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Oral Direct-Acting Antiviral Therapy for Hepatitis C

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Chronic hepatitis C infection is a major cause of chronic liver disease, cirrhosis and liver cancer in much of the Western world [1,2] and poses a significant public health problem. The early institution of appropriate treatment to prevent the complications of hepatitis C is the cornerstone of management.

At the start of the 1990s, treatment with interferon alpha monotherapy was considered to be the gold standard of treatment for hepatitis C infection despite a poor cure rate of less than 20% [3]. This bleak statistic reinforced a negative perception, among clinicians, of the natural history of the disease and led to the widespread view of infection with the hepatitis C virus (HCV) as being a (largely) incurable, chronic condition with inevitable progression to cirrhosis and, possibly, hepatocellular carcinoma in a significant percentage of patients [4]. However, the advent of two new drugs in the 1990's hailed a paradigm shift in clinicians' attitudes towards hepatitis C infection, from both a therapeutic and prognostic perspective, when it was shown that: firstly, the addition of ribavirin to standard interferon monotherapy significantly increased the rate of sustained virological response (SVR) to around 40-45% [5]; and, secondly, the combination of pegylated interferon and ribavirin boosted SVR rates to around 55% for patients with HCV genotype 1 and 80% for patients with HCV genotypes 2 and 3 [6,7].

Initial hopes, that combined pegylated interferon and ribavirin therapy might turn out to be the long sought-after "holy grail" in the treatment of hepatitis C, were quickly tempered when it was subsequently found that combination therapy required long durations of treatment (up to 72 weeks) to achieve significant efficacy; had limited efficacy in the treatment of patients with HCV genotype 1; and was associated with multiple significant adverse effects [8]. These therapeutic limitations prompted a search for new, direct-acting antiviral agents for the effective treatment of hepatitis C infection. Recent advances in our understanding of the significance of host factors and the role of HCV structural and non-structural proteins in the pathogenesis of hepatitis C infection, in addition to the creation of the HCV replicon system which has allowed researchers to study HCV replication *in vitro* [9,10], have led to the design, discovery and early-phase development of these much sought-after new drugs [11,12]. These drugs include the HCV-specific protease inhibitors which have been shown to have a compound synergistic effect, resulting in SVR rates of 70%, when used in combination with both pegylated interferon and ribavirin [13-15].

HCV Genome

HCV is a single-stranded RNA virus, and a member of the genus Hepacivirus, belonging to the Flaviviridae family of viruses [16]. The HCV genome contains approximately 9600 base nucleotides with a single open reading frame encoding a large pre-protein, of around 3100 amino acids in length, which subsequently undergoes proteolytic cleavage resulting in the generation of the gamut of HCV structural and non-structural proteins [17] (Figure 1).

The HCV structural proteins include the core protein, C, which forms the viral nucleocapsid, and the viral envelope glycoproteins, E1 and E2, which carry receptors crucial for the adsorption to, and invasion of, host cells by HCV [18]. The HCV non-structural proteins consist of the NS1, NS2, NS3, NS4A, NS4B, NS5A and NS5B proteins, some of which perform multiple functions essential for HCV viral replication which will be discussed in further detail below [18].

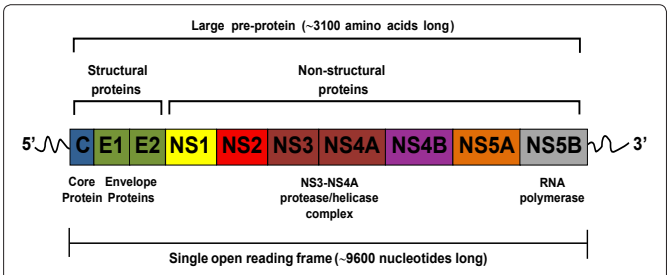

Figure 1: Schematic representation of the HCV genome.

NS3-NS4A

NS3, a protein with both protease and helicase activity, together with NS4A, a cofactor of NS3, form the non-covalent heterodimeric NS3-NS4A protease/helicase complex, which is responsible for proteolytic cleavage of the large pre-protein (encoded by the HCV genome) at 4 active sites, resulting in the release of the NS4A, NS4B, NS5A and NS5B non-structural proteins [19]. The NS3-NS4A protease/helicase complex has long been considered one of the most promising therapeutic targets for HCV drug discovery, due to its crucial role in HCV viral replication both *in vivo* (in primates) and *in vitro* [20], however the development of selective NS3-NS4A inhibitors has been fraught with multiple technical and scientific challenges. Although the currently available NS3-NS4A inhibitors are highly effective in suppressing HCV viral replication [21], they have also been shown to be associated with the development of treatment resistance [22], and viral breakthrough, due to the inadvertent natural selection of drug-resistant mutations. However, the risk of developing treatment resistance with NS3-NS4A inhibitors can be significantly reduced by the concomitant use of both pegylated interferon and ribavirin [23].

NS5B

NS5B, an RNA-dependent RNA polymerase lacking an exonuclease domain, is a critical enzyme in HCV viral replication due

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to its crucial role in the assembly of a functional replication complex [24]. Its complete lack of any genomic proofreading activity, due to the lack of an exonuclease domain, results in extremely low levels of transcriptional fidelity and the subsequent generation of multiple mutant strains of HCV (also known as HCV quasispecies) [25]. There are two distinct categories of NS5B inhibitors currently in clinical development: the nucleos(t)ide analogue inhibitors and the non-nucleos(t)ide analogue inhibitors [21]. The former category of direct-acting antiviral agents target the active site of NS5B by mimicking the natural substrate of NS5B, causing disruption of RNA replication, while the latter category target the allosteric binding sites of NS5B by locking NS5B in an inactive conformation thereby preventing the initiation of RNA replication [26,27]. One of the most promising NS5B inhibitors currently undergoing clinical trials is mericitabine (formerly known as RG7128), an oral nucleos(t)ide analogue inhibitor, which has been shown to have a good safety profile, with minimal adverse effects, and excellent efficacy against all HCV genotypes, with no viral resistance reported to date [28].

NS5A

NS5A, a non-structural protein with pleiotropic roles in the pathogenesis of HCV infection, has been shown to synergistically enhance the transcriptional activity of NS5B [29,30]. The experimental drug candidate daclatasvir (formerly known as BMS-790052), the first of the NS5A inhibitors to undergo clinical trials, has been shown to have excellent efficacy in low doses, with a significant initial treatment response, against all HCV genotypes *in vitro*, but has also been shown to have a low genetic barrier to resistance [31,32]. Studies evaluating the efficacy of daclatasvir in combination with other direct-acting antiviral agents will be discussed in further detail below.

Cyclophilins

Various host factors are known to be crucial in the pathogenesis of HCV infection. Cyclophilins, an evolutionarily conserved family of ubiquitous proteins with peptidyl-prolyl isomerase activity found in all organisms [33], have been shown to play a major regulatory role in HCV viral replication, with cyclophilin B, in particular, being germane as a direct stimulatory regulator of NS5B [34]. Alisporivir (formerly known as Debio 025), an oral first-in-class cyclophilin inhibitor, has recently been shown to have good efficacy against HCV viral replication, in addition to a favourable pharmacokinetic and safety profile, with nil immunosuppressive effects reported in patients [33].

Rationale for Combination Antiviral Therapy

Combination antiviral therapy has long been the mainstay of treatment for human immunodeficiency virus (HIV) and hepatitis B infection [35]. There has been much speculation, among both clinicians and researchers, that adopting a similar therapeutic approach in the treatment of HCV infection, using a combination of two or more of the various classes of direct-acting oral antiviral agents (i.e. the NS3-NS4A inhibitors, NS5A inhibitors and NS5B inhibitors), either alone or in combination with ribavirin, might result in significant synergistic enhancement of the known therapeutic effects of the individual drug classes [12]. Moreover, this novel putative approach to HCV therapy is also thought to be particularly attractive due to the excellent oral bioavailability, and good pharmacokinetic and safety profiles, of the various drug classes, with no significant drug-drug interactions and minimal adverse effects reported with their individual use [12,21]. Despite a recent deluge in published studies on the efficacy of various combinations of direct-acting oral antiviral agents in the treatment of HCV infection, the overwhelming majority of these studies have been conducted in patients with HCV genotype 1, with very few studies specifically evaluating their efficacy in patients with HCV genotypes 2 and 3. The results from some of the most promising studies, many of which were recently presented at the International Liver Congress™ 2012 (ILC 2012), the 47th annual meeting of the European Association for the Study of the Liver, will be briefly summarized and presented below. The following is in no way intended to be an exhaustive review of the literature on direct-acting antiviral agents in the treatment of hepatitis C but, rather, seeks to highlight some of the most recent, and significant, findings from this burgeoning field of research.

Studies in Patients with HCV Genotype 1

The landmark INFORM-1 proof of concept phase 1 trial, the first published study of oral combination antiviral therapy for the treatment of HCV infection, which evaluated the efficacy of the concomitant use of the NS3-NS4A inhibitor, danoprevir, with the NS5B nucleos(t)ide inhibitor, mericitabine, in the treatment of 88 patients with HCV genotype 1, found that a 13 day course of treatment with this particular combination of direct-acting antiviral agents resulted in undetectable levels of HCV RNA in over 60% of treatment-naïve subjects [36] however SVR was not an outcome measure in this particular study. Significantly, there were no reports of treatment resistance in any of the subjects enrolled in this study.

The first proof of concept study to evaluate the efficacy of an NS3-NS4A inhibitor, asunaprevir (formerly known as BMS-650032), in combination with an NS5A inhibitor, daclatasvir, in the treatment of HCV infection was the preliminary open-label study conducted by Lok et al. [37] in a small cohort of 21 patients with HCV genotype 1 (previously refractory to conventional therapy with pegylated interferon and ribavirin), which found that a 24 week course of treatment with this particular combination of direct-acting antiviral agents resulted in a overall rate of SVR, 12 weeks following study completion (SVR_{12}), of 35%, but a 100% SVR_{12} rate in subjects with HCV genotype 1b, consistent with the results of a previous small study on the efficacy of the same combination of direct-acting antiviral agents in the treatment of patients with HCV genotype 1b [38].

However, a recent study presented at ILC 2012, which evaluated the efficacy of a specific 24 week regimen of combined asunaprevir and daclatasvir therapy in the treatment of 43 patients with HCV genotype 1b (previously refractory to, or intolerant of, conventional therapy), found that a regimen of asunaprevir (60mg once daily) and daclatasvir (200mg twice daily) resulted in an overall SVR_{12} rate of only 77%, with a 91% SVR_{12} rate in subjects previously refractory to conventional therapy as compared to a 64% SVR_{12} rate in subjects intolerant of conventional therapy [39]. The reasons for the apparent discrepancies between the various reported results of the overall SVR_{12} rate in patients with HCV genotype 1b, receiving direct-acting antiviral combination therapy comprising asunaprevir and daclatasvir, remain unclear and are yet to be fully elucidated. However, possible explanations may include the small sample size of previous studies and differences in both the dosing regimens and study cohorts between the various studies.

Preliminary results from the ELECTRON phase 2 trial, which evaluated the safety and efficacy of the NS5B nucleos(t)ide inhibitor, GS-

7977, in combination with ribavirin in the treatment of 50 patients with HCV infection across all genotypes, have been extremely encouraging. The study found that this particular combination of antiviral therapy resulted in an 88% SVR_4 rate in treatment-naïve subjects with HCV genotype 1, with no reported viral breakthrough [40]. Moreover, this combination of antiviral therapy was also shown to have a relatively good safety profile and was well tolerated by subjects.

The open-label SOUND-C2 phase 2b trial, which evaluated the efficacy of the NS3-NS4A inhibitor, BI 201335, in combination with the NS5B non-nuclos(t)ide inhibitor, BI 207127, in the treatment of 362 treatment-naïve patients with HCV genotypes 1a or 1b, found that this particular combination of antiviral therapy resulted in an SVR_{12} rate of 39% [41]. This study, which also compared the efficacy of various regimens of the above combination of direct-acting antiviral agents, either alone or in combination with ribavirin, also found that the adjunct use of ribavarin resulted in an SVR_{12} rate of up to 68% depending on the treatment regimen assigned to subjects.

Another recent phase 2 trial, the results of which were presented at ILC 2012, which compared the safety and efficacy of 2 "quad" treatment regimens comprising the NS3-NS4A inhibitor, GS-9451, in combination with both the NS5A inhibitor, GS-5885, and the NS5B non-nucleos(t)ide inhibitor, tegobuvir (formerly known as GS-9190), and ribavirin in the treatment of 140 treatment-naïve patients with HCV genotypes 1a or 1b, found that all "quad" treatment regimens of combination antiviral therapy were generally well tolerated by subjects, with favourable safety profiles, and highly efficacious in treating HCV infection caused by both genotypes, with a higher SVR_{12} rate seen in the cohort of subjects assigned to the treatment regimen containing the higher dose of GS-5885 [42]. Of note, subjects with HCV genotype 1a were observed to have a higher rate of viral breakthrough than those with HCV genotype 1b.

Two recent studies, conducted by investigators mostly from the same collaborative research group, which evaluated the efficacy of ABT-450/r (the NS3-NS4A inhibitor, ABT-450, administered in conjunction with ritonavir, a CYP3A4 inhibitor used in the treatment of HIV infection [35]) in combination with various other direct-acting antiviral agents in the treatment of patients with HCV infection, were also presented at ILC 2012 [43,44]. The first study, which evaluated the efficacy of a 12 week course of treatment with ABT-450/r in combination with both the NS5B non-nucleos(t)ide inhibitor, ABT-333, and ribavirin in 50 patients with HCV genotype 1, found that this particular combination of antiviral therapy resulted in an extremely high SVR_{12} rate of around 95% in treatment-naïve subjects [44]. This study, which also compared the efficacy of 2 treatment regimens (differing in the dosing of ABT-450/r) of the aforementioned combination of antiviral therapy, also found that there was no significant difference in the SVR rates observed in treatment-naïve subjects assigned to the different treatment regimens. The second study, a small pilot study which evaluated the efficacy of a 12 week course of treatment with ABT-450/r in combination with the NS5B non-nucleos(t)ide inhibitor, ABT-072, in 11 treatment-naïve patients with HCV genotype 1, all of whom possessed the CC variant of the IL28B genetic polymorphism (which is thought to be the best predictor of SVR, with treatment, in patients with HCV genotype 1 infection [45]), found that this particular combination of direct-acting antiviral agents resulted in a SVR_{12} rate of over 90%, with only 2 reported relapses during the follow-up period [43].

One of the most promising studies presented at ILC 2012 was a study, which evaluated the safety and efficacy of a 24 week course of treatment with the NS5A inhibitor, daclatasvir, in combination with the NS5B nucleos(t)ide inhibitor, GS-7977, in the treatment of 88 treatment-naïve patients with HCV infection across all genotypes, which found that this particular combination of direct-acting antiviral agents was well tolerated and, more importantly, resulted in an outstanding overall SVR_{12} rate of over 95%. This study, which also compared the efficacy of various regimens of daclatasvir and GS-7977, either alone or in combination with ribavirin, also found that the adjunct use of ribavirin had no synergistic therapeutic effect, but was associated with adverse effects, and that a once-daily regimen of the aforementioned combination of direct-acting antiviral agents, alone, resulted in SVR_{12} rates of 100%, in subjects with HCV genotype 1, and over 90% in subjects with HCV genotypes 2 and 3 [46].

Studies in Patients with HCV Genotypes 2 and 3

As mentioned above, the overwhelming majority of studies on the efficacy of combination antiviral therapy for the treatment of HCV infection have been conducted in patients with HCV genotype 1. Indeed, studies in the literature conducted specifically in patients with HCV genotypes 2 and 3 are few and far between. However, one such study is the VITAL-1 phase 2b trial, the results of which were also recently presented at ILC 2012. This international study, which evaluated the efficacy of the cyclophilin inhibitor, alisporivir, in combination with ribavirin in the treatment of 340 treatment-naïve patients with HCV genotypes 2 or 3, found that this combination of antiviral therapy resulted in an SVR_{12} rate of just over 90% [47].

Conclusion

The current mainstay of treatment for HCV infection, conventional therapy with pegylated interferon and ribavarin, has several significant therapeutic limitations including limited efficacy in the treatment of patients with HCV genotype 1 and an association with multiple significant adverse effects, as previously noted. The last decade has seen quantum leaps in our understanding of both the structure and function of the HCV genome and the role of host factors, and the various structural and non-structural HCV proteins, in the pathogenesis of HCV infection. This has, in turn, led to the recent legion of published literature on the safety and efficacy of various oral direct-acting antiviral agents (either as monotherapy or in combination therapy) in the treatment of HCV infection. Although most of these promising new agents are still in various early phases of drug development, there is genuine cause for optimism, especially for those patients who are either refractory to, or unable to, tolerate pegylated interferon and/or ribavirin, that at least some of these agents will have been approved for widespread clinical use by the end of the next decade.

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