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MONOGRAPH

VANIPREVIR

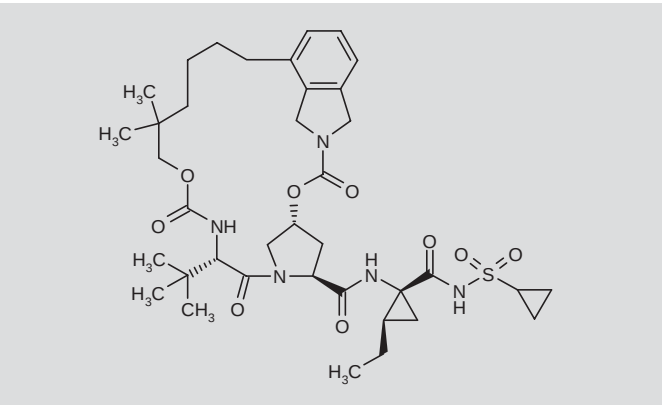
Prop INN; USAN

HCV NS3/4A Protease Inhibitor
Treatment of Hepatitis C

MK-7009

(5*R*,7*S*,10*S*)-10-*tert*-Butyl-*N*-[1(*R*)-[*N*-(cyclopropylsulfonyl)carbamoyl]-2(*R*)-ethylcyclopropyl]-15,15-dimethyl-3,9,12-trioxo-3,5,6,7,9,10,11,12,14,15,16,17,18,19-tetradecahydro-1*H*-2,23:5,8-dimethano-4,13,2,8,11-benzodioxatriazacyclohenicosine-7-carboxamide

InChI: 1S/C38H55N5O9S/c1-7-25-18-38(25,33(46)41-53(49,50)27-14-15-27)40-31(44)29-17-26-20-43(29)32(45)30(36(2,3)4)39-34(47)51-22-37(5,6)16-9-8-11-23-12-10-13-24-19-42(21-28(23)24)35(48)52-26/h10,12-13,25-27,29-30H,7-9,11,14-22H2,1-6H3,(H,39,47)(H,40,44)(H,41,46)/t25-,26-,29+,30-,38-/m1/s1



$C_{38}H_{55}N_5O_9S$
Mol wt: 757.936
EN: 441129

SUMMARY

Protease inhibitors are among a number of new classes of direct-acting antivirals for hepatitis C which, when combined with conventional peginterferon alfa/ribavirin or with other classes of direct-acting antivirals, offer the potential to vastly improve disease outcomes for affected individuals. Early evaluations imply that a number of these agents are well tolerated and produce encouraging rapid declines in plasma viral load. Concerns relate to the rapid development of drug resistance, with resulting viral rebound. Vaniprevir (MK-7009) is a novel inhibitor of genotype 1 proteases with some potency against genotype 2 currently undergoing phase II trials.

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SYNTHESIS*

Vaniprevir can be prepared by two related strategies as follows:

In one method, intramolecular ring-closing metathesis of diene (I) using Neolyst-M1 or Zhan 1B catalysts in CH_2Cl_2 produces the macrocyclic triamide (II), which undergoes methyl ester hydrolysis with LiOH in THF/H_2O at 40 °C, giving carboxylic acid (III). Subsequent coupling of acid (III) with the vinylcyclopropylamine derivative (IV) in the presence of HATU, DMAP and DIEA in CH_2Cl_2 at 40 °C yields the corresponding amide (V), which is finally reduced by catalytic hydrogenation over Pd/C in EtOAc (1). Scheme 1.

In an alternative strategy, reduction of the macrocyclic alkene (II) by means of H_2 and Pd/C in EtOAc yields the saturated macrocycle (VI), which by hydrolysis with LiOH in THF/MeOH/H_2O furnishes carboxylic acid (VII). Finally, macrocyclic free acid (VII) is condensed with the ethylcyclopropylamine derivative (VIII) by means of HATU, DMAP and DIEA in DMF (1, 2). Scheme 1.

The synthetic precursor diene (I) is prepared as follows:

Benzylic bromination of 3-bromo-*o*-xylene (IX) with NBS in the presence of benzoyl peroxide in refluxing CCl_4 or chlorobenzene at 80 °C gives 1-bromo-2,3-bis(bromomethyl)benzene (X), which then cyclizes with benzylamine in the presence of $KHCO_3$ in acetonitrile to yield 2-benzyl-4-bromoisoindoline (XI). Alkenylation of compound (XI) with tributyl(vinyl)tin (XII) by means of $Pd(PPh_3)_4$ in toluene provides the isoindoline (XIII), which is then deprotected by means of 1-chloroethyl chloroformate (ACE-Cl) in refluxing 1,2-dichloroethane to afford 4-vinylisoindoline (XIV). Finally, coupling of 4-vinylisoindoline (XIV) with *N*-Boc-*trans*-4-hydroxyproline methyl ester (XV), previously activated with CDI, in DMF at 60 °C results in carbamate (XVI) (1, 2). Scheme 2.

Alternatively, debenzylation of 2-benzyl-4-bromoisoindoline (XI) by means of ACE-Cl in chlorobenzene at 90 °C, followed by condensation of the deprotected isoindoline (XVII) with *N*-Boc-*trans*-4-hydroxyproline methyl ester (XV), previously treated with CDI, by

means of DIEA in DMF at 50 °C furnishes carbamate (XVIII). Then, alkenylation of the aryl bromide (XVIII) with potassium vinyltrifluoroborate (XIX) and Et_3N in refluxing EtOH affords the vinyl isoindoline derivative (XVI) (1). Scheme 2.

Finally, Boc-deprotection of proline derivative (XVI) by means of HCl in dioxane gives the corresponding amine (XX), which is then coupled with the L-*tert*-leucine carbamate derivative (XXI) in the presence of EDC, DIEA and HOAt or HOBt in DMF to yield the building block diene (I) (1, 2). Scheme 2.

The L-*tert*-leucine carbamate (XXI) is prepared by alkylation of ethyl isobutyrate (XXII) with 4-bromo-1-butene (XXIII) and LDA in HMPA, followed by ester group reduction with $LiAlH_4$ in Et_2O, affording 2,2-dimethyl-5-hexen-1-ol (XXIV) (3). After treatment of hexenol (XXIV) with triphosgene by means of DIEA and NaOH in 1,4-dioxane, the resulting chloroformate is condensed with L-*tert*-leucine (XXV) to give carbamate (XXI) (2). Scheme 2.

Building blocks (IV) and (VIII) are prepared as follows:

Condensation of benzaldehyde (XXVI) with ethyl glycinate hydrochloride (XXVII) by means of Na_2SO_4 and Et_3N in *tert*-butyl methyl ether gives 2-(benzylideneamino)acetic acid ethyl ester (XXVIII), which cyclizes with 1,4-dibromo-2(*E*)-butene (XXIX) in the presence of *t*-BuOLi in dry toluene to yield, after imine hydrolysis, the cyclopropylamine derivative (XXX). Protection of amine (XXX) with Boc_2O furnishes the racemic *N*-Boc amino ester (XXXI), which is subjected to kinetic resolution by means of enzymes such as Acalase®, Savinase® or Esperase® in DMSO to afford unreacted (1*R*,2*S*)-isomer (XXXII). Saponification of ethyl ester (XXXII) using LiOH in THF/MeOH gives the cyclopropanecarboxylic acid derivative (XXXIII), which, after activation with CDI in refluxing THF, is coupled with cyclopropanesulfonamide (XXXIV) (prepared by treatment of cyclopropanesulfonyl chloride [XXXV] with NH_3 in THF) in the presence of DBU to yield the *N*-acylsulfonamide (XXXVI). Alternatively, the sulfonamide intermediate (XXXIV) can be prepared by condensation of 3-chloropropanesulfonyl chloride (XXXVIII) with *tert*-butylamine in THF to give *N*-*tert*-butyl-(3-chloro)propylsulfonamide (XXXIX), which then cyclizes to the cyclopropyl derivative (XL) by treatment with *n*-BuLi in THF at –78 °C. Removal of the *N*-*tert*-butyl group in intermediate (XL) using CF_3CO_2H then furnishes cyclopropanesulfonamide (XXXIV). After *N*-Boc group cleavage in (XXXVI) by means of CF_3CO_2H in DCM, acidification with HCl in Et_2O affords intermediate (IV) (4). Intermediate (VIII) can be obtained by vinyl group reduction in (IV) with H_2 and Pd/C in EtOAc. Intermediate (VIII) can also be obtained by reduction of vinylcyclopropylamine derivative (XXXVI) with H_2 over Ru/C in MeOH to yield *N*-Boc-ethylcyclopropylamine (XXXVII), which is finally *N*-deprotected by means of HCl in CH_2Cl_2 (5). Scheme 3.

BACKGROUND

Hepatitis C virus (HCV) is a positive-sense RNA virus of the *Flaviviridae* family which chronically infects 1-3% of the human population (approx. 180 million people). HCV infection is a major public health concern worldwide, and in the U.S. represents the most common chronic blood-borne infection and is the major reason for liver transplant and liver-related death (6, 7). Infection outcomes for HCV are highly variable, ranging from spontaneous clearance among approximately 30% of untreated individuals to chronic infection. Infected individuals are often asymptomatic and may unknowingly transmit the virus to others via blood-borne products; accordingly, HCV infections tend to recur among at-risk individuals. There is currently no vaccine and no post-exposure prophylaxis.

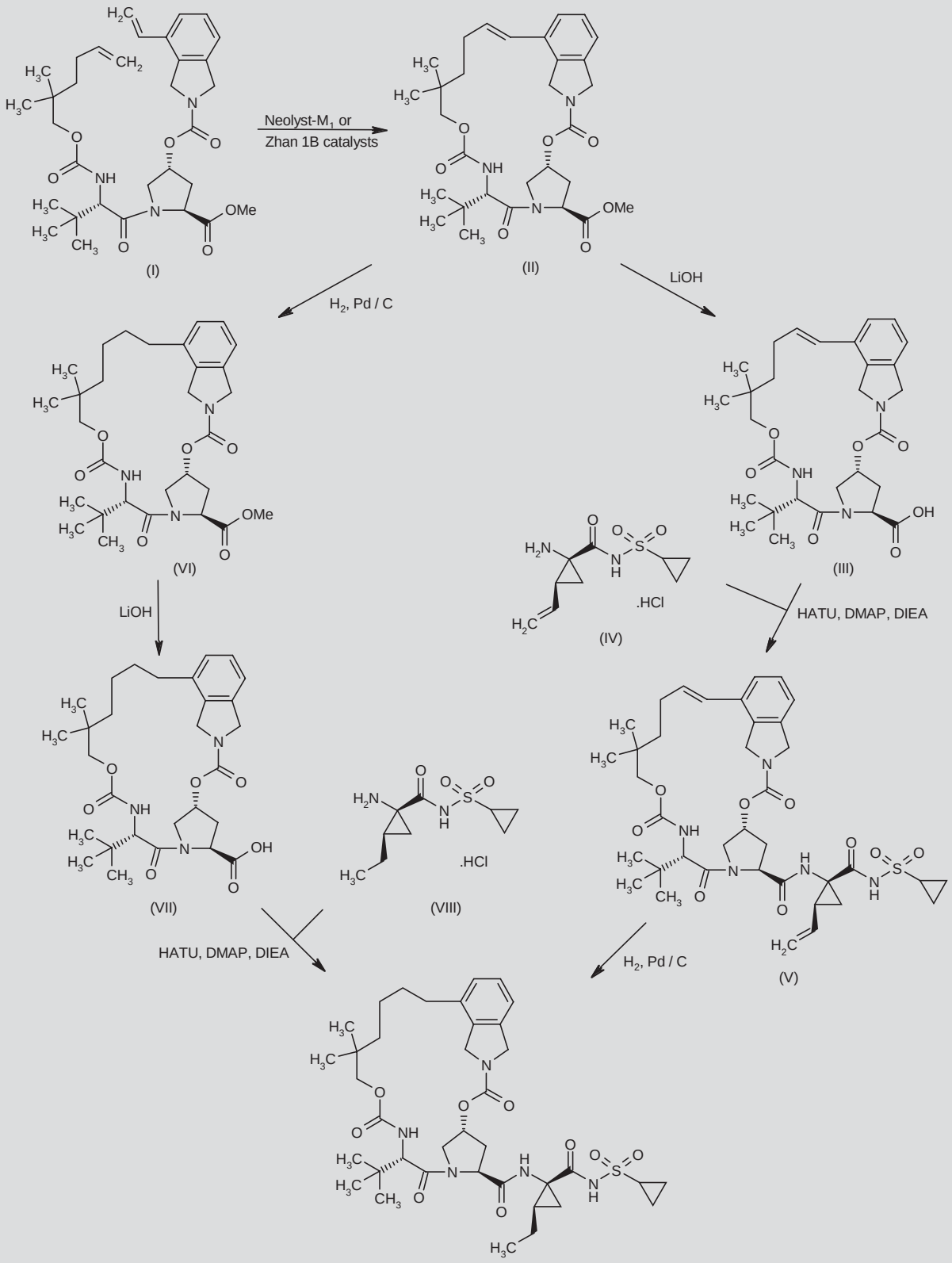
HCV shows significant genetic heterogeneity and is currently classified into six major genotypes and numerous subtypes (8-10). The genetic barrier to viral adaptation is affected by genotype-specific constraints. For example, using codon 155 of the HCV protease, subtype 1a requires one nucleotide change (A<u>G</u>G to A<u>A</u>G) to encode for the change from arginine (R) to lysine (K), whereas for subtype 1b, two steps are required –firstly <u>C</u>GG to <u>A</u>GG, followed by A<u>G</u>G to A<u>A</u>G– in order to achieve the same result at the level of amino acid residues. Differences in the diversity observed at sites along proteins for particular genotypes support that the number and type of naturally occurring variations observed within one genotype or subtype are not necessarily present for another genotype (11-13) (Table I). Accordingly, protease inhibitors do not appear to be equally effective against all HCV genotypes. In some examples the consensus for one HCV genotype/subtype is the reported drug resistance mutation. Review of the public HCV databases (EuHCVdb and Los Alamos) indicates that many compensatory sites are also naturally polymorphic among proteases from several HCV subtypes (14). The genetic barrier to emergence of drug variants which may then acquire compensatory changes and persist in post-therapy would therefore also be predicted to differ between genotypes.

The determinants for drug success are multifaceted and include drug potency (binding affinity), drug plasma levels (half-life, potential and need for ritonavir boosting of protease inhibitors, target organ exposure, dosing regimen, food intake requirements), tolerability (drug–drug interactions, safety profile, side effects, adherence, pill burden) and the ability to combine with other drugs to increase the genetic barrier to resistance and reduce viral fitness.

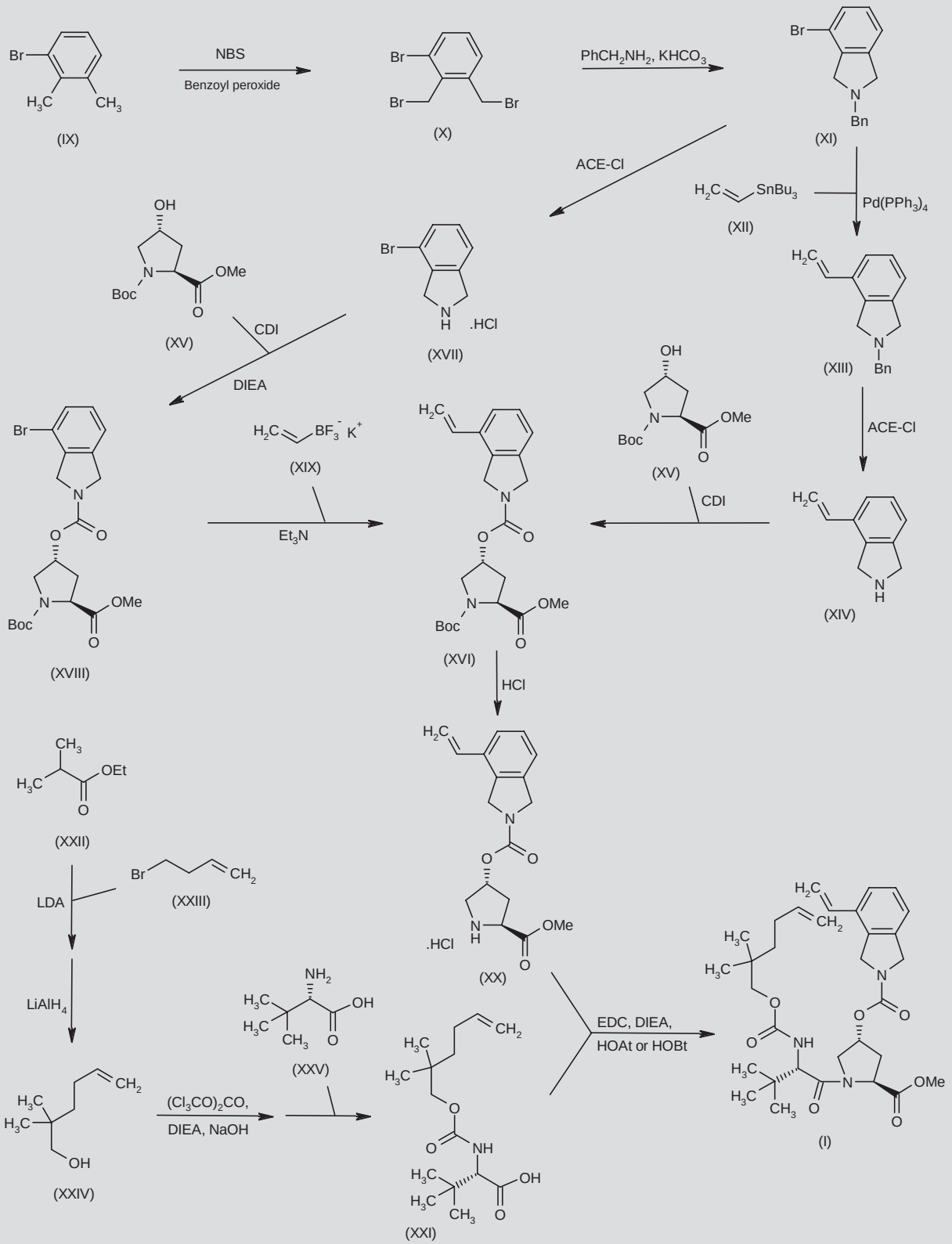
Treatment guidelines for HCV infection recommend adherence to the most effective regimens to minimize the opportunity for drug-selected variants to acquire additional compensatory mutations that allow them to persist post-therapy. These risks are abrogated, but not removed, with triple therapy. For example, in previously untreated patients infected with genotype 1, the use of combination therapy was associated with high-level telaprevir-resistant variants among a subset of those failing to achieve sustained virological response (15). Recent data from the SUCCESS study (16) somewhat counter this argument by confirming in a large prospective cohort the benefits of treating individuals who show slow initial response to standard treatment for more than 48 weeks for 72 weeks to give them the best chance of achieving sustained virological response. For this particular group of patients, studies examining the persistence of variants selected under therapy are particularly warranted.

Once-weekly injection of the combination of peginterferon alfa plus oral administration of ribavirin is the current standard of care for individuals with HCV infection (17, 18). Achievement of a sustained virological response (or undetectable levels of HCV RNA at 24 weeks after cessation of therapy) is associated with improved overall clinical outcomes of reduced morbidity and mortality (18).

Scheme 1. Synthesis of Vaniprevir



Scheme 2. Synthesis of Precursor Diene (I)



Scheme 3. Synthesis of Building Blocks (IV) and (VIII)

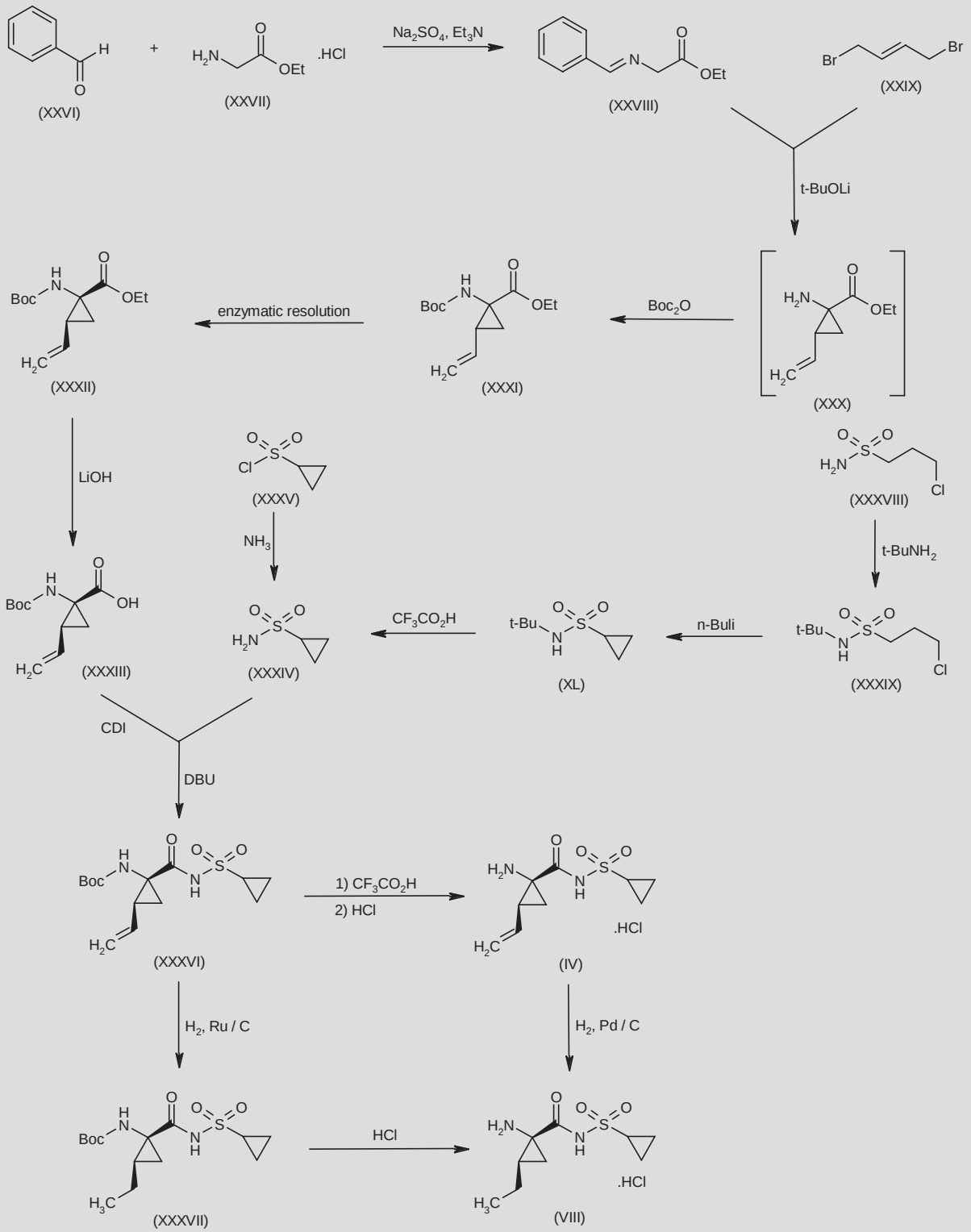


Table I. *Prevalence of naturally occurring drug-resistant variants according to genotype.*

Drug resistance site	Consensus amino acid (codon)/variant(s) detected					
	Genotype 1a		Genotype 1b		Genotype 3a	
R155	R(AGR)/T	1/155	R(CGG)	0/33	R(AGG)	0/117
A156	A(GCC)	0/155	A(GCT)	0/33	A(GCT)	0/117
D168	D(GAC)/E	2/152	D(GAC)	0/33	Q(CAG)/R	2/117

Sites are associated with resistance to vaniprevir or reduced EC_{50} results (> 50-fold concentration) relative to wild type in replicon assay. "R" in codon = A+G (from 11).

Achievement of an early virological response (defined as a 2 $\log_{10}$ decrease in the HCV RNA level or an undetectable HCV RNA level by week 12 of therapy) is considered a robust predictor of treatment success to standard of care therapy (18). Furthermore, a rapid virological response by week 4 is likely to be a primary indicator of treatment success for standard of care therapy (19). Another significant factor in determining treatment response is the genotype of the infecting virus. Sustained viral response varies from site to site, but is generally achieved in approximately 40-45% of HCV genotype 1-infected patients and in approximately 70-80% of genotype 2- and 3-infected patients (18). Thus, response rates are poor among patients with the common genotype (HCV genotype 1), and especially those who have advanced disease or coinfection with HIV (20). Recently, single nucleotide polymorphisms (SNPs) in the *IL28B* region have been associated with rates of sustained virological response to standard of care treatment among HCV-infected individuals (21, 22), but this genetic region does not entirely explain treatment outcomes. The exact mechanisms by which *IL28B* influences spontaneous and treatment resolution of HCV infection remain an enigma, but the effect of *IL28B* polymorphism on the sustained virological response rates using direct-acting antiviral combinations is not expected to be as large as that observed with standard of care. Nonetheless, the impact of *IL28B* SNPs on controlling for HCV is likely to remain an important covariate for future analyses of infection outcomes and personalization of HCV regimens.

Other limitations with the current standard therapeutic approach include the i.v. mode of administration for interferon, the high cost of treatment, adherence and the associated plethora of side effects. Side effects resulting in treatment discontinuation are observed in approximately 10% of patients and include dose-dependent influenza-like symptoms, cytopenia, nausea, severe dermatological conditions (alopecia, dermatitis), anorexia and neuropsychiatric effects, including depression and suicidal ideation (23). No effective alternative treatments are routinely available for individuals who fail to achieve a sustained virological response with peginterferon/ribavirin therapy.

Although novel interferon-based products continue to be developed, interest is focusing on different classes of anti-HCV drugs. Small-molecule inhibitors that act against viral proteins integral to HCV replication have been shown to be effective in demonstrating clinical proof of concept. To date, the direct-acting antiviral compounds most advanced in development are the HCV protease NS3/4A inhibitors (seven drugs in development) and the RNA-dependent HCV polymerase NS5B inhibitors (three nucleoside and seven non-nucleoside inhibitors in development). Other compounds of interest directly targeting HCV replication include entry inhibitors, p7 inhibitors, NS4A-binding drugs and inhibitors of NS4B RNA binding, and NS5A inhibitors.

The specificity of action of small molecules against viral targets predisposes to the development of resistance mutations. Every direct-acting antiviral compound from each of the NS3/4A protease, NS5B polymerase and NS5A inhibitor classes has been shown to select for specific resistance variants (24). Resistance profiles cluster according to and within each direct-acting antiviral drug class. This is encouraging because a lack of resistance across drug classes provides opportunity for overcoming resistance by combining drugs from different classes. Accordingly, direct-acting antiviral compounds in combination therapy with standard of care are associated with vastly improved rates of sustained virological response to treatment.

Analysis of the x-ray crystal structure of the NS3 enzyme reveals a shallow hydrophobic catalytic site located on the surface of the protein, which has placed considerable constraint on development options for competitive inhibitors of this protease. Various novel approaches have been used to design preorganized, depeptidized macrocyclic inhibitors linking the P2-P4 groups and P1-P3 residue. The chemical design of NS3 inhibitors under development falls into two groups: linear (e.g., telaprevir, boceprevir) and macrocyclic (e.g., vaniprevir, danoprevir, TMC-435350, BI-201335) (25) (Table II).

The rates of sustained virological response with all antiviral regimens are influenced by the ease with which the replicating virus is able to escape therapeutic pressure. The broad genetic diversity of HCV is generated by a high rate of viral replication (estimated at 10^{12} virion particles/day) and poor fidelity of the RNA polymerase (estimated at 10^{-3}-10^{-5} mutations/nucleotide/genome replication) (26). Furthermore, genetic variants coexist as minority populations among the dominant wild type in HCV, resulting in quasi-species (27). Drug-resistant mutations describe amino acid mutations from the dominant or wild-type population which produce conformational changes that interfere with the drug–target interaction. Drug resistance mutations are selected in that their frequency in the viral population increases relative to replicative success under treatment. Selection is dependent on the genetic barrier (the number of nucleotide changes needed for the virus to acquire clinical resistance to an antiviral regimen) and the cost to replicative fitness. The frequency or presence of NS3-resistant mutations during treatment is likely to be influenced by other pressures, including those exerted by the

Table II. *Drug resistance sites for NS3/4A protease inhibitors in development for HCV infection.*

Compound	Resistance mutations (NS3) V36A/M	Q41R	F43C/S	T54A/S	V55A	Q80K/R	S138T	R155K/Q/T	A156S/T/V	D168A/T/V	V170A/T	Phase
α-Ketoamide (linear)												
Telaprevir		*			*						*	III
Boceprevir		*										III
Macrocyclic												
TMC-435350		*	*						*			II
Danoprevir		*	*				*		*			II
BI-201335												II
Vaniprevir									*			II

Grey boxes indicate one or more of the variants at site associated with drug resistance. *Putative resistance sites based on in vitro analysis including the use of replicon assays. Reviewed in references 11, 13, 40-43.

host immune response (including at baseline), the constraints of each genotype and by other co-prescribed drugs.

Drug resistance to both linear and macrocyclic NS3 inhibitors is conferred by single-site mutations at A156 and R155 (Table II, Fig. 1). These mutations are predicted to occur outside the substrate envelope for NS3/NS4A. Crystallization studies indicate that the P2 groups of boceprevir, danoprevir and TMC-435350 all protrude extensively from the substrate envelope to contact residues A156 and R155. Thus, mutations in this area are better able to selectively weaken drug binding without compromising viral binding, and therefore avoid a cost in replication to drug adaptation (28). As with other class-specific mutations, these two variants appear susceptible to other drug classes, and patients with NS3 linear *and* macrocyclic inhibitor resistance variants (R155K) can respond to peginterferon/ribavirin and achieve a sustained virological response.

The two most clinically advanced of the protease inhibitors (telaprevir and boceprevir) are both linear ketoamide compounds which covalently bind to the active-site serine of the protease in a slowly reversible manner. For therapy-naïve and -experienced patients with genotype 1 infection, treatment with telaprevir in combination with peginterferon/ribavirin increased the rates of rapid, early and sustained virological responses more than peginterferon/ribavirin alone (PROVE-1, PROVE-2 and PROVE-3 trials; see 29-31). The principal adverse events reported for telaprevir in the PROVE-2 trial were pruritus and rash, and clinical monitoring for dermatological reactions for telapravir recipients is therefore recommended (32). For therapy-naïve HCV-infected individuals (and especially those with genotype 1) boceprevir with peginterferon/ribavirin improved the sustained virological response by 20-25% compared with those receiving peginterferon/ribavirin only (SPRINT-1 trial). However, treatment discontinuations were more frequent among patients receiving boceprevir-based therapy (33-35). It is likely that both telaprevir and boceprevir will soon be available for clinical use.

Modified macrocyclic NS3 inhibitors which maintain a ketoamide moiety as a serine trap for the enzyme, but which also contain a cyclic sulfone moiety at the P4 site, are also at the early stages of evaluation, including vaniprevir (36). Vaniprevir (MK-7009) is a new macrocyclic NS3/4A protease inhibitor. The compound was developed based on leads provided by a modeling approach that identified the potential for alternative macrocyclic inhibitors that utilized a P2-P4 macrocyclic linker as opposed to a P1-P3 linker common in other macrocyclic protease inhibitors. The P2 to P4 constraint noncovalently binds to the NS3/4A active site (Fig. 1).

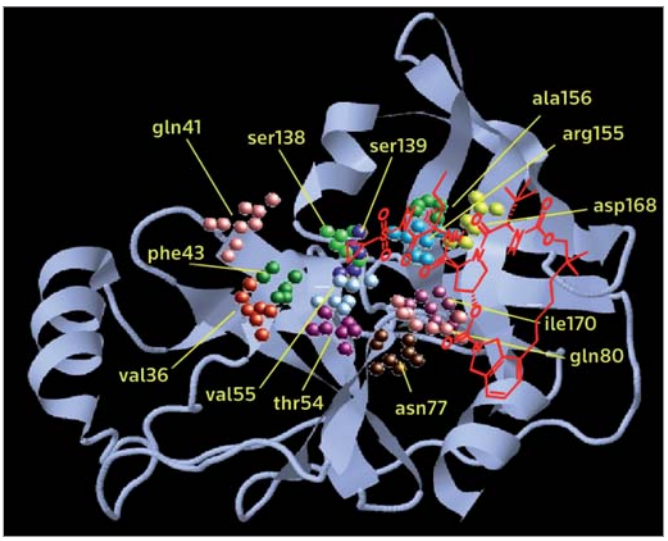


Figure 1. Drug-resistant mutations highlighted in the HCV NS3a alpha chain highlight the vulnerability of vaniprevir to mutations in residues arg155, ala156 or asp168. The HCV NS3 alpha chain is displayed with reported drug resistance amino acid mutations (including sites listed in Table II) highlighted using a space-filling model. The macrocyclic inhibitor vaniprevir is superimposed. Vaniprevir binds to the active site of NS3 centered on Ser139. The NS3 alpha chain is rendered using the protein data bank (http://www.pdb.org/pdb; 1RTL) by the Jmol 3d molecular viewer (Jmol: an open-source Java viewer for chemical structures in 3D. http://www.jmol.org/).

PRECLINICAL PHARMACOLOGY

Merck & Co. recently released details of the preclinical evaluation of vaniprevir, which is scheduled for further progression to phase III trials in healthy volunteers and HCV-infected patients. The following information is sourced from publications released by Merck's research departments (2, 37).

In vitro evaluation showed that vaniprevir inhibits HCV genotype 1a and 1b NS3/4A protease activity at subnanomolar concentrations (K_i = 0.07 and 0.06 nM, respectively) and inhibits genotype 2a and 2b enzymes at low nanomolar concentrations (K_i = 1.0 and 1.4 nM, respectively). These results compare favorably with those obtained using the same assay with another macrocyclic protease inhibitor, ciluprevir (K_i = 0.81 and 7 nM, respectively, for genotype 1a and 2a), and the most clinically advanced linear inhibitors, telaprevir (K_i = 130 and 54 nM, respectively, for genotype 1a and 2a) and boceprevir (K_i = 21 and 25 nM, respectively, for genotype 1a and 2a) (37).

The potency of vaniprevir demonstrated using in vitro assessment of viral proteases is consistent with cell-based activity against both genotype 1 and 2 replicon cell lines. Vaniprevir was marginally less potent against a genotype 2a replicon (EC_{50} = 15 nM for genotype 2a compared to 5 nM for genotype 1b). Again, these results are encouraging when compared to the activity observed for telaprevir (EC_{50} = 1100 nM for genotype 1b) and boceprevir (EC_{50} = 480 nM for genotype 1b) when the authors used the same replicon system (37).

The potency of vaniprevir has also been tested against a small panel of mutant NS3 replicons resistant to other protease inhibitor drugs. Data showing > 2-fold increases in the EC_{50} suggest that vaniprevir has poor potency against the known protease inhibitor-resistant sites R155K (EC_{50} = 350 nM), A156T (EC_{50} = 200 nM) and D168Y (EC_{50} = 430 nM), but good potencies against Q41R (EC_{50} = 5.2 nM), F43S (EC_{50} = 7.8 nM) and wild-type genotype 1b (EC_{50} = 1.6 nM) (37). In patients with virological breakthrough in phase II studies resistance at positions 155 and 168 has been identified. The consequences of these shifts in activity against mutant proteases will need to be established through clinical studies, which will take account of complexities arising from the viral and host genetic constraints unique to each infected individual.

Additive/synergistic inhibition observed in combination studies in replicon assays suggests a likely benefit of utilizing vaniprevir in combination with interferon alfa-2b and/or with an HCV RNA-dependent RNA polymerase inhibitor (e.g., the nucleoside analogue MK-0608) (37).

Potencies based on a transient cell-based protease activity (phenotype) assay as a measure of activity against NS3/4A patient isolates with either genotype 1b or 1a indicated good activity, with EC_{50} values all under 30 nM.

A very large window with respect to cellular toxicity was observed, with the 50% cytotoxic concentration (CC_{50}) being > 50 μM.

In vitro testing of vaniprevir activity against various human proteases, namely cathepsins, chymase, pancreatic elastase 1, neutrophil elastase 2c and chymotrypsin, showed that it was not generally inhibitory, indicating good selectivity of action. In contrast, boceprevir and telaprevir showed detectable effects on some of these proteases. The lack of significant activity against the cytochrome P450 isoforms examined is an attractive attribute for a drug targeting significant liver exposure and beneficial for combination therapy with other drugs with regards to reduced potential for drug interactions.

PHARMACOKINETICS AND METABOLISM

The pharmacokinetic profile of vaniprevir has been evaluated in chimpanzees, rats, dogs and rhesus monkeys. Doses of vaniprevir of 2 mg/kg i.v. were given to rats, dogs and rhesus monkeys, followed by quantification of drug levels in plasma, and it was found that vaniprevir can achieve moderate to high plasma clearance, a moderate volume of distribution and a short half-life (54-78 min). Oral administration of 10 mg/kg to chimpanzees showed that at 12 h post-dosing the plasma exposure was poor (5.2 μM.h) and the liver concentration was excellent (31 μM.h), being 1,500-fold higher than needed for potency based on the replicon system, and levels were maintained post-dosing for up to 24 h. Oral administration of 5 mg/kg to rats, dogs and monkeys revealed poor bioavailability and plasma exposure, but across all species tested the drug maintained significant potency against genotype 1b replicon EC_{50} systems for at least 24 h after dosing.

CLINICAL STUDIES

On the basis of this favorable profile, vaniprevir was selected for clinical development and is currently being evaluated in controlled phase I and II clinical trials in both healthy volunteers and HCV-infected patients (Table III). Efficacy, safety, tolerability and dosing convenience are likely to be key features in determining the progressive clinical development of this drug.

The initial phase I trial examined the safety and efficacy of vaniprevir monotherapy in 40 individuals who were chronically infected with HCV genotype 1 and revealed substantial decreases in viral load from baseline with different doses of the drug during an 8-day period (Table IV). The maximum viral decline observed in this study (in the 700-mg twice daily arm) again compared favorably with maximum viral declines in phase I monotherapy trials for other NS3/4A protease inhibitors (Table V). No serious adverse events were observed up to 14 days following cessation of vaniprevir monotherapy, but some subjects did report other adverse events, including constipation (n = 4), diarrhea (n = 4), nausea (n = 4), headache (n = 7) and bradycardia (n = 1) (Table IV).

A recent phase II trial investigated the efficacy of 28 days' administration of different doses of vaniprevir combined with peginterferon/ribavirin in previously untreated chronically infected participants (Table III) (38). All patients subsequently continued peginterferon/ribavirin for an additional 44 weeks. Virological breakthrough associated with R155K mutations resistant to vaniprevir was reported for nine patients. Four of the nine had a virological breakthrough during vaniprevir dosing, while the other five patients experienced treatment failure post-dosing during peginterferon/ribavirin treatment. Of these, one patient with genotype 1a had high levels of R155K, whereas two patients with genotype 1b had no or low levels of vaniprevir-resistant variants. The variant R155K has been reported to have a high degree of replicative fitness for genotype 1a, and has been detected as the dominant quasi-species in a treatment-naïve patient, raising concerns that drug resistance may not necessarily be associated with reduced viral fitness in the individual in vivo situation (39). As discussed earlier, for genotype 1b, R155K is constrained by a higher genetic barrier, in that two nucleotide changes at the R155 codon are necessary to achieve an amino acid change (38). Loss of potency using vaniprevir against the R155K variant has already been demonstrated in vitro using replicon systems (see above).

Table III. *Vaniprevir (MK-7009) human trials.*

Phase	Identifier	Subjects	Objective	Regimen	Completion
I	NCT00518622	Chronic GT1	Safety and efficacy of MK-7009	Monotherapy 8 days	May 2009
I	NCT01010906	Hepatic subjects	Influence of hepatic insufficiency on the pharmacokinetics of MK-7009	Monotherapy single dose	June 2010
I	NCT00954993	Chronic GT1-TE	Pharmacokinetics and HCV RNA following administration of MK-7009	MK-7009 + peginterferon alfa/ribavirin 28 days	February 2011*
II	NCT00704184	Chronic GT1-TN	Safety and effectiveness of MK-7009 administered with peginterferon alfa/ribavirin	MK-7009 + peginterferon alfa/ribavirin 28 days	April 2010
II	NCT00704405	Chronic GT1-TE	Safety and effectiveness of MK-7009 administered with peginterferon alfa/ribavirin	MK-7009 + peginterferon alfa/ribavirin 24 weeks ± placebo and peginterferon alfa/ribavirin 24 weeks OR MK-7009 + peginterferon alfa/ribavirin 48 weeks	November 2012
II	NCT00880763	Japanese chronic GT1	Safety and effectiveness of MK-7009 administered with peginterferon alfa/ribavirin	MK-7009 + peginterferon alfa/ribavirin 28 days	March 2012
II	NCT00943761	Chronic TE (no SVR)	MK-7009 in patients after participation in other MK-7009 studies	MK-7009 + peginterferon alfa/ribavirin 48 weeks	June 2014
II	NCT00895882	Chronic GT1-TN	Safety, tolerability and efficacy of different regimens of MK-7009 administered with preginterferon alfa/ribavirin	MK-7009 + peginterferon alfa/ribavirin 12 weeks + same dosage 12 weeks or placebo MK-7009 + peginterferon alfa/ribavirin 12 weeks	January 2013*

*Not yet recruiting. All studies randomized and placebo-controlled except for NCT01010906 (active control), NCT00954993 (uncontrolled) and NCT00943761 (nonrandomized and uncontrolled). GT, genotype; TN, treatment-naïve; TE, treatment-experienced.

Table IV. *Antiviral activity of vaniprevir at different doses in phase I trial (NCT00518622).*

Dose (mg)	Interval	HCV RNA decline* (log_{10} IU/mL)	Adverse effects^ (no. participants affected/at risk)
25	b.i.d.	1.9	2/3
75	b.i.d.	2.5	3/6
250	b.i.d.	2.8	6/6
500	b.i.d.	3.3	4/5
700	b.i.d.	4.6	2/6
125	o.d.	1.8	5/5
600	o.d.	2.3	1/4
Placebo	b.i.d.	−0.1	3/5

*Mean HCV RNA decline from baseline. q.d., once a day; b.i.d., twice a day for 8 days. ^No serious adverse events reported up to 14 days after completion of therapy. The description of common adverse event (not serious) and number of participants reporting them were: constipation (n = 3), diarrhea (n = 4 + 2 from placebo group), nausea (n = 4 + 1 from placebo group), headache (n = 7). One subject reported bradycardia.

CONCLUSIONS

The development of direct-acting antiviral drugs, including the macrocyclic NS3/4A protease inhibitor vaniprevir, offers the potential for improved outcomes with combination therapy. Genetic barriers to pharmacological success must consider the constraints from genotype/subtype diversity and other selective pressures. More research is needed to understand the circumstances leading to the emergence of direct-acting antiviral-specific resistant variants and the impact drug resistance has on treatment efficacy. We should expect more development of new compounds such as vaniprevir and of optimum drug combinations that aim to avoid selection of resistant strains, enhance the effectiveness of treatment, reduce the duration of treatment and potentially improve tolerability.

Improvements in sustained virological response with novel combination therapy inclusive of direct-acting antiviral classes are providing reasons for optimism among individuals with HCV. The progressive development of vaniprevir to the clinical phase reflects the impressive attributes of this compound in terms of pharmacokinetic profile, potency and resistance profiles as measured in vitro and in vivo. More data from a clinical setting are needed to predict its use and implications in the new era of direct-acting antiviral combination therapy. Our ability to predict treatment outcomes with vaniprevir will be improved by: 1) understanding the contribution of specific combinations of host *and* viral genetics to treatment failure; and 2) prospectively monitoring the mutational kinetics of HCV quasispecies following treatment failure. These two processes should inform each other and increase our ability to individualize therapy.

As new antiviral drugs emerge, the management of patients with combined therapy will become increasingly challenging and demand better understanding of adverse drug reactions, drug interactions and the genetic makeup of individuals receiving treatment.

Table V. *Antiviral activity of NS3/4A protease inhibitors in phase I monotherapy trials.*

Protease inhibitor	HCV RNA decline (log_{10} IU/mL)*	Dose (mg)	Interval	Dosing duration (days)
Telaprevir	4.4	750	t.i.d.	14
Boceprevir	2.1	400	t.i.d.	14
TMC-435350	3.9	200	q.d.	5
Danoprevir	3.8	200	t.i.d.	14
BI-201335	4.2	240	q.d.	14
Vaniprevir	4.6^	700	b.i.d.	8

*Mean/median maximum HCV RNA decline of 3-14 days (from 40, 41). Note that the boceprevir monotherapy trial involved standard of care nonresponders only. Trials had 6-12 subjects in the specific dose arm shown. q.d., once a day; b.i.d., twice a day; t.i.d., three times a day. ^Study participants included Caucasian, African American or Hispanic American ethnicity and all were infected with HCV genotype 1 (predominantly 1a).

Based on equivalent parameters of assessment, the data for vaniprevir compare favorably against its competitor drugs telapravir and boceprevir. However, data on the adverse events associated with clinical exposure to vaniprevir are limited but will be an important consideration that determines future prescribing patterns. We have yet to develop models that can accurately predict the metabolic pathway from the structure of the drug and whether haptenization of proteins and immunogenicity will occur. Thus, the full spectrum and severity of adverse events with vaniprevir will only be revealed when data from more extensive clinical testing become available.

SOURCE

Merck & Co., Inc. (US).

DISCLOSURES

The authors state no conflicts of interest.

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