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Hepatitis C: It's a Long Way to New Therapy, It's a Long Way to Go . . .

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The conference room was packed during the annual American Association for the Study of Liver Diseases meeting of November 2002, when 2 speakers reported the antiviral efficacy of a drug belonging to a brand new class of hepatitis C virus (HCV) inhibitors in patients with chronic hepatitis C. HCV RNA load reductions of 2 to 3 log IU/mL were seen after 2 days of treatment with BILN 2061 (Boehringer-Ingelheim), an HCV serine protease inhibitor. The National Institutes of Health Consensus Conference on the Management of Hepatitis C¹ had just officially recognized the combination of peginterferon- α and ribavirin as the standard of care for hepatitis C, and little could be offered to the approximately 20% of HCV genotype 2- and 3-infected patients or to the 50% of genotype 1-infected patients who were not clearing the infection with this combination. The BILN 2061 presentations therefore triggered a wave of hope and enthusiasm in the hepatology community. An announcement that a new drug was able to reduce HCV load by 3 logs within a few hours after oral administration was posted on the Internet the same day and was rapidly taken up by the medical and general media. The day after the meeting, patients were already calling, bidding to receive the new drug "that cured hepatitis C."

What happened next? One year later, the proof of concept that BILN 2061 significantly inhibited HCV replication in vitro and in a small number of HCV-infected patients was published in *Nature* by Lamarre et al.² Exactly 2 years after the initial report, the current issue of *GASTROENTEROLOGY* publishes the full results of the 3 clinical trials involving HCV genotype 1-infected patients treated with BILN 2061 for 48 hours.³ These carefully conducted studies, involving patients with mild hepatitis, advanced fibrosis, or compensated cirrhosis, are presented together, giving the reader a thorough view of

the antiviral effectiveness, pharmacodynamics, and tolerability of BILN 2061 in this group of patients. The results show impressive antiviral efficacy, with viral load reductions of the order of 2 to 3 log IU/mL in all patients at doses of 200 mg or more, together with excellent tolerability after 2 days of treatment.³ Never-treated patients and nonresponders or responder/relapsers to previous interferon- α -based therapy responded equally well to BILN 2061.³ This was not surprising, given the antiviral mechanisms of interferon α and ribavirin, the putative mechanisms of virological failure of interferon-based treatment,⁴ and the completely different mechanism of antiviral action of BILN 2061, which specifically inhibits the function of HCV NS3 serine proteinase, a key viral enzyme involved in HCV polyprotein processing.³ What this report does not show is the antiviral effect of BILN 2061 on genotypes other than 1, and it is noteworthy that lesser in vitro sensitivity of HCV genotypes 2 and 3 proteases to BILN 2061 was recently reported.⁵ The sustainability of the antiviral response was not studied, and neither was the incidence and importance of viral resistance during longer BILN 2061 administration. Finally, this report confirms persistent rumors of cardiac toxicity with BILN 2061 in animals.³ This explains why no more patients have received this drug in the past 2 years, and it is hampering further human studies with longer administration.

A very large number of specific HCV inhibitors originating from both large, well-established drug companies and smaller biotechnology companies are currently at the preclinical or early clinical development stage (see review⁶). Although all functional HCV structures theoretically represent potential targets for specific inhibitors, 3 are particularly promising: the HCV internal ribosome entry site (IRES, the HCV RNA structure that drives polyprotein translation), the NS3 serine proteinase, and NS5B RNA-dependent RNA polymerase (the enzyme that catalyzes RNA replication).⁶ Approaches

using nucleotide base complementarity to inhibit IRES function have given promising *in vitro* results.⁶ In contrast, early human studies of ribozyme and antisense oligonucleotide-based approaches have been disappointing.⁷ The proof of concept that RNA silencing could have antiviral efficacy in humans remains to be obtained. Alternative approaches are targeting the 3-dimensional functional structure of the HCV IRES, combined with ribosome units and host cell factors involved in the translational process. Several approaches are being followed to inhibit the NS3 serine proteinase, including the development of peptide-based molecules and of peptidomimetic molecules such as BILN 2061.³ The design of low-molecular-weight NS3 serine proteinase inhibitors is particularly challenging, however, because the enzyme's active site is long, shallow, and exposed, offering little grasp for small inhibitor molecules.⁸⁻¹⁰ HCV RNA-dependent RNA polymerase, like other viral polymerases, can be inhibited by nucleoside/nucleotide analogs that directly target the catalytic site and also by nonnucleoside inhibitors that bind to an allosteric site on the enzyme surface some distance from the active site, possibly distorting its fine geometry and function.¹¹ Most drugs currently about to enter clinical trials belong to the RNA-dependent RNA polymerase inhibitor category.

The BILN 2061 story perfectly illustrates the challenges and issues of drug development in general and new HCV drug development in particular. The design and clinical development of specific inhibitors of HCV replication indeed raise serious issues, including antiviral efficacy, toxicity, and viral resistance and their place in the future standard therapy for chronic hepatitis C. HCV inhibitors are generally selected by systematic screening of a very large number of small molecules, according to their predicted physical ability to fix to a functional site of an HCV target structure (RNA or protein) and to inhibit its vital function in the virus life cycle. Before entering clinical studies, such drugs need to prove their antiviral efficacy in preclinical models. Yet there is no consensus on the sequence of experiments required before launching clinical development. Some drugs were shown to have a potent antiviral action in surrogate models before being administered to chimpanzees. This was, for instance, the case of NM 283 (Idenix/Novartis), a specific HCV RNA-dependent RNA polymerase inhibitor currently being tested in phase II trials after it showed antiviral efficacy both on bovine viral diarrhea virus (a virus that belongs to the same Flaviviridae family as HCV) in cell culture and on HCV itself in experimentally infected chimpanzees.¹² Other drugs have been selected for their ability to inhibit functional *in vitro*

models of their target structure and/or the replicon system, a cell culture model of HCV replication, in the presence of HCV IRES and nonstructural proteins. This was the case with BILN 2061.^{2,3}

Many potent antiviral molecules have been found to be potentially toxic (or even lethal) or to have adverse effects that would be unacceptable in the context of hepatitis C infection.^{13,14} This emphasizes the absolute need for thorough preclinical toxicity studies. A substantial number of potentially highly active candidate drugs have already disappeared from the HCV drug pipeline because of worrisome preclinical toxicity profiles. BILN 2061 itself, although it has potent antiviral activity both *in vitro* and *in vivo* during short-term administration, has not entered any new clinical trials because of suspected cardiac toxicity in animals.³ Given the urgent need for new HCV therapies and the fact that the infected patient is the "gold standard" model and the key to subsequent development, drugs with potent antiviral efficacy in appropriate *in vitro* models, together with an acceptable preclinical toxicity profile, should rapidly enter clinical evaluation to establish their potential benefit in chronic hepatitis C.

Even if they are effective and well tolerated, specific HCV inhibitors may well come up against viral resistance during mid- to long-term administration. HCV is a highly variable virus that behaves as a viral quasispecies in infected individuals (see review¹⁵). Individual HCV quasispecies are likely to harbor minor populations bearing amino acid substitutions in target proteins, potentially conferring various levels of resistance to specific inhibitors. Drug-resistance studies of human immunodeficiency virus and hepatitis B virus^{16,17} show that resistant populations gradually become fitter upon drug administration, generally by accumulating additional substitutions during their replication. They ultimately become predominant in the quasispecies mixture because the replication of sensitive HCV variants is driven down to very low or undetectable levels by drug administration. Selection of resistant variants by the drug is followed by virological relapse, and the viral load gradually returns to baseline or near-baseline values. Several arguments strongly suggest that resistant HCV variants will be quickly selected if specific inhibitors are administered alone: these include the quasispecies distribution of HCV populations,¹⁵ the very rapid HCV replication kinetics *in vivo*,¹⁸ and the recent demonstration that both HCV protease and polymerase inhibitors are able to select resistant variants *in vitro*.¹⁹⁻²¹ For these reasons, monotherapy must be avoided in favor of combination strategies.

The foreseeable advent of specific HCV inhibitors raises the issue of their position among available treatments for chronic hepatitis C. The goal of HCV therapy is permanent viral eradication, and this is currently achieved in a substantial proportion of patients receiving the peginterferon/ribavirin combination. The goal of future therapies is therefore at best to increase the sustained viral eradication rate and at worst to improve the tolerability and acceptability of therapy for patients in whom the peginterferon/ribavirin combination is currently effective. One intriguing question is whether a combination of potent inhibitors of HCV replication could achieve sustained viral clearance without the help of an immunomodulatory drug. This would imply that reducing viral replication below a certain threshold would allow the natural immune response to gradually eliminate infected cells. It is possible, however, that only patients who eradicate the infection during current standard therapy are immunologically equipped to clear infected cells during exclusively antiviral therapy. If so, there is little hope that the global cure rate will be improved by replacing current therapies by combinations of HCV inhibitors. Should specific HCV inhibitors therefore be combined with peginterferon- α and ribavirin, or could HCV inhibitors replace ribavirin in combination with peginterferon? In addition to its moderate and transient antiviral effect early during therapy,²² ribavirin's main antiviral action involves the prevention of breakthroughs during therapy and relapses after therapy, through mechanisms that remain unknown.²³ In other words, ribavirin sustains the virological response to peginterferon. If this effect is related to a direct mechanism of ribavirin and not to a pharmacological interaction with interferon, then ribavirin or, ideally, less toxic ribavirin-like molecules will still be needed to sustain the response to HCV inhibitors. Alternatively, could HCV inhibitors replace interferon in combination with ribavirin? Interferon- α has been shown to be a potent inhibitor of viral replication *in vitro*,^{24,25} and mathematical modeling suggests that one of its principal modes of action is the blockade of virus production.¹⁸ It is not currently known whether subsequent clearance of infected cells is ensured solely by the natural action of the host immune system or whether it is accelerated by the immunomodulatory properties of interferon. In the latter scenario, peginterferon or even more potent interferon molecules will still be needed to treat chronic hepatitis C.

All these crucial issues and questions will need to be addressed in future clinical trials by following a careful step-by-step approach. On the basis of past experience in antiretroviral drug development, it is foreseeable that industry-sponsored trials will remain pivotal but that the increasing number of available molecules will increase the opportunities for investi-

gator-initiated studies aimed at establishing the best therapeutic strategy for the individual patient. In this respect, the authors of this report, together with the clinical investigators and the sponsor, should be congratulated for the quality of their preclinical and clinical work, thanked for the hopes they have generated, and acknowledged for bringing us back down to earth, albeit with a bump. It's a long way to new therapy, it's a long way to go. . .

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