genotype of HCV infection determines treatment duration and PegIFN requirement. Genotype 1 is treated with sofosbuvir plus PegIFN and RBV for 12 weeks, or if ineligible for PegIFN, sofosbuvir plus RBV for 24 weeks. Genotype 2 is treated with sofosbuvir plus RBV for 12 weeks, and genotype 3 has the same treatment extended to 24 weeks. Genotype 4 is treated with sofosbuvir plus PegIFN and RBV for 12 weeks. Sofosbuvir is the first drug in its class and the first drug that does not necessarily require concomitant use of PegIFN for the treatment of chronic HCV infection.[6]

Review of Clinical Trials

Several clinical trials have evaluated the safety and efficacy of these two new medications. One of the clinical trials evaluated by the FDA in considering the approval of simeprevir was the ASPIRE trial.[10] ASPIRE was a phase IIb, randomized, double-blind trial. This trial evaluated the safety and efficacy of simeprevir plus PegIFN/RBV in comparison to PegIFN/RBV alone in patients with HCV genotype 1 who have previously failed to respond to treatment with PegIFN/RBV. Four hundred sixty-two patients began the trial and were divided into seven groups. All groups received PegIFN/RBV for 48 weeks. Each group also received either 100 mg or 150 mg simeprevir once daily for 12, 24 or 48 weeks, or no simeprevir, designated as the control group. The primary end point of the study was the proportion of patients maintaining SVR at 24 weeks after end of treatment (SVR24). In the simeprevir groups, 60.6 to 80.0 percent of patients achieved SVR24 compared to 22.7 percent of the control group, demonstrating efficacy of the addition of simeprevir over PegIFN/RBV alone. In the 150 mg simeprevir groups, 72.9 percent of patients achieved SVR24 compared to 65.6 percent of patients in 100 mg simeprevir groups, supporting the choice of 150 mg capsules. Duration of treatment with simeprevir showed no improvement in SVR24 rate beyond 12 weeks (68.2% in 12-week groups, 69.2% in 24-week groups and 70.2% in 48-week groups) supporting the use of simeprevir for only 12 weeks.

The ASPIRE trial further looked at the efficacy of simeprevir versus the control group in patients with prior null response, prior partial response and relapse to previous treatment with PegIFN/RBV.[10] In prior null response patients (patients that did not achieve SVR in previous treatment with PegIFN/RBV), SVR24 rates were 37.5 to 58.8 percent in simeprevir groups versus 18.8 percent in control group. In prior partial response patients (patients that had reduced HCV RNA in previous treatment with PegIFN/RBV but did not achieve SVR), SVR24 rates were 47.8 to 86.4 percent in simeprevir groups versus 8.7 percent in control group. In prior relapse patients (patients that achieved SVR at end of treatment (EOT) with PegIFN/RBV but had detectable HCV RNA 24 weeks later), SVR24 rates were 76.9 to 88.9 percent in simeprevir groups versus 37 percent in control group. This indicates potential efficacy in patients with prior treatment failure. The safety profile in simeprevir groups was similar to that of the control group (PegIFN/RBV alone). All groups had similar total incidence of adverse events (AEs) and severe AEs, with fatigue, headache, pruritus, influenza-like illness and neutropenia most frequently reported. Groups treated with simeprevir had higher frequency of rash (26.5% versus 18.2%) and pruritus (34.1% versus 16.7%) than PegIFN/RBV groups, although severity of these AEs was similar.

Another clinical trial evaluating simeprevir was the PILLAR trial.[11] PILLAR was a phase IIb, randomized, double-blind trial that assessed the safety and efficacy of simeprevir plus PegIFN/RBV in comparison to PegIFN/RBV alone in treatment-naïve patients with HCV genotype 1. The trial began with 386 patients divided into five groups. All groups received PegIFN/RBV for 48 weeks unless the patient was eligible to end all treatment at 24 weeks. Each group also received either 75 mg or 150 mg simeprevir once daily for 12 or 24 weeks, or no simeprevir, designated as the control group. If a simeprevir-treated patient achieved rapid virologic response (RVR; defined by HCV RNA undetectable at week 4) and had undetectable HCV RNA at weeks 12, 16 and 20, PegIFN/RBV could be stopped at week 24 rather than finishing the full 48 weeks. The primary end point was the proportion of patients maintaining SVR at week 72 of the trial (SVR72), with treatment ending at week 48 or earlier. There was a statistically significant difference in SVR72 between the 150 mg, 12-week simeprevir group versus control

group (77.9% versus 64.9%, P<0.05) and between the 150 mg, 24-week simeprevir group versus control group (84.8% versus 64.9%, p<0.05), indicating that the addition of 150 mg daily simeprevir leads to greater success in therapy than PegIFN/RBV alone. No differences in SVR rates were noted between different durations of simeprevir treatment. RVR was achieved by 68.0 to 75.6 percent of simeprevir patients versus 5.2 percent of patients in the control group. Of simeprevir-treated patients, 79.2 to 86.1 percent were eligible to complete all treatment at week 24. This is significant because it allowed these patients to cut treatment duration in half, saving money, time and inconvenience. Adverse events and serious AEs were similar across all groups. The most frequently reported AEs were fatigue, influenza-like illness, pruritus, headache and nausea, typically associated with PegIFN/RBV therapy. This trial did not show higher frequency of rash and pruritus in simeprevir groups compared to PegIFN/RBV alone.

The FDA approval of the other new HCV treatment medication, sofosbuvir, was dependent on the FISSION trial and the POSITRON trial, among others.[9,12] The FISSION trial was a phase III, randomized, open-label trial.[12] This study evaluated the safety and efficacy of sofosbuvir plus RBV in comparison to PegIFN/RBV alone in treatment-naïve patients with HCV genotype 2 or 3. At the beginning of the trial, 499 patients were randomized to receive either 400 mg sofosbuvir once daily plus RBV for 12 weeks or to receive PegIFN/RBV for 24 weeks. The primary end point was defined as the proportion of patients maintaining SVR at 12 weeks after EOT (SVR12). The primary end point was achieved in 67 percent of patients in the sofosbuvir/RBV group versus 67 percent in the PegIFN/RBV group, indicating that sofosbuvir/RBV therapy is not inferior in efficacy to PegIFN/RBV therapy. This is significant in that it allows patients the option to receive treatment with only oral medications. Patients with genotype 2 achieved SVR12 in 97 percent of sofosbuvir/RBV group versus 78 percent of PegIFN/RBV group, supporting the efficacy of sofosbuvir/RBV therapy in genotype 2 HCV. Patients with genotype 3 HCV achieved SVR12 in 56 percent of sofosbuvir/RBV group versus 63 percent of PegIFN/RBV group, indicating sofosbuvir/RBV for 12 weeks is not as effective as PegIFN/RBV for 24 weeks in these patients. The FDA recommends using sofosbuvir/RBV for 24 weeks (not 12 weeks) in patients with genotype 3.[8] Adverse events in this trial occurred more frequently in PegIFN/RBV group than the sofosbuvir/RBV group, and serious AEs were low among all groups. The most common AEs in all groups were fatigue, headache, nausea and insomnia.[12]

Another clinical trial used by the FDA in consideration of sofosbuvir was the POSITRON trial.[9] POSITRON was a phase III, randomized, double-blinded trial. This trial evaluated the safety and efficacy of sofosbuvir/RBV compared to RBV alone in patients with HCV genotype 2 or 3 unable to take PegIFN. The trial began with 278 patients randomized to receive RBV and sofosbuvir 400 mg once daily for 12 weeks or to receive RBV and placebo for 12 weeks. The primary end point was SVR12. The primary end point was achieved in 78 percent of sofosbuvir/RBV group versus 0 percent of RBV group. At EOT (12 weeks prior to this measurement), 100 percent of sofosbuvir/RBV group versus 0 percent of RBV group had achieved SVR. This difference in SVR in the sofosbuvir/RBV group was due to viral relapse after EOT. All cases of relapse that were reported occurred within 12 weeks after EOT, with no new cases of relapse reported between 12 to 24 weeks after EOT. Treatment with sofosbuvir/RBV led to SVR12 in 93 percent of patients with HCV genotype 2 versus 61 percent of patients with HCV genotype 3. Adverse events occurred more frequently in the sofosbuvir/RBV group than the RBV group, particularly fatigue, insomnia and anemia. The rate of AEs was overall low in both groups. The rate of serious AEs was similar between the two groups. Although the use of RBV alone is not a valid treatment option for the treatment of chronic HCV, giving the impression that this study is invalid, treatment with RBV alone may be the only option for patients who cannot tolerate PegIFN, especially in patients with non-genotype 1 HCV infection. This study supports the idea that treatment with sofosbuvir/RBV has better efficacy for patients with genotype 2 and 3 than RBV alone.[9]

Pharmacists' Impact

A look at the new medications' AEs and drug interactions can help a health care provider decide if these new medications would be right for his or her patient. Pharmacists can help counsel on the new drugs' adverse reactions, helping to ensure a safe introduction of these new medications.

There are two major AEs with simeprevir: teratogenicity and sun sensitivity. Male and female patients taking simeprevir with RBV must use two forms of birth control while on the therapy and for six months after discontinuation.[24] Female patients must be monitored monthly via pregnancy tests. Severe rashes may develop with simeprevir. These rashes occur with sun exposure and usually develop within the first four weeks of treatment. Patients should be encouraged to contact a health care provider if any sort of redness, rash or conjunctivitis occurs. Severe cases may require hospitalization, so patients should be urged to wear sunscreen and protective clothing (such as hats) if they are going to be outside for long periods of time. As it contains a sulfonamide moiety, simeprevir should be used with caution in those patients with sulfa allergies. Simeprevir is contraindicated with various medications ranging from over-the-counter supplements such as St. John's Wort to prescription hypertension medications. Patients should provide health care providers with a complete drug list, and pharmacists should monitor use of these contraindicated medications in patients taking simeprevir.

Similar to simeprevir, sofosbuvir requires two forms of birth control and pregnancy monitoring due to use with RBV, along with two negative pregnancy tests prior to beginning therapy.[7] Other sofosbuvir side effects include central nervous system effects such as depression, insomnia and nausea with headache and fatigue being the most common. Abnormalities in lab values including bilirubin, creatinine kinase, and lipases were also seen in patients taking sofosbuvir. The main drug interaction involved in sofosbuvir use is seen in potent intestinal P-glycoprotein inducers such as St. John's

Wort or rifampicin. These medications can alter the concentrations of sofosbuvir; therefore, their concurrent use is discouraged.

Neither of the medications has recommended renal or hepatic dosing adjustments at this time, due to insufficient evidence in these patient populations.[7,8] Each therapy is to be given over 12 weeks (with the exception of sofosbuvir in genotype 3), while monitoring different levels for dosing adjustments. Hemoglobin levels should be monitored with use of sofosbuvir due to ribavirin co-administration.[8] In patients with no history of cardiac disease, it is recommended that ribavirin be reduced to 600 mg/day with hemoglobin levels less than 10 g/dL and discontinued with levels less than 8.5 g/dL. In patients with a history of stable cardiac disease, ribavirin levels should be reduced with any drop in hemoglobin levels of over 2 g/dL over a four-week period or discontinued if hemoglobin levels drop below 12 g/dL. Hepatitis C virus RNA levels are monitored with use of simeprevir.[25] It is recommended to discontinue simeprevir, as well as PegIFN and RBV, if HCV RNA levels reach 25 IU/mL or greater at any point during the therapy duration.

Cost should be considered when weighing risks versus benefits of these therapies. Both therapies have gained media attention for their cost, many noting that these medications should be saved for those with severe cases of hepatitis C.[26] Simeprevir's 12 week therapy runs at $66,360, while sofosbuvir is priced at $84,000.[27,28]

Place in Therapy

The American Association for the Study of Liver Diseases (AASLD) provides working guidelines for the treatment of HCV infection that expound upon the place in therapy for these two new medications.[29] Because these medications were approved relatively recently, the AASLD has created web-based 2014 guidelines that are continuously being modified as new data become available. In general, the AASLD 2014 guidelines recommend the use of sofosbuvir with RBV as first-line treatment in nearly all patients (treatment-naïve and treatment-experienced patients with genotype 1 through 6), also including PegIFN when appropriate. The guidelines find sofosbuvir with RBV to have the best balance of high efficacy and AEs. Simeprevir with RBV/PegIFN is listed by the AASLD as an alternative regimen for treating genotype 1 and 4 (despite lack of FDA approval for use in genotype 4). The AASLD guidelines no longer recommend the use RBV/PegIFN alone for nearly all patients, claiming that therapy with RBV/PegIFN alone is inferior to the addition of sofosbuvir or simeprevir, due to lower efficacy, higher rate of serious AEs, and longer treatment duration when RBV/PegIFN is used alone. The guidelines prefer simeprevir over telaprevir and boceprevir, claiming comparatively lower efficacy, lower tolerability, and higher pill burden for telaprevir and boceprevir. Sofosbuvir and simeprevir may be used more prominently for the treatment of HCV infection as these guidelines are finalized.

Conclusion

Hepatitis C virus is one of the most common blood-borne pathogens and can lead to serious long-term complications.[1] Simeprevir and sofosbuvir have been shown to be effective and safe in multiple studies for the treatment of chronic HCV infection in both treatment-naïve and treatment-experienced patients.[7,8] Simeprevir is approved for the treatment of genotype 1 HCV and requires concurrent RBV and PegIFN.[7] Sofosbuvir can treat genotype 1 through 4 HCV with concurrent RBV and sometimes PegIFN.[8] The side effect profile, drug interactions and cost of each of these new medications must be weighed against the potential benefit to the patient in treating chronic HCV infection.

References

1. Moyer VA. Screening for hepatitis C virus Infection in adults: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med.* 2013;159:349-57.
2. Ashtari S, Vahedi M, Pourhoseingholi MA, Zali MR. Evaluated outcomes in patients with chronic hepatitis C. *Gastroenterol Hepatol.* 2013;6:S58-S64.
3. Victrelis (boceprevir) capsules for oral use [package insert]. Whitehouse Station, NJ: Merck and Co, Inc; 2011 [revised 2014 Feb].
4. Incivek (telaprevir) tablets, for oral use [package insert]. Cambridge, MA: Vertex Pharmaceuticals; 2011 [revised 2013 Oct].
5. U.S. Food and Drug Administration [Internet]. Silver Spring (MD): U.S. Food and Drug Administration. FDA approves new treatment for hepatitis C virus; 2013 Nov 22 [updated 2013 Nov 26; cited 2014 Feb 10]; [about 3 screens]. Available from: www.fda.gov/newsevents/newsroom/pressannouncements/ucm376449.htm.
6. U.S. Food and Drug Administration [Internet]. Silver Spring (MD): U.S. Food and Drug Administration. FDA approves Sovaldi for chronic hepatitis C; 2013 Dec 6 [updated 2013 Dec 9; cited 2014 Feb 10]; [about 3 screens]. Available from: www.fda.gov/newsevents/newsroom/pressannouncements/ucm377888.htm.
7. Olysio (simeprevir) capsules, for oral use [package insert]. Titusville, NJ: Janssen Therapeutics; 2013 [revised 2013 Dec].
8. Sovaldi (sofosbuvir) tablets, for oral use [package insert]. Foster City, CA: Gilead Sciences; 2013 [revised 2013 Dec].
9. Jacobson IM, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, et al. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med.* 2013;368(20):1867-77.
10. Zeuzem S, Berg T, Gane E, Ferenci P, Foster GR, Fried MW, et al. Simeprevir increased rate of sustained virologic response among treatment-experienced patients with HCV genotype-1 infection: a phase IIb trial. *Gastroenterology.* 2014;146(2):430-41.
11. Fried MW, Buti M, Dore GJ, Flisiak R, Ferenci P, Jacobson I, et al. Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naïve genotype 1 hepatitis C: the randomized PILLAR study. *Hepatology.* 2013;58(6):1918-29.
12. Lawitz E, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, et al. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med.* 2013;368(20):1878-87.
13. Pawlotsky JM, Dahari H, Neumann AU, Hezode C, Germanidis G, Lonjon I, et al. Antiviral action of ribavirin in chronic hepatitis C. *Gastroenterology.* 2004;126:703-14.
14. Thompson AJ, Patel K. New agents for the treatment of hepatitis c virus- focus on telaprevir. *Virus Adaptation and Treatment.* 2012:4;75-84.
15. Lauer, GM. Immune response to hepatitis C virus (HCV) infection and the prospects for an effective HCV vaccine or immunotherapies. *J Infect Dis.* 2013;207(S1)S7-12.
16. Bittar C, Jardim ACG, Yamasaki LHT, Carareto CMA, Pinho JRR, Lemey P, et al. On hepatits C virus evolution: the interaction between virus and host towards treatment outcome. *PLoS ONE.* 2013;8:e62393.
17. Diedrich G. How does hepatitis c virus enter cells? *FEBS Journal.* 2006; 273:3871-85.
18. Pearlman BL, Traub N. Sustained virologic response to antiviral therapy for chronic hepatitis C infection: a cure and so much more. *Clin Infect Dis.* 2011;52(7)889-900.
19. Center for Disease Control and Prevention [Internet]. Atlanta (GA): Center for Disease Control and Prevention. Hepatitis C Information for the Public; 2013 May 6 [Cited 2013 Feb 26]. Available from: www.cdc.gov/hepatitis/c/.

20. Majidzadeh K, Morovvati A, Soleimani M, Langeroudi AG, Merat SH, Jabbari H. Development and application of an in-house line probe assay for hepatitis C virus genotyping. *Hepat Mon.* 2013;13(5):e6767.
21. Weiwei X, Pan D, Yang Y, Liu H, Yao X. Molecular modeling study on the resistance mechanism of HCV NS3/4ª serine protease mutants R155K, A156V and D168A to TMC435. *Antiviral Res.* 2012;93:126-37.
22. Zhu H and Briggs JM. Mechanistic role of NS4A and substrate in the activation of HCV NS3 protease. *Proteins.* 2011;79:2428-43.
23. Boyce SE, Tirunagari N, Niedziela-Majka A, Perry J, Wong M, Kan E, et al. Structural and regulatory elements of HCV NS5B polymerase – beta loop and C-terminal tail – are required for activity of allosteric thumb site II inhibitors. *PLoS One.* 2014;9(1):1-12.
24. Gilead [Internet]. Foster City, CA: Gilead; Dec 6, 2013. U.S. Food and Drug Administration approves Gilead's Sovaldi™ (Sofosbuvir) for the treatment of chronic hepatitis C. Available from: www.jnj.com/news/all/OLYSIO-simeprevir-Receives-FDA-Approval-for-Combination-Treatment-of-Chronic-Hepatitis-C.
25. Olysio [Internet]. New Brunswick, NJ: Johnson & Johnson; Nov 22, 2013. Olysio Dosing. Available from: www.olysio.com/hcp/duration.
26. Appleby, J. Healthier patients may have to wait for costly hepatitis C drugs [Internet]. National Public Radio. March 12, 2014. Available from: www.npr.org/blogs/health/2014/03/12/289436931/healthier-patients-may-have-to-wait-for-costly-hepatitis-c-drugs.
27. Olysio [Internet]. New Brunswick, NJ: Johnson & Johnson; Nov 22, 2013. OLYSIO™ (simeprevir) Receives FDA approval for combination treatment of chronic hepatitis. Available from: www.investor.jnj.com/releasedetail.cfm?releaseid=809393.
28. Gilead [Internet]. Foster City, CA: Gilead; Dec 6, 2013. U.S. Food and Drug Administration approves Gilead's Sovaldi™ (Sofosbuvir) for the treatment of chronic hepatitis C. Available from: www.gilead.com/news/press-releases/2013/12/us-food-and-drug-administration-approves-gileads-sovaldi-sofosbuvir-for-the-treatment-of-chronic-hepatitis-c.
29. American Association for the Study of Liver Diseases [Internet]. Alexandria (VA): American Association for the Study of Liver Diseases and the Infectious Diseases Society of America; c2014. Recommendations for testing, managing, and treating hepatitis C; [updated 2014 Mar 21; cited 2014 Apr 14]; [about 5 screens]. Available from: www.hcvguidelines.org/full-report-view.