せ，肝内鉄過剰を誘導すること[37]，HCV蛋白複合体を発現するトランスジェニックマウスにおいて，鉄過剰はミトコンドリア障害と肝細胞癌発生のリスクとなること[38]，HCV蛋白質がROSを介して体内の鉄代謝を調節するヘプシジンの転写を抑制し，肝内の鉄過剰を引き起こすことが報告されている[39]。

2　インスリン抵抗性

肝硬変では，末梢組織でのインスリン感受性の低下の代償として膵β細胞から過剰のインスリンが分泌され，高インスリン血症が引き起こされる。疫学的にはHCV感染による肝硬変の方が，その他の原因による肝硬変より糖尿病の合併率が高いとされる[40]。動物実験においてもHCVコア蛋白はインスリンシグナル伝達経路に作用し，インスリン抵抗性を惹起する[41, 42]。また，インスリン抵抗性は肝脂肪化や肝線維化と密接に関連し[43, 44]，IFN治療効果にも影響している[45]。

Ⅶ　HCV治療効果を規定する因子

IFN治療効果を規定するウイルス側因子として，ウイルス量や遺伝子型だけでなく，コア領域の

表1　インターフェロン（IFN）の治療効果に影響する因子

効きやすい群	ウイルス側因子	効きにくい群
少ない	HCV RNA量	多い
2a/2b（2型）	ウイルス型	1b（1型）
野生型	コアアミノ酸変異（aa70，aa91）	変異型
変異数 ≧ 2	ISDR変異	変異数 ≦ 1
変異数 ≧ 6	IRRDR変異	変異数 ≦ 5
効きやすい群	宿主側因子，薬剤因子	効きにくい群
男性	性別	女性
軽度	線維化	進展
野生型	IL28B SNP	変異型
十分	薬剤投与量	不十分
なし	脂肪肝	あり
なし	インスリン抵抗性	あり

IFNの治療効果にはさまざまなウイルス因子や宿主側因子，薬剤因子などが関連しているとされる。

ISDR：interferon sensitivity determining region

IRRDR：interferon/ribavirin-resistance determining region

IL28B SNP：（single nucleotide polymorphisms of interleukin-28B）（著者作成）

70番目と91番目のアミノ酸変異[46]，NS5A中央部に存在するinterferon sensitivity determining region（ISDR）変異[47]，NS5AのISDR後方に存在するinterferon/ribavirin-resistance determining region（IRRDR）変異[48]が報告されている。さらに，宿主側因子として，19番染色体のIL28B遺伝子近傍に，IFN治療効果に強く関連する一塩基多型（single nucleotide polymorphism；SNP）が同定された[49～51]。その他，宿主側因子として女性，肝線維化，肝脂肪化[52]，インスリン抵抗性[45]など，さまざまな因子がIFN治療効果と関連している（表1）。一方，IFN freeの治療であるダクラタスビル＋アスナプレビル併用療法において，これまでIFN治療効果と関連のあった，ウイルス側因子や宿主側因子は治療効果と関連がなかったと報告されている[53]。DAAs製剤は薬剤耐性変異の問題があり，さらなるウイルス因子の解明が治療効果の向上に必要である[15]。

おわりに

HCVは一度感染すると高率に慢性化し，肝硬変，肝細胞癌の原因となるが，ウイルス蛋白質が宿主免疫応答や発癌などに関わっていることが示唆されている。今後DAAにより高いウイルス排除が期待されるが，線維化や発癌の機序など不明な点も多く，これらが解明されれば新規薬剤の開発につながり，予後改善に寄与すると考えられる。

（馬渡誠一・宇都浩文・井戸章雄）

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肝臓病学

Hepatology

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C型肝炎治療の転換期

2013年は，相次ぐDAA（direct anti-viral agents）の開発でC型肝炎の治療法が大きく転換していくことを予感させる1年となった。DAAはC型肝炎ウイルスの感染ライフサイクルの基礎的知見に基づいて設計され，ウイルスのNS（non-structural）蛋白を標的として抗ウイルス効果を発揮する薬剤である。現在DAAは，NS3を標的とするプロテアーゼ阻害薬，NS5Bを標的としたポリメラーゼ阻害薬，ウイルス粒子の形成を阻害するNS5A阻害薬の3種を中心に開発が進められている（図1）。

DAAはインターフェロン（IFN）と併用して抗ウイルス効果を高めるのみならず，複数のDAAを併用することで，IFNを用いることなくウイルス排除を目指す治療法としての開発も急速に進みつつある。第2世代のNS3プロテアーゼ阻害薬であるシメプレビルは2013年に世界に先駆けて我が国において保険適用となり，これを受けて日本肝臓学会編『C型肝炎治療ガイドライン』が改訂され，第2版となった。また，IFNを使わずに複数のDAAを併用することによるC型慢性肝炎治療の第Ⅱ相，第Ⅲ相試験のポジディブな結果も学会ならびに学術雑誌に発表され始めている。

C型慢性肝炎のみならず，2013年はB型慢性肝炎，肝細胞癌のガイドラインも公開・改訂された年となった。肝細胞癌に対する分子標的薬の第Ⅲ相治験において，ここ数年ポジティブな結果を出した薬剤はなく，ソラフェニブ以外の新規分子標的薬が使用可能となる見通しは立っていない。また，B型慢性肝炎に対する核酸アナログの新規承認も，ここ数年見られてはいない。しかし，この現状により既存の薬剤の実臨床での知見が蓄積され，

最も注目されるTOPICとその臨床的意義

TOPIC 1

シメプレビル導入による『C型肝炎治療ガイドライン(第2版)』の改訂

治療効果が高く，重篤な副作用が少ない第2世代プロテアーゼ阻害薬シメプレビルの国内第Ⅲ相試験の発表と保険適用を受けて，シメプレビルを併用したペグインターフェロン，リバビリン療法が1型高ウイルス量の抗ウイルス療法の標準的治療となった。

この1年間の主なTOPICS

1 シメプレビル導入による『C型肝炎治療ガイドライン(第2版)』の改訂
2 DAAによるIFNを使用しないC型肝炎治療の開発
3 『科学的根拠に基づく肝がん診療ガイドライン 2013年版』の発表
4 肝性腹水・浮腫に対するバソプレシンV_2受容体拮抗薬
5 『B型肝炎治療ガイドライン』の発表

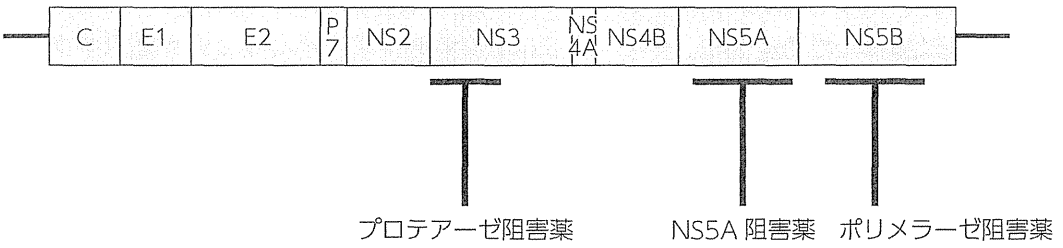


図1 HCVゲノムの構造とDAA

適応，治療効果，副作用などが明確になったことで，整理された形でガイドラインに反映されたと考えることもできる。

C型肝炎，B型肝炎，肝細胞癌の3疾患は，現在の肝疾患治療において最も治療必要度の高い疾患であり，その治療内容に関して熟知しておくことが望ましい。本稿では，2013年に新たに治療成績が発表された薬剤，適応となった薬剤や，新しい治療薬の登場によるガイドラインの変更点を中心として，5つのトピックスを選定し，それぞれについて概説する。

TOPIC 1 ▶シメプレビル導入による『C型肝炎治療ガイドライン』の改訂

難治例である1型高ウイルス量症例に対して，2011年に第1世代プロテアーゼ阻害薬であるテラプレビル（TPV：テラビック®）が保険適用となり，ペグインターフェロン（PEG-IFN）α2bおよびリバビリン（RBV）と併用することで治療成績は飛躍的に改善し，初回治療例では約70％の高いウイルス排除（sustained virological response；SVR）が可能になった。しかし，TPVは貧血，血小板減少をはじめとする血球減少，腎障害，高尿酸血症，消化器症状など，副作用が高度であり，またStevens-Johnson症候群，薬剤性過敏症症候群などの全身症状を伴う重篤な皮膚障害の発現のおそれがあることから，使用条件として皮膚科専門医と連携した肝臓専門医が治療を行うこととされ，使用できる施設が限られていた。

2013年6月の日本肝臓学会総会にて第2世代プロテアーゼ阻害薬であるシメプレビル（SMV：ソブリアード®）の国内第Ⅲ相試験の結果が発表され[1)~3)]，9月に承認された。SMV＋PEG-IFNα＋RBV3剤併用療法の国内臨床試験では，従来のRBV併用PEG-IFN療法と比較して，治療期間が24週に短縮されたにもかかわらず，初回治療例，前治療再燃例において約90％の高いSVR率が得られ，副作用も差を認めなかった。この結果に基づき，『C型肝炎診療ガイドライン（第2版）』[4)]ではSMVについての項が追加され，1型高ウイルス症例に対する推奨ならびに治療フローチャート（**図2・3**）が大幅に改訂された。1型高ウイルス症例では，初回治療，前治療再燃，前治療無効のいずれの条件においても，認容性が許せば，SMV＋PEG-IFNα＋RBV3剤併用療法が第一選択として推奨されることになった。

SMVはTPVのように治療施設の制限がないことから，より多くのC型慢性肝炎症例に対して抗ウイルス療法による恩恵がもたらされると期待される。一方で，SMVは世界に先駆けて日本で承認されたことから，治験段階で判明していなかった合併症が市販後に明らかになる可能性も危惧され，この点を考慮した慎重な対応が肝臓専門医に求められるであろう。

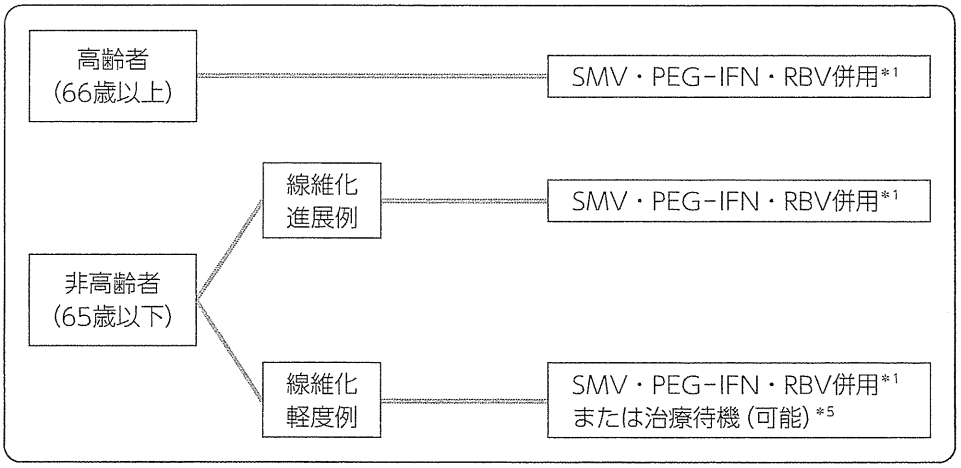
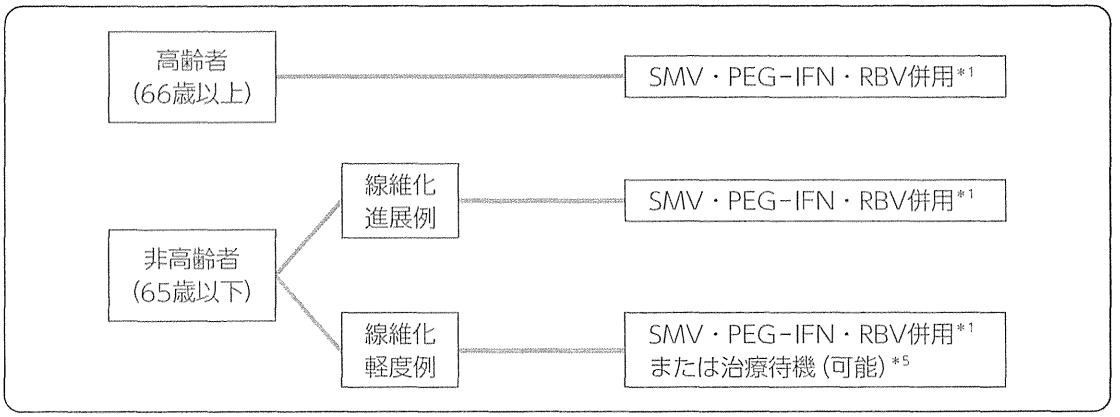


図2　C型慢性肝炎ゲノタイプ1型・高ウイルス量症例治療の原則（初回治療） *2*3*4

*1 TVR・PEG-IFN・RBV併用も使用可能（高齢者ではTVRを1500mg/日に減量して投与）。
*2 IL28B測定が可能であれば参考とする。
*3 前治療がPEG-IFN（IFN）単独治療の場合，あるいはPEG-IFN（IFN）・RBV併用療法施行例で前治療歴不明の場合は，初回治療の方針に従う。
*4 うつ症状合併ではIFN-β・RBV併用も考慮に入れる。
*5 ALT値異常例では肝庇護療法またはPEG-IFN（IFN）少量長期。

（文献[4)]より引用）

(1) 前治療再燃



(2) 前治療無効*4

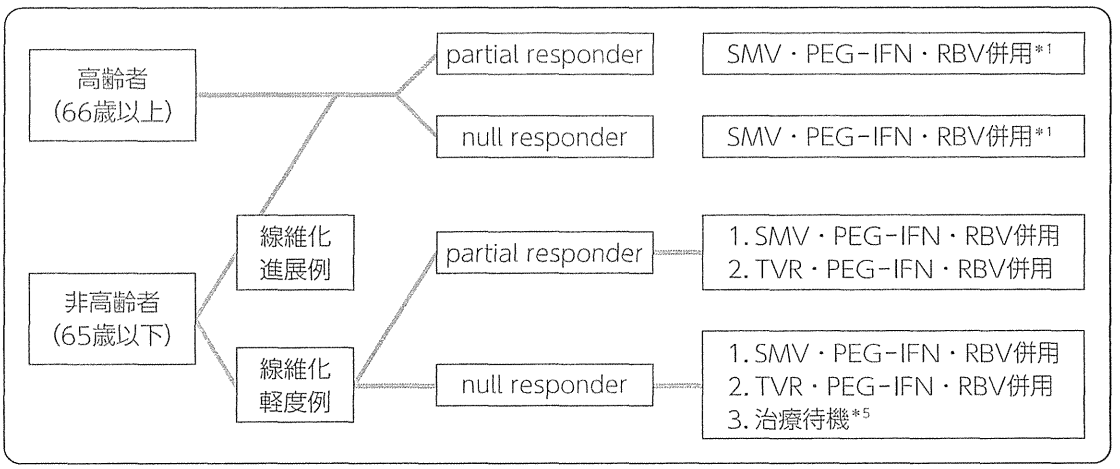


図3 C型慢性肝炎ゲノタイプ1型・高ウイルス量症例治療の原則(既治療) *2*3

*1 TVR・PEG-IFN・RBV併用も使用可能(高齢者ではTVRを1500mg/日に減量して投与)。
*2 既治療は,前治療にPEG-IFN(IFN)・RBV併用療法を施行していることを指す。
*3 うつ症状合併ではIFN-β・RBV併用も考慮に入れる。
*4 前治療でウイルス陰性化を認めなかった症例(無効例)で,12週のHCV RNA減少量が不明な場合はnull responderの方針に準じる。
*5 ALT値異常例では肝庇護療法またはPEG-IFN(IFN)少量長期。 (文献4)より引用)

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TOPIC 2 ▶ DAAによるIFNを使用しないC型肝炎治療の開発

難治性である1型高ウイルス量症例の治療としてIFNを使用せず,プロテアーゼ阻害薬,NS5A阻害薬,NS5B阻害薬など,経口のDAAの組み合わせによる抗ウイルス療法の臨床試験が進捗中であり,2013年には複数のポジティブな第Ⅱ相試験の結果が雑誌に掲載された。

日本人を対象としたNS5A阻害薬daclatasvirとプロテアーゼ阻害薬asunaprevirの併用療法の

第Ⅱa相試験の結果が発表[1]された。前治療無効あるいはIFN療法不適格・不耐容の慢性肝炎43例を対象とし，24週の内服により無効例90.5%，不適格・不耐容例でも63.6%のSVR率が得られた。副作用として下痢，鼻咽頭炎，頭痛，ALT/AST上昇などを認め，高ビリルビン血症により1例，ALT/AST上昇により2例が治療中止となった。第Ⅲ相試験の結果も2013年のAASLD（米国肝臓学会議）で発表され，無効例80.5%，不適格・不耐容例では87.4%と高いSVR率であった。現時点ではこのDAAの組み合わせが最も速く実地医療で使用可能になると予想されている。

さらに，海外で行われたNS5B阻害薬であるBMS-791325を加えた3剤併用療法の第Ⅱa相試験の結果も発表されている[2]。前治療歴のない肝硬変を除く慢性肝炎66例を対象とした検討であるが，3剤の12週または24週併用でSVR率は90%以上と，さらに向上がみられている。

またNS5B阻害薬sofosbuvirとNS5A阻害薬ledipasvirの併用療法の第Ⅱ相試験結果も発表された[3]。前治療歴のない肝硬変を除く慢性肝炎60例を対象にした検討では，RBVの有無にかかわらず8～12週間の内服により95～100%の高いSVR率が得られた。プロテアーゼ阻害薬を含むIFN治療で無効・再燃を来した慢性肝炎・肝硬変40例を対象にした場合でも12週間の内服で95～100%のSVRが達成されている。重篤な副作用は少なく認容性にも優れていることから，第Ⅲ相臨床試験での結果が待たれる。

プロテアーゼ阻害薬とNS5B阻害薬の併用療法に関しては，プロテアーゼ阻害薬faldaprevirとNS5B阻害薬deleobuvirの併用療法の第Ⅱb相試験[4]，NS5B阻害薬ABT-333とプロテアーゼ阻害薬ABT-450とritonavirの合剤（ABT-450/r），RBVの4剤併用療法の第Ⅱa相試験[5]の報告が見られた。前者では前治療歴のない症例を対象とし，RBVを含むプロトコールではゲノタイプ1bに限るとSVRは56～85%であった。後者では肝硬変を除く未治療または無効例の慢性肝炎50例を対象とし，12週の内服で初回治療例では93～95%，無効例では47%のSVR率が得られており，今後第Ⅲ相試験での結果が期待される。

2014年以降，様々なDAAの組み合わせによるIFNフリーの抗ウイルス療法の結果が発表され，承認，実地医療における使用への展開が期待される。現時点でC型肝炎症例に対しシメプレビルを併用したIFN療法で治療を開始するのか，IFNフリーの複数のDAAによる抗ウイルス療法の承認を待って治療するのか結論を出すのは難しい。現時点での抗ウイルス療法の適応，肝炎の沈静化や肝癌発癌抑制効果の必要性，DAAによる耐性ウイルスの出現リスクなどを個々の症例に応じて十分に考慮した上で決定することが求められる。

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TOPIC 3 ▶『科学的根拠に基づく肝がん診療ガイドライン2013年版』の発表

第2版である『科学的根拠に基づく肝がん診療ガイドライン2009年版』の作成・発表以降，肝細胞癌の診断領域では肝細胞特異性造影剤であるGd-EOB-DTPA（gadolinium ethoxybenzyl diethylenetriaminepentacetic acid）造影剤（プリモビスト®），超音波造影剤ソナゾイド®が本格的に臨床の場に導入された。また，治療領域では分子標的薬であるソラフェニブ（ネクサバール®）が肝細胞癌に対して適応拡大となり，これらの薬剤に対するエビデンスが集積してきた。2013年版[1]ではこの点を踏まえ，CQ（clinical question）や推奨の改訂が行われている。特にGd-EOB-DT-

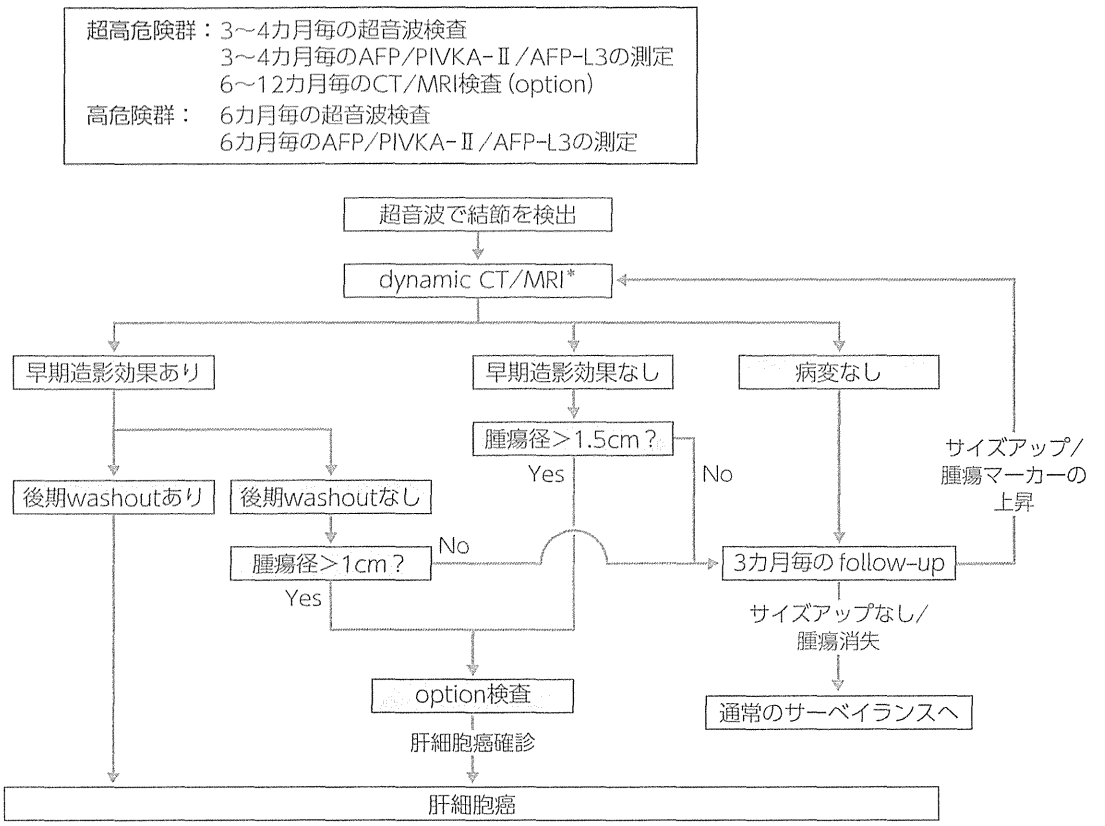

図4　肝細胞癌診断アルゴリズム

*超音波の描出不良等を理由に超音波で結節の描出がなくてもCT/MRIを撮影する場合もある。腎機能低下例，造影剤アレルギー例などでは造影超音波検査も考慮される。（文献[1]より引用）

PA造影MRIは，多血性である典型的肝細胞癌の前段階である乏血性の早期肝細胞癌結節の描出能を劇的に向上させており，またMRI，CT装置自体の進歩により小結節の段階で検出される症例も少なくない。この現状に照らし合わせ，診断アルゴリズム（図4）[1]では，乏血性結節に対してoption検査（Gd-EOB-DTPA造影MRI，造影超音波検査，血管造影下CT，腫瘍生検）で精査を行う腫瘍径が2cmから1.5cmに引き下げられた。

治療アルゴリズムは2009年版では2007年6月までのエビデンスを基に作成されたため，ソラフェニブの生存期間改善効果を示したRCT[2)3)]は盛り込まれていなかったが，2013年版はこのエビデンスを盛り込んだ治療アルゴリズム（図5）[1]に改訂された。ソラフェニブは肝障害度Aで，腫瘍数が4個以上，遠隔転移・脈管侵襲を有する肝細胞癌の治療選択肢の1つとして位置づけられた。

また，体幹部低位放射線療法，粒子線療法といった線量集中性の高い治療技術が出現してきたことを踏まえ，放射線療法の項目も更新されている。他の局所療法の適応困難な肝細胞癌，例えば門脈腫瘍栓や下大静脈腫瘍栓，巨大腫瘍などが治療対象候補として挙げられている。治療アルゴリズムには放射線療法は掲載される段階ではないものの，今後治療成績のエビデンスが集積することで，肝癌治療における位置づけが次第に明らかになっていくことが期待される。

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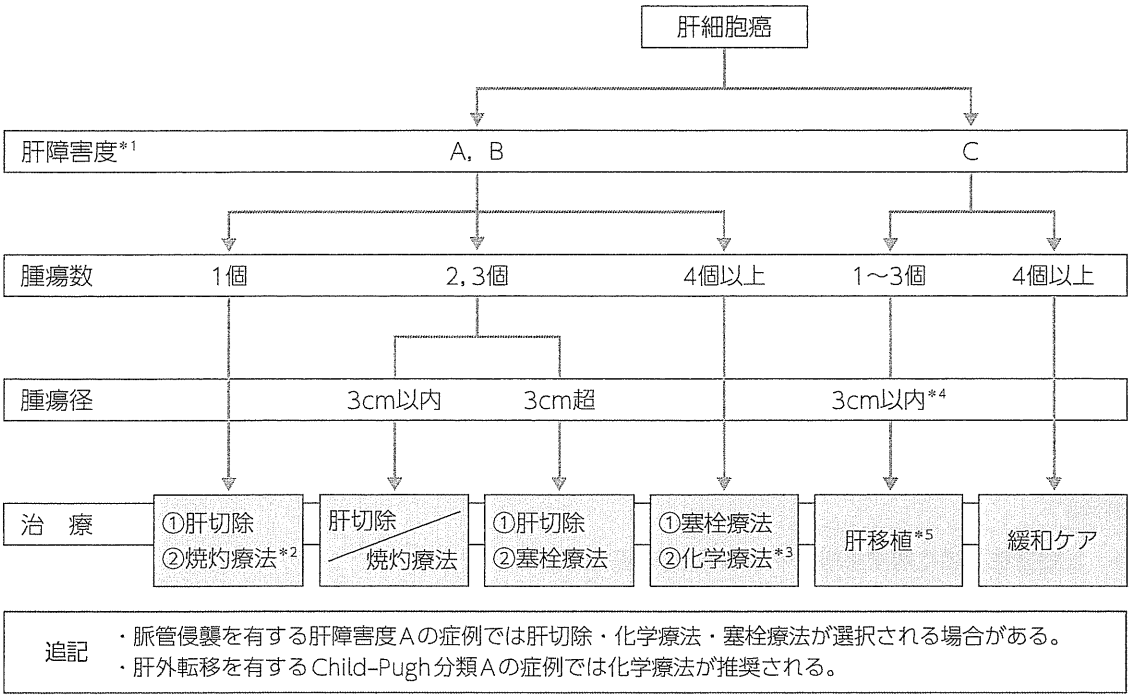

図5　エビデンスに基づく肝細胞癌治療アルゴリズム

*1 内科的治療を考慮する時はChild-Pugh分類の使用も可。
*2 腫瘍径3cm以内では選択可。
*3 経口投与や肝動注などがある。
*4 腫瘍が1個では5cm以内。
*5 患者年齢は65歳以下。

（文献[1]より引用）

TOPIC 4 ▶肝性腹水・浮腫に対するバソプレシンV_2受容体拮抗薬

非代償性肝硬変では腹水や浮腫を来すことがあるが，塩分制限に加え，抗アルドステロン性利尿薬であるスピロノラクトン，ループ利尿薬であるフロセミドなどの利尿薬が治療の主体となっている。難治例では利尿薬の増量に加え，BCAA (branched-chain amino acids) 顆粒製剤，アルブミン製剤の併用も行うが，肝予備能低下例では治療効果は限局的となることも多い。

肝硬変では血漿バソプレシン濃度の上昇が認められ，水排泄障害を来し，稀釈性低Na血症となる病態が知られている。選択的バソプレシンV_2受容体拮抗薬は，遠位尿細管のバソプレシンV_2受容体に選択的に結合し，腎集合管における水吸収を阻害するが，選択的バソプレシンV_2受容体拮抗薬であるtolvaptan（サムスカ®）は肝性腹水において強力な水利尿効果が得られ，低Na血症の改善[1]のみならず腹水を減少させる効果もあることが報告[2]された。

この結果を受け，tolvaptanは2013年9月に「肝硬変における体液貯留」に関しても適応拡大となった。通常は抗アルドステロン利尿薬やループ利尿薬と併用して使用する。重篤な肝障害の合併や，肝予備能の低下による薬物血中濃度の上昇などを来す可能性もあり，少量から開始し，肝機能検査を頻回に行い，安全性を確認した上で使用していくことが望ましい。

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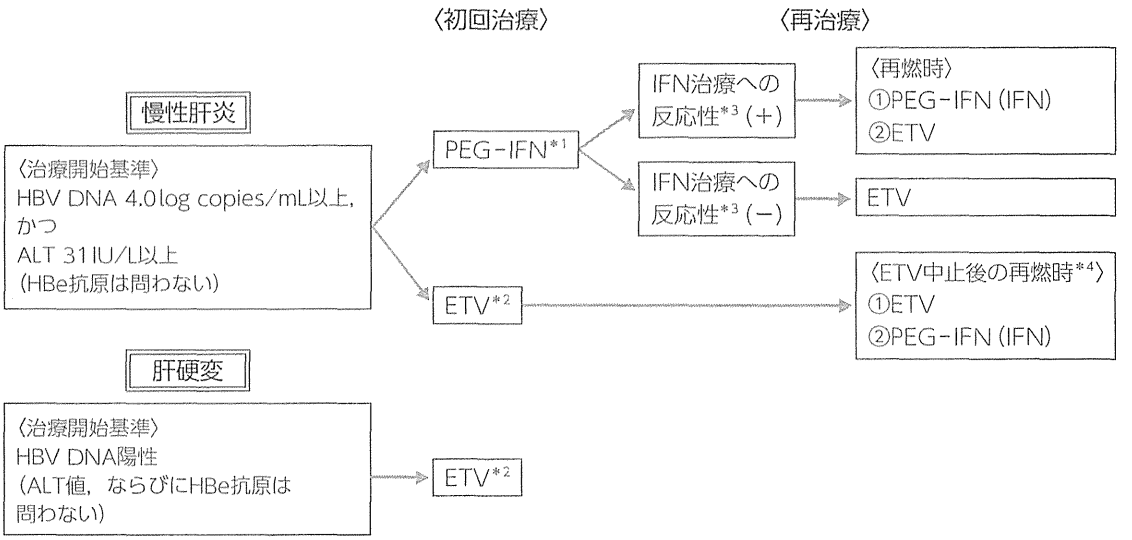

図6　B型慢性肝炎に対する抗ウイルス療法の基本方針

*1 HBe抗原セロコンバージョン率やHBV DNA陰性化率が必ずしも高くはないこと，個々の症例における治療前の効果予測が困難であること，予想される副反応などを十分に説明すること。
*2 挙児希望がないことを確認した上で，長期継続投与が必要なこと，耐性変異のリスクがあることを十分に説明すること。
*3 ALT正常化，HBV DNA量低下 (HBs抗原量低下)，さらにHBe抗原陽性例ではHBe抗原陰性化を参考とし，治療終了後24～48週時点で判定する。
*4 ETV中止後再燃時の再治療基準；HBV DNA 5.8log copies/mL以上，またはALT 80IU/L以上。

(TOPIC 5の文献[1]より引用)

TOPIC 5 ▶『B型肝炎治療ガイドライン』の発表

実臨床における肝炎治療の標準化と充実を図るため，C型肝炎に引き続き，2013年に日本肝臓学会より『B型肝炎治療ガイドライン』も発表[1]された。慢性肝炎を中心とするB型肝炎ウイルス感染者の治療目標が明記されており，治療対象や治療薬の選択について詳細に記載されている。加えて急性肝炎，劇症肝炎ならびにB型肝炎ウイルスの再活性化への対応も掲載されている。

慢性肝炎の治療の長期目標として，肝炎の沈静化やHBV-DNAの陰性化だけではなく，HBs抗原の消失をめざすべきと掲げている。核酸アナログ製剤であるエンテカビル(バラクルード®)は副作用が少なくウイルス低下効果は高いことから多くの症例に使用されているが，長期内服が必要であることやHBs抗原陰性化率が低いことなど解決すべき点も残されている。従来のIFN療法では年齢や線維化が治療効果規定因子であったが，2011年にPEG-IFNが承認され治療効果が高まったことから，年齢，線維化と治療効果との関連が乏しくなった。そのため肝硬変へ達していない慢性肝炎状態では積極的にPEG-IFNを用いた抗ウイルス療法を検討していく治療方針(図6)[1]が提示されている。近年増加しているゲノタイプAではPEG-IFNの治療効果が高いため特に推奨されている。

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Original article

A randomized trial of daclatasvir with peginterferon alfa-2b and ribavirin for HCV genotype 1 infection

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Background: Daclatasvir-containing regimens have the potential to address limitations of current regimens combining peginterferon alfa and ribavirin with first-generation protease inhibitors for treatment of chronic HCV genotype 1 infection.

Methods: In this randomized, double-blind study, 27 Japanese treatment-naive patients received once-daily daclatasvir 10 mg or 60 mg or placebo, each combined with peginterferon alfa-2b/ribavirin; 18 prior null (n=9) or partial (n=9) responders received the same daclatasvir-containing regimens without a placebo arm. Daclatasvir recipients with protocol-defined response (HCV RNA<15 IU/ml at week 4, undetectable at week 12) were treated for 24 weeks; those without protocol-defined response and placebo recipients continued treatment to week 48.

Results: Sustained virological response 24 weeks post-treatment (SVR_{24}) was achieved by 66.7%, 90.0% and 62.5% of treatment-naive patients in the daclatasvir 10 mg, 60 mg and placebo groups, respectively. Prior non-responders had more frequent virological failure; 22.2% and 33.3% of daclatasvir 10 mg and 60 mg recipients, respectively, achieved SVR_{24}. Adverse events were similar across groups and were typical of peginterferon alfa-2b/ribavirin. Pyrexia, headache, alopecia, decreased appetite and malaise were the most common adverse events; two daclatasvir recipients discontinued due to adverse events.

Conclusions: Daclatasvir 60 mg combined with peginterferon alfa-2b and ribavirin achieved a high rate of SVR_{24} in treatment-naive patients with HCV genotype 1 infection, with tolerability similar to that of peginterferon alfa-2b/ribavirin alone. However, regimens with greater antiviral potency are needed for prior non-responders.

Introduction

The advent of direct-acting antivirals (DAAs) marks a significant advance in the treatment of chronic HCV infection. Regimens containing the non-structural protein 3 (NS3) protease inhibitors telaprevir and boceprevir, as well as multiple investigational agents, have demonstrated significantly increased rates of sustained virological response (SVR) compared with peginterferon alfa/ribavirin (alfa/RBV) alone [1]. SVR rates of 68–75% have been achieved with alfa/RBV combined with boceprevir or telaprevir in treatment-naive patients with HCV genotype 1 infection [2,3], although efficacy is lower in patients who previously failed alfa/RBV therapy [4,5].

Initial experience with regimens containing telaprevir or boceprevir has, however, identified several limiting characteristics that emphasize the need for continued development of alternative DAAs. Telaprevir- and boceprevir-containing regimens have complicated

dosing schedules and are associated with frequent adverse events such as rash and anaemia [2–5]. In addition, telaprevir and boceprevir have frequent drug–drug interactions with other medications, potentially limiting utility for patients with concomitant medical conditions, and their efficacy has been established only in HCV genotype 1 infection [6,7]. Compounds from alternative mechanistic classes offer potential for greater and broader antiviral potency to include HCV genotypes 2, 3 and 4, as well as improved tolerability, more convenient dosing schedules, reduced risk of drug resistance and reduced potential for drug–drug interactions [8]. Agents of new mechanistic classes with non-overlapping resistance profiles allow development of DAA combinations that may be effective for patients resistant to current NS3 protease inhibitors.

Daclatasvir (BMS-790052) is a first-in-class non-structural protein 5A (NS5A) replication complex inhibitor with picomolar potency and activity against HCV genotypes 1 to 6 [9]. Daclatasvir has a human pharmacokinetic profile consistent with once-daily dosing and has shown potent antiviral activity in Phase I clinical studies [10]. Daclatasvir has been well tolerated in combination with alfa/RBV in clinical studies, with an adverse event profile similar to that of alfa/RBV alone [11,12]. In a previous Phase II study in patients with chronic HCV genotype 1 infection, 83% of patients achieved SVR following a 48-week regimen of daclatasvir 60 mg once daily combined with standard peginterferon alfa-2a/RBV (alfa-2a/RBV) [12].

We assessed the efficacy and safety of daclatasvir in combination with peginterferon alfa-2b (alfa-2b; PegIntron) and RBV in Japanese patients with chronic HCV genotype 1 infection, including HCV treatment-naive patients and patients who previously failed to achieve SVR following alfa/RBV therapy (null and partial responders). The response-guided design assessed whether a shorter 24-week course of therapy was sufficient for daclatasvir recipients who achieve early virological milestones.

Methods

Study design

In this five-arm, double-blind, randomized Phase IIa study (ClinicalTrials.gov identifier NCT01016912), enrolled patients were either naive to treatment with interferons and DAAs active against HCV or had prior non-response to alfa/RBV, defined as failing to achieve a 2 $\log_{10}$ reduction of HCV RNA at week 12 (null responder) or having never achieved undetectable serum HCV RNA after at least 12 weeks of therapy (partial responder) [13]. Treatment-naive patients were randomly assigned (1:1:1) to receive once-daily oral daclatasvir 10 or 60 mg or placebo, each in combination with subcutaneous alfa 60 to 150 μg once weekly and twice-daily oral RBV 600 to 1,000 mg/day. Alfa and RBV doses were determined by body weight in accordance with Japanese label recommendations. Prior non-responders were randomly assigned (1:1) to receive the same daclatasvir-containing regimens but there was no placebo arm for this group because of the known very poor responsiveness of these populations to retreatment with alfa/RBV.

Patients were treated for 24 or 48 weeks. Randomized treatment assignment was double-blind and placebo-controlled for daclatasvir in the first 24 weeks. The study was unblinded at week 24 and conducted subsequently as open label. Patients receiving daclatasvir-containing regimens stopped treatment at week 24 if they achieved a protocol-defined response (PDR), defined as HCV RNA below the assay limit of quantitation (<15 IU/ml) at week 4 and undetectable at week 12; daclatasvir recipients without PDR continued treatment to week 48. All placebo recipients were treated for 48 weeks.

The study protocol and informed consent were approved by an independent ethics committee and institutional review boards at each participating site prior to study initiation. The study was designed and conducted by the sponsor (Bristol-Myers Squibb) in collaboration with the principal investigators, and was conducted in compliance with the Declaration of Helsinki, local regulatory requirements and Good Clinical Practice, as defined by the International Conference on Harmonisation.

Patients

Patients were enrolled in six academic clinical research centers in Japan between December 2009 and February 2010. Enrolled patients were men and women, aged 20–70 years, with chronic HCV genotype 1 infection and HCV RNA≥10^5 IU/ml. Women of childbearing potential must have been using effective methods of contraception due to the contraindication of RBV for women who are pregnant or who may become pregnant.

Patients were excluded if they had evidence of liver cirrhosis by laparoscopy, imaging studies or liver biopsy within 24 months prior to screening, history or evidence of hepatocellular carcinoma or other chronic liver disease; coinfection with HBV or HIV; haemoglobinopathies or other diagnoses associated with increased risk of anaemia; or other medical, psychiatric or social reason rendering the individual inappropriate for study participation. Patients were also excluded if they had been exposed to any investigational drug or placebo within 4 weeks prior to dosing, or had any previous exposure to new or investigational HCV therapeutic agents. Exclusionary laboratory parameters included alanine aminotransferase ≥5× upper limit of normal,

total bilirubin ≥2 mg/dl, international normalized ratio ≥1.7, albumin ≤3.5 g/dl, haemoglobin <12 g/dl, white blood cell count $<4\times10^9$/l, absolute neutrophil count $<1.5\times10^9$/l, platelet count $<100\times10^9$/l or creatinine clearance <50 ml/min. Prohibited medications included proton pump inhibitors and moderate or strong inducers or inhibitors of CYP3A4.

Safety and efficacy assessments

Assessments that included HCV RNA, physical examination, adverse events, laboratory tests, pregnancy test and concomitant medications, were conducted at screening, study day 1 (baseline), weeks 1, 2, 4, 6, 8 and 12, then every 4 weeks until the end of therapy, and post-treatment weeks 4, 12 and 24. Twelve-lead electrocardiograms were recorded at screening and on-treatment weeks 4, 12, 24 and 48. Serum HCV RNA was determined centrally using the COBAS TaqMan HCV Auto assay (Roche Diagnostics KK, Tokyo, Japan), lower limit of quantitation =15 IU/ml. HCV genotype was determined at a central laboratory by PCR amplification and sequencing. *IL28B* genotype was determined by PCR amplification and sequencing of the rs12979860 single-nucleotide polymorphism.

Efficacy end points

The primary efficacy end point was the proportion of patients with HCV RNA undetectable at weeks 4 and 12 on-treatment (extended rapid virological response [eRVR]). Secondary end points included the proportions of patients with undetectable HCV RNA at week 4 (rapid virological response [RVR]), week 12 (complete early virological response [cEVR]) and post-treatment weeks 12 (SVR_{12}) and 24 (SVR_{24}).

HCV resistance testing was performed on stored specimens by PCR amplification and population sequencing of the HCV NS5A domain. Resistance testing was performed on all samples at baseline and on samples indicative of virological failure when HCV RNA was ≥1,000 IU/ml. Virological failure was defined as either <2 $\log_{10}$ HCV RNA decrease from baseline at week 12, virological rebound (HCV RNA detectable on treatment after previously undetectable or ≥1 $\log_{10}$ increase in HCV RNA from nadir) or detectable HCV RNA at end of therapy or post-treatment in patients with undetectable HCV RNA at end of therapy (relapse).

Sample size and statistical analysis

With the target sample size of eight patients per treatment group, a safety event occurring at an incident rate of 19% with 80% probability could be detected. Randomization was conducted by the sponsor at a central randomization centre. Patients were randomly allocated to treatment groups; investigators received treatment kit assignments by fax from the randomization centre for eligible screened patients. Categorical variables were summarized using counts and percentages; continuous variables were summarized with univariate statistics. CIs were two-sided with an 80% confidence level. CIs for binary end points were exact binomial, whereas the CIs for continuous end points were based on the normal distribution. All statistical analyses were conducted using SAS/STAT Version 8.02 (SAS Institute, Cary, NC, USA).

Results

Patient characteristics and disposition

A total of 51 patients were screened; 6 were excluded due to abnormal thyroid function, history of cholecystectomy, ventricular arrhythmia or white blood cell count $<4\times10^9$/l (3 patients), respectively. Twenty-seven treatment-naive patients and 18 prior non-responders met study criteria and were randomized and treated (Table 1). All patients were Japanese; other than an imbalance in gender distribution and older age in non-responders, baseline characteristics were similar across treatment groups. Although the study permitted any HCV genotype 1 subtype, all enrolled patients had genotype 1b, reflecting the high proportion of this subtype in Japan [14]. The non-responder group included nine null responders and nine partial responders, with similar distributions in the two treatment arms. Prior non-responders were primarily (16/18 patients) *IL28B* genotypes CT or TT as expected for this population; 18 of the 27 treatment-naive patients were genotype CC, consistent with the overall distribution of *IL28B* genotypes in Japan [15,16]. However, there was an imbalance of *IL28B* genotypes (CC versus CT/TT) among the three treatment-naive groups, with six, one and two patients with non-CC genotypes in the daclatasvir 10 mg, daclatasvir 60 mg and placebo groups, respectively.

The 24-week double-blind phase was completed by 38 of 45 patients. Two treatment-naive patients, one each from the daclatasvir 10 mg and 60 mg groups, discontinued due to neutropenia (week 12) and depression (week 20), respectively. Five patients discontinued due to lack of efficacy (viral breakthrough), including one treatment-naive patient (daclatasvir 10 mg group) and four non-responders (one in the daclatasvir 10 mg group and three in the daclatasvir 60 mg group).

Four patients (three in the daclatasvir 10 mg group and one in the daclatasvir 60 mg group) discontinued open-label treatment between weeks 36 and 45 due to lack of efficacy (viral breakthrough). Eleven patients had reduction of alfa-2b dose, including two, six and three patients in the placebo, daclatasvir 10 mg and daclatasvir 60 mg groups, respectively. Thirty-three patients had RBV dose reductions, including 7, 14

Table 1. Baseline demographic and disease characteristics

Baseline parameter	Treatment-naive			Non-responders	
	Placebo + alfa-2b/RBV (*n*=8)	DCV 10 mg + alfa-2b/RBV (*n*=9)	DCV 60 mg + alfa-2b/RBV (*n*=10)	DCV 10 mg + alfa-2b/RBV (*n*=9)	DCV 60 mg + alfa-2b/RBV (*n*=9)
Median age, years (range)	50 (42–66)	51 (21–68)	55 (36–66)	58 (48–67)	63 (42–70)
Male, *n* (%)	4 (50)	2 (22)	6 (60)	3 (33)	3 (33)
HCV genotype 1b, *n* (%)	8 (100)	9 (100)	10 (100)	9 (100)	9 (100)
Mean HCV RNA, $\log_{10}$ IU/ml (SD)	6.9 (0.54)	6.6 (0.44)	6.5 (0.81)	6.8 (0.54)	6.8 (0.57)
Response to prior alfa/RBV					
Null response, *n* (%)	N/A	N/A	N/A	4 (44)	5 (56)
Partial response, *n* (%)	N/A	N/A	N/A	5 (56)	4 (44)
IL28B genotype (rs12979860)					
CC, *n*	6	3	9	0	1
CT, *n*	2	6	1	7[a]	7
TT, *n*	0	0	0	1	1

[a]*IL28B* genotype not available for one non-responder recipient of daclatasvir (DCV) 10 mg. N/A, not available; alfa-2b/RBV, peginterferon alfa-2b/ribavirin.

and 12 patients in the placebo, daclatasvir 10 mg and daclatasvir 60 mg groups, respectively.

Virological response

In treatment-naive daclatasvir recipients, HCV RNA levels declined rapidly after initiation of therapy, with HCV RNA becoming undetectable by week 4 (RVR) in 77.8% and 80% of patients in the daclatasvir 10 mg and 60 mg groups, respectively, compared with none in the placebo group (Table 2). The primary efficacy end point, eRVR, was achieved by 66.7% and 80.0% of patients in the daclatasvir 10 mg and 60 mg groups, respectively, versus 0% in the placebo group.

PDR was achieved by 7 of 9 (77.8%) and 10 of 10 (100%) treatment-naive patients in the daclatasvir 10 mg and 60 mg groups, respectively; these patients completed treatment after 24 weeks. HCV RNA was undetectable at the end of therapy (week 24) in 10 of 10 (100%) daclatasvir 60 mg recipients with PDR and in 6 of 7 (85.7%) daclatasvir 10 mg recipients with PDR. SVR_{24} was achieved by 6 of 7 (85.7%) daclatasvir 10 mg recipients with PDR and by 9 of 10 (90.0%) daclatasvir 60 mg recipients with PDR. Overall, in the combined group of treatment-naive patients with PDR (24 weeks of therapy) or without PDR (48 weeks) SVR_{24} was achieved by 66.7% and 90.0% of patients receiving daclatasvir 10 mg and 60 mg, respectively, compared with five of eight (62.5%) treatment-naive placebo recipients after 48 weeks of therapy. Two treatment-naive daclatasvir 10 mg recipients failed to achieve PDR; neither achieved SVR following 48 weeks of therapy.

Viral suppression was less pronounced in prior non-responders. The primary efficacy end point, eRVR, was achieved by 55.6% and 22.2% of patients in the daclatasvir 10 mg and 60 mg groups, respectively; SVR_{24} was achieved by 22.2% and 33.3% of these patients. PDR was achieved by 55.6% and 33.3% in the daclatasvir 10 mg and 60 mg groups, respectively (Table 2). Although all eight non-responders with PDR had undetectable HCV RNA through the end of therapy (week 24), among these patients only two of five (40.0%) daclatasvir 10 mg recipients and two of three (66.7%) daclatasvir 60 mg recipients achieved SVR_{24}, with the remaining patients experiencing post-treatment relapse. PDR was not achieved by four of nine and six of nine non-responder recipients of daclatasvir 10 mg and 60 mg, respectively; at the end of post-treatment follow-up, none of four and one of six of these patients achieved SVR_{24}.

In treatment-naive patients, HCV RNA was undetectable at week 12 (cEVR) and post-treatment week 24 (SVR_{24}) in 100% of daclatasvir recipients with *IL28B* genotype CC (rs12979860; three of three and nine of nine daclatasvir 10 mg and 60 mg recipients, respectively). Response rates were lower in patients with *IL28B* genotype CT: SVR_{24} was achieved by three of six treatment-naive patients with genotype CT in the daclatasvir 10 mg group; the single daclatasvir 60 mg recipient who failed to achieve SVR_{24} was also genotype CT.

Virological failure

Virological failure of all types was less frequent in treatment-naive patients than in non-responders. Treatment-naive recipients of daclatasvir 60 mg had the lowest rate of virological failure and no on-treatment viral breakthrough. Breakthrough occurred in one treatment-naive patient receiving daclatasvir 10 mg and in one placebo recipient, and one daclatasvir 10 mg recipient had detectable HCV RNA at the end of treatment (Table 2). Four treatment-naive patients

Table 2. Virological outcomes

	Treatment-naive			Prior non-responders	
	Placebo (*n*=8)	DCV 10 mg (*n*=9)	DCV 60 mg (*n*=10)	DCV 10 mg (*n*=9)	DCV 60 mg (*n*=9)
All patients					
HCV RNA undetectable week 4 (RVR)	0/8 (0; 0.0, 25.0)	7/9 (77.8; 51.0, 93.9)	8/10 (80.0; 55.0, 94.5)	5/9 (55.6; 30.1, 79.0)	3/9 (33.3; 12.9, 59.9)
HCV RNA undetectable week 12 (cEVR)	5/8 (62.5; 34.5, 85.3)	7/9 (77.8; 51.0, 93.9)	10/10 (100; 79.4, 100.0)	5/9 (55.6; 30.1, 79.0)	5/9 (55.6; 30.1, 79.0)
HCV RNA undetectable weeks 4 and 12 (eRVR)	0/8 (0; 0.0, 25.0)	6/9 (66.7; 40.1, 87.1)	8/10 (80.0; 55.0, 94.5)	5/9 (55.6; 30.1, 79.0)	2/9 (22.2; 6.1, 49.0)
HCV RNA undetectable, EOT	7/8 (87.5; 59.4, 98.7)	7/9 (77.8; 51.0, 93.9)	10/10 (100; 79.4, 100.0)	5/9 (55.6; 30.1, 79.0)	5/9 (55.6; 30.1, 79.0)
SVR_{24}	5/8 (62.5; 34.5, 85.3)	6/9 (66.7; 40.1, 87.1)	9/10 (90.0; 66.3, 99.0)	2/9 (22.2; 6.1, 49.0)	3/9 (33.3; 12.9, 59.9)
Viral breakthrough[a]	1/8 (12.5)	1/9 (11.1)	0/10 (0)	4/9 (44.4)	4/9 (44.4)
Post-treatment relapse[a]	2/8 (25.0)	1/9 (11.1)	1/10 (10.0)	3/9 (33.3)	2/9 (22.2)
Patients with PDR					
HCV RNA<15 IU/ml at week 4, undetectable at week 12 (PDR)	0/8 (0; 0.0, 25.0)	7/9 (77.8; 51.0, 93.9)	10/10 (100; 79.4, 100.0)	5/9 (55.6; 30.1, 79.0)	3/9 (33.3; 12.9, 59.9)
HCV RNA undetectable, EOT[a]	-	6/7 (85.7)	10/10 (100)	5/5 (100)	3/3 (100)
SVR_{24}[a]	-	6/7 (85.7)	9/10 (90.0)	2/5 (40.0)	2/3 (66.7)
Post-treatment relapse[a]	-	1/7 (14.3)	1/10 (10.0)	3/5 (60.0)	1/3 (33.3)

Data are end point (*n*/total *n* [%; 80% CI]) unless otherwise indicated. [a]Data are end point (*n*/total *n* [%]). cEVR, complete early virological response; DCV, daclatasvir; EOT, end of treatment; eRVR, extended rapid virological response; PDR, protocol-defined response; RVR, rapid virological response; SVR_{24}, sustained virological response 24 weeks post-treatment.

relapsed post-treatment, including two placebo recipients and one in each daclatasvir group. In non-responders, four patients in each treatment group experienced viral breakthrough and five relapsed post-treatment (three receiving daclatasvir 10 mg, two receiving daclatasvir 60 mg; Table 2).

NS5A-L31M/V and/or NS5A-Y93H, which are the predominant genotype 1b NS5A polymorphisms associated with daclatasvir resistance, were detected at baseline in three of the seven daclatasvir recipients with virological failure [17]. NS5A-L31M/V–Y93H variants were detected post-failure in the four treatment-naive daclatasvir recipients with virological failure. Emerging NS5A variants were more variable in the 13 prior non-responders who failed treatment, and included L31I/M/V–Y93H, R30Q/A92K, ΔP32 and L31F–ΔP32. Most patients with virological failure had non-CC *IL28B* genotypes, including all 4 treatment-naive daclatasvir recipients, 1 of 3 treatment-naive placebo recipients and 11 of 13 non-responders (data missing for 1 patient).

Safety

The most frequent adverse events were pyrexia, headache, alopecia, decreased appetite and malaise (Table 3). There were no consistent differences in adverse events between groups receiving placebo or either dose of daclatasvir. Frequencies of grade 3 or 4 adverse events were comparable across treatment groups; the majority of events were cytopenias. There was one serious adverse event (gastroenteritis of moderate intensity) in a non-responder treated with daclatasvir 10 mg combined with alfa-2b/RBV; the event occurred during treatment week 24 and resolved within 8 days without treatment, coincident with the end of study therapy. There were no deaths.

Two adverse events led to discontinuation of study treatment in naive patients: neutropenia (daclatasvir 10 mg + alfa-2b/RBV) and depression (daclatasvir 60 mg + alfa-2b/RBV group); both events resolved post-treatment without intervention. There were no consistent differences in haematological or laboratory abnormalities between groups receiving placebo or daclatasvir (Table 4); most abnormalities were mild or moderate in intensity (grade 1 or 2).

Discussion

Clinical outcomes with current telaprevir- and boceprevir-containing regimens can be limited by frequent virological failure, poor tolerability, complicated dosing schedules and drug–drug interactions with other medications [2–5]. Our results and other clinical findings suggest that daclatasvir, in combination with alfa/RBV and/or other DAAs, may offer a viable alternative to regimens containing first-generation NS3 protease inhibitors [12,18,19].

Table 3. Adverse events occurring in >25% of patients in any treatment group

	Treatment-naive			Non-responders	
Grade 1–4 adverse event	Placebo (*n*=8)	DCV 10 mg (*n*=9)	DCV 60 mg (*n*=10)	DCV 10 mg (*n*=9)	DCV 60 mg (*n*=9)
Pyrexia	5	8	9	6	8
Headache	5	6	4	5	3
Alopecia	2	3	7	2	3
Decreased appetite	1	3	3	5	2
Malaise	2	7	2	2	1
Pruritus	3	3	2	1	5
Anaemia	5	2	2	4	0
Nasopharyngitis	2	5	3	0	2
Lymphopenia	1	2	3	3	2
Rash	3	2	5	0	1
Diarrhoea	0	3	4	3	1
Injection site pruritus	2	3	3	2	1
Fatigue	3	0	3	3	1
Neutropenia	1	4	2	2	0
Back pain	1	0	3	5	0
Stomatitis	1	3	3	1	1
Abdominal discomfort	1	2	3	1	1
Constipation	3	1	1	1	2
Nausea	3	1	1	2	0
Dysgeusia	0	0	3	3	0
Insomnia	1	2	3	0	0
Cheilitis	0	2	3	0	0
Arthralgia	3	2	0	0	0

Data are *n*. DCV, daclatasvir.

Table 4. On-treatment haematological and laboratory abnormalities

	Treatment-naive, placebo (*n*=8)		Treatment-naive, DCV 10 mg (*n*=9)		Treatment-naive, DCV 60 mg (*n*=10)		Non-responders, DCV 10 mg (*n*=9)		Non-responders, DCV 60 mg (*n*=9)	
Event	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4
Anaemia	5	1	5	0	6	1	6	3	3	0
Neutropenia	5	1	7	4	8	2	7	2	5	0
Leukopenia	5	0	7	1	5	0	5	2	4	0
Thrombocytopenia	3	0	4	0	5	0	1	0	5	0
Lymphopenia	2	1	3	2	4	3	3	3	4	2
Low albumin	2	0	3	0	3	0	5	0	3	0
Elevated ALT	0	0	1	1	0	0	0	0	1	0
Elevated AST	0	0	2	1	0	0	0	0	0	0
Elevated bilirubin	3	0	5	1	5	0	5	0	1	0
Elevated lipase	1	0	2	0	2	0	0	0	2	0

Data are *n*. ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCV, daclatasvir.

This study demonstrates that the combination of daclatasvir and alfa-2b/RBV provides more rapid virological response than alfa-2b/RBV alone. A high proportion of treatment-naive patients receiving daclatasvir and alfa-2b/RBV achieved PDR and qualified for 24 weeks of therapy, including all 10 patients receiving daclatasvir 60 mg. PDR was generally predictive of SVR_{24}: 15 of 17 treatment-naive daclatasvir recipients with PDR subsequently achieved SVR_{24}. In the control arm, 62.5% of patients achieved SVR_{24} following 48 weeks of therapy with alfa-2b/RBV. Rates of SVR_4, SVR_{12} and SVR_{24} correlated well at the 60 mg dose of daclatasvir in both treatment-naive patients and non-responders, suggesting that late relapses are infrequent with this regimen.

The overall SVR_{24} rate of 90% in treatment-naive patients receiving the daclatasvir 60 mg regimen compares favourably with SVR rates reported for telaprevir

and boceprevir in global registration studies, and was achieved with a shorter six-month therapeutic regimen [2,3]. This 90% response rate is similar to the 83% and 90% SVR_{24} rates achieved with regimens combining telaprevir with alfa-2b/RBV in generally similar Japanese populations of patients with HCV genotype 1b infection and *IL28B* genotype TT (rs8099917) or CC (rs12979860) [20,21]. Outcomes in treatment-naive patients in the present study are comparable to the 100% SVR_{24} rate (8/8 patients) achieved in a parallel study where a similar patient population received daclatasvir 60 mg combined with alfa-2a/RBV [18]. However, cross-study comparisons and small patient numbers do not support definitive conclusions concerning outcomes with alfa-2a versus alfa-2b. The 90% SVR_{24} rate achieved with the daclatasvir 60 mg regimen in treatment-naive patients is comparable to the 83% SVR_{24} rate achieved with 48 weeks of treatment with daclatasvir 10 or 60 mg + alfa-2a/RBV in US/European patients with predominantly HCV genotype 1a infection [12]. In a recent Phase IIb study with a response-guided design similar to that applied in this study, 87% of patients with genotype 1b infection achieved SVR_{12} after 24 or 48 weeks of therapy with daclatasvir 60 mg in combination with alfa-2a/RBV; however, SVR_{12} was achieved by a lower percentage of patients (58%) with genotype 1a [19]. For daclatasvir 10 mg recipients in the present study, the overall SVR_{24} rate was 66.7%. This lower rate, compared with results achieved with daclatasvir 60 mg, was attributable to a reduced early virological response and a higher rate of virological failure, and provides additional support for selection of the 60 mg dose of daclatasvir for further evaluation in Phase III studies.

Results suggest that *IL28B* genotype may influence outcomes with this regimen, although data are limited. All 12 treatment-naive daclatasvir recipients with CC genotype achieved SVR_{24}, compared with 3 of 7 (43%) patients with non-CC genotype. However, three of the four treatment-naive patients with non-CC genotypes who failed to achieve SVR received the lower 10 mg dose of daclatasvir, which may have been a factor in non-response. Further study is needed to determine the possible influence of *IL28B* genotype on outcomes with this regimen. Data from other studies in which DAAs were combined with alfa/RBV suggest that the magnitude of *IL28B* effect is generally reduced with more potent regimens [22]. Only one patient in the non-responder cohort had CC genotype, precluding assessment of *IL28B* effects in this population.

Because of higher rates of on-treatment and post-treatment virological failure, a lower proportion of patients with prior non-response to alfa/RBV achieved PDR and SVR_{24} compared with treatment-naive patients. SVR_{24} was achieved by 33.3% of prior non-responders receiving daclatasvir 60 mg, comparable to results achieved with telaprevir- or boceprevir-containing regimens after 48 weeks of therapy [4,5]. Results suggest that virological failure in this study was predicted primarily by host alfa/RBV responsiveness. The non-responder population in this study had previously shown poor response to alfa/RBV; 50% were prior null responders. All but one prior non-responder had *IL28B* non-CC genotypes, which may have contributed to their initial failure with alfa/RBV as well as to the high virological failure rate in the present study. Together, these results suggest that alternative regimens are needed for non-responders to address their interferon non-responsiveness. In this regard, two studies in prior null responders have evaluated regimens containing two DAAs with or without alfa/RBV. SVR rates exceeding 90% were achieved in genotype-1b-infected patients with a regimen combining daclatasvir with the NS3 protease inhibitor asunaprevir [23,24], and in genotype-1a-infected patients using a quadruple regimen of daclatasvir, asunaprevir and alfa/RBV [24].

Virological failure was infrequent in treatment-naive patients and occurred primarily in patients receiving daclatasvir 10 mg. The single failure in treatment-naive patients receiving daclatasvir 60 mg was post-treatment relapse in the only patient from this group with non-CC *IL28B* genotype. As expected, virological failure was more frequent in non-responders; failure was experienced by similar proportions of patients receiving the 10 mg and 60 mg doses of daclatasvir. Daclatasvir-resistant HCV variants were detected in all patients with virological failure, most frequently the combination of NS5A-L31V-Y93H which confers high-level daclatasvir resistance *in vitro* [17]. This resistance pattern is consistent with that observed in other clinical studies of daclatasvir [12,24].

Safety profiles of the study regimens were generally similar and comparable to that typically seen with alfa/RBV [25]. There was no marked difference in the patterns of adverse events or laboratory abnormalities between treatment groups, with no evidence suggesting that daclatasvir at either dose contributed significantly to overall regimen tolerability or safety. The observed safety profile of daclatasvir is consistent with results of previous studies of daclatasvir monotherapy [10], daclatasvir combined with alfa/RBV [11,12,19] and daclatasvir combined with other DAAs [23,24,26]. The single serious adverse event (gastroenteritis) was considered treatment-related by the investigator, but the relative contributions of daclatasvir and alfa-2b/RBV to the event cannot be assessed.

Limitations of this study include the relatively small sample size, which precludes quantitative comparisons of efficacy outcomes and definitive conclusions

regarding the possible contribution of daclatasvir to low-frequency safety signals. Only Japanese research sites were included; therefore, possible effects of patient ethnicity cannot be assessed, such as potential contributions of the high prevalence of *IL28B* CC genotype in Japan. In addition, the enrolled population was exclusively HCV genotype 1b and thus not representative of genotype 1 populations in Western countries. Finally, interpretation of study results cannot be extended to cirrhotic patients, who were excluded from the study.

Together, efficacy and safety results suggest that some weaknesses of current regimens that combine an NS3 protease inhibitor with alfa/RBV can be addressed with regimens that utilize a potent DAA with an alternative mechanism of action. The combination of daclatasvir 60 mg and alfa-2b/RBV elicited rapid clearance of detectable HCV RNA and achieved SVR_{24} in a large proportion of treatment-naive patients with HCV genotype 1b infection, with safety and tolerability similar to that of alfa/RBV alone. For non-responders, the efficacy of this regimen is similar to that seen with boceprevir or telaprevir in combination with alfa/RBV, although daclatasvir may have tolerability advantages and relatively lower risk of drug–drug interactions [27–29]. For non-responders and other difficult-to-treat patients, daclatasvir is being assessed as part of more potent regimens containing one or two additional DAAs with or without alfa/RBV. Overall, the results of this study support current Phase III development of daclatasvir 60 mg combined with alfa/RBV in larger treatment-naive populations infected with a broader range of HCV genotypes.

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