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MONOGRAPH

DACLATASVIR DIHYDROCHLORIDE

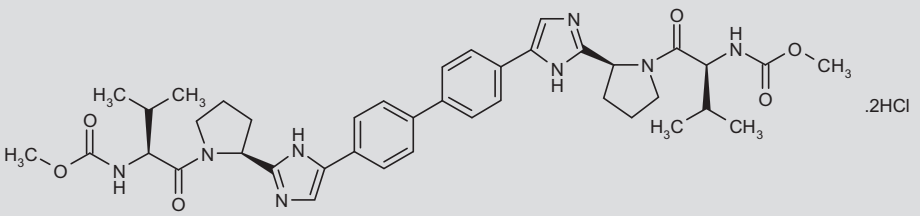
Treatment of Hepatitis C Virus
HCV NS5A Inhibitor

Prop INNM; USAN

BMS-790052

N,*N*'-(Biphenyl-4,4'-diyl)bis(1*H*-imidazol-5,2-diyl)bis[pyrrolidin-2(*S*),1-diyl]bis[3-methyl-1-oxobutan-2(*S*),1-diyl]bis(carbamic acid methyl ester) dihydrochloride

InChI: 1S/C40H50N8O6.2ClH/c1-23(2)33(45-39(51)53-5)37(49)47-19-7-9-31(47)35-41-21-29(43-35)27-15-11-25(12-16-27)26-13-17-28(18-14-26)30-22-42-36(44-30)32-10-8-20-48(32)38(50)34(24(3)4)46-40(52)54-6;;/h11-18,21-24,31-34H,7-10,19-20H2,1-6H3,(H,41,43)(H,42,44)(H,45,51)(H,46,52);2*1H/t31-,32-,33-,34-;;/m0../s1



$C_{40}H_{52}Cl_2N_8O_6$
Mol wt: 811.797
EN: 468969

SUMMARY

Hepatitis C virus (HCV) is a major chronic disease and infected individuals are at risk of developing multiple and highly severe liver complications, such as cirrhosis or liver carcinoma. The current standard of care for the treatment of HCV is a combination of pegylated interferon alfa and ribavirin, a poorly tolerated regimen with limiting side effects and low sustained virological response rates in genotype 1-infected patients. Therefore, the exploration of alternative treatment regimens is of utmost importance. Daclatasvir dihydrochloride (BMS-790052) is a novel, small-molecule, first-in-class inhibitor of HCV replication that targets non-structural protein 5A (NS5A). It has shown impressive clinical efficacy, high selectivity, picomolar activity and broad genotype coverage in vitro. Daclatasvir is safe and generally well tolerated, and it has a pharmacokinetic profile suggestive of once-daily administration. With its extraordinary potency and non-overlapping resistance profile, daclatasvir has the potential to be an important component in future combination strategies for HCV therapy.

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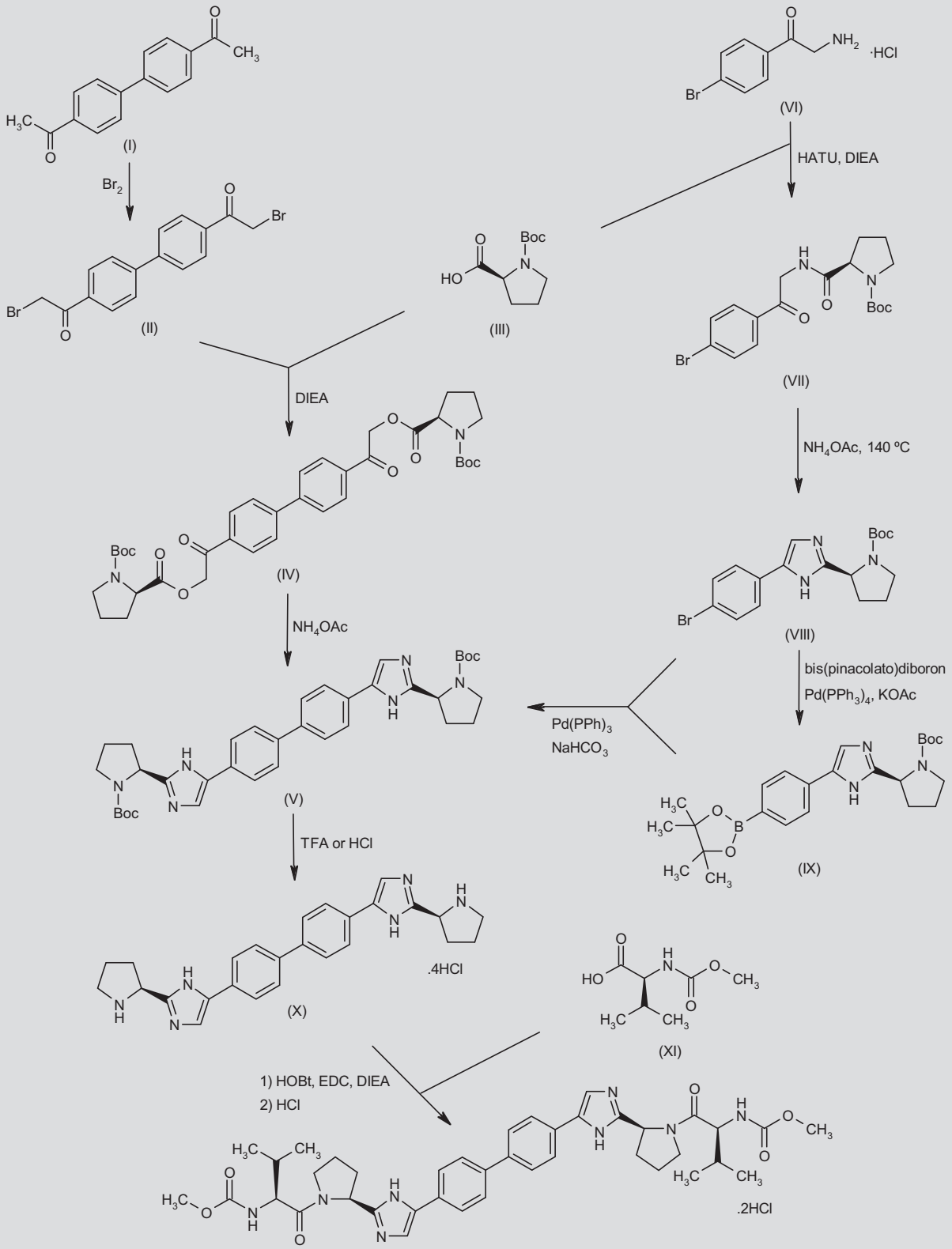
SYNTHESIS*

Daclatasvir can be prepared by two alternative methods:

Bromination of 4,4'-diacetylbiphenyl (I) with Br_2 in CH_2Cl_2 generates 4,4'-bis(bromoacetyl)biphenyl (II), which is then condensed with *N*-Boc-L-proline (III) in the presence of DIEA in acetonitrile to give the diester (IV). Subsequent cyclization of the keto ester (IV) with NH_4OAc in toluene at 95-100 °C affords the bis(imidazole) precursor (V) (1-3). In an alternative method, coupling of *N*-Boc-L-proline (III) with 2-amino-4'-bromoacetophenone (VI) by means of HATU and DIEA in DMF provides the keto amide (VII), which undergoes cyclization to the imidazolyl-pyrrolidine (VIII) upon heating to 140 °C with NH_4OAc in xylenes in a sealed tube. Treatment of the aryl bromide (VIII) with bis(pinacolato)diboron in the presence of $Pd(PPh_3)_4$ and KOAc in dioxane at 80 °C gives rise to the boronate ester (IX). Then, Suzuki coupling between bromide (VIII) and boronate (IX) using $Pd(PPh_3)_4$ and $NaHCO_3$ in DME/H_2O at 80 °C also affords the bis(imidazolyl)biphenyl precursor (V) (1). Deprotection of compound (V) with either TFA in CH_2Cl_2 (1) or HCl in MeOH or *i*-PrOH/H_2O at 50 °C (1-3) provides the *N*-unsubstituted-bis(pyrrolidine) compound (X), which is then acylated with *N*-(methoxycarbonyl)-L-valine (XI) by means of EDC, HOBt·H_2O and DIEA in acetonitrile, followed by treatment with HCl in EtOH (1-3). Scheme 1.

Scheme 1. Synthesis of Daclatasvir



BACKGROUND

Up to 200 million individuals worldwide are infected with hepatitis C virus (HCV), a major cause of chronic liver disease (4, 5). Individuals affected with HCV are at considerable risk of developing cirrhosis, end-stage liver disease and hepatocellular carcinoma (6). HCV is an enveloped, positive-strand RNA virus and is the only member of the *Hepacivirus* genus of the *Flaviviridae* family (7). HCV is classified into six major genotypes, each with multiple subtypes based on sequence diversity (7, 8).

Currently, optimal therapy for HCV treatment relies upon a combination of weekly subcutaneous (s.c.) injection of peginterferon alfa and twice-daily oral doses of ribavirin (4, 9, 10). Treatment can last for periods ranging from 24 to 72 weeks depending on genotype (9, 10). The inadequacies of this treatment are evident in the poor tolerability and the significant incidence of side effects (9-13). Moreover, its effectiveness is highly genotype-dependent (11-13). For genotypes 1a and 1b, accounting for approximately 60% of global infections, long-term efficacy or a sustained virological response is only achieved in around 50% of chronically infected individuals (11, 14).

There are an increasing number of small-molecule inhibitors targeting specific viral proteins entering late-stage clinical development (12, 13, 15-17). The most advanced direct-acting HCV antiviral drug candidates (direct-acting antiviral agents, or DAAs) target enzymatic activities of the HCV non-structural proteins NS3 (serine protease) and NS5B (RNA-dependent RNA polymerase) (15, 17). Although these compounds are currently being developed as add-on therapy to the standard of care and have demonstrated potent antiviral activity, trials have also revealed the rapid emergence of resistant viral variants (15, 18-21).

In this context, daclatasvir dihydrochloride (BMS-790052) is a potent small-molecule inhibitor of HCV RNA replication that targets HCV NS5A, unlike most HCV inhibitors under clinical evaluation (22, 23). In fact, daclatasvir is the compound that established proof of concept for HCV NS5A inhibition as a treatment for HCV infection (24). NS5A is an essential component of the HCV replication complex, is not known to possess any enzymatic activities thus far, and its role in viral replication remains poorly defined (25, 26).

Daclatasvir is the most potent HCV inhibitor reported to date. It has demonstrated remarkable antiviral efficacy in HCV genotype 1-infected subjects at single oral doses, and proved to be well tolerated in a phase I clinical study. Preliminary in vitro results and its clinical profile make daclatasvir a potentially valuable component of any interferon-free treatment regimen (22). However, given the high degree of genetic variability in infected individuals, it is unlikely that daclatasvir or any other DAA alone will be successful as a monotherapy for HCV infection (27). In this context, the latest clinical results suggest that daclatasvir may have high antiviral potential in combination with other DAAs and/or peginterferon alfa/ribavirin for difficult-to-treat individuals (28-30).

PRECLINICAL PHARMACOLOGY

In vitro, daclatasvir demonstrated potent inhibitory activity towards all genotypes tested, including genotypes 1a and 1b, with EC_{50} values within the picomolar range (22, 23, 31). In combination studies, daclatasvir displayed additive to synergistic effects with interferon alfa/ribavirin, an inhibitor of NS3 protease, and both nucleoside and allosteric inhibitors of NS5B polymerase, which is indicative of the potential of this molecule as a candidate for combination therapy with other HCV therapeutic agents. Daclatasvir has also been tested against other viruses and did not inhibit a panel of several RNA and DNA viruses, including, among others, HIV, herpes simplex virus (human herpesvirus, HHV), influenza and poliovirus, with EC_{50} values of > 10 μM, which confirms its specificity for HCV. More significantly, daclatasvir displayed similar potency in multiple cell types, suggesting that the function or functions of NS5A inhibited by daclatasvir are highly conserved in different cellular environments (22, 31).

Daclatasvir exhibited no significant effects in an extensive battery of in vitro receptor binding and enzymatic assays designed to assess promiscuity (22).

PHARMACOKINETICS AND METABOLISM

Kinetic studies with DAAs including telaprevir and daclatasvir against HCV demonstrated an especially rapid first-phase viral decline slope. In fact, mean HCV $t_{1/2}$ estimates during peginterferon alfa, telaprevir and daclatasvir treatment were 2.8, 1.5 and 0.6 hours, respectively, meaning that the HCV half-life is significantly shortened during DAA therapy compared to interferon therapy (32).

In a double-blind, randomized, placebo-controlled, single-ascending-dose study, daclatasvir was administered orally at six dose levels to healthy, non-HCV-infected subjects. The compound was readily absorbed, with dose-proportional exposure over the studied dose range and all subjects had drug concentrations greater than the protein binding-adjusted EC_{90} for genotypes 1a and 1b, as measured in the replicon assay, at and beyond 24 hours post-dose (22).

Comparable results were observed in a similar study, where daclatasvir was administered orally to subjects with genotype 1 chronic HCV at doses of 1, 10 and 100 mg (22, 33-35).

In HCV-infected patients, daclatasvir gave a mean plasma elimination half-life of 10-14 hours, and plasma drug levels were similar to those in non-HCV-infected individuals (22, 34, 35). In parallel, a multiple-ascending-dose study in treatment-naive subjects infected with genotype 1 HCV exhibited similar results; peak plasma concentrations were achieved approximately 1-2 hours post-dose and steady state was achieved by day 4, consistent with a terminal half-life of approximately 13-15 hours (36).

The initial pharmacokinetic profile suggested the possibility for once-daily administration (22, 34, 35), and this was confirmed in subsequent multiple-dose studies (36), where the optimal dose range for maximal antiviral effect beyond the first phase of viral decline was studied.

Coadministration of daclatasvir and the HCV NS3 inhibitor asunaprevir (BMS-650032) in healthy subjects did not result in a clinically meaningful pharmacokinetic interaction, and this is not anticipated to occur when both drugs are administered to HCV-infected patients (37).

SAFETY

Daclatasvir was safe and well tolerated after single oral doses up to 100 mg in HCV-infected patients (22, 33) and 200 mg in healthy

non-HCV-infected subjects (22). Similarly, daclatasvir was shown to be well tolerated in a multiple-ascending-dose study in both healthy and treatment-naive subjects infected with genotype 1 HCV, with a safety profile similar to that of placebo (36). Specifically, there were no deaths, no serious adverse events reported, nor discontinuations due to adverse events or clinically relevant adverse events (22, 30, 36). In fact, headache was the most frequent adverse event seen in both single- and multiple-ascending-dose studies in genotype 1 HCV-infected subjects (22, 36).

Daclatasvir was generally well tolerated when administered in combination with asunaprevir plus peginterferon alfa/ribavirin for 12 weeks (28, 30). However, mild to moderate diarrhea was a common adverse event in 70% of the subjects (29). The safety/tolerability profile for the quadruple treatment was similar to that for peginterferon alfa/ribavirin alone (30, 38).

CLINICAL STUDIES

A double-blind, randomized, placebo-controlled, multiple-ascending-dose study was designed to evaluate the antiviral activity, safety, tolerability and pharmacokinetics of daclatasvir in treatment-naive subjects infected with genotype 1 HCV. Patients infected with genotype 1b HCV generally demonstrated greater antiviral responses than patients with HCV genotype 1a. However, the antiviral effect was not sustained for the entire 14 days of dosing and some subjects experienced viral rebound on or before day 7 (36).

A randomized, open-label phase IIa study to evaluate the antiviral activity and safety of daclatasvir and asunaprevir with or without peginterferon alfa/ribavirin revealed that double therapy including daclatasvir and asunaprevir can lead to sustained virological response (SVR) in difficult-to-treat individuals. Quadruple therapy suppressed the emergence of resistance variants, resulting in a 100% rate of SVR12, with SVR12 defined as undetectable HCV RNA at week 12 post-treatment (29).

Comparable results were observed in a double-blind phase IIa study enrolling treatment-naive subjects infected with genotype 1 HCV using a combination of daclatasvir and peginterferon alfa/ribavirin (30, 38).

Patients receiving two DAAs alone, daclatasvir and asunaprevir, experienced viral breakthrough while on treatment. However, the addition of peginterferon alfa/ribavirin resulted in further suppression of HCV RNA in most of these patients (29).

The genotypic and phenotypic characteristics of viral breakthrough were studied in a clinical trial. The results showed that HCV NS3 protease and NS5A variants reported to confer resistance to daclatasvir and asunaprevir, respectively, were detected at the time of breakthrough. No breakthrough was observed in patients infected with genotype 1b HCV or receiving quadruple therapy including two DAAs and peginterferon alfa/ribavirin (39).

Plasma HCV RNA levels were measured for up to 6 days after administration and a significant antiviral effect was observed at 24 hours post-dose with both the 10- and 100-mg doses. In fact, the dose of 100 mg exhibited a prolonged response in genotype 1b-infected subjects, which was maintained at 144 hours (22, 33-35). Furthermore, even a 1-mg dose of daclatasvir produced an HCV viral load reduction 24 hours after drug administration (22).

A phase III trial in treatment-naive subjects with HCV genotypes 1 and 4 evaluating daclatasvir in combination with peginterferon alfa-2a and ribavirin is scheduled to commence in December 2011 (ClinicalTrials.gov Identifier: NCT01448044).

DRUG INTERACTIONS

Coadministration of daclatasvir and asunaprevir in healthy subjects did not exhibit any pharmacokinetic interaction (37). However, administration of daclatasvir and asunaprevir, with and without peginterferon alfa/ribavirin displayed a very interesting virological response after 12 weeks post-treatment in HCV-infected subjects (28, 29). Similar results were observed when only daclatasvir was administered in combination with peginterferon alfa/ribavirin (30).

SOURCES

Bristol-Myers Squibb (US); being codeveloped with Pharmasset in combination with the nucleotide polymerase inhibitor PSI-7977.

DISCLOSURES

The author states no conflicts of interest.

REFERENCES

1. Bachand, C., Belema, M., Deon, D.H. et al. (Bristol-Myers Squibb Co.). *Hepatitis C virus inhibitors*. EP 2049522, JP 201050013, WO 2008021927.
2. Pack, S.K., Geng, P., Smith, M.J., Hamm, J. (Bristol-Myers Squibb Co.). *Process for synthesizing compounds useful for treating hepatitis C*. CN 101778841, EP 2178863, JP 2010535784, US 2009043107, US 7728027, WO 2009020825.
3. Kim, S., Gao, Q., Yang, F. (Bristol-Myers Squibb Co.). *Crystalline form of methyl ((1S)-1-(((2S)-2-(5-(4'-(2-((2S)-1-((2S)-2-((methoxycarbonyl)amino)-3-methylbutanoyl)-2-pyrrolidinyl)-1H-imidazol-5-yl)-4-biphenylyl)-1H-imidazol-2-yl)-1-pyrrolidinyl)carbonyl)-2-methylpropyl)carbamate dihydrochloride salt*. CN 101778840, EP 2183244, JP 2010535785, US 2009041716, WO 2009020828.
4. Kim, W.R. *Global epidemiology and burden of hepatitis C*. Microbes Infect 2002, 4: 1219-25.
5. Shepard, C.W., Finelli, L., Alter, M.J. *Global epidemiology of hepatitis C virus infection*. Lancet Infect Dis 2005, 5(9): 558-67.
6. Lauer, G.M., Walker, B.D. *Hepatitis C virus infection*. N Engl J Med 2001, 345(1): 41-52.
7. Lemon, S.M., Walker, C., Alter, M.J., Yi, M. *Hepatitis C virus*. In: Fields Virology, 5th Ed., Vol 1. D.M. Knipe et al. (Ed.). Lippincott-Raven Publishers, Philadelphia 2007, 1254-304.
8. Simmonds, P. *Genetic diversity and evolution of hepatitis C virus-15 years on*. J Gen Virol 2004, 85(Pt. 11): 3173-88.
9. Hoofnagle, J.H., Seeff, L.B. *Peginterferon and ribavirin for chronic hepatitis C*. N Engl J Med 2006, 355(23): 2444-51.
10. Pearlman, B.L. *Extended-therapy duration for chronic hepatitis C, genotype 1: The long and the short of it*. World J Gastroenterol 2008, 14(23): 3621-7.
11. Chevaliez, S., Pawlotsky, J.M. *Hepatitis C virus: Virology, diagnosis and management of antiviral therapy*. World J Gastroenterol 2007, 13(17): 2461-6.
12. Schinazi, R.F., Bassit, L., Gavegnano, C. *HCV drug discovery aimed at viral eradication*. J Viral Hepatol 2010, 17(2): 77-90.

13. Thompson, A., Patel, K., Tillman, H., McHutchison, J.G. *Directly acting antivirals for the treatment of patients with hepatitis C infection: A clinical development update addressing key future challenges.* J Hepatol 2009, 50(1): 184-94.
14. Magiorkinis, G., Magiorkinis, E., Paraskevis, D. et al. *The global spread of hepatitis C virus 1a and 1b: A phylodynamic and phylogeographic analysis*. PLoS Med 2009, 6(12): e1000198.
15. Asselah, T., Benhamou, Y., Marcellin, P. *Protease and polymerase anhibitors for the treatment of hepatitis C.* Liver Int 2009, 29(Suppl. 1): 57-67.
16. Moucari, R., Marcellin, P. *Hepatitis C: The role of new interferons in the era of STAT-C*. Nat Rev Gastroenterol Hepatol 2009, 6(9): 509-11.
17. Parfieniuk, A., Jaroszewicz, J., Flisiak, R. *Specifically targeted antiviral therapy for hepatitis C virus*. World J Gastroenterol 2007, 13(43): 5673-81.
18. De Francesco, R., Migliaccio, G. *Challenges and successes in developing new therapies for hepatitis C.* Nature 2005, 436(7053): 953-60.
19. Manns, M.P., Foster, G.R., Rockstroh, J.K., Zeuzem, S., Zoulim, F., Houghton, M. *The way forward in HCV treatment – Finding the right path.* Nat Rev Drug Discov 2007, 6(12): 991-1000.
20. Kieffer, T.L., Kwong, A.D., Picchio, G.R. *Viral resistance to specifically targeted antiviral therapies for hepatitis C (STAT-Cs).* J Antimicrob Chemother 2010, 65(2): 202-12.
21. Sarrazin, C., Zeuzem, S. *Resistance to direct antiviral agents in patients with hepatitis C virus infection.* Gastroenterology 2010, 138(2): 447-62.
22. Gao, M., Nettles, R.E., Belema, M. et al. *Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect.* Nature 2010, 465(7294): 96-100.
23. Belema, M., Nguyen, V.N., Romine, J.L. et al. *Discovery of potent HCV inhibitors targeting the NS5A protein.* 240th ACS Natl Meet (Aug 22-26, Boston) 2010, Abst MEDI 256.
24. Romine, J.L., Laurent, D.R., Leet, J.E. et al. *Inhibitors of HCV NS5A: From iminothiazolidinones to symmetrical stilbenes*. ACS Med Chem Lett 2011, 2(3): 224-9.
25. Huang, Y., Staschke, K., De Francesco, R., Tan, S.L. *Phosphorylation of hepatitis C virus NS5A nonstructural protein: A new paradigm for phosphorylation-dependent viral RNA replication?* Virology 2007, 364(1): 1-9.
26. Macdonald, A., Harris, M. *Hepatitis C virus NS5A: Tales of a promiscuous protein*. J Gen Virol 2004, 85(Pt. 9): 2485-502.
27. Fridell, R.A., Qiu, D., Wang, C., Valera, L., Gao, M. *Resistance analysis of the hepatitis C virus NS5A inhibitor BMS-790052 in an in vitro replicon system*. Antimicrob Agents Chemother 2010, 54(9): 3641-50.
28. Lok, A.S., Gardiner, D.F., Lawitz, E. et al. *Combination therapy with BMS-790052 and BMS-650032 alone or with pegylated interferon and ribavirin (pegIFN/RBV) results in undetectable HCV RNA through 12 weeks of therapy in HCV genotype 1 null responders*. Hepatology 2010, 52(4): Abst LB-8.
29. Lok, A., Gardiner, D., Lawitz, E. et al. *Quadruple therapy with BMS-790052, BMS-650032 and PEG-IFN/RBV for 24 weeks results in 100% SVR12 in HCV genotype 1 null responders*. J Hepatol [46th Annu Meet Eur Assoc Study Liver (EASL) (March 30-April 3, Berlin) 2011] 2011, 54(Suppl. 1): Abst 1356.
30. Pol, S., Ghalib, R.H., Rustgi, V.K. et al. *First report of SVR12 for a NS5A replication complex inhibitor, BMS-790052 in combination with PEG-IFNa-2A and RBV: Phase 2a trial in treatment-naïve-HCV-genotype-1 subjects.* J Hepatol [46th Annu Meet Eur Assoc Study Liver (EASL) (March 30-April 3, Berlin) 2011] 2011, 54(Suppl. 1): Abst 1373.
31. Gao, M. Fridell, R., O'Boyle, D. II et al. *HCV NS5A inhibitor: From screen hit to clinic.* 19th Conf Asian Pac Assoc Study Liver (APASL) (Feb 13-16, Hong Kong) 2009, Abst FP117.
32. Dahari, H., Guedj, J., Cotler, S.J., Layden, T.J., Perelson, A.S. *Higher hepatitis C virus (HCV) clearance rates during treatment with direct acting agents compared to interferon-alpha*. Hepatology 2010, 52(4): Abst 826.
33. Nettles, R., Chien, C., Chung, E. et al. *BMS-790052 is a first-in-class potent hepatitis C virus (HCV) NS5A inhibitor for patients with chronic HCV infection: Results from a proof-of-concept study.* Hepatology [Liver Meet (Oct 31-Nov 4, San Francisco) 2008] 2008, 48(4, Suppl.): Abst LB12.
34. Nettles, R.E., Chien, C., Chung, E. et al. *BMS-790052 is a first-in-class potent hepatitis C virus (HCV) NS5A inhibitor for patients with chronic HCV infection: Results from a proof-of-concept study.* 19th Conf Asian Pac Assoc Study Liver (APASL) (Feb 13-16, Hong Kong) 2009, Abst PE365.
35. Nettles, R.E., Chien, C., Chung, E. et al. *BMS-790052 is a first-in-class potent hepatitis C virus (HCV) NS5A inhibitor for patients with chronic HCV infection: Results from a proof-of-concept study.* 20th Conf Asian Pac Assoc Study Liver (APASL) (March 25-28, Beijing) 2010, Abst PP321.
36. Nettles, R.E., Sevinsky, H., Chung, E. et al. *BMS-790052, a first-in-class potent hepatitis C virus NS5A inhibitor, demonstrates multiple-dose proof-of-concept in subjects with chronic GT1 HCV infection*. Hepatology 2010, 52(4): Abst 1881.
37. Bifano, M., Sevinsky, H., Bedford, B.R. et al. *Co-administration of BMS-790052 and BMS-650032 does not result in a clinically meaningful pharmacokinetic interaction in healthy subjects*. Hepatology 2010, 52(4): Abst 827.
38. Pol, S., Everson, G., Ghalib, R. et al. *Once-daily NS5A inhibitor (BMS-790052) plus peginterferon-alpha-2a and ribavirin produces high rates of extended rapid virologic response in treatment-naïve HCV-genotype 1 subjects: Phase 2a trial-Bristol-Myers Squibb study AI444014*. J Hepatol 2010, 52(4): Abst 1189.
39. McPhee, F., Hernandez, D., Yu, F. et al. *Characterization of virologic escape in HCV genotype 1 null responders receiving a combination of the NS3 protease inhibitor BMS-650032 and NS5A inhibitor BMS-790052.* J Hepatol [46th Annu Meet Eur Assoc Study Liver (EASL) (March 30-April 3, Berlin) 2011] 2011, 54(Suppl. 1): Abst 63.