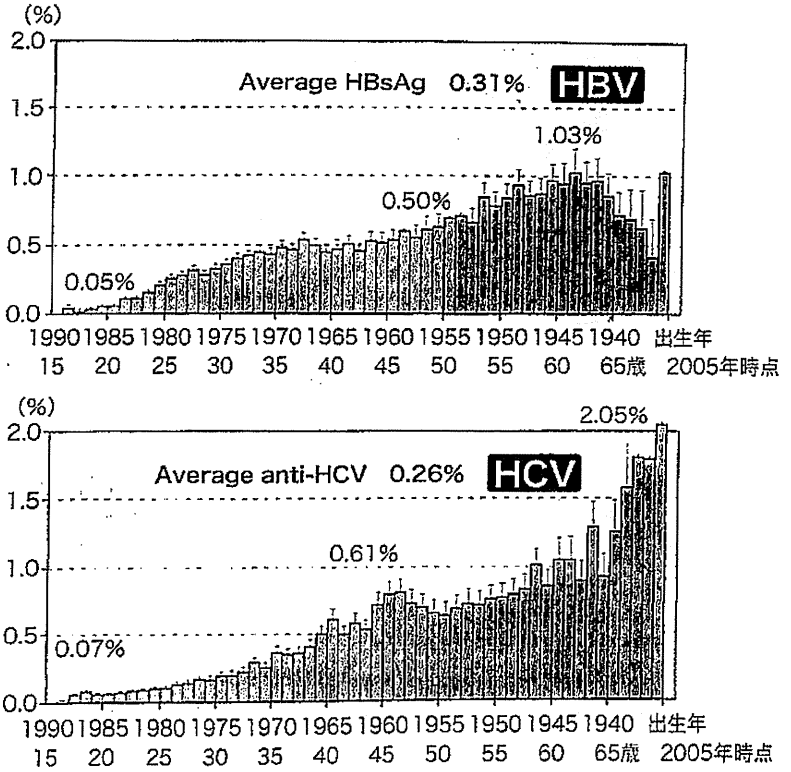

図3-2　初回供血者集団における年齢階級別にみたHBs抗原陽性率とHCV抗体陽性率(2000年以後)
日本赤十字社　初回供血者　2001.1～2006.12　N=3,748,422（Intervirology 2011；54：185–195）

界中の輸血後肝炎の主な原因はC型肝炎ウイルスであったこと，特に米国における輸血後肝炎の90％はHCVによるものであったことをWHOは報告[7]している．わが国においても同様の状況であったと推定され，1999年10月から導入された核酸増幅検査(Nucleic acid amplification test：NAT)により，輸血に伴うHCV感染はほぼ駆逐されたといえる状況となっている[8]．

輸血以外の水平感染によるHCV新規感染についてこれまでに得られた疫学的調査結果を示す．

広島県赤十字血液センターにおける1994年6月から2004年4月までの供血者418,269人(総献血本数1,409,465本)を対象とした前向き調査[9]では，期間内に複数回献血をした218,797人(861,842人年)のうち新たなHCV感染が確認されたのは16例，HCV新規発生率は10万人年あたり1.86人(95％CI：1.06～3.01人/10万人年)と推定された．女性のHCV新規発生率は10万人年あたり2.77人(男性：同1.08人)と，統計学的な有意差は認められないものの，高い値を示す傾向が認められ，さらに20歳代と50歳代(3.21人/10万人年，6.02人/10万人年)でも高い傾向が認められている．

また，血液透析患者を対象とした多施設前向き調査成績[10]では，3カ月以上の観察が可能であった2,114人の血液透析患者のうちHCV感染の新規発生数は16例であり，人年法によるHCV新規感染率は1,000人年あたり3.3人(95％CI：1.7～4.9人/1,000人年)と推

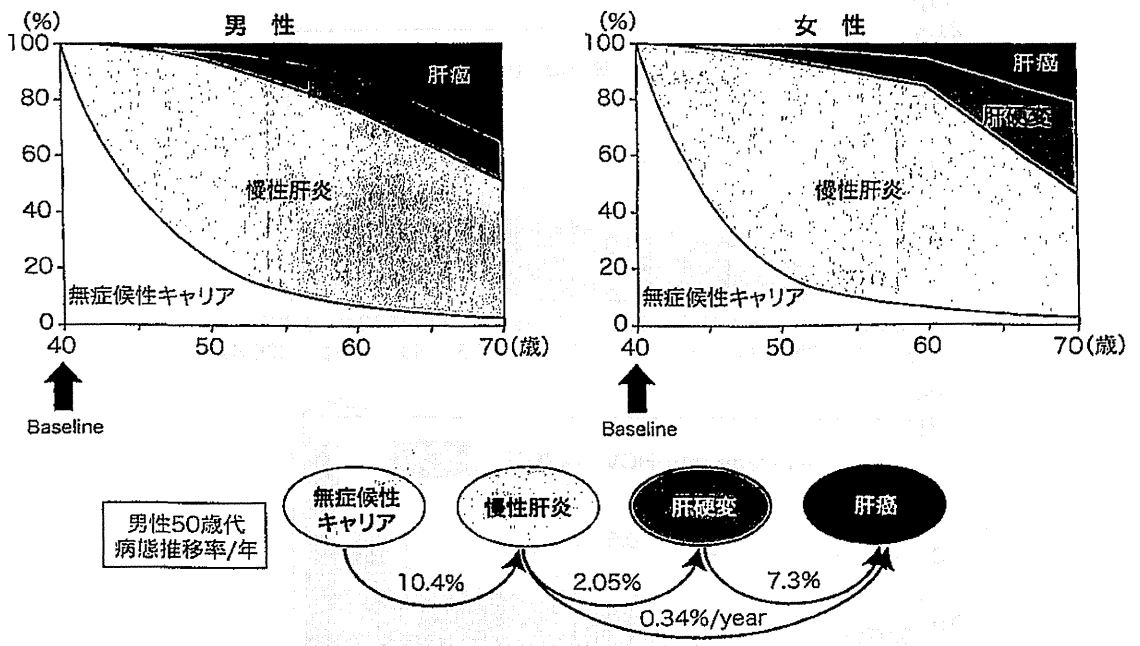

図4-1　HCVキャリアの自然史(natural course)生涯肝発癌率　Markovモデルによる推定各病態への累積罹患率—抗ウイルス療法を行わない場合—(J Med Virol 70 : 378–386, 2003)

計された.

一般集団ではHCV感染の新規発生はごく稀であるが，血液を介する感染の可能性があるハイリスク集団におけるHCV発生率は供血者集団と比較して10^2倍程度高い頻度を示すことが明らかとなっている．女性の中高年齢層の新規感染確認調査，ハイリスクと考えられる集団のHCV感染防止対策は引き続き重要と考えられる.

一方，HCVの母子感染に関する1990年代の調査から報告された新規発生率は2～10％[11,12]と幅が大きく，調査地域や対象妊婦の背景因子の相異などにより異なることが明らかとなっている．感染成立には，分娩方法や児の免疫能，出産時の母体のHCV RNA量などが関与していることが示唆されるが，一般にはHCVの母子感染率は低率と考えられている.

6 肝炎ウイルス感染の病態の推移—マルコフモデルによる推定—

HCVキャリアの病態推移について，長期臨床経過のデータをもとに数理疫学的手法(マルコフ確率モデル)を用いて治療介入の有無別に検討した結果を図4に示す.

抗ウイルス療法などの積極的治療が行われていなかった1990年代に通院していたHCVキャリア942例を対象に40歳を起点として推計した結果，治療介入のない場合，男性では55歳を過ぎるころから，女性では60歳を過ぎる頃から肝発癌率が上昇し始め，70歳時点の累積肝発癌率は男性では約38％，女性では約20％に達することが明らかとなった(図4-1)[13].

さらに1991～2001年に献血を契機に見いだされ医療機関を受診したHCVキャリア1,018例について40歳を起点として推計

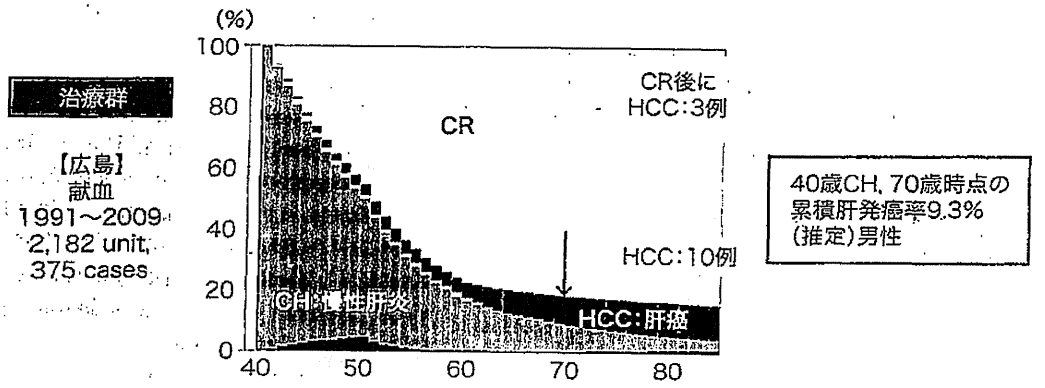

図4-2　献血を契機にみつかったHCVキャリアの病態推移
Markovモデルによる数理疫学的推定(男性)
(厚労省肝炎等克服緊急対策研究事業
「肝炎ウイルス感染状況・長期経過と予後調査及び治療導入対策に関する研究」班)

した結果，抗ウイルス治療介入を施行した375例では，70歳時点には累積CR(complete response)率は82.1%，累積肝発癌率は9.3%となった(図4-2)．CR後に肝発癌例が3例見いだされているものの，現在，臨床試験が進行中あるいは認可申請中のDAAが広く適用されることにより，著効率がさらに上がることから累積肝発癌の割合は減少すると予測される．

7　今後の課題

わが国では，本項で述べてきたようにHCVキャリア率あるいはキャリア数の増加は認められていない．その原因としてはわが国の一般集団におけるHCV新規感染が低率であることに加え，コホート効果により高年齢層のHCVキャリア率がより低い年齢層の値にスライドすることにより，全体でのHCVキャリア率が低下したことが考えられる．しかし一方，アメリカでは20～30歳代を中心としたHCV新規感染率の上昇が報告されていることや，わが国においても女性20歳代50歳代の新規感染率が高い報告があることから，2010年代の各種集団における新規感染率調査を行う必要性があると推察される．

わが国の肝炎・肝癌対策としては，すでに感染しているHCVキャリアを見いだし，適切な治療へ導入するための肝炎ウイルス検診の推進，未検査率の高い職域集団への介入が求められており，加えて，治療に至っていないキャリアへの対策が急務である．治療効果の高い新薬の導入を見据えた，組織的なHCVキャリアの掘り起こしとフォローアップ事業等の新たな治療導入対策への取り組みが必要とされている．

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ウイルス肝炎の自然経過と疫学の up-to-date

ウイルス肝炎の疫学の最近の変化
現況と未来像

片山惠子　田中純子

Summary

- わが国のC型肝炎およびB型肝炎に関する疫学について，これまでの調査成績をもとに紹介した．
- 肝がんの原因の約8割がHCVおよびHBVの持続感染によるものであること，HBVキャリア数とHCVキャリア数の推計，肝炎ウイルス感染の新規発生率および感染状況を示した．
- 効果的な肝炎・肝がん対策として，潜在する肝炎ウイルスキャリアには肝炎ウイルス検査の受検勧奨を，感染が判明したキャリアには医療機関への受診・継続受診の勧奨や抗ウイルス療法等の治療介入を行うことが必要である．

世界保健機関（World Health Organization：WHO）の推計によると，世界全体でのC型肝炎ウイルス（hepatitis C virus：HCV）の持続感染率（キャリア率）は平均約2%であり，毎年300～400万人がHCVに新規感染し，持続感染している人は約1.5億人，年間35万人以上がHCV関連疾患（慢性活動性肝炎，肝硬変や肝がん）で死亡していると試算[1]している．一方，B型肝炎ウイルス（hepatitis B virus：HBV）の持続感染者（HBVキャリア）は2.4億人，約60万人がHBV関連肝疾患により毎年死亡すると報告されている[2]．日本のHCV抗体陽性率は1.5%未満，HBs抗原陽性率は2%未満の低い地域に属している．

わが国では，社会全般における肝炎ウイルス感染の発生要因が徐々に減少し，現在，若い世代における肝炎ウイルスキャリア率は低い値を示すにいたっている．本稿では，わが国のC型肝炎およびB型肝炎に関する疫学調査成績をもとに，現況と予想される未来像と課題について示したい．

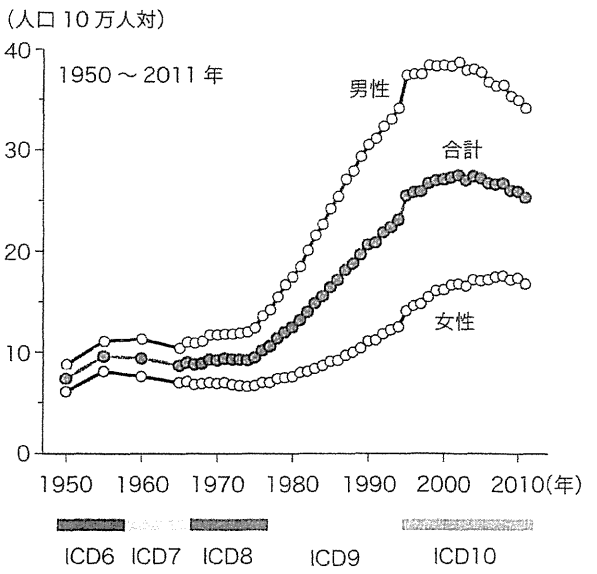

図1　わが国における肝がんによる死亡者数の推移
［人口動態統計2012年より作成］

キーワード：肝炎ウイルスキャリア，肝がん死亡，キャリア数，新規発生率，肝炎ウイルス検査
かたやま けいこ，たなか じゅんこ：広島大学大学院医歯薬保健学研究院疫学・疾病制御学

表1　肝がん(肝および肝内胆管の悪性新生物)による死亡率(人口10万人対)の高い都道府県

	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
1位	佐賀	福岡	佐賀	和歌山	佐賀	福岡	佐賀	佐賀	佐賀	佐賀	佐賀	佐賀	佐賀	佐賀
	37.3	38.3	42.4	41.8	43.3	42.9	41.7	44.3	43.1	47.5	45	49.8	46.9	47.6
2位	福岡	佐賀	福岡	佐賀	福岡	佐賀	福岡	島根	和歌山	山口	和歌山	和歌山	福岡	福岡
	35.6	37.4	41	39.9	41.4	41.4	41.4	40.6	42.6	41.6	43.9	41.7	41.5	40.4
3位	大阪	広島	広島	広島	和歌山	和歌山	広島	福岡	徳島	和歌山	福岡	福岡	山口	徳島
	34.2	33.8	38	39.8	40.5	40.6	39.9	40.5	40.3	41.6	43	40.1	41.4	39.8
4位	和歌山	大阪	大阪	福岡	徳島	広島	和歌山	広島	福岡	福岡	山口	広島	和歌山	山口
	33.8	33.2	37.8	38.7	39.5	40.3	39.6	39.2	39.8	41.4	38.3	39.9	39.9	39.4
5位	広島	和歌山	和歌山	島根	広島	大阪	山口	山口	広島	広島	大阪	山口	山梨	広島
	33	32.3	36.6	38.5	39.4	39	38.4	38.9	39.6	39.9	37.7	39.7	38.2	37.7
6位	徳島	鳥取	山口	大阪	大阪	徳島	大阪	愛媛	愛媛	長崎	広島	鳥取	島根	和歌山
	31.4	31.6	36.3	37.4	37.1	37.9	37.6	38.9	38.8	38.9	37.6	38.9	38.1	37.6
7位	高知	愛媛	長崎	山口	島根	愛媛	大分	大阪	大阪	愛媛	山梨	徳島	広島	高知
	30.6	31.6	35.4	36.6	35.7	37.8	37.3	38.4	38	37.9	37.1	37.7	38.1	36.2
8位	鳥取	兵庫	兵庫	徳島	山口	兵庫	愛媛	徳島	高知	高知	徳島	大分	愛媛	大阪
	30.5	31.2	34.5	35.7	35.7	36.1	36.8	37.9	36.4	37.7	36.4	37.7	37.4	34.9
9位	山口	山口	山梨	高知	兵庫	島根	山梨	和歌山	兵庫	大阪	大分	山梨	徳島	愛媛
	30.4	31.2	33.9	35.7	34.7	36	36.4	35.8	35.5	37.2	36.3	37.1	37	34.8
10位	兵庫	山梨	岡山	兵庫	大分	山口	島根	大分	島根	徳島	高知	島根	熊本	山梨，島根，熊本
	29.6	30.7	33.6	34	33.3	34.8	36.2	35.6	35.3	36.6	35.9	37	36.7	34.6

下線：中国・四国・九州地域
～1994年：ICD9，1995年～：ICD10

肝がん死亡とその成因

わが国の悪性新生物による死亡は360,963人(人口10万対286.6)であり[3]，死因の第1位(28.7%)を占めている(2012年)．そのうち，「肝」(肝および肝内胆管)の悪性新生物による死亡は30,690人(男性20,060人，女性10,630人)と，2011年と比べ約1,200人減少したが，依然として死亡数は臓器別の上位4番目(男性)，6番目(女性)に位置している．肝がんによる死亡者数の推移をみると，1950年代はじめから1970年代半ばまで人口10万人あたり10人前後(死亡実数は1万人以下)と横ばいであったが，その後2002年には27.5人(人口10万対)まで急増した(図1)．男性は女性の約2倍の死亡率を示し，近年は男性では若干の減少，女性では横ばい状態を保っている．

人口動態統計資料と調査成績[4]とをもとに，病因ウイルス別に推定した肝がんの成因について示す(図2)．HBVの持続感染に起因する肝がん死亡は，1980年代から現在にいたるまで10万人対3～4人とほぼ一定の値を示している．一方で，1980年代から2000年代にかけて肝がんによる死亡が増加した原因は非A非B型に起因するものと推定できるが，1992年以降そのほとんどがHCVの持続感染に起因するものであることが明らかとなっている．また，2000年以降の動向をみると，非B非C型に由来する肝がんによる死亡の割合が肝がん死亡全体の10～15%を占めて増加傾向にあり，その原因に

2007	2008	2009	2010	2011
佐賀	佐賀	佐賀	佐賀	佐賀
46.1	45.9	45.7	41.1	
和歌山	福岡	和歌山	広島	和歌山
41.2	40.1	39.3	38.4	
福岡	愛媛	高知	和歌山	広島
40.2	37.7	38.1	37.1	
広島	長崎	長崎	愛媛	福岡
38.2	37.1	37.5	37	
鳥取	広島	福岡	福岡	愛媛
37.2	36.9	37.3	36.8	
大分	島根	愛媛	島根，大分	山口
36.9	36.8	36.7	36.6	
島根，徳島	和歌山	山口	高知	大分
36.2	35.9	36.1	35.6	
高知	高知	徳島	鳥取	長崎
35.8	35.5	35.9	35	
山口	山口	島根	山梨，長崎	島根
35.7	34.8	35.1	33.5	
愛媛	大阪	大分	山口	高知
35.6	34.6	33.8	32.9	

［人口動態統計，1993～2011年より］

ついては今後の研究や調査が必要となっている．

一方，都道府県別に肝がん死亡率（人口10万人対）の順位をみると，中国・四国・九州地域が肝がん死亡率の上位を占めており（表1），上位県では効果的な肝がん対策を行うことが求められている．

わが国の肝がん死亡の約8～9割はHCVあるいはHBVの持続感染に起因し，その多くはHCVによる持続感染であることから，肝炎ウイルス感染予防と肝炎ウイルスの持続感染者（キャリア）対策が重要である．肝炎ウイルスキャリア率とキャリア数の把握は，治療の推進と開発とともに肝炎対策の柱となる．

一般集団における肝炎ウイルス感染状況：2000年以後の初回供血集団からみた検討

一般集団におけるHCVおよびHBVの感染状況を把握するため，全国で統一された試薬と診断基準により判定している日本赤十字社血液センターにおける2001年から2006年の6年間の初回供血者集団（3,748,422人）の資料からHCV抗体陽性率とHBs抗原陽性率（HBVキャ

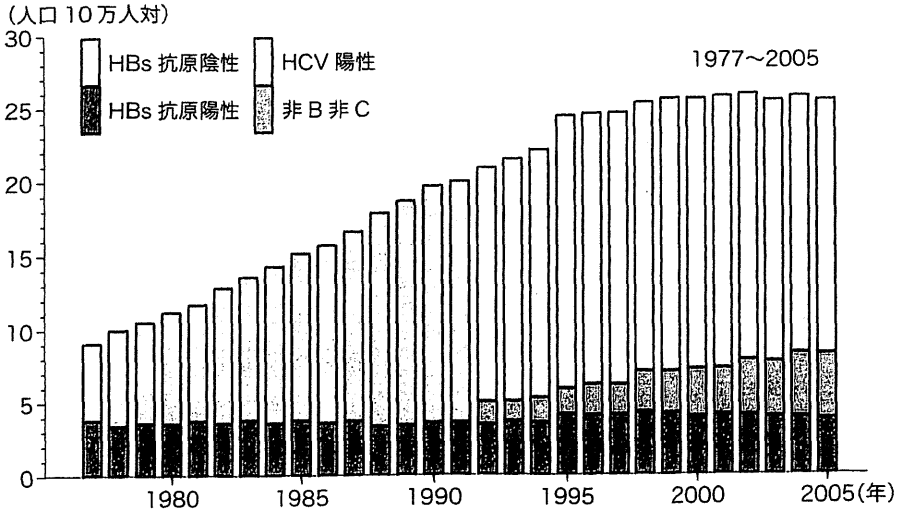

図2　病因別にみた肝がんによる死亡数の経年的推移

厚生労働省 肝炎等克服緊急対策研究事業「急性肝炎も含めた肝炎ウイルス感染状況・長期経過と治療導入対策に関する研究」班より．

［Yoshizawa H, Tanaka K：International Kilmer Conference Proceedings, vol 8, p247-264, 2004より引用，改変］

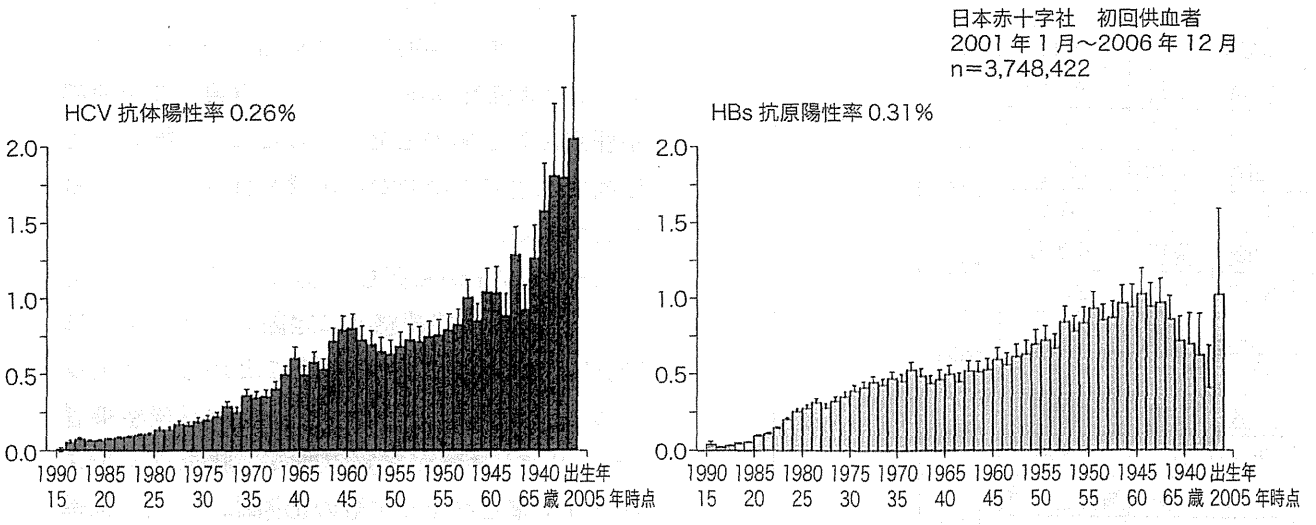

図3　初回供血者集団における年齢階級別にみたHCV抗体陽性率とHBs抗原陽性率(2000年以後)

[文献5)より引用]

リア率)を算出した(図3)[5]．HCV抗体陽性率は全体平均で0.26%と低いが，高年齢層では2%を超える一方，若年齢集団ではきわめて低い陽性率を示している．また，HBs抗原陽性率は全体平均で0.31%と低いが，団塊の世代で約1%と他の年齢層と比較すると高い値を示すことが特徴的である．

これらの肝炎ウイルスキャリア率は，献血を契機に感染が判明した率であり，言い換えるとそれまでに感染を知らないまま社会に潜在している肝炎ウイルスキャリア率と考えることができる．

C型肝炎ウイルスがクローニングされて以後，さまざまな機会での肝炎ウイルス検査が進んできた．2000年代になっても，いまだ感染を知らないまま社会に潜在する肝炎ウイルスキャリア数を推計し，肝がんへ進行する可能性のある人数規模や年齢・地域偏在を明らかにすることは，治療戦略や肝がん対策を構築するうえでの重要な基礎資料となる．

初回供血者集団から得られた上記の成績を用いて推計を試みたところ，2005年時点では，HCVキャリア数は807,903人(95% CI：68.0-97.4万人)，HBVキャリア数は903,145人(95% CI：83.7-97.0万人)となった．この値は，自身が「感染を知らないまま潜在しているキャリア」の推計数に相当していると考えられる．社会での肝炎ウイルスキャリアの存在状態には，「感染を知らないまま潜在しているキャリア」の他に，「患者としてすでに通院・入院しているキャリア」，「感染を知ったが受診しないでいる，あるいは継続受診にいたっていないキャリア」，「新規感染によるキャリア」の4つの存在状態があり，これらの把握のためにさまざまなアプローチによる検討が厚生労働省研究班[6]で行われている．

肝炎ウイルス検査受診者の検査後の動向調査

肝炎対策基本法に基づく肝炎・肝がん対策が進んでおり，効果の高い治療薬の開発が期待できる現時点の課題は，肝炎ウイルス検査受診率の向上と併せて，検査後の医療機関受診率と治療導入率の推進である．

肝炎ウイルス検査後のキャリアの動向を把握する目的で7自治体(107市区町村)が肝炎ウイルス検査受検者5,944人を対象として実施した調査について，厚生労働省研究班で解析を行った．その結果[7]，肝炎ウイルス検査で「陽性」

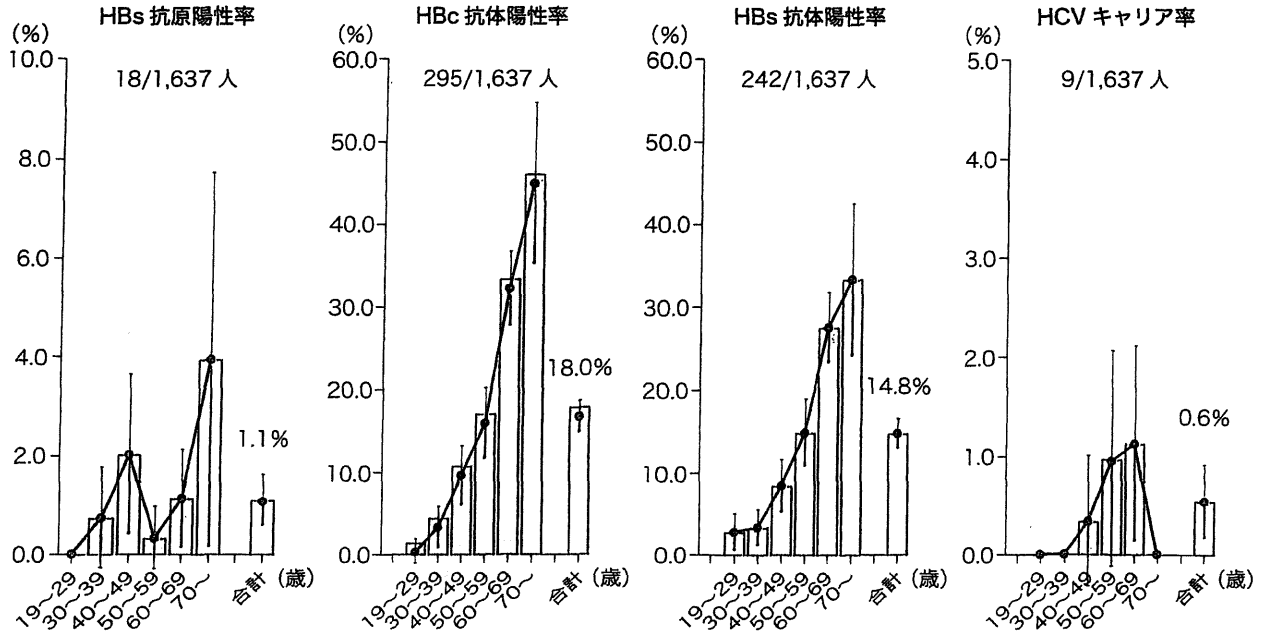

図 4　職域集団における肝炎ウイルス検査結果
n=1,637, 男：女=1,391：246, 平均年齢 49.3±14.9 歳(19〜81 歳 median 50 歳)

と判定された 2,177 人のうち，「検査の受検自体を忘れていた者」が 14.3%存在し，受検したことは覚えているが結果を間違って「陰性」と認識していたものが全体の 8%存在していることが明らかとなった．この結果から推定される医療機関受診率は 66.2%と低率であること，また，医療機関を受診しなかった理由では，「必要がないと思う」31.7%，「どこを受診するのかわからない」11.9%，「受診する機会がなかった」11.2%等であったことから，検査結果の通知時には，受診勧奨のための具体的な情報提供が必要であることが示唆されている．

一般集団における肝炎ウイルス感染状況：職域集団からみた検討

これまでの厚生労働省疫学班の調査研究から，職域集団における肝炎ウイルス検査がなかなか進んでいないこと，すなわち検査受検率が低いことが明らかとなっている．

職域の定期検診時に合わせて，肝炎ウイルス検査を実施する方式，つまり出前検査による「肝炎ウイルス検査」を実施した広島県の調査成績を示す(図 4)．

サービス業および運輸業に属する集団 1,637 人(男性 1,391 人，女性 246 人；平均年齢：49.3±14.9 歳，19〜81 歳)を対象に行った調査では，HBs 抗原陽性率は全体で 1.1%(95% CI：0.59-1.60 %)，HBc 抗体陽性率 18.0 %(95 % CI：16.2-19.9 %)，HBs 抗体陽性率 14.8 %(13.1-16.5%)となった．HBs 抗原陽性率は 20 歳代では 0%と低く，70 歳代では 3.9%と高い値を示している．年齢階級が高い集団では HBV 曝露率は 18.3%(16.4-20.1%)と高値となった．今回見出された肝炎ウイルスキャリア 27 人(HCV 9 人，HBV 18 人)のうち，11 人は初めて検査を受け，感染を指摘されていた．現在，16 人が受診し適切なフォローアップが行われている．

おわりに

わが国では，C 型肝炎ウイルスがクローニン

グされて以後，輸血用血液のスクリーニングにHCV抗体検査をいち早く導入し，また医療機関や住民検診に積極的に肝炎ウイルス検査を取り入れるなど感染防止対策を講じてきた．また，肝炎対策基本法や医療費助成制度の制定や無料検査を取り入れ，積極的に肝がん対策を行ってきている．

その結果，供血者等一般集団におけるC型肝炎ウイルスの新規感染率(incidence)は，10万人年あたり1.86人[8]と低く，HCVキャリアの発生頻度はまれであることが示されている．しかし，観血的治療を頻回に実施する血液透析患者集団においては1,000人年あたり3.3人[9]と高く，ハイリスク集団における感染防止対策は引き続き重要であることも忘れてはならない．

一方，HBVについては，母子感染防止事業が効果的に運用されていることから，1986年以後に出生した若年世代のHBVキャリア率はきわめて低い値を示しており，次世代のHBV母子感染はほぼ消滅することが期待されている．今後，水平感染のHBVgenotype別にみた頻度と感染後の病態に関する研究が課題としてあげられる．

現在，次々に開発される治療効果の高い抗ウイルス薬の導入を見据えると，肝炎ウイルス検査の推進と同時に治療にいたっていないキャリアへの対策が重要である．また，抗ウイルス治療に対する医療費助成制度が整っている状況からみると，手術前検査等さまざまな機会に行われている肝炎ウイルス検査の結果を受検者に適切に通知し，「陽性」と判定されている場合には，一度は肝臓専門医を紹介することが必要である．かかりつけ医と肝臓専門医，自治体との連携により肝炎・肝がん対策を推し進めることにより，国民の健康増進につながる結果をもたらすことが期待できる．

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Research Article

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Dual oral therapy with daclatasvir and asunaprevir for patients with HCV genotype 1b infection and limited treatment options

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See Editorial, pages 643–645

Background & Aims: Improved therapeutic options for chronic hepatitis C virus (HCV) infection are needed for patients who are poor candidates for treatment with current regimens due to anticipated intolerability or low likelihood of response.

Methods: In this open-label, phase 2a study of Japanese patients with chronic HCV genotype 1b infection, 21 null responders (<2 $\log_{10}$ HCV RNA reduction after 12 weeks of peginterferon/ribavirin) and 22 patients intolerant to or medically ineligible for peginterferon/ribavirin therapy received dual oral treatment for 24 weeks with the NS5A replication complex inhibitor daclatasvir (DCV) and the NS3 protease inhibitor asunaprevir (ASV). The primary efficacy end point was sustained virologic response at 12 weeks post-treatment (SVR_{12}).

Results: Thirty-six of 43 enrolled patients completed 24 weeks of therapy. Serum HCV RNA levels declined rapidly, becoming undetectable in all patients on therapy by week 8. Overall, 76.7% of patients achieved SVR_{12} and SVR_{24}, including 90.5% of null responders and 63.6% of ineligible/intolerant patients. There were no virologic failures among null responders. Three ineligible/intolerant patients experienced viral breakthrough and four relapsed post-treatment. Diarrhea, nasopharyngitis, headache, and ALT/AST increases, generally mild, were the most common adverse events; three discontinuations before week 24 were due to adverse events that included hyperbilirubinemia and transaminase elevations (two patients).

Conclusions: Dual therapy with daclatasvir and asunaprevir, without peginterferon/ribavirin, was well tolerated and achieved high SVR rates in two groups of difficult-to-treat patients with hepatitis C virus genotype 1b infection.

Keywords: Daclatasvir; Asunaprevir; Hepatitis C; Antiviral.

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Abbreviations: HCV, hepatitis C virus; DAA, direct-acting antiviral; PegIFN-α/RBV, peginterferon alfa and ribavirin; SVR, sustained virologic response; HIV, human immunodeficiency virus; NS5A, non-structural protein 5A; NS3, non-structural protein 3; ALT, alanine aminotransferase; ULN, upper limit of the normal reference range; INR, international normalized ratio; CYP3A4, cytochrome P450 3A4.

Introduction

Therapies for chronic hepatitis C virus (HCV) infection have improved markedly over the past decade. The recent approval of the first direct-acting antivirals (DAAs) was an important milestone in the evolution of HCV therapy, establishing that DAAs can enhance regimen efficacy and provide durable viral clearance. These new agents in combination with peginterferon and ribavirin (PegIFN-α/RBV) achieve overall sustained virologic response (SVR) rates of approximately 70% in treatment-naïve patients with HCV genotype 1 infection [1,2].

Despite these advances, current treatment options remain inadequate for some patients. Patients with prior null response to PegIFN-α/RBV (<2 $\log_{10}$ decline in HCV RNA after 12 weeks) have a particularly acute need for further therapeutic improvements. Null responders generally respond poorly to retreatment with PegIFN-α/RBV; fewer than 10% achieve SVR [3]. Retreatment of null responders with PegIFN-α/RBV combined with telaprevir or boceprevir increases SVR rates to approximately 30–38%, suggesting that addition of a DAA to PegIFN-α/RBV increases efficacy, but that more potent regimens are still urgently needed [4,5]. There are also many patients who cannot be treated with current therapies; this group includes patients with prior intolerance to PegIFN-α/RBV and patients who are ineligible for PegIFN-α/RBV -containing therapy for medical reasons.

There is precedence for use of combination antiviral regimens to treat human immunodeficiency virus (HIV) infections;

Research Article

evidence is mounting that DAA regimens can also provide durable clearance of HCV infections. Thus, there is a strong rationale for exploration of dual DAA regimens, without PegIFN-α/RBV. In combination, DAAs with different molecular targets can increase regimen potency and raise the barrier to resistance, potentially eliminating the need for PegIFN-α/RBV and providing a viable therapy for patients who are anticipated to be poorly responsive or intolerant to current PegIFN-α/RBV-containing regimens. The improved tolerability and convenience that can be anticipated with dual DAA regimens suggests that they may also benefit treatment-naïve patients and other groups. Previous studies of DAA-only regimens, or DAAs combined with RBV, have demonstrated marked antiviral effects in treatment-naïve and experienced patients, including null responders, supporting the further evaluation of dual DAA therapy reported here [6–10].

Daclatasvir (DCV; BMS-790052) is a first-in-class, highly selective NS5A replication complex inhibitor with picomolar potency and broad genotypic coverage; asunaprevir (ASV; BMS-650032) is a potent NS3 protease inhibitor active against genotypes 1 and 4. Daclatasvir and asunaprevir have different modes of action and resistance-associated variants, and in combination show increased antiviral potency *in vitro* and a high genetic barrier to resistance [11,12]. Daclatasvir and asunaprevir had no clinically meaningful pharmacokinetic interaction in healthy volunteers [13]. Initial efficacy evaluations of daclatasvir and asunaprevir (DUAL therapy) showed potent antiviral effects and SVR rates ⩾90% in Japanese and US/European null responders with HCV genotype 1b infection [7,8].

We present final results of an open-label trial evaluating DUAL oral therapy with daclatasvir and asunaprevir in Japanese patients with chronic HCV genotype 1b infection. Initial results from a sentinel cohort of 10 patients with prior null response to PegIFN-α/RBV have been reported [7]. The present report combines these data with results for 11 additional null responders, together with results for 22 patients with prior intolerance to PegIFN-α/RBV or who were medically ineligible for PegIFN-α/RBV-containing therapy.

Materials and methods

Study design

This open label, phase 2a study (AI447-017; clinicaltrials.gov identifier NCT01051414) was conducted in two populations of patients with HCV genotype 1 infection, including null responders (<2 $\log_{10}$ decline of serum HCV RNA levels after 12 weeks of prior PegIFN-α/RBV), and PegIFN-α/RBV ineligible/intolerant patients. The latter group discontinued prior therapy with PegIFN-α/RBV due to intolerance after <12 weeks, or patients were treatment-naïve but poor candidates for PegIFN-α/RBV for medical reasons such as advanced age or complications of depression, anemia, myelosuppression, diabetes, or cardiovascular or renal dysfunction.

Patients were enrolled in two cohorts of null responders and two cohorts of PegIFN-α/RBV ineligible/intolerant patients. One cohort of each population included intensive sampling for pharmacokinetic analyses; both cohorts of each population were combined for efficacy and safety assessments. The sentinel cohort of null responders, reported previously, provided 4-week safety data for review by the study Safety Committee, prior to initiation of the other cohorts [7]. The primary efficacy end point was the proportion of patients with undetectable HCV RNA at 12 weeks post-treatment (SVR_{12}). Key secondary end points included the proportions of patients with HCV RNA undetectable at week 4, week 12, the end of treatment, and post-treatment week 24 (SVR_{24}).

Written informed consent was obtained from all patients. The study was approved by institutional review boards at each site and was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice Guidelines, and local regulatory requirements.

Patients

Eligible patients were men and women aged 20–75 years with HCV genotype 1 infection ⩾6 months and HCV RNA ⩾10^5 IU/ml. Women of childbearing potential were using adequate contraception. Patients were excluded if they had evidence of liver cirrhosis within 24 months of screening by laparoscopy, imaging studies, or liver biopsy; a history of hepatocellular carcinoma, other chronic liver disease, variceal bleeding, hepatic encephalopathy, or ascites requiring diuretics or paracentesis; co-infection with hepatitis B virus or HIV; other clinically significant medical conditions; exposure to any investigational drug or placebo within 4 weeks, or any previous exposure to NS5A or NS3 protease inhibitors.

Exclusionary laboratory findings included alanine aminotransferase (ALT) >5× upper limit of normal (ULN), total bilirubin ⩾2 mg/dl, direct bilirubin >1.5× ULN, international normalized ratio (INR) ⩾1.7, albumin ⩽3.5 g/dl, hemoglobin <9.0 g/dl, white blood cells <1500/mm^3, absolute neutrophils <750/mm^3, platelets <50,000/mm^3, and creatinine >1.8× ULN. Prohibited concomitant medications included CYP3A4 inducers or moderate/strong CYP3A4 inhibitors, non-study medications with anti-HCV activity, prescription or herbal products not prescribed for treatment of a specific condition, proton pump inhibitors, and erythropoiesis-stimulating agents. Prescribed H2 receptor antagonists were administered ⩾2 h after and ⩾10 h prior to daclatasvir; other acid modifying agents were administered ⩾2 h prior and ⩾2 h after daclatasvir.

Study drug dosing

Patients received 24 weeks of treatment with daclatasvir 60 mg once daily (two 30 mg tablets), combined with asunaprevir 200 mg twice daily, with 24 weeks of post-treatment follow-up. In the sentinel cohort of null responders, asunaprevir was initially administered as three 200 mg tablets twice daily (600 mg BID), subsequently reduced to 200 mg BID during treatment following reports from another study of greater and more frequent aminotransferase elevations with the higher dose [14].

Patients with HCV RNA <15 IU/ml on or after week 4 continued treatment to week 24; patients discontinued treatment if HCV RNA decreased <2 $\log_{10}$ IU/ml from baseline on or after week 2. Patients with viral breakthrough on or after week 2, or quantifiable HCV RNA (⩾15 IU/ml) on or after week 4, either discontinued treatment or weight-based PegIFN-α/RBV was added (null responders only), for up to 48 additional weeks, at the discretion of the investigator based on anticipated tolerability. Viral breakthrough was defined as confirmed ⩾1 $\log_{10}$ IU/ml increase from nadir of HCV RNA, or HCV RNA ⩾15 IU/ml after confirmed undetectable. Post-treatment relapse was defined as confirmed HCV RNA ⩾15 IU/ml during follow-up in patients with undetectable HCV RNA at the end of treatment.

Safety and efficacy assessments

HCV RNA, physical examinations, adverse events, laboratory parameters, and concomitant medications were assessed at screening, study days 1 (baseline), weeks 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24, and post-treatment weeks 4, 8, 12, and 24. Twelve-lead electrocardiograms were recorded at all visits except weeks 3 and 6.

Serum HCV RNA levels were determined at a central laboratory using the Roche COBAS® TaqMan® HCV Auto assay, (Roche Diagnostics KK, Tokyo, Japan), lower limit of quantitation 15 IU/ml. HCV genotype and subtype and *IL28B* genotype (rs12979860) were determined by PCR amplification and sequencing. Baseline liver fibrosis was assessed by serum blood markers (APRI; AST and Platelet Ratio Index) [15]. HCV resistance-associated polymorphisms were analyzed in stored baseline samples from all patients and post-failure samples from patients with viral breakthrough or post-treatment relapse. Polymorphisms were analyzed by PCR amplification and population sequencing of the HCV NS3 protease and NS5A domains.

Statistical analysis

Categorical variables were summarized using counts and percents; continuous variables were summarized with univariate statistics.

Table 1. Baseline demographic and disease characteristics.

Parameter		Null responders n = 21	Ineligible/intolerant n = 22
Age, median yr (range)		61 (31-70)	68 (47-75)
Male, n (%)		8 (38.1)	6 (27.3)
HCV genotype 1b, n (%)		21 (100)	22 (100)
IL28B genotype, n (%)			
(rs12979860)	CT	18 (85.7)	6 (27.3)
	CC	3 (14.3)	16 (72.7)
HCV RNA, mean $\log_{10}$ IU/ml (SD)		6.8 (0.47)	6.6 (0.64)
ALT, mean U/L (SD)		57.9 (24.86)	45.7 (25.79)
APRI score			
	Score >2, n (%)	3 (14.3)	1 (4.5)
	Median (range)	0.96 (0.24-3.41)	0.57 (0.40-2.79)
PegIFN-α/RBV ineligible, n (%)		n.a.	18 (81.8)
PegIFN-α/RBV intolerant, n (%)		n.a.	4 (18.2)

n.a., Not available.

Results

Patient characteristics and disposition

Forty-nine patients were screened of which six failed to meet entry criteria; 21 null responders and 22 ineligible/intolerant patients were enrolled and treated (Table 1). The enrolled population was generally older (median 62 years), consistent with HCV epidemiology in Japan, and primarily female (67%); all patients were Japanese. No patient had prior exposure to HCV DAAs. Although any HCV genotype 1 subtype was permitted, all enrolled patients had genotype 1b infection, reflecting the high proportion of this subtype in Japan [16]. Null responders were primarily *IL28B* genotype CT (rs12979860) as expected [17]; ineligible/intolerant patients were primarily genotype CC, consistent with the distribution of *IL28B* genotypes in Japan [18]. Eighteen ineligible/intolerant patients were treatment-naïve and considered ineligible for PegIFN-α/RBV due to anticipated difficulty in completing therapy due to advanced age (≥70 years) (seven patients), cytopenia (two), depression (two), hypertension (one), or other reasons (six), consistent with common clinical practice in Japan. Four patients had prior PegIFN-α/RBV intolerance due to cytopenia (two patients), depression (one), or other reasons (one). Baseline HCV RNA and ALT levels were similar across patient groups. Although patients with cirrhosis by imaging criteria were excluded, four enrolled patients had APRI scores >2 at baseline, indicating probable cirrhosis [15].

Thirty-six of 43 enrolled patients completed 24 weeks of therapy (Fig. 1). Two null responders discontinued study medication due to hyperbilirubinemia (week 2) and aminotransferase elevation (week 12), respectively. One null responder achieved very low HCV RNA (50 IU/ml) at week 4; however, stringent protocol-defined rules required discontinuation from DAA-only therapy and addition of PegIFN-α/RBV to the dual DAA regimen at week 6. Study drugs were discontinued in four ineligible/intolerant patients due to aminotransferase elevation (week 16), viral breakthrough (week 16), or patient request (weeks 8 and 16); all four patients remained on study for assessment of SVR.

Virologic response

High rates of virologic response were seen at all time points in both study populations (Table 2). Overall, 77% of patients achieved SVR_{12} and SVR_{24}. HCV RNA was undetectable in more ineligible/intolerant patients than null responders at week 4, suggesting a more rapid initial antiviral effect, but HCV RNA was undetectable in similar proportions of both populations at week 12 and the end of treatment. Rates of SVR_{24} were higher in null responders (91%) than in ineligible/intolerant patients (64%) due to virologic failures in the latter group (3 breakthroughs and 4 relapses). Assessment of virologic response by *IL28B* genotype (rs12979860) showed slightly greater responses at weeks 2, 3, and 4 in patients with genotype CC; however, similar proportions of patients with genotypes CC and CT achieved SVR_{24} (Fig. 2). All four patients with possible cirrhosis based on APRI score achieved SVR_{24}.

HCV RNA declined rapidly after initiation of therapy in all patients (Fig. 3). Mean reductions of HCV RNA from baseline at week 4 were 5.6 and 5.4 $\log_{10}$ IU/ml in null responders and ineligible/intolerant patients, respectively; HCV RNA was undetectable by week 8 in all patients on therapy. In the ineligible/intolerant group, initial virologic response in the four intolerant patients was similar to that of the cohort overall; three of these patients subsequently achieved SVR_{24} and one relapsed. The null responder who discontinued at week 2 with hyperbilirubinemia had low-level HCV RNA at discontinuation and undetectable HCV RNA at all post-treatment assessments. The null responder who added PegIFN-α/RBV at week 6 received 46 weeks of quadruple therapy and HCV RNA remained undetectable 24 weeks post-treatment. Among the four ineligible/intolerant patients who discontinued study drugs before week 24, HCV RNA was undetectable at discontinuation (weeks 8 or 16) in three patients and remained undetectable in the two patients who completed post-treatment follow-up.

Viral breakthrough and relapse

No null responders experienced virologic breakthrough or relapse (Table 2). Three ineligible/intolerant patients experienced viral breakthrough at weeks 10 or 16 after ≥4 weeks with undetectable

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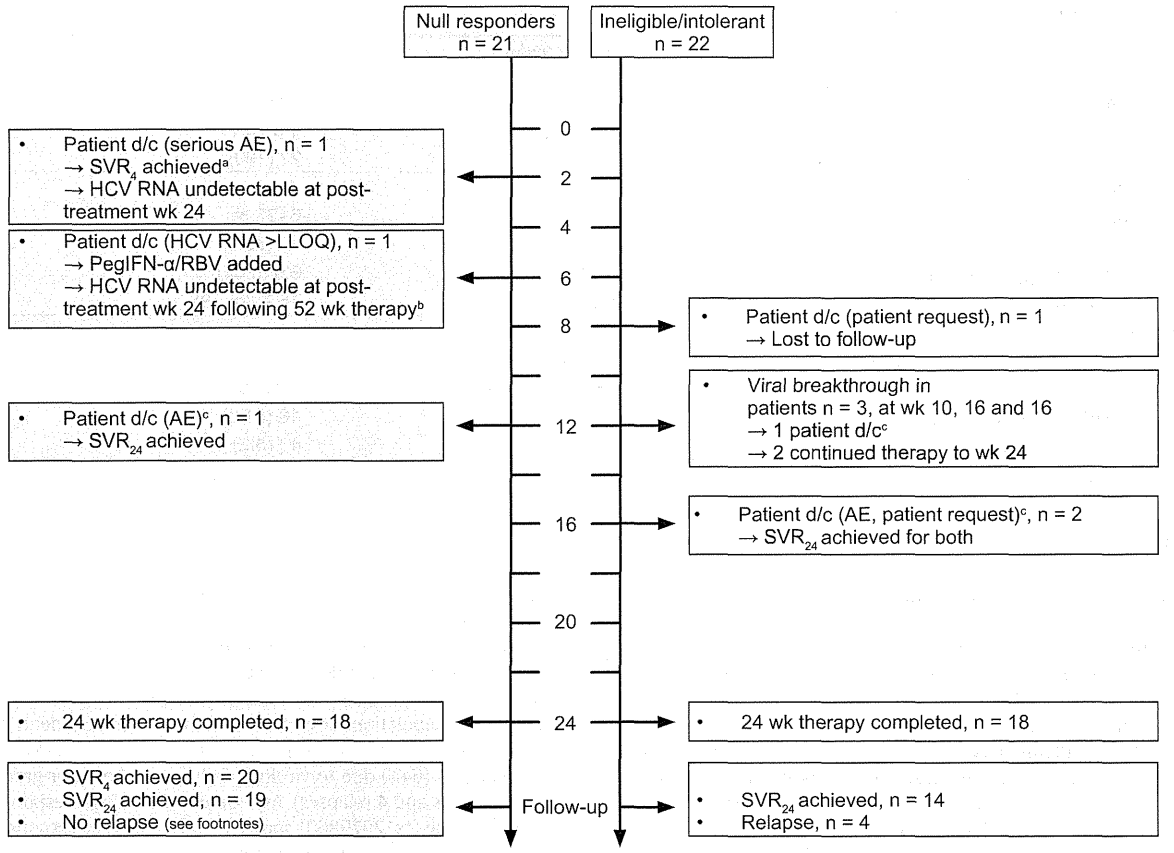

Fig. 1. Patient disposition. Patient flow through treatment and follow-up is shown. d/c, Discontinuation of study medication; SVR_4, SVR_{12} and SVR_{24}, sustained virologic response 4, 12 or 24 weeks post-treatment. [a]On-study follow-up continued to post-treatment week 4; HCV RNA remained undetectable at post-treatment week 24 after study discontinuation, reported as failure for SVR_{24} per statistical protocol requirements; [b]HCV RNA was undetectable at post-treatment week 24 after study discontinuation due to addition of PegIFN-α/RBV, reported as failure for SVR per statistical protocol requirements; [c]on-study follow-up to assess SVR continued after discontinuation of study drugs.

Table 2. Virologic outcomes.

n (%)	Null responders, n = 21	Ineligible/intolerant, n = 22
HCV undetectable		
Wk 4 (RVR)	11 (52.3)	19 (86.4)
Wk 12 (cEVR)	19 (90.5)	20 (90.9)
End of treatment	19 (90.5)	19 (86.4)
SVR_4	20 (95.2)[1]	15 (68.2)[2]
SVR_{12}	19 (90.5)[1]	14 (63.6)[2]
SVR_{24}	19 (90.5)[1]	14 (63.6)[2]
Viral breakthrough	0	3 (13.6)
Post-treatment relapse	0	4 (18.2)

Intention to treat (missing = failure) analysis. End of treatment is week 24 or last on-treatment visit for patients who discontinued early.
RVR, rapid virologic response; cEVR, complete early virologic response; SVR_4, SVR_{12}, and SVR_{24}, sustained virologic response 4, 12 or 24 weeks post-treatment.
[1]Two patients discontinued from the study before completion of follow-up. One patient received added PegIFN-α/RBV per protocol criteria and is counted as failure for SVR_4, SVR_{12}, and SVR_{24} for DAA only therapy; one patient had missing HCV RNA data for follow-up weeks 12 and 24 and is counted as failure for SVR_{12} and SVR_{24} per statistical protocol.
[2]One patient was lost to follow-up for assessment of SVR_{12} and SVR_{24}.

serum HCV RNA, and four patients relapsed at post-treatment week 4 (three patients) or 12 (one patient) after ⩾18 weeks with undetectable HCV RNA. All three patients with viral breakthrough were *IL28B* genotype CT (rs12979860), compared with 6/22 ineligible/intolerant patients overall. Three patients who relapsed were *IL28B* genotype CC; one was genotype CT.

Resistance-associated polymorphisms in NS5A and/or NS3 protease were found pretreatment in 33/43 patients overall, most of whom achieved SVR. Daclatasvir and asunaprevir resistance-associated variants were detected post-failure in all seven patients with virologic failure (Table 3). The NS5A-Y93H variant pre-existed in 10/43 study patients, of which five (50%) experienced virologic failure and five (50%) achieved SVR. NS5A-L31 and NS3-D168 substitutions emerged in all failures, but were not detected pretreatment except for NS5A-L31M in one patient.

In general, patients with virologic failure had concurrent asunaprevir and daclatasvir trough concentrations below median values, but within the expected range (Fig. 4). Notably, most patients with trough concentrations below median values achieved SVR. There were no strong associations between virologic failure and pretreatment parameters that included gender, age, baseline HCV RNA level, *IL28B* genotype, reason for PegIFN-

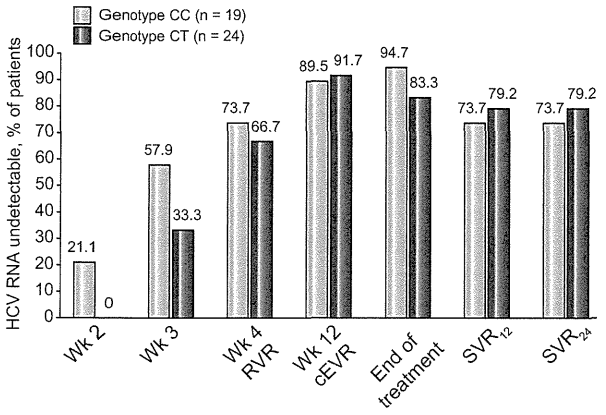

Fig. 2. Outcomes by *IL28B* genotype. Virologic outcomes at milestone time points are shown for the overall population by *IL28B* (rs12979860) genotype. End of treatment is week 24 or the last on-treatment visit for patients who discontinued early. RVR, rapid virologic response; cEVR, complete early virologic response; SVR_{12} and SVR_{24}, sustained virologic response 12 or 24 weeks post-treatment.

α/RBV ineligibility, and fibrosis stage. Adherence to treatment, assessed by pill counts at study visits, was high in six of the seven patients with virologic failure.

Safety

The most frequently reported adverse events were generally mild headache, nasopharyngitis, aminotransferase elevations, and diarrhea (Table 4). The most frequent grade 3 or 4 laboratory abnormalities were serum aminotransferase elevations. There were six serious adverse events in five patients, including grade 2/3 pyrexia (three patients), grade 2 exacerbation of hypochondriasis, and grade 2 gastroenteritis (unrelated to study drugs) with grade 4 hyperbilirubinemia (described in detail previously)

Table 3. Resistance-associated polymorphisms in patients with virologic failure.

	Patient		NS5A				NS3	
			L31	Q54	P58	Y93	Q80	D168
Viral breakthrough	1	Baseline	L/M			Y/H		
		Post-VBT	M		A	H		A
	2	Baseline		Y		Y/H	L	
		Post-VBT	M	Y		H		V
	3	Baseline		Y		H		
		Post-VBT	M	Y		H		V
Post-treatment relapse	4	Baseline			P/S	Y/H		
		Post-relapse	M			H		A
	5	Baseline			L			
		Post-relapse	M		L	H		V/D
	6	Baseline						
		Post-relapse	V			H		V
	7	Baseline				H		
		Post-relapse	V/M			H		V

[7]. All three pyrexia events resolved after 4–10 days with continued study treatment; the hypochondriasis persisted for approximately six months and resolved after completion of study treatment. In the patient who discontinued with hyperbilirubinemia, bilirubin normalized four weeks post-treatment [7]. Serum aminotransferases normalized by four weeks post-treatment in the two patients who discontinued for elevations.

Discussion

High rates of SVR_{24} were achieved after 24 weeks of dual oral DAA therapy in null responders and PegIFN-α/RBV ineligible or

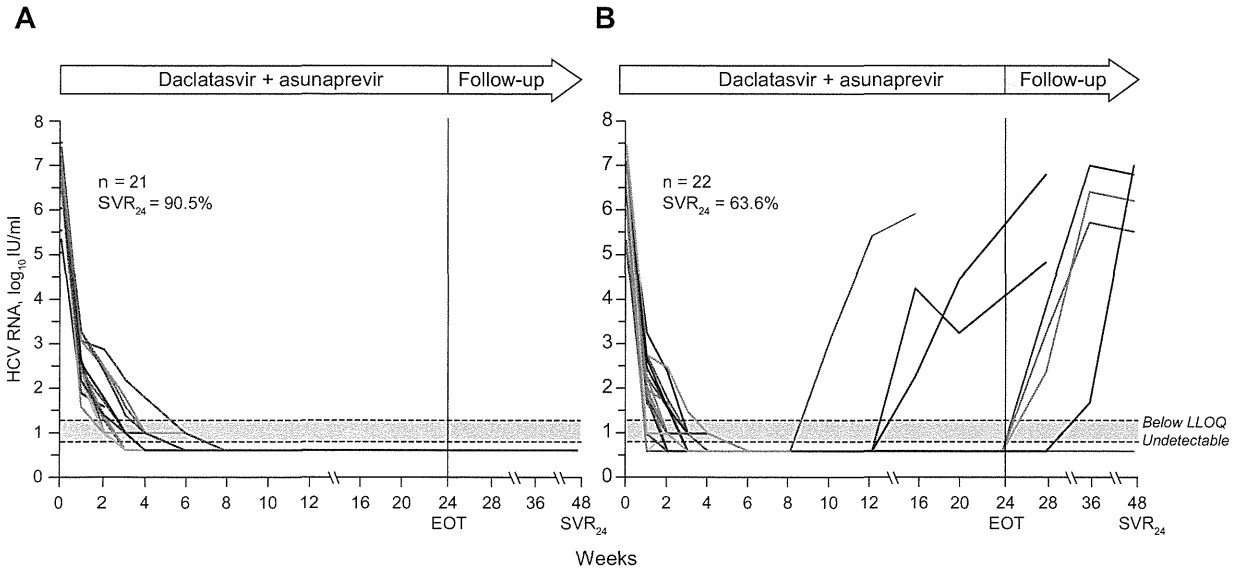

Fig. 3. HCV RNA levels, individual patients. Serum HCV RNA levels over time are shown for each patient. (A) Null responders; (B) ineligible/intolerant patients. EOT, end of treatment; SVR_{24}, sustained virologic response 24 weeks post-treatment; LLOQ, lower limit of quantitation = 15 IU/ml.

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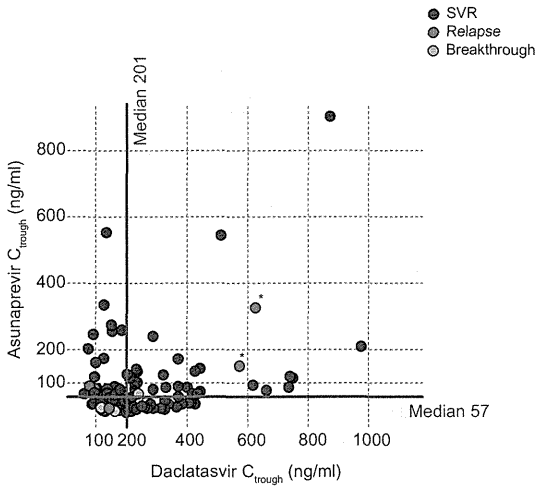

Fig. 4. Daclatasvir and asunaprevir trough plasma concentrations. Available trough plasma concentrations of asunaprevir and daclatasvir for individual patients are plotted and color-coded according to each patient's virologic outcome. Multiple determinations are shown for some patients. *Indicates values from a single patient with documented non-compliance.

Table 4. Most frequent adverse events and laboratory abnormalities.

Event, n (%)		Null responders (n = 21)	Ineligible/ intolerant (n = 22)
Adverse events occurring in ≥3 patients in either group	Headache	8 (38)	6 (27)
	Nasopharyngitis	6 (29)	8 (36)
	ALT increase	6 (29)	6 (27)
	Diarrhea	9 (43)	2 (9)
	AST increase	6 (29)	4 (18)
	Pyrexia	3 (14)	5 (23)
	Eosinophilia	1 (5)	4 (18)
	Abdominal discomfort	3 (14)	2 (9)
	Malaise	2 (10)	3 (14)
	Constipation	2 (10)	3 (14)
	Back pain	3 (14)	1 (5)
	Decreased appetite	0	3 (14)
Grade 3 or 4 lab abnormalities	ALT	2 (10)	2 (9)
	AST	1 (5)	2 (9)
	Lymphocytes	2 (10)	1 (5)
	Phosphorus	1 (5)	1 (5)
	Bilirubin, total	1 (5)	0
	Leukocytes	1 (5)	0

intolerant patients, representing two populations that are particularly difficult to treat due to limited therapeutic options. SVR rates were comparable at post-treatment weeks 4, 12, and 24; only one relapse occurred more than 4 weeks post-treatment. The 90.5% SVR rate in null responders is substantially higher than the generally poor response to PegIFN-α/RBV retreatment and the 37% SVR rate reported for genotype 1b null responders treated with PegIFN-α/RBV and telaprevir [4,19]. Therefore, therapy of this population with daclatasvir and asunaprevir appeared to overcome the poor interferon responsiveness, which may be less relevant to the efficacy of this DAA-only regimen. The SVR rate of 63.6% in ineligible/intolerant patients, although lower than results in null responders, is the first demonstration of a potentially effective treatment for these patients who currently have no therapeutic options. High SVR rates in both populations were achieved despite multiple adverse predictors of response to PegIFN-α/RBV therapy, including older age, high viral load, and a high proportion of *IL28B* genotype CT in the null responders.

Detectable HCV RNA was cleared rapidly; viral suppression was greater at all time points compared to reported results with PegIFN-α/RBV combined with telaprevir or TMC435 in genotype 1 null responders [4,20]. The slightly greater early viral suppression in ineligible/intolerant patients may reflect the higher frequency of *IL28B* CC genotype in this group. In the overall population, early virologic response was greater in patients with CC genotype, although this difference disappeared by week 12. Potentially, CC genotype may increase early viral suppression by increasing responsiveness to endogenous interferons that are released as a result of the rapid antiviral activity of the dual DAA therapy, allowing reversal of HCV-induced immunosuppression [21].

These results in patients with HCV genotype 1b differ from those reported for genotype 1a. In a similar study of US/European null responders, 2/9 patients with genotype 1a achieved SVR with daclatasvir + asunaprevir dual therapy, compared with 10/10 patients with genotype 1a who received quadruple therapy combining daclatasvir and asunaprevir with PegIFN-α/RBV [8]. This difference suggests that viral genotype can influence responses to DAA regimens, and outcomes can be optimized by individualized therapy that considers viral genotype.

The two populations included in this study represent substantial numbers of patients worldwide. Approximately 10% of HCV genotype 1-infected patients receiving PegIFN-α/RBV have a null response [22]. The cumulative prevalence of PegIFN-α/RBV null responders and the frequent failure of retreatment with current regimens, together suggest that a large population of null responders is awaiting improved therapies. The population of PegIFN-α/RBV ineligible or intolerant patients has not been extensively studied but may be substantial. In the IDEAL study, 23.2% of the 4469 patients screened were considered ineligible for PegIFN-α/RBV therapy; of these, 30.3% had hematologic or psychiatric conditions that may not preclude DAA-only regimens [23]. In registration trials, 9.7–14% of patients receiving PegIFN-α/RBV discontinued study treatment due to intolerance [24,25]. Moreover, these clinical trial data are likely to underestimate the true size of the ineligible and intolerant populations in community practice.

Virologic failures occurred relatively late in therapy after extended periods with undetectable HCV RNA. All seven patients with virologic failure had emergent NS5A and NS3 mutations that together confer high-level resistance to both daclatasvir and asunaprevir *in vitro* [11,12]. Pretreatment, NS5A-Y93H was detected in five of the seven patients with virologic failure and in five additional patients who achieved SVR, suggesting that pre-existing Y93H is loosely associated with virologic failure but is not an absolute predictor. Pharmacokinetics may also have contributed; nearly all patients with virologic failure had trough plasma concentrations of daclatasvir and asunaprevir below their respective median values. However, SVR was achieved by most patients with trough drug levels below the median, and by

several patients who discontinued study treatment after 2–16 weeks. Thus, the relationship of drug exposure to virologic outcome remains uncertain; further study is needed to define on-treatment predictors of outcome and the optimal duration of therapy.

Current data do not fully explain the observed differences in rates of virologic failure and SVR, between the two study populations. *IL28B* genotype was the primary difference between the two populations pretreatment. All three breakthroughs occurred in ineligible/intolerant patients with the unfavorable *IL28B* CT genotype; however, null responders had no breakthroughs, despite a much higher frequency of this genotype. Differing proportions of patients with concurrent pre-existing resistance-associated polymorphisms and low plasma drug concentrations may have contributed to differing rates of virologic failure between the two populations. Analysis of baseline parameters failed to identify other factors that may have influenced outcomes. However, these analyses were limited by the relatively small study population and may have been confounded by unreported non-adherence or baseline parameters not quantified absolutely, such as the stage of liver fibrosis. This issue requires further study in larger populations to confirm the apparent difference in outcomes and to identify factors predictive of virologic failure.

The adverse event profile of the study regimen was generally more favorable than that typically observed with PegIFN-α/RBV-containing regimens [26]. There were no significant hematologic or psychiatric abnormalities; the most common adverse events were non-specific in nature and generally mild to moderate in intensity. Mild diarrhea was experienced by 26% of study patients, consistent with previous studies of asunaprevir and other drugs of this class [4,6,14]. The four observed grade 3/4 ALT elevations resolved with continued therapy or after discontinuation and were not associated with significant clinical events. A role for study drugs in the reported serious adverse events cannot be ruled out except for gastroenteritis; however, four of the six events resolved spontaneously with continued treatment. The case of hyperbilirubinemia with gastroenteritis was complicated by multiple confounding factors, and the contribution of study drugs is uncertain [7].

In conclusion, dual oral therapy with daclatasvir and asunaprevir elicited rapid clearance of detectable HCV RNA and achieved high rates of SVR in two difficult-to-treat patient populations. These results confirm initial findings that HCV genotype 1b infections can be cured with daclatasvir combined with asunaprevir, without PegIFN-α/RBV [7,8]. Thus, this regimen has potential to offer effective treatment to null responders who have previously shown little or no response to PegIFN-α/RBV, and to PegIFN-α/RBV ineligible/intolerant patients who have no current treatment options. Further research will assess the benefits of this and other DAA combinations in larger and more diverse patient populations, but the promise of all oral and well-tolerated HCV therapy is on the horizon.

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Conflicts of interest

K Chayama has received research grants and consulting fees from Bristol-Myers Squibb, Dainippon Sumitomo Pharma, Mitsubishi Tanabe Pharma, Daiichi Sankyo, Toray Industries, Otsuka Pharmaceutical Company, and GlaxoSmithKline KK. Hiroki Ishikawa, Hideaki Watanabe, Wenhua Hu, Timothy Eley, Fiona McPhee, and Eric Hughes are employees of Bristol-Myers Squibb. All other authors have no conflicts to report.

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Research Article

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Characterization of virologic escape in hepatitis C virus genotype 1b patients treated with the direct-acting antivirals daclatasvir and asunaprevir

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See Editorial, pages 643–645

Background & Aims: Daclatasvir and asunaprevir are NS5A and NS3 protease-targeted antivirals currently under development for treatment of chronic hepatitis C virus infection. Clinical data on baseline and on-treatment correlates of drug resistance and response to these agents are currently limited.

Methods: Hepatitis C virus genotype 1b Japanese patients (prior null responders to PegIFN-α/RBV [n = 21] or PegIFN-α/RBV ineligible or intolerant [n = 22]) were administered daclatasvir/asunaprevir for 24 weeks during a phase 2a open-label study. Genotypic and phenotypic analyses of NS3 and NS5A substitutions were performed at baseline, after virologic failure, and post-treatment through follow-up week 36.

Results: There were three viral breakthroughs and four relapsers. Baseline NS3 polymorphisms (T54S, Q80L, V170M) at amino acid positions previously associated with low-level resistance (<9-fold) to select NS3 protease inhibitors were detected in four null responders and three ineligibles, but were not associated with virologic failure. Baseline NS5A polymorphisms (L28M, L31M, Y93H) associated with daclatasvir resistance (<25-fold) were detected in five null responders and six ineligibles. All three viral breakthroughs and 2/4 relapsers carried a baseline NS5A-Y93H polymorphism. NS3 and NS5A resistance-associated variants were detected together (NS3-D168A/V, NS5A-L31M/V-Y93H) after virologic failure. Generally, daclatasvir-resistant substitutions persisted through 48 weeks post-treatment, whereas asunaprevir-resistant substitutions were no longer detectable. Overall, 5/10 patients with baseline NS5A-Y93H experienced virologic failure, while 5/10 achieved a sustained virologic response.

Conclusions: The potential association of a pre-existing NS5A-Y93H polymorphism with virologic failure on daclatasvir/asunaprevir combination treatment will be examined in larger studies. The persistence of treatment-emergent daclatasvir- and asunaprevir-resistant substitutions will require assessment in longer-term follow-up studies.

Keywords: Asunaprevir; Daclatasvir; Drug resistance; Direct-acting antivirals; Hepatitis C; Peginterferon-sparing.

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Abbreviations: DAA, direct-acting antiviral; HCV, hepatitis C virus; SVR, sustained virologic response; GT, genotype; PegIFN-α/RBV, peginterferon alfa and ribavirin; DCV, daclatasvir; ASV, asunaprevir; LLOQ, lower limit of quantitation; PCR, polymerase chain reaction; FU, follow-up; RAV, resistance-associated variant; BL, baseline; VBT, viral breakthrough; SD, standard deviation.

Introduction

The introduction of direct-acting antivirals (DAAs) targeting hepatitis C virus (HCV) NS3 protease activity has substantially increased sustained virologic response (SVR) in chronic HCV genotype 1 (GT1) infection. In combination with peginterferon-alfa and ribavirin (PegIFN-α/RBV), treatment with the recently approved protease inhibitors boceprevir or telaprevir results in SVR rates of around 70–75% in treatment-naive patients [1,2]. Despite these improvements, SVR rates vary by genotype and remain suboptimal in some patients, such as null responders to PegIFN-α/RBV [3], and patients for whom PegIFN-α/RBV is poorly tolerated or medically contraindicated. Furthermore, PegIFN-α/RBV is associated with frequent side effects [3], and the addition of these DAAs results in elevated rates of anemia and additional events such as dysgeusia (boceprevir), or rash, pruritus, and nausea (telaprevir) [4,5].

Daclatasvir (DCV) and asunaprevir (ASV) are currently undergoing clinical development for HCV infection. DCV (BMS-790052) is a first-in-class, highly selective NS5A replication complex inhibitor with picomolar potency and broad HCV genotypic coverage [6] that has demonstrated antiviral efficacy and good tolerability in combination with PegIFN-α/RBV [7]. ASV (BMS-650032) is a selective inhibitor of NS3 protease with antiviral activity *in vitro* against GT1 and GT4 [8]; it has also been shown to be

efficacious and generally well tolerated in combination with PegIFN-α/RBV [9]. Clinical interest is increasingly focusing on exploring DAA-only regimens without PegIFN-α/RBV, whose potential benefits might include better tolerability and compliance, and a reduced duration of therapy. One recent PegIFN-α/RBV-sparing study of DCV plus ASV (AI447017) has examined the efficacy and safety of this combination for 24 weeks in a small cohort of ten GT1b null responders, in whom an SVR rate of 90% was observed [10]. The study was then expanded to include an additional cohort of null responders and a group of patients ineligible to receive, or intolerant of, PegIFN-α/RBV [11].

As with other antiviral agents, the efficacy of DCV and ASV can be compromised by the development of drug resistance. *In vitro* data suggest that DCV and ASV should provide additive or synergistic activity that enhances the genetic barrier to resistance [8]. Here we characterize virologic escape observed on DCV plus ASV treatment in the expanded AI447017 study [11]; its associations with baseline characteristics, including *IL28B* genotype and HCV polymorphisms; and an assessment of on- and off-treatment genotypic changes in NS5A and NS3 protease and their phenotypic consequences.

Patients and methods

Study design and patients

This was an open-label, phase 2a study (AI447017; clinicaltrials.gov identifier NCT01051414) evaluating the antiviral activity and safety of DCV plus ASV in 43 patients with HCV GT1 infection. Patients comprised (a) 21 PegIFN-α/RBV null responders (<2 $\log_{10}$ decline in plasma HCV RNA after 12 weeks) and (b) 22 patients who discontinued previous PegIFN-α/RBV within 12 weeks for intolerance or were considered medically poor candidates for PegIFN-α/RBV for reasons such as advanced age, complications of depression, anemia, myelosuppression, diabetes, or cardiovascular or renal dysfunction. Patients enrolled in four cohorts; two each of null responders and ineligible/intolerant patients. The initial sentinel cohort of null responders has been described previously [10]. All enrolled patients were infected with GT1b.

Patients received DCV 60 mg once daily with ASV 200 mg twice daily for 24 weeks, with a further 48 weeks of post-treatment follow-up. ASV dosing in the expanded study was reduced from the 600 mg twice-daily administration used in the sentinel cohort, following reports of hepatic enzyme elevations at this dose, in another clinical study [12].

The full study design, including inclusion/exclusion criteria, and safety/efficacy endpoints, is described elsewhere [11]. Briefly, eligible patients were men and women aged 20–75 years with HCV GT1 infection ⩾6 months and HCV RNA ⩾10^5 IU/ml. Patients were excluded if they had evidence of liver cirrhosis within 24 months of screening; a history of hepatocellular carcinoma, other chronic liver disease, variceal bleeding, hepatic encephalopathy, or ascites requiring diuretics or paracentesis; co-infection with hepatitis B virus or HIV; or other clinically significant medical conditions.

Laboratory assessments

Plasma samples for resistance testing were collected at baseline and study weeks 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 and post-treatment weeks 4, 8, 12, 24, 36, and 48. HCV RNA was determined at a central laboratory using the Roche COBAS® TaqMan® HCV Auto assay (Roche Diagnostics KK, Tokyo, Japan) with a lower limit of quantitation (LLOQ) of 15 IU/ml. HCV genotype and subtype and *IL28B* genotype (rs12979860 single-nucleotide polymorphism) were determined by polymerase chain reaction (PCR) amplification and sequencing.

Genotypic and phenotypic analysis of clinical samples

Testing was performed on all baseline samples and on samples indicative of slow virologic response at week 1 or virologic failure with HCV RNA levels ⩾1000 IU/ml. Virologic failure, for the purpose of the study, was defined as an HCV RNA level (a) ⩾LLOQ at week 4 (futility rule), (b) >1 $\log_{10}$ IU/ml above nadir or ⩾LLOQ after confirmed undetectable (virologic breakthrough), or (c) ⩾LLOQ at any follow-up visit after being undetectable at the end of treatment (relapse).

Population sequencing of PCR amplicons was performed using methods described elsewhere [13–15]. For clonal analysis, amplicons were cloned into the TOPO vector and transformed into TOP10 *Escherichia coli* using a commercially available kit (TOPO® TA-cloning® kit, Invitrogen, Carlsbad, CA) according to manufacturer's instructions, with ⩾20 individual colonies expanded and sequenced for each analysis.

Phenotypic analyses of resistance-associated substitutions were performed by employing *in vitro* HCV replicon systems according to previously published methodologies [15–17].

Results

Viral response to DCV and ASV

Overall, plasma HCV RNA was undetectable in 77% (33/43) of patients at 24 weeks post-treatment. SVR was higher among the null responders than in the PegIFN-α/RBV ineligible population; all viral breakthroughs (n = 3) and relapses (n = 4) occurred in the ineligible/intolerant subpopulation. Three patients discontinued the study without subsequent SVR or virologic failure (Tables 1 and 2) [11].

Null responders

Virologic response.

Rapid and similar decreases in plasma HCV RNA levels were observed among patients who initiated treatment with ASV 600 mg (Fig. 1A) or ASV 200 mg (Fig. 1B). Mean reduction in HCV RNA at week 1 was comparable for both groups (−4.4 *vs.* −4.3 $\log_{10}$ IU/ml, respectively). Of the patients still receiving treatment (P-6 discontinued at day 16 due to an AE), all but one patient (P-13) had HCV RNA <15 IU/ml at week 4 and 52% had undetectable HCV RNA at this time.

Baseline analysis. Baseline *IL28B* genotype and naturally occurring polymorphisms associated with ASV or DCV resistance (resistance-associated variants [RAVs]) are shown in Table 1. As anticipated for this prior null responder population, the majority (18/21) were non-CC *IL28B*. The NS5A polymorphism Y93H (24-fold DCV resistance [13]) was observed in three patients. Other polymorphisms conferring minimal (two- to three-fold) DCV resistance were detected in two patients (NS5A-L28M-R30Q and NS5A-L31M). Polymorphisms associated with minimal to low-level resistance to select NS3 protease inhibitors (one patient, NS3-T54S-Q80L; one patient, NS3-Q80L-V170I/M; two patients, NS3-Q80L) [4,5,18] were also observed.

Baseline polymorphisms and *IL28B* genotype did not appear to influence either the week 1 response or SVR rate (Fig. 2A). Five patients had RNA levels ⩾1000 IU/ml after 1 week, of whom one (P-21) had significantly slower initial HCV RNA declines when compared with mean reductions (standard deviation [SD]) in HCV RNA for null responders on the study (−3.4 *vs.* −4.35 ± 0.49 $\log_{10}$ IU/ml). This patient had a CC *IL28B* genotype and an NS5A polymorphism (Q54L; no fold-change in DCV resistance). The other four patients had polymorphisms that have been associated with DCV and NS3 protease inhibitor low-level resistance [13,19]—specifically NS5A-Q54H/Q-Q62Q/E-Y93H/Y with NS3-T54S-Q80L (P-1, no fold-change to DCV/ASV), NS3-Q80L-V170I/M (P-2, no fold-change to ASV), NS5A-R30Q