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Attacking the Hepatitis C Virus with New Mechanisms of Action: Drugs in the Pipeline

A number of strategies for drug development have focused on the combination of antiviral therapy in the form of pegylated interferon with approaches to modulate and/or induce a cellular immune response [4]. These strategies have been based on the successful outcome of combination therapy of interferon or pegylated interferon with ribavirin, which has been the standard of care for treatment of hepatitis C in the US and the EU since 1998 [5-7]. Challenges facing our current treatment of HCV include lack of efficacy in patients with difficult-to-treat disease, such as patients with cirrhosis or infected with HCV genotype 1 (who represent a majority of US HCV infections), the toxicity of combination therapy, the expense and difficulty of therapy and the poor reception of these treatments by many patients. The development of new hepatitis C antiviral agents is critical to our management of this disease. A number of approaches are under investigation, including long-acting interferons; immunomodulators such as Interleukin-12, IL-10 and IL-2, histamine, thymosin alpha-1, IMPDH inhibitors, TNF antagonists, oral interferon like molecules, ribavirin and ribavirin analogues, and

hepatoprotectants; antifibrotics; specific HCV-derived enzyme inhibitors, drugs that either block HCV antigen production from RNA or prevent normal processing of HCV proteins; and other molecular approaches to treating HCV, such as ribozymes, short interfering RNAs, antisense oligonucleotides, immune blockers and therapeutic vaccines.

NEW IFNs AND IFN-DELIVERY SYSTEMS

A number of different technologies are being studied which might offer alternative strategies for sustained IFN release into the circulation. These include the following:

- disposable infusion pumps which hold a 3-day reservoir of drug
- controlled release injectables which would utilise a polymer matrix (e.g., a thermosensitive gel or a sucrose acetate isobutyrate-based system) delivered either im. or sc.
- a polyaminoacid-based oral delivery system
- encapsulation in liposomes

Oral Interferon-Like Molecules

A number of compounds are

known to induce interferon alpha and cytokines, including high molecular weight agents such as double stranded RNA and oligonucleotides [23]. Because these compounds are not well absorbed with oral delivery and/or metabolized by the gastrointestinal tract, a more reasonable approach is to use low molecular weight molecules such as resiquimod. This compound is similar to imiquimod, which is currently approved as a topical agent for the treatment of external genitalia and perianal warts [25].

The only data available regarding safety and efficacy of this oral interferon inducer is limited to that presented in 2003 in Abstract form [26], and in data submitted for publication which is derived primarily from two multicenter trials, one from the United States and one from the European Union (Patton H, Pockros PJ, et al., unpublished data). In the U.S. trial, resiquimod was tested at 0.01 mg/kg, both as a single dose and bi-weekly. At a higher dosage of 0.02 mg/kg used in the European trial the drug induced numerous cytokines including 2-5-AS, IL-12, IL-1-RA, IL-6, interferon alpha, TNF-alpha, and neopterin. A mean of approximately 1.5 log fall in HCV RNA was obtained by day 24 of biweekly therapy, with 3 of 11 patients obtaining a >2

log fall in HCV RNA, and 2 of 11 obtaining a >3 log fall in HCV RNA. Unfortunately, the typical interferon like side effects were seen in all patients.

IMMUNOMODULATORS AND ANTI-FIBROTICS

Interleukin-12

Recombinant human interleukin-12 (IL-12), is a 70kD heterodimeric immunomodulatory

chronic hepatitis C [16]. Unfortunately, however, in the 12-month follow-up study there was an increase in HCV RNA levels which led to titers >120 mEg/ml in some cases with significant ALT flares in 2 patients.

Gamma Interferon

Gamma interferon has activity both as an immunomodulator [34], and as an anti-fibrotic cytokine in both the murine and human hepatic stellate cells [35]. Data from a preliminary

hydrogenase activity is a well-known effect of ribavirin induced by its monophosphate form [27]. Because of this, it would be sensible to study compounds which inhibit the IMPDH enzyme but do not have the adverse effect of hemolysis associated with ribavirin therapy. Two such compounds have been studied to date. One agent (VX-497, Vertex Pharm Inc., Cambridge, MA) has been shown to be safe in patients with hepatitis C. VX-497 is an orally bioavail-

pression effects requiring dose reduction in many patients.

Ribavirin Analogues

In a search for analogues which would have the beneficial effects of ribavirin (RBV) without the adverse events, 2 key observations have been made which are critical to drug development: 1) that RBV monotherapy is not effective at inducing a sustained virologic clearance, and 2) that the hemolytic anemia caused by RBV has limited its dosing. The exact mechanism of action of RBV is unknown, although four proposed mechanisms have been described in detail [27]. The most important of these is the immune mediated activity which predisposes the Th2 to Th1 response. This in turn stimulates

release of interferon gamma and TNF-alpha from the cytotoxic T lymphocyte which stimulates immune clearance of HCV virus. As well, ribavirin also is rapidly taken up by the hepatocyte and phosphorylated. The monophosphorylated form of RBV acts as an inhibitor of IMPDH, which may be an important mechanism of its action as noted above. The triphosphorylated form of RBV is a direct though mild inhibitor of HCV RNA dependent RNA polymerase which may also be an important mechanism of action. As well, the triphosphorylated form of RBV acts as a RNA mutagen creating defective HCV RNA particles which are unable to replicate.

Both ribavirin and levovirin induce a Th1 cytokine bias favoring an antiviral response at similar concentrations. In chronic HCV infection, it is known that interferon induced clearance is associated with increased cytotoxic T lymphocyte response and interferon induced clearance is also associated with release of gamma interferon and diminished release of IL-10. Both of these immune re-

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cytokine which stimulates proliferation of activated virus specific cytotoxic T lymphocytes and NK cells, as well as stimulating interferon alpha production.

225 patients who were non-responders to interferon alpha or combination interferon alpha plus ribavirin, were randomized to receive 500 ng/kg IL-12 or placebo subcutaneously twice weekly for 12 weeks, and then unblinded. Only 1% of non-responsive patients enrolled for treatment had a sustained virologic response to therapy (2 of 160), whereas 3% (7 of 225) developed severe adverse events probably related to treatment, which resulted in early termination of the trial.

Interleukin-10 and Interleukin-2

Interleukin (IL) 10 is a cytokine which down-regulates the proinflammatory response and therefore is thought to have a possible modulatory effect on hepatic fibrosis. In 2000, a pilot trial of 24 patients was performed by Nelson et al., suggesting that IL-10 treatment reduces fibrosis in patients with

study of long term treatment with gamma interferon 1b and low dose prednisolone suggested this compound might be useful in patients with idiopathic pulmonary fibrosis [36]. Subsequent phase III trial data of interferon gamma 1b for idiopathic pulmonary fibrosis failed to reach significance. The results of a small pilot trial using gamma interferon as an anti-fibrotic in data presented in 2003 [41]. A large, phase 2b multicenter randomized, controlled trial using hepatic fibrosis as the primary endpoint in patients with advanced fibrosis or cirrhosis due to HCV has been completed and did not show any benefit of gamma interferon in reaching this endpoint.

RIBAVIRIN-LIKE MOLECULES

MPDH (Inosine-5'-monophosphate dehydrogenase) Inhibitors

Inhibition of the host inosine-5'-monophosphate de-

able small molecule which is a potent IMPDH inhibitor. The results of a randomized, double-blind, placebo-controlled, multicenter trial of VX-497 plus interferon in treatment of naïve patients showed no significant additive reduction in HCV RNA and one patient with a significant rise in ALTs [28]. A second known IMPDH inhibitor is mycophenolate mofetil (CellCept, Hoffman-La Roche, Inc.). Mycophenolate Mofetil (MMF), is a well-known immunosuppressant compound currently used for management following organ transplantation. Because the compound has potent IMPDH inhibition, it has been studied as adjuvant therapy in patients treated with standard or PEG-interferon alpha-2a with or without ribavirin [29;30]. No data regarding sustained virologic response has been published to date. Because of the significant hemolytic effects related to bone marrow suppression, it is unlikely, in my opinion, that MMF will be suitable in combination with pegylated interferons, which by themselves have significant bone marrow sup-

sponses have been mimicked in normal individuals receiving levovirin. Because the bioavailability of levovirin is less than that of RBV, the dose range for a phase II trial currently in progress is somewhat higher than that previously studied with RBV alone. No data are available regarding efficacy.

A second RBV analogue currently under study is viramidine, the amidine inversion of RBV. This prodrug is rapidly converted into RBV by the enzyme adenosine deaminase, as the liver is exposed to the first pass effect of oral dosing of the drug [43]. The strategy behind the use of this prodrug is simply to favor the concentration of ribavirin in hepatocytes over the rest of the body, including erythrocytes. In chimpanzees, this compound has been shown to concentrate in the liver, with a liver/RBC ratio 3- to 6-fold higher than that seen with RBV. In a phase I trial, viramidine showed similar adverse events as ribavirin, however, there was a diminished drop in hemoglobin over that seen with RBV (1.4 g/dl versus 2/5 g/dl) when it is used in a conventional weight based dosing manner [44;45]. The advantage of viramidine over levovirin is that it will not be lacking any of the properties of RBV.

MOLECULAR THERAPIES

Ribozymes

Stabilised ribozymes, small catalytic RNA molecules directed against a highly-conserved region (5'UTR) of the HCV genome, are currently being tested as a novel therapeutic approach for HCV infection [52]. A ribozyme might effectively block the translation of viral gene products and subsequent replication in the host cell [53]. A pi-

lot trial of an active ribozyme preparation (Heptazyme™) has been completed in humans and has demonstrated that the agent is safe and well-tolerated. The results of the virological data of that study have not been released, although it is widely believed the compound was not very effective and was toxic in animals. The possible synergistic effects of the combined Heptazyme™ and Type 1 consensus IFN have been studied in a Phase 2 trial to compare three doses of Heptazyme™ with or without consensus IFN. No data are available regarding efficacy or safety at this time.

Antisense oligonucleotides

Antisense drugs block the synthesis of disease-causing proteins by preventing translation of viral RNA. In the case of HCV, an antisense oligodeoxynucleotide directed against a specific HCV sequence effectively inhibits viral gene expression [54]. A number of HCV-infected patients have been infused with this antisense oligonucleotide in a Phase I/II trial at our center to demonstrate safety and tolerability (ISIS 14803) [55]. The data from this trial indicate that treatment with ISIS 14803 results in significant reductions of HCV RNA (1 log or more) in some patients. A frequent and marked elevation of ALT occurred in association with a fall in HCV RNA in these cases, for currently unexplained reasons. The efficacy and safety of the compound is being further studied in a larger Phase II trial. No data are available for release at this time.

Protease inhibitors

The NS2 and NS3 protease enzymes cleave the nonstructural proteins of the HCV polypeptide into functional proteins. Thus, inhibition of the NS2/3 proteases would block efficient viral

replication. The x-ray crystallography structure of the proteases have been elucidated, allowing for the development of small molecule inhibitors. A serine protease inhibitor has been studied and reported in man in a phase I/II trial in HCV infected patients. This compound (BILN 2061) caused a 2 - 3 log¹⁰ reduction of HCV RNA in all patients when given for a treatment duration of only 48 hours.[56] Many of the cases became HCV RNA undetectable in that brief period of treatment. Unfortunately, the compound has reportedly caused cardiotoxicity in this dose range and therefore must be studied at a lower concentration or must be replaced by alternative protease inhibitors. The key outcome of this pivotal trial is the proof-of-principle that a viral enzyme inhibitor has been extremely effective against HCV. It is very likely that viral resistance would occur after a relatively short period of use of any enzyme inhibitor given as monotherapy. Therefore, one can expect to see development programs of these compounds in combination with pegylated interferons with or without RBV.[57]

HCV polymerase inhibitors

Based on the fundamental knowledge of the action of HCV enzymes during viral replication, a number of viral enzyme inhibitors are being developed. The HCV RNA polymerase inhibitors are to be the first enzyme inhibitors that were tested in patients. Phase I safety trials have been completed with a number of compounds. One of these (JTK-003, AkrosPharma, Inc.) has been studied in a randomised, doubleblind, placebo-controlled, ascending dose, multi-centre trial of patients with HCV. Data from this trial have not been made publicly available at this time. Although a

helicase inhibitor has been in development, it has not yet been placed in human trials.

Small interfering RNA compounds

Small interfering RNAs (siRNAs) are a new class of drug which inhibit gene expression by blocking specific mRNAs.[58] The 5'UTR is the most conserved region in the HCV genome across all genotypes and is therefore the ideal target for siRNA inhibition. An siRNA has been shown to specifically inhibit HCV RNA replication in a replicon model.[58] To date, no siRNA has been tested in man. It is possible that siRNA based gene therapy may be a promising approach to anti-viral therapy for HCV.

Summary

- The most important lessons learned regarding the use of immunomodulators can be drawn from our sizable knowledge base evaluating ribavirin
- The most likely of these changes will be the use of a ribavirin analogue, at the earliest by 2007
- Although a number of the other compounds discussed may ultimately show benefit, I believe this will take a longer period of time.
- New therapeutic approaches to HCV include antisense therapy, hammerhead ribozymes, oral IFNs and specific enzyme inhibitors, such as protease, helicase and polymerase.
- Ultimately, we need oral drugs that are easy to take, non-toxic, inexpensive and efficacious.

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