The Discovery and Development of Boceprevir: A Novel, First-generation Inhibitor of the Hepatitis C Virus NS3/4A Serine Protease

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Abstract
An estimated 2–3% of the world’s population is infected with hepatitis C virus (HCV), making it a major global health problem. Consequently, over the past 15 years, there has been a concerted effort to understand the pathophysiology of HCV infection and the molecular virology of replication, and to utilize this knowledge for the development of more effective treatments. The virally encoded non-structural serine protease (NS3) is required to process the HCV polyprotein and release the individual proteins that form the viral RNA replication machinery. Given its critical role in the replication of HCV, the NS3 protease has been recognized as a potential drug target for the development of selective HCV therapies. In this review, we describe the key scientific discoveries that led to the approval of boceprevir, a first-generation, selective, small molecule inhibitor of the NS3 protease. We highlight the early studies that reported the crystal structure of the NS3 protease, its role in the processing of the HCV polyprotein, and the structural requirements critical for substrate cleavage. We also consider the novel attributes of the NS3 protease-binding pocket that challenged development of small molecule inhibitors, and the studies that ultimately yielded milligram quantities of this enzyme in a soluble, tractable form suitable for inhibitor screening programs. Finally, we describe the discovery of boceprevir, from the early chemistry studies, through the development of high-throughput assays, to the phase III clinical development program that ultimately provided the basis for approval of this drug. This latest phase in the development of boceprevir represents the culmination of a major global effort to understand the pathophysiology of HCV and develop small molecule inhibitors for the NS3 protease.

Keywords: Biochemistry; Synthesis; Sustained virologic response.

Introduction
An estimated 2–3% of the world’s population is infected with hepatitis C virus (HCV), hence there is a need for better understanding of the pathophysiology and treatment of HCV.

During the late 1990s and throughout the 2000s, interferon (IFN) formed the cornerstone of treatment for HCV infection, and global efforts to optimize IFN-based treatment strategies were paralleled by basic research efforts focused on the biology of HCV and the molecular virology of its replication and infection. In 2011, with the launch of the first direct-acting antiviral agents, boceprevir and telaprevir, these strands of research ultimately converged.

HCV belongs to the Flaviviridae family of single-stranded RNA viruses. It has a genome length of approximately 9.6 kb, which is translated into a polyprotein of 3010–3033 amino acid residues. When processed, this polyprotein generates at least 10 mature structural and non-structural viral proteins (Fig. 1A).\(^1\) The non-structural 3 (NS3) serine protease cleaves the viral polyprotein to release the non-structural proteins, which then form the viral RNA replication machinery. The NS3 protease is pivotal in the processing of the viral polyprotein, and is therefore critical for HCV replication.\(^2\,^3\)

In this article, we review the scientific discoveries that led to the identification of the NS3 protease as a rational drug target for anti-HCV therapies. In this paper, we focus on the studies that contributed to our present knowledge of the structure and replication of HCV, and the subsequent development of boceprevir as a selective small molecule inhibitor of the NS3 protease.

Insights into the structure and proteolysis of the HCV NS3 protease: implications for drug development

Structure of the NS3 protease

The X-ray crystal structure of the NS3 protease domain (amino acids 2–180) in complex with a synthetic NS4A activator peptide was described by Kim and colleagues in 1997 (Fig. 2).\(^4\) The NS3/4A complex adopts a chymotrypsin-like fold with two structural domains: the C-terminal domain contains a six-stranded β-barrel with hydrophobic residues localized at the core of the barrel, and the N-terminal domain contains eight β strands, including one contributed by the NS4A peptide. These two β-barrel domains are separated by a deep cleft that harbors the catalytic triad (His57, Asp81, and Ser139), with a geometrical arrangement similar to other serine proteases.\(^5\) A zinc ion at the C-terminal domain distal...
from the active site may play a structural rather than catalytic role. The crystal structure of the full-length NS3 protein molecularly linked to the NS4A peptide was solved by Yao and colleagues. This crystal structure provides an atomic view of the local and global structural rearrangement that involves the protease and helicase domains during polyprotein processing.

The NS4A polypeptide is believed to serve dual functions: the hydrophobic N-terminal 20 amino acids are thought to anchor the NS3/4A complex to the host cell membrane, while the central portion provides the structural platform within one of the \( \beta \)-barrels of the NS3 protease domain, required for protease activation and stabilization. In the absence of NS4A, the NS3 domain is able to cleave the NS5A/B but not the NS4B/NS5A site. Co-expression of NS4A with NS3 restores the ability to cleave NS4B/NS5A, and also enhances the processing at NS5A/5B.

**Biochemistry of proteolysis**

The mechanism of NS3-mediated proteolysis resembles that of other chymotrypsin-like serine proteases. Polypeptide substrates form an extended anti-parallel \( \beta \)-strand along the edge of the protease \( \beta \)-barrel, with one strand contributed by the protease and the other by the substrate. The catalytic amino acid triad within the active site of the enzyme orchestrates a series of covalent and acid-based catalytic events.
reactions following a “ping-pong” mechanism. Upon binding of the polypeptide substrate to the enzyme, the nucleophilic oxygen of Ser139 in the enzyme binds covalently to the carbonyl carbon of the substrate peptide scissile bond. This requires coordination of His57 and Asp81, and results in the formation of a tetrahedral intermediate (E-T1), followed by release of the N-terminus half of the peptide. During this transitional state, the negatively charged oxygen (the oxyanion) of the carboxylate tetrahedral intermediate moves to the previously vacant hole (the “oxyanion hole”), and forms hydrogen bonds with the backbone amides of Ser138 and Gly137, the Nε2 of the catalytic His57, and the side chain of the Lys136.

Dissociation of the N-terminal peptide permits binding of water and hydrolysis of the acyl-enzyme intermediate. This hydrolysis causes formation of a second tetrahedral intermediate (E-T2), which is again stabilized by the oxyanion hole. The final acid-base catalysis is mediated by His57 in conjunction with Asp81, releasing the C-terminus half of the peptide. In general, mechanism-based inhibitors exhibit a biphasic kinetic profile similar to that seen during natural proteolysis of the peptide substrates. The “inhibition” is carried out in a two-step kinetic process: the initial inhibitor–enzyme binding is followed by stabilization of the covalent interaction during the transition state.

A pivotal observation by Steinkühler and colleagues – that the N-terminal cleavage products of substrate peptides corresponding to the NS4A/NS4B, NS4B/NS5A, and NS5A/NS5B are potent inhibitors of the NS3 protease – provided a key starting point in the design of peptide inhibitors and ultimately the development of small molecule compounds.

**Challenges and opportunities in structure drug design**

The substrate binding site of the HCV NS3 protease is relatively shallow, solvent-exposed, and lacks the loops and other structural determinants present in other serine proteases for substrate/enzyme interactions. Several of the loops that interact with the P4, P3, and P2 moieties, and thus help to delineate a well-defined substrate-binding pocket in other serine proteases, are either shortened or missing in the NS3 protease. Viral substrates compensate for this shallow binding pocket with an extended substrate spanning 10 amino acid residues (P6 to P4, distal to the scissile bond) (Fig. 1B), thus allowing many hydrophobic and electrostatic interactions between protein and substrate.

Based on the interactions between NS3 and viral substrates, one property of effective inhibitors may be the need for a large molecular size (the P6–P4 peptide is a decamer) to ensure substrate binding. However, large molecules complicate drug development because of their poor physicochemical properties and limited bioavailability. Therefore, it was a considerable challenge to design small molecule inhibitors with sufficient binding affinity for the shallow binding cleft of the NS3 protease, while maintaining the drug-like properties required for oral delivery.

Binding of peptide substrate appears to modulate the conformation of both the proximal region near the active site and also the distal amino acid residues, including those in the helicase domain. This inherent flexibility of an “induced-fit” mechanism also presents challenges for structure-based drug design. In addition, in the context of therapeutic utility, mutations that confer drug resistance can affect the favorable induced-fit binding, and thus these conformational changes can play a role in engendering viral resistance.
HCV can be divided into six genotypes, with genotype 1 being the predominant species in North America. The genetic diversity at the NS3 active site between different genotypes presents another challenge for small molecule inhibitors (Fig. 3). Amino acid heterogeneity around the NS3 binding pocket across genotypes may mean that inhibitors exhibit vastly different potencies against different genotypes. Inhibitors with pan-genotypic activity need to be able to bypass interactions with those residues that exhibit amino acid variation.

A wealth of information on serine proteases provides insight into the mechanisms of proteolysis and the potential design of mechanism-based inhibitors. For example, one approach is to exploit the inherent binding affinity of the products released from proteolysis. An atomic view capturing the NS3-mediated cis cleavage at the NS3/4A junction showed that the NS3 C-terminal sequence, consisting of residues 626–631, forms 12 H-bonds and creates a contact surface of approximately 500 Å² near the protease active site. Inhibition by the P-side product corresponding to the NS4A/NS4B and NS5A/5B junctions was observed in vitro. These observations led to the strategy of using the P or P' product fragments as the scaffold for building chemical warheads/serine traps.

Biochemistry and molecular virology: translating science into molecular tools for drug discovery

Novel single-chain HCV NS3/4A protease

NS4A forms part of the jI-barrel structure of the NS3 protease, ensuring the correct alignment of the catalytic triad and hence enhancing proteolytic efficiency during polyprotein processing. During the research, various strategies were employed to generate milligram quantities of this enzyme complex in a tractable form that could be used for compound screening, biochemical studies, and X-ray crystallography. Early attempts to produce full-length NS3_1–631/4A_1–54 expressed in eukaryotic systems yielded relatively low quantities of active material, which was generally insoluble, making it inappropriate for biochemical and structural analyses. Expression of the NS3_1–181 domain alone in Escherichia coli yielded greater quantities of protein with better solubility characteristics, but the resultant enzyme still displayed weakened proteolytic activity. Improved proteolytic activity was achieved by using a synthetic 12-amino acid NS4A peptide (amino acids 22–33) in trans to complex with the NS3 protease. However, the overall catalytic efficiency of this enzyme remained 10-fold lower than that of the full-length NS3_1–631/4A_1–54.

The final piece in the puzzle was provided by Taremi and colleagues, who reported a single-chain construct encoding the N-terminal NS4A_1–22 fragment linked to the NS3_1–181 or NS3_1–631 protease domain through a flexible GSGS linker. This construct had improved solubility, and generated an active tractable reagent in sufficient quantities for compound screening and structural studies. It also allowed intercalation of the NS4A peptide into the NS3 protease domain (as suggested by the crystal structure) and resulted in a >15-fold improvement in the second order rate constant relative to the protease domain supplemented with a synthetic NS4A peptide. Overall, the NS4A_1–32-GSGS-NS3_1–181 comprised approximately 5% of the intracellular protein following purification, and displayed kinetic parameters similar to those of the full-length NS3_1–631/4A_1–54 generated in insect cells.

Continuous spectrophotometric enzyme assay

The objective of the rational NS3/4A drug design program was to identify mechanism-based inhibitors that could form a stable covalent bond with the catalytic serine residue in the NS3/4A protease. The formation of this covalent bond is characterized by the slow formation of a fully reversible transition state tetrahedral intermediate. To assess potency of these “slow-binding” inhibitors accurately, it was imperative to have a continuous assay that could conveniently and accurately evaluate the time-dependent equilibrium binding of each candidate inhibitor. Traditional methods such as ELISAs or HPLC-based assays are considered discontinuous, time-consuming, and impractical for high-throughput screening. Zhang and colleagues reported a series of spectrophotometric substrates suitable for detailed continuous kinetic analyses. One of these substrates was a depsipeptide derived from the P side of the NS5A/5B junction and containing a C-terminal carboxyl group esterified with a chloromorphol alcohol. This substrate was cleaved efficiently by the NS3 protease, was resistant to non-enzymatic hydrolysis, displayed adequate aqueous solubility, and allowed sensitive detection of the proteolytic product without compromising the NS3 activities. Hence, this substrate was used to develop an automated microtiter-plate assay for the rapid evaluation of candidate inhibitors.

Replicon cell-based assay of boceprevir activity

During the initial stages of the boceprevir drug-discovery program, structure–activity relationship (SAR) studies were based primarily on enzyme assays because of the lack of a robust and reliable cell-culture system. In 1999, Lohmann and colleagues reported the first cell-culture system, using a human hepatoma cell line (Huh7) transfected with an HCV RNA derived from an infectious genotype 1b isolate that contained tissue culture-adapted mutations. This replicon system was based on a subgenomic HCV RNA encoding the non-structural HCV proteins. Transfection of this replicon RNA into Huh7 cells resulted in an autonomous viral replicon without the production of infectious viruses, making it an ideal tool for inhibitor evaluation. The replicon system was adapted into a 384-well format for high-throughput screening of small molecule inhibitors.

Discovery of boceprevir: a novel HCV NS3 protease inhibitor

Chemistry

The search for a small molecular inhibitor of the HCV NS3 protease began by screening compound libraries. Traditional high-throughput screening of compound libraries failed to identify chemical leads that could be followed-up by optimization of medicinal chemistry structure activity. Given no viable lead from screening, a structure-based design approach was adopted. The first compounds synthesized were based on an HCV protease substrate, incorporating reversible electrophilic traps that could potentially bind covalently to catalytic Ser139. Introducing a ketoamide moiety generated an undecapeptide (Analogue 1) (Fig. 4) that inhibited NS3 enzyme activity at low nanomolar concentrations ($K_{i} = 1.9$ nM). However, this lead compound lacked almost all the desired drug-like characteristics, and was unsuitable for clinical
Fig. 4. Structure activity synthesis of boceprevir.
was identified, which
Since mammalian
in vitro
5
in vivo
9
1.4 nmol/L;
EC
8 nmol/L; EC
5
5
Ki
11 nmol/L) than
5
5
values of 200–400 nmol/L in
50
as a primary ketoamide improved bio-
and
Ki
90
5
5
m
59x748
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in Analog 6, which exhibited improved enzyme-binding
demonstrate potency in the sub-micromolar range.
630 nmol/L) (Fig. 4). This was one of the first analogs to
demonstrate potency in the sub-micromolar range. Replacement of leucine with cyclopropylalanine at P2 resulted in
Analog 6, which exhibited improved enzyme-binding potency (Ki* = 50 nmol/L; Fig. 4).29
Most of the initial SAR studies during the early stages were
guided by enzyme-binding assays because of the lack of
robust cellular assays. The introduction of the replicon cell-
based assay was pivotal in further refining SAR, and allowed
identification of compounds that were active in both binding
and cellular assays. Unfortunately, Analog 6 had a half-
molar effective concentration (EC50) of >5000 nmol/L in
the replicon-based cellular assay, despite its promising
enzyme-binding affinity. It was unclear how to interpret
these results. One hypothesis was that Analog 6 was polar,
containing many amide bonds that prevented it crossing cell
membranes. By introducing peptide isosteres, modifying the
side chains of amino acids, and methylating amide nitrogens
to depeptidize the molecule, the methylated Analog 7 was
identified; this demonstrated similar solubility and enzyme
activity to Analog 6 (Ki* = 60 nmol/L) but with much
improved cellular activity in the replicon assay (EC50 =
950 nmol/L). Comparison of Analogs 6 and 7 revealed that
addition of a secondary amide at P2 yielded compounds that
retained good enzyme activity and exhibited favorable
potency in the replicon-based cell-culture assay. Further
optimization of this secondary amide was investigated via
the addition of proline analogs at P2 (Fig. 4).
Cyclization of the N-methyl group to a P2 cyclopropyl
group generated the cyclopropylated Analog 8, which had
greater enzyme-binding affinity (Ki* = 11 nmol/L) than
Analog 6. Introduction of a gem dimethyl group at the
cyclopropyl proline resulted in Analog 9, and was associated
with improved enzyme-binding affinity and increased potency
in the replicon-based cell-culture assay (Ki* = 1.4 nmol/L;
EC50 = 90 nmol/L).30
Having established that Analog 9 showed desirable binding
and cellular activities, its oral pharmacokinetics were next evaluated. In rats, administration of Analog 9 (10 mg/kg)
was associated with poor plasma exposure, with an area
under the plasma concentration curve (AUC) of 0.23 µmol/L/
h and oral bioavailability of 1%. These data indicated that the
compound was poorly absorbed, suggesting that it was
peptidic and needed further reduction in molecular weight.
To reduce molecular weight to an acceptable level and improve oral pharmacokinetics, a series of compounds were
synthesized, with the most active being analogs with small groups. Truncation of the Analog-9 containing norvaline at P1’ with
a primary amide (to produce Analog 11) significantly reduced enzyme activity (Ki* = 100 nmol/L). However, this analog
improved oral absorption resulting in increased oral exposure (AUC = 2.52 µmol/L/h). Thus, truncating compounds
at P1’ as a primary ketoamide improved bioavailability but detrimentally affected binding affinity. Modifications at P1, at P3, and at P3 caps were therefore reconsidered to identify groups that make stronger lipophilic
contact with the enzyme, consistent with an improved binding profile. A systematic modification of P1 by the
introduction of carbocyclic alanine derivatives led to the
discovery of a cyclobutyl methyl group as the optimal P1 moiety based on its excellent contact at the S1 pocket. The
resulting Analog 12 exhibited favorable enzyme-binding and
cellular activity (Ki* = 8 nmol/L; EC50 = 700 nmol/L). In
addition, Analog 12 also demonstrated improved oral phar-
macokinetics with an AUC of 1.5 µmol/L/h when administered
at a dose of 10 mg/kg. Finally, P3 SAR modifications in
combination with changes at the P3 capping group identified
tert-butyl glycine at P3 and tert-butyl urea at the P3 cap,
resulting in Analog 13 (SCH503034, boceprevir)31; this had improved selectivity against human neutrophil elastase,
and retained activity in enzyme and cellular replicon-based
assays. The molecular interactions between boceprevir and
the NS3/4A protease are depicted in Fig. 5 (A, B).

In vitro characterization of boceprevir
Evaluation of boceprevir in the continuous enzyme assay
revealed time-dependent inhibition of enzymes 1–6 NS3/4A
proteases in a progress curve analysis yielding overall Ki
values of 10–104 nmol/L (Table 1).32–34 Since mammalian
serine proteases perform many important biological func-
tions, especially in relation to digestion, blood clotting, and
complement cascade during antibody neutralization, boce-
previr was evaluated against a panel of other human proteases (e.g. human neutrophil elastase, human neutrophil
cathepsin G, and human liver cathepsins H and L). Boceprevir was found to be selective for inhibition of the
HCV protease. A selectivity ratio for the HCV NS3 protease
ranging from 4 to >7000 was observed when boceprevir was
evaluated against a number of human serine and cysteine
proteases (Table 1).
In the HCV replicon cell assay, boceprevir potently reduced
HCV RNA levels with EC50 values of 200–400 nmol/L in
genotypes 1, 2, and 5 replicons (Table 1).32 Incubation of
replicon-bearing HuH7 cells for 72 hours with 2.5 µmol/L
boceprevir reduced HCV RNA level by 1.5 logs, and prolonged
exposure for up to 14 days resulted in replicon RNA levels
falling by approximately 4.0 logs, equivalent to <1 copy of
HCV RNA remaining per cell.32 No cytoxicity was observed
in human hepatoma cells or stimulated peripheral blood
mononuclear cells.

Preclinical pharmacokinetics and metabolism of
boceprevir
Boceprevir had a favorable oral pharmacokinetic profile based
on preclinical in vivo and in vitro evaluations. It was readily
absorbed in rats, dogs, and mice, with acceptable plasma exposure, and its bioavailability ranged from 24–34% with
a half-life of 4.2 hours in rats and 1.1 hour in dogs. The
compound was found to be partitioned in the liver of rats

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The primary route of metabolism was reduction by aldo-keto reductase (which reduced the ketoamide to hydroxyl amide) and to a lesser extent, oxidation by CYP3A5. Boceprevir did not inhibit cytochrome P450, and was negative in the Ames mutagenicity assay. It was moderately protein-bound in animals, with a human plasma protein binding of 85%. Boceprevir was readily hydrated, and existed as a mixture of two interconvertible diastereomers at P1 in an approximate 2:1 ratio.

**Clinical development of boceprevir**

The boceprevir phase III clinical program consisted of two international randomized trials in patients with HCV genotype...
1 infection. Previously untreated patients were enrolled in SPRINT2 (n = 1097) and treatment-experienced patients were enrolled in RESPOND2 (n = 403). Each trial included a control arm in which patients received peginterferon plus ribavirin (P/R) for 4 weeks (lead-in) then P/R plus placebo for 44 weeks, and an experimental arm in which patients received P/R lead-in followed by P/R plus boceprevir for 44 weeks. Each trial also included a response-guided therapy (RGT) arm, in which individual treatment duration was tailored based on virologic response. In these studies, treatment with boceprevir significantly improved rates of sustained virologic response (SVR) compared with treatment with P/R alone across all patient groups (Table 2).35,36

### Lead-in therapy

In both SPRINT-2 and RESPOND-2, the use of a 4-week lead-in phase with P/R before the introduction of boceprevir was thought to be associated with several theoretical benefits.35,36 The use of P/R to reduce HCV RNA levels before introducing boceprevir may help mitigate the risk of viral breakthrough or resistance, which might otherwise occur with direct antivirals.35 In the phase II SPRINT-2 study, use of lead-in therapy was associated with moderately lower rates of virologic breakthrough (defined as a 2 log increase in HCV RNA from nadir and >50,000 IU/mL on two consecutive occasions) compared with initiation of treatment with P/R plus boceprevir (4% vs 9%, P = 0.057). Population sequen-
In this study, 63 of 80 patients achieved sustained virologic response (defined as undetectable HCV RNA 24 weeks after completion of treatment; PR = peginterferon alfa-2b (1.5 µg/kg/week) plus ribavirin (600–1400 mg/day); NR = not reported; SVR = sustained virologic response (defined as undetectable HCV RNA 24 weeks after completion of treatment); RGT = response-guided therapy.

Table 2. Virologic response rates in the boceprevir phase III registration studies SPRINT-235 and RESPOND-236

<table>
<thead>
<tr>
<th>Treatment arm</th>
<th>EOTR</th>
<th>Relapse</th>
<th>SVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control arm (n = 363): (PR)</td>
<td>53% (191/363)</td>
<td>22% (39/176)</td>
<td>38% (137/363)</td>
</tr>
<tr>
<td>RGT arm (n = 368)5</td>
<td>71% (261/368)</td>
<td>9% (24/257)</td>
<td>63% (233/368)</td>
</tr>
<tr>
<td>“Fast responders”: (PR)2/(BPR)24, total 28 weeks</td>
<td>76% (277/366)</td>
<td>9% (24/265)</td>
<td>66% (242/366)</td>
</tr>
<tr>
<td>“Slow responders”: (PR)2/(BPR)24/(PR)30, total 48 weeks</td>
<td>96% (156/162)</td>
<td>72% (59/82)</td>
<td></td>
</tr>
<tr>
<td>Fixed duration arm (n = 366): (PR)4/(BPR)44, total 48 wk</td>
<td>9% (24/265)</td>
<td>66% (242/366)</td>
<td></td>
</tr>
<tr>
<td>RESPOND-2: treatment-experienced, genotype 136</td>
<td>70% (15/111)</td>
<td>59% (95/162)</td>
<td></td>
</tr>
<tr>
<td>Control arm (n = 80): (PR)</td>
<td>31%</td>
<td>32% (8/25)</td>
<td>21% (17/80)</td>
</tr>
<tr>
<td>RGT arm (n = 162)5</td>
<td>70%</td>
<td>15% (17/111)</td>
<td>59% (95/162)</td>
</tr>
<tr>
<td>“Fast responders”: (PR)2/(BPR)32, total 36 weeks</td>
<td>86% (64/74)</td>
<td>66% (107/161)</td>
<td></td>
</tr>
<tr>
<td>“Slow responders”: (PR)2/(BPR)32/(PR)32, total 48 weeks</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR for 4 wks then BOC + PR for 44 wks (N = 161)</td>
<td>77%</td>
<td>12% (14/121)</td>
<td>66% (107/161)</td>
</tr>
</tbody>
</table>

BOC = boceprevir (800 mg three times daily with food); EOTR = end of treatment response (defined as undetectable HCV RNA at completion of treatment); PR = peginterferon alfa-2b (1.5 µg/kg/week) plus ribavirin (600–1400 mg/day); NR = not reported; SVR = sustained virologic response (defined as undetectable HCV RNA 24 weeks after completion of treatment); RGT = response-guided therapy.

The objective of this approach was to minimize treatment duration and thereby limit drug exposure and treatment-related adverse events. In SPRINT-2, 44% (162/368) of patients randomized to RGT were eligible for the reduced treatment duration, and of these 96% (156/162) achieved SVR. Similarly, in RESPOND-2, 46% (74/162) of patients randomized to RGT were eligible for reduced treatment duration, and the SVR rate in this cohort was 86% (64/74). Thus, in both SPRINT-2 and RESPOND-2, >40% of subjects enrolled in the RGT arms achieved a reduced treatment duration without any impact on SVR rates. RGT has now been adopted as the recommended treatment approach with boceprevir, with week 8 of therapy being the key decision point used to define treatment duration.

**Conclusions**

Boceprevir in combination with P/R significantly increases the rates of SVR compared with P/R alone, with an approximate two-fold improvement in SVR in previously untreated patients, and approximate three-fold improvement in treatment-experienced patients. These improved clinical outcomes for patients with HCV represent a significant advance in the treatment of HCV, and have been derived from efforts to understand the pathophysiology of HCV, and the molecular virology and biochemistry of HCV replication processes. The advances in the basic science of HCV made during the past 2 decades will continue to bear fruit as second- and third-generation direct-acting antiviral agents undergo clinical evaluation both in conjunction with IFN and in combination as IFN-free regimens. It is hoped that these next generations of antivirals will offer pan-genotype activity with shorter...
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treatment duration, and meet the ultimate goal of IFN-free regimens for all patients.

Conflict of interest
AYMH and SV are employees of Merck Research Laboratories, Kenilworth, NJ

Author contributions
Manuscript writing (AYMH, SV).

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