New therapeutic approaches to hepatitis C virus

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Abstract Year 201X will see a huge battle against hepatitis C virus (HCV) infection. HCV, a leading cause of end stage diseases and hepatocellular malignancies, is a negative legacy of the past in many regions worldwide, and has long been refractory to conventional treatments. The most effective peginterferons and ribavirin-based antiviral therapies can eliminate the virus in only half of patients treated, and the treatments are often poorly tolerated. Recently, the development of an HCV cell culture system has become a turning point of basic research. At present, novel therapeutic agents with different mechanisms of action are under development or on clinical trials. Some of these drugs have been proven to be effective when used with the conventional treatments, and may constitute antiviral therapies without being used in combination with interferons. This article reviews the current status of pre-clinical drug development, ongoing clinical trials, and near future perspectives in the field of HCV therapeutics.

Keywords Protease inhibitor · Polymerase inhibitor · Cyclosporine · HCV replicon system

Introduction

Hepatitis C virus (HCV), infecting 170 million people worldwide, is characterized by chronic liver inflammation and fibrogenesis leading to end-stage liver failure and hepatocellular malignancy [1]. Since the first report in 1986, interferon-alpha and -beta formulations have continued to be a mainstay against HCV infection [2]. However, even combination treatments of ribavirin plus peginterferon can achieve sustained viral response (SVR) rates of only 40–50% [3–6]. Furthermore, these therapies carry a substantial risk of serious side effects, and in quite a considerable proportion of patients require premature therapy discontinuation [7]. Given these situations, the development of safe and effective therapies against HCV has been our first priority.

Basics of HCV infection and replication

Difficulties in eradicating HCV in the past were largely attributable to the lack of useful virus cell cultures and of small animal models. These problems have been largely overcome by the development of the HCV subgenomic replicon [8–11] and HCV-JFH1 cell culture systems [12]. Furthermore, the Huh7-derived subclones of host cell lines have allowed production of higher viral titers and higher permissiveness for HCV [13, 14]. These cell culture systems now allow us to study the complete HCV life cycle: virus-cell attachment and entry, translation, protein processing, RNA replication, virion assembly and virus release (Fig. 1).

HCV belongs to the family *Flaviviridae*, which have positive strand RNA genomes of ~10 kilo-bases that encode a polyprotein of ~3,000 amino acids (Fig. 2). The

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Fig. 1 Life cycle of HCV **Fig. 2** Structure of HCV genome and its functions

protein is post- or co-translationally processed by cellular and viral proteases into at least 10 mature proteins. The viral nonstructural proteins accumulate in the endoplasmic reticulum (ER) and direct genomic replication and viral protein synthesis [15, 16].

Drug development and clinical trials of anti-HCV drugs

There are various compounds and therapies being tested to treat hepatitis C. In 2008, 154 clinical phases I through III trials on 78 drugs (excluding interferon-alpha, peginterferons and ribavirin), are registered in NIH *Clinicaltrials.gov* (Table 1). A class of drugs that is under most extensive development is STAT-C (specifically targeted antiviral therapy for HCV), which are designed to target certain

viral proteins or their functional epitope. On the other hand, a distinct feature of anti-HCV drug development is that various host-targeted drugs are tested, which include cyclophilin inhibitors, nitazoxanide, anti-steatosis drugs, immune modulators and new interferon formulations. A number of compounds for these targets are in early pre-clinical development phases, of which only one in ~1,000 compounds proceed to clinical human trials. Of those drugs that make it to human testing only 1 in 5 will be approved for clinical use. When new drugs are tested they will be required to demonstrate superiority over the conventional standard of care (SOC) therapy, which presently is the peginterferon and ribavirin combination. Clinical testing of new drugs is a cost- and time-consuming process, which takes an average of 8.5 years from phase I through approval.

Table 1 Excerpt: ongoing clinical trials of anti-HCV agents (exclude interferon-alpha, peginterferons, and ribavirin)

STAT-C	
Protease inhibitors	
Telaprevir	Phase III
Boceprevir	Phase III
TMC435350	Phase II
MK7009	Phase II
Polymerase inhibitors	
R1626	Phase II
VCH916	Phase II
RO5024048	Phase II
ABT-333	Phase II
NS5A inhibitor, A-831	Phase II
Interferon formulations	
Albupheron	Phase III
cIFN + RBV	Phase III
Omega interferon	Phase II
Locteron	Phase II
Interferon lambda (IL-29)	Phase II
Immune modulators	
Thymosin alpha 1	Phase III
KRN7000	Phase II
SCV-07	Phase II
MDX-1106	Phase I
Vaccines	
IC41	Phase II
CHRONVAC-C	Phase II
TG4040	Phase I
Host-targeted	
Cyclosporins	
Debio-025	Phase II
SCY-635	Phase Ib
Nitazoxanide	Phase III
Viramidine	Phase II
Fluvastatin + IFN/RBV	Phase II
Celgosivir	Phase II
Bavituximab	Phase I
Anti-steatosis	
PPAR-gamma agonists	Phase II–IV
Metformin	Phase IV

Protease inhibitors

The HCV proteins are post- or co-translationally processed by host and viral proteases. The nonstructural NS3 protein is a bifunctional protein that has serine protease and helicase (Fig. 2). NS3 serine protease, which the protease inhibitors target, cleaves junctions between NS3 and NS4A in *cis* and subsequently cleaves junctions between the other

nonstructural proteins in *trans*. In addition to the effects on the viral proteins, NS3 protease targets host proteins, TRIF and Cardif (a.k.a. VISA, IPS-1 or MAVS) that mediate innate antiviral signaling to induce production of endogenous interferon in response to cellular virus replication [17]. Based on the findings, targeting of NS3 protease not only inhibits virus expression but also may help impaired cells recover their ability to produce antiviral proteins.

Protease inhibitors were designed from crystal structures of the NS3 protease domain [18]. The first in vivo proof-of-concept report was that of BILN2061 [19], which showed rapid and potent antiviral effects by 4-day twice daily oral administration. Further development of the drug, however, has been halted because of cardiotoxicity in pre-clinical animal tests. At present, two protease inhibitors are in late phase II and III, telaprevir (VX-950) and boceprevir (SCH405054).

Telaprevir

A 14-day short-term administration showed that the plasma HCV titer decreased 1.1-log by peginterferon-alpha2a alone, 4.0-log by telaprevir, and 5.5-log by a combination of peginterferon and telaprevir [20–22]. Two phase II trials, PROVE1 and PROVE2, have been independently conducted [23, 24]. In PROVE 1, 61% achieved SVR by the triple combination therapy for 24 weeks and 67% for 48 weeks, while only 41% achieved SVR standard of care treatment [25]. In PROVE 2, 68 and 62% achieved SVR by 12- or 24-week triple combination therapy, while 48% achieved standard of care [24]. The above results from the two trials showed that treatment period of 24 weeks is sufficient to improve the outcome of the triple combination by over 20% compared to the conventional 48-week peginterferon and ribavirin treatment and that the combination with ribavirin is necessary to achieve optimal results.

Emergence of resistant mutations has been reported in vitro [26, 27] and in vivo [20, 28]. After 14 days of telaprevir monotherapy, 50–75% of serum quasiespecies acquired resistant mutations [27]. The viral breakthrough occurred in 5 of 163 patients during 12 weeks of the triple combination therapy and in 24 out of 78 patients who were treated with telaprevir and peginterferon. The telaprevir-resistant mutations were present in most breakthrough cases [24]. A phase III trial is currently being conducted in Japan.

Polymerase inhibitors

Polymerase inhibitors target viral NS5B RNA-dependent RNA polymerase. There are both nucleoside and non-nucleoside type agents. In vitro studies showed that the

nucleoside type polymerase inhibitors have a higher barrier to viral drug resistance than the non-nucleoside type [29]. Treatment of patients with polymerase inhibitors developed no viral resistance after 14-day monotherapy with R1626 or NM283.

R1626 is a prodrug of NS5B polymerase inhibitor R1479. A 4-week administration of R1626 in combination with peginterferon alfa-2a plus ribavirin in HCV genotype 1 patients showed that HCV RNA was undetectable in 69 and 74% of patients at the end of treatment with R1626 plus peginterferon and triple combination, respectively. In contrast, standard of care therapy eliminated HCV-RNA in only 5% within 4 weeks. There was no evidence of development of viral resistance. Grade 4 neutropenia developed in 78, 39, and 10% of the dual, triple and standard of care, respectively, and was the main reason for dose reductions. There are other polymerase inhibitors in early clinical and preclinical development, PF-00868554 [30], BILB 1941 [31], AG-021541 [32]. Two polymerase inhibitors, NM283 [33] and HCV796 [34], have been cancelled or halted in their clinical trials for gastrointestinal adverse effects and for hepatotoxicity, respectively [35].

New interferon formulations

New formulations of type I interferons and their relatives have been developed. These include Albuferon [36], IL-29, Locteron and interferon-omega [37]. Because these type-I interferons share a common cell surface receptor, IFNAR, most new formulations are designed in combination with controlled release drug delivery devices.

IL-29 (interferon lambda)

An interferon-related cytokine IL-29, also known as interferon-lambda 1, inhibits virus replication by inducing a cellular antiviral response similar to that activated by interferon-alpha/beta. Because IL-29 binds to its unique receptor, this cytokine might also be useful therapeutically in combination with interferons to treat HCV infection. In vitro studies have shown that IL-29 has potent and specific anti-viral activities against HCV and other flaviviruses [38]. A combination of IL-29 with interferon-alpha or interferon-gamma induced antiviral gene expression was more effective than when used alone and was more effective at blocking vesicular stomatitis virus and HCV replication in vitro [39]. A phase I study of a pegylated IL-29 formulation showed that the drug was safe and well tolerated.

Locteron is a controlled-release formulation of unpegylated recombinant interferon-alpha2b in microspheres,

which enables injections at a rate of every two weeks. *Locteron* has been evaluated in 27 volunteers [40]. After injection of *Locteron*, serum interferon-alpha2b remained elevated through 14 days. The serum half-life of *Locteron* was more than 2-fold that of peginterferon-alpha2b. A phase IIa study for treatment naïve HCV patients has shown that the early virological response (EVR, less than two-log drop of virus titer in 12 weeks) was 88 to 100% with doses of 320 through 640 mg per 2 weeks (EASL 2008).

Immune modulators

Toll-like receptor (TLR) agonists

TLRs are transmembrane receptors that recognize pathogen-associated molecular patterns of pathogens and mediate protective responses against microorganisms. Single-stranded viral RNA and bacterial DNA containing unmethylated CpG motifs are the ligands for TLR7 and TLR8 and 9 respectively [41, 42]. Binding of PAMP onto TLRs recruits an appropriate adaptor protein to the TIR domain and leads to the activation of NF- κ B and IRF3 resulting in the induction of high and sustained levels of interferons and related genes. Isatoribine is a selective agonist of TLR7. A 7-day treatment with intravenous isatoribine caused significant reduction of plasma HCV RNA in patients [43, 44]. Viral load reduction occurred in patients infected with HCV genotype 1 as well as non-1 genotypes. IMO-2125, a TLR9 agonist, has started phase II for study in prior null-responder HCV patients [45].

Thymosin alpha 1

A phase III study of thymosin alpha 1 used in combination with interferon and ribavirin showed only a modest improvement in treatment outcome and liver histology. Final results from the complete study have found that thymosin alpha 1 in combination with pegylated interferon failed to produce significant results over pegylated interferon alone (46).

Cyclosporins and cyclophilin inhibitors

Cyclosporin A (CsA) is a neutral cyclic undecapeptide that was isolated from the fungus *Hypocladium inflatum* *gams*. CsA has been used widely for post-transplantation immune modulation and various autoimmune and inflammatory diseases. It has been reported that CsA suppressed HIV replication in vitro by blocking the function of cellular cyclophilin A to bind to viral gag protein [47, 48]. We and

another group have reported that CsA substantially and specifically inhibits intracellular HCV replication in vitro [49, 50] using HCV replicon [10]. The 50% replicon inhibitory concentration of CsA is ~ 0.5 $\mu\text{g/ml}$, which is within clinically achievable concentrations. The antiviral effect of CsA is mediated by intracellular ligand proteins, cyclophilins [51]. Non-immunosuppressive cyclosporin analogues, cyclosporin D [52] and NIM811 [53] induced a similar suppression of HCV as well as of HIV replication [54].

DEBIO-025, a non-immunosuppressive cyclosporine analogue, inhibited as efficiently as CsA in vitro [55]. Clinical trial of DEBIO-025 monotherapy in HCV/HIV coinfection patients showed serum viral decline by 3.6- $\log_{10}$ 14-day oral treatment with 1200 mg twice daily [56]. In vitro synergic effects of Debio 025 have been reported when used in combination with STAT-C agents, polymerase or protease inhibitors [57]. Results from a double-blind, placebo-controlled study of Debio 025 in combination with peginterferon- α 2a in HCV patients showed that their combination achieved a 4.6–4.8 $\log_{10}$ early HCV-RNA decline, compared to a 2.5 $\log_{10}$ decline in peginterferon monotherapy.

Nitazoxanide

Nitazoxanide was originally developed and widely used as an antiparasite agent for cryptosporidiosis and helminthic infections. This drug has been recently shown to be effective against a wide range of organisms, including bacteria and viruses [58]. Nitazoxanide and its active metabolite, tizoxanide (TIZ), suppressed HBV and HCV replication in vitro [59]. Nitazoxanide was equally effective on lamivudine- or adefovir-resistant HBV mutants and on telaprevir-resistant HCV mutants [60]. Monotherapy of nitazoxanide in HCV genotype 4, low virus titer patients showed that 7 of 23 patients (30.4%) became serum HCV-RNA negative during therapy and 17.4% achieved SVR. Adverse events were similar to the placebo group [61]. Combination therapy of nitazoxanide, peginterferon plus ribavirin showed that the SVR rate was 79% in triple combination therapy, which was significantly higher than that of the standard of care patients of 50% [62]. Combination treatment with nitazoxanide plus peginterferon had a still higher SVR rate of 61%. The therapies were well tolerated except for higher rates of anemia in the groups receiving ribavirin. An in vitro study demonstrated a resistance to nitazoxanide by prolonged treatment, which was directed by adaptation of host cells but not by virus mutation [59]. Currently, a phase II trial is ongoing.

Other host-targeted drugs

NA255 prevents the de novo synthesis of sphingolipids, major lipid raft components, by inhibiting serine palmitoyltransferase activity. NA255 inhibited HCV replication by disrupting anchoring of HCV nonstructural proteins on the lipid rafts. [63].

Bavituximab, a chimeric antibody against exposed anionic phospholipids, showed antiviral action against a wide variety of viruses. Bavituximab selectively binds to virus-infected cells in which anionic phospholipids are exposed on the cell surface presumably by virus replication-induced ER-stress response [64]. A phase I clinical study is being prepared [65].

Celgosivir is an alpha-glucosidase I inhibitor that has been shown to inhibit the replication of various enveloped viruses. In vitro studies using flaviviruses, bovine viral diarrhea virus (BVDV) as well as hepatitis C virus, showed that celgosivir had potent and specific activities [66, 67].

Conclusion

Given the current situation of the absence of singly effective, proven antiviral agents against HCV other than interferon formulations, combinations of interferon with agents that possess potential antiviral effects will continue to dominate the therapy. Various anti-HCV agents have been developed and have shown antiviral activities and safety of use. These situations suggest that the next field of anti-HCV therapy will be interferon treatment potentiated by combinations of certain drugs. Continuing the search for more potent, singly effective, and less toxic antiviral drugs is mandatory to improve clinical anti-HCV chemotherapeutics.

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