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Figure legends

Fig. 1. Comparison of *IFNλs* expression levels between chronic hepatitis C patients with rs12979860 CC or CT/TT. (a) Baseline mRNA levels of *IL29*, *IL28A,* and *IL28B* in PBMCs expressed relative to the internal control (/int.cont.). (b) Fold changes in *IL29*, *IL28A,* and *IL28B* expression in PBMCs stimulated for 8 h with poly(I:C) (10 μg/ml) after a 12-h pretreatment with IFNα-2b (100 IU/ml). Columns represent means ± SEM.

Fig. 2. Impact of *IFNλs* expression levels on therapy response in chronic hepatitis C patients. Fold changes in *IL29*, *IL28A*, and *IL28B* expression in PBMCs stimulated with IFNα-2b and poly(I:C). IFNλ induction levels were compared between (a) SVR (sustained virological responders), relapsers, and NR (non-virological responders) for peg-IFNα/ RBV (P/R) therapy. (b) VR (virological responders) and NR in patients with distinct IL28B genotypes (rs12979860 CC or CT/TT). (c) SVR for P/R, SVR for protease inhibitor (PI) plus P/ R triple therapy, and non-SVR for the triple therapy. Columns represent means ± SEM.

Fig. 3. Impact of *IFNλ4* on *IFNλs* expression and therapy response. Relationship of *IFNλ4* expression with (a) baseline expression of *IFNλs*, (b) *IFNλs* induction and (c) therapy response were compared in chronic hepatitis C patients with distinct *IL28B* genotypes (rs12979860 CC or CT/TT). The *IL28B*-unfavorable (CT/TT) group were subdivided into undetectable (−) or detectable (+) *IFNλ4* mRNA patients. (a) Baseline expressions of *IL29*, *IL28A*, and *IL28B* in PBMC. (b) Fold changes in *IL29*, *IL28A*, and *IL28B* expression in PBMCs stimulated f with IFNα-2b and poly(I:C). (c) Virological non-response rates for PEG-IFNα/ RBV therapy. Columns represent means ± SEM.

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Fig. 4. Manipulating *IFNλ4* expression regulates *IL28B* induction and promoter activity.

(a) Fold inductions of *IL28B* mRNA in BLCs transfected with *IFNλ4* and treated with IFNα (100U/ml). (b) Fold inductions of *IL28B* mRNA in HEK293T cells co-transfected with *IFNλ4* and IRF7 (control, 100ng, 500ng, 1000ng). Induction rates were expressed as fold change relative to control-transfected cells. (c) Fold inductions of *IL28B* promoter activity in HEK293/IL28B-Luc cells transfected with IFN*λ4* and treated with IFNα (0, 10, 100, 1000 IU/ml). (d, e) Fold inductions of *IL28B* promoter activity in HEK293/IL28B-Luc cells co-transfected with IFNλ4 and (d) IRF7 (control, 200ng, 500ng) or (e) p50:p65 (control, 200ng). Luciferase activities and cell viabilities were expressed as fold change relative to untreated or control-transfected cells. The error bars indicate standard deviation. *P<0.05.

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Table 1. Characteristics of patients analyzed for IFNλ expression levels.

Characteristic	(n = 50)
Age median (range), year	64 (29-79)
Sex, n (%) male/female	19 (38) / 31 (62)
ALT median (range), IU/L	22 (5-157)
γGTP median (range), IU/L	23 (10-343)
LDL-C median (range), mg/dL	100 (38-169)
Hemoglobin median (range), g/dL	13.4 (9.3-16.8)
Platelet count median (range), $\times 10^4/\mu L$	15.5 (5.2-23.6)
Fibrosis stage, n (%)	
F1,2 / F3,4	28 (70) / 12 (30)
Viral load median (range), log IU/mL*	6.8 (4.8-7.6)
HCV core 70 a.a. n(%)†	
wild / mutant / ND	15 (30) / 21 (42) / 14 (28)
HCV core 91 a.a. n (%)	
wild / mutant / ND	18 (36) / 18 (36) / 14 (28)
ISDR substitutions, n (%)‡	
0,1 / 2≦ / ND	26 (52) / 6 (12) / 18 (36)
IL28B SNP (rs8099917), n (%)	
TT / TG, GG	27 (54) / 23 (46)
IL28B SNP (rs12979860), n (%)	
CC / CT, TT	24 (48) / 26 (52)
IL28B SNP (ss469415590), (%)	
TT / ΔG	24 (48) / 26 (52)
Effect of previous therapy, n (%)	
SVR / Relapse / NR	18 (36) / 14 (28) / 18 (36)

ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; LDL-C, low-density lipoprotein cholesterol; HCV, Hepatitis C virus; ISDR, IFN sensitivity determining region; SVR, sustained virological responder; VR, virological responder; NR, non-responder; ND, not determined.

*HCV viral load was analyzed among Relapsers and Non-responders.

†HCV core amino acid (aa) 70R and 91L are considered wild type, while substituted amino acids are considered mutants.

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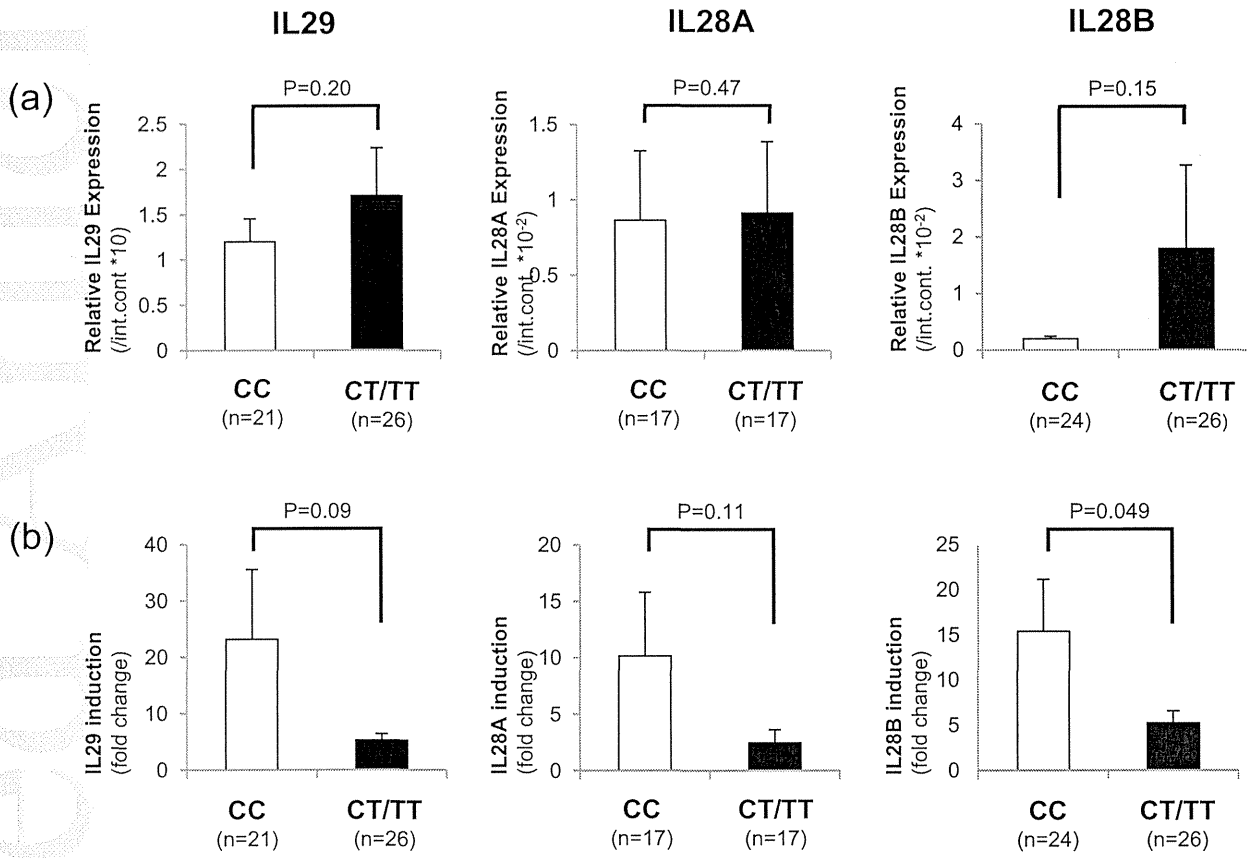

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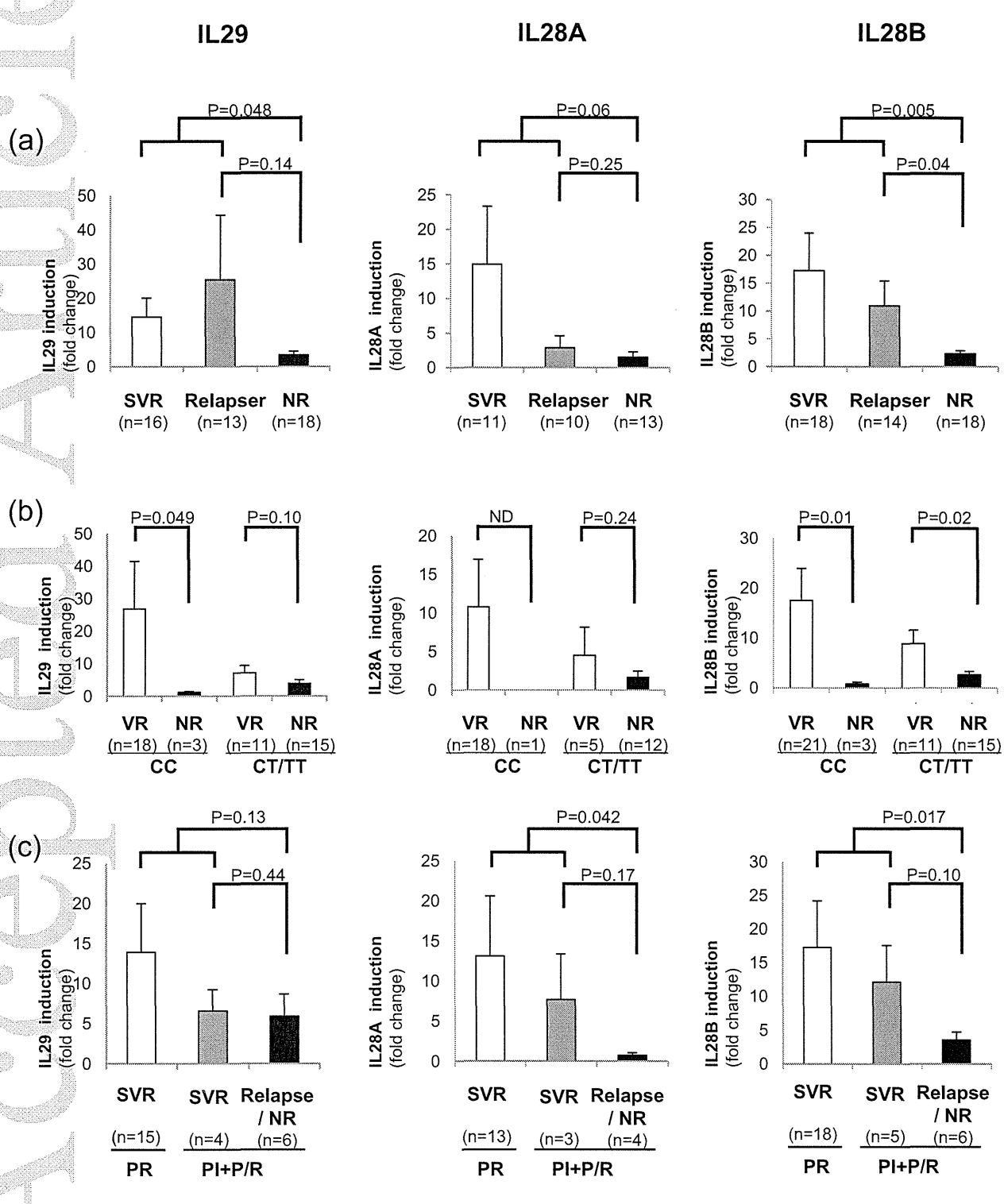

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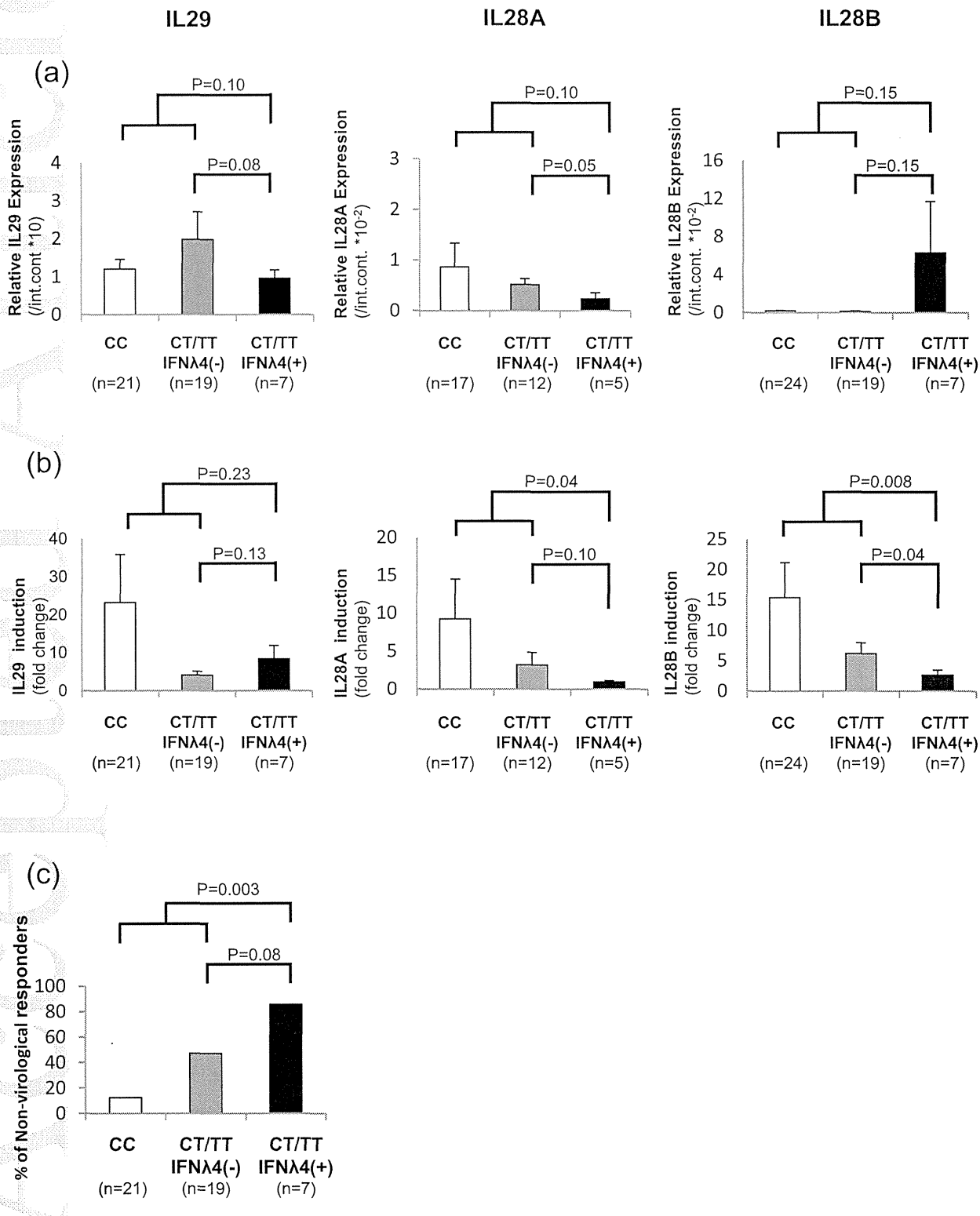

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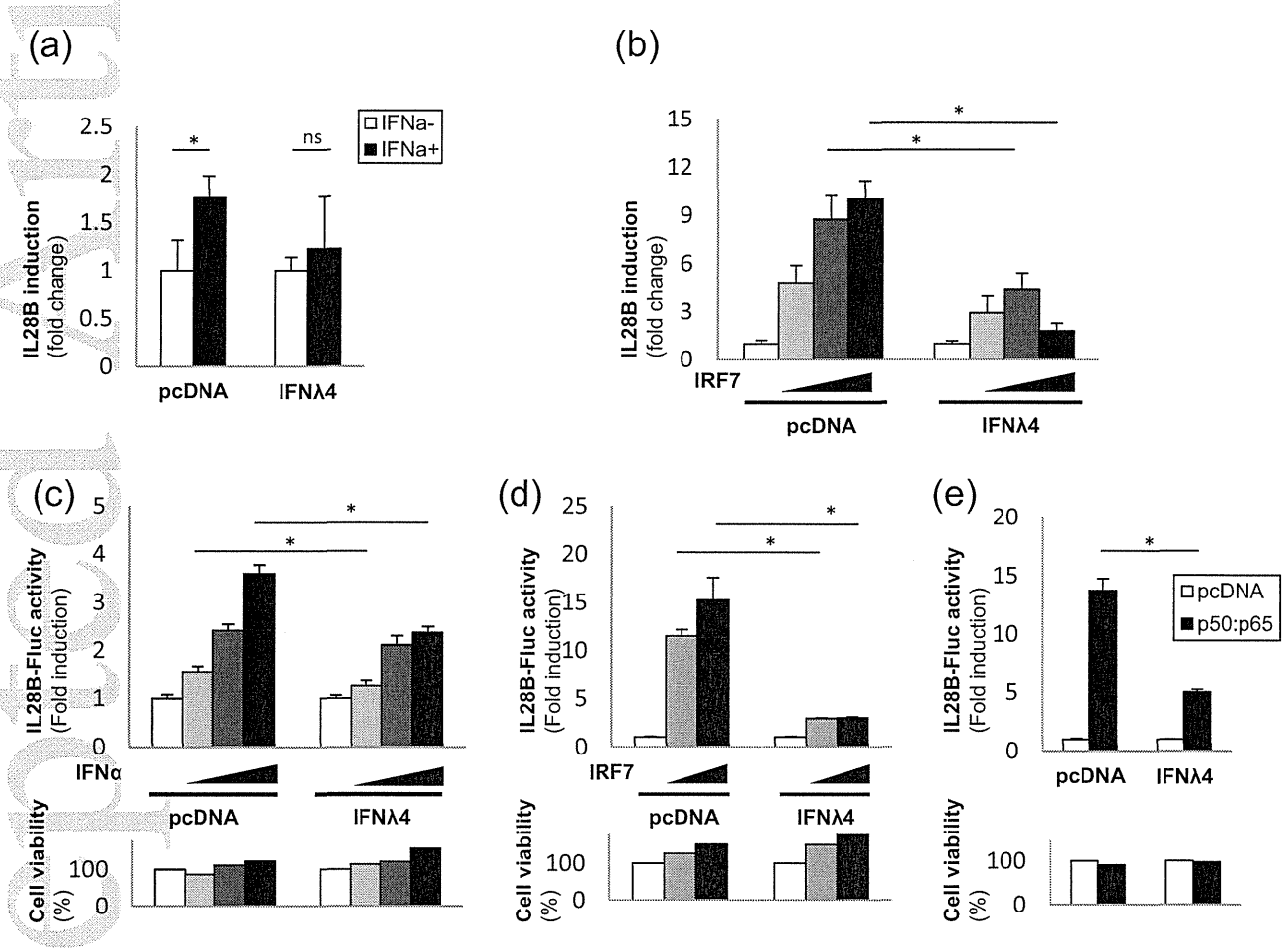

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Review Article

New molecularly targeted therapies against advanced hepatocellular carcinoma: From molecular pathogenesis to clinical trials and future directions

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Hepatocellular carcinoma (HCC) can be lethal due to its aggressive course and lack of effective systemic therapies for advanced disease. Sorafenib is the only systemic therapy that has demonstrated an overall survival benefit in patients with advanced HCC, and new agents for treatment of advanced HCC are needed. The multiple pathways involved in HCC oncogenesis, proliferation and survival provide many opportunities for the development of molecularly targeted therapies. Molecular targets of interest have expanded from angiogenesis to cancer cell-directed oncogenic signaling pathways for treatment of advanced HCC. Agents targeting vascular endothelial growth factor receptor, epidermal growth factor receptor, fibroblast growth factor receptor, platelet-derived growth factor receptor, c-mesenchymal-epithelial transition factor-1 and mammalian target of rapamycin signaling have been actively explored. This article focuses on the evaluation of molecular agents targeting pathogenic HCC and provides a review of recently completed phase III drug studies (e.g. involving sorafenib, sunitinib, brivanib, linifanib, erlotinib, everolimus, ramucirumab or orantinib) and ongoing drug studies (e.g. involving lenvatinib, regorafenib, tivantinib or cabozantinib) of molecularly targeted agents in advanced HCC, including a brief description of the biologic rationale behind these agents.

Key words: clinical trials, hepatocellular carcinoma, molecularly targeted therapy, novel agents, sorafenib

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is the sixth most common cancer and the third most common cause of cancer-related deaths worldwide.[1] Because a considerable number of patients are diagnosed when the disease becomes advanced, only approximately a third of all HCC patients are eligible for potentially curative treatments, such as resection or transplantation.[2] Surgical resection or transplantation provides good survival rates (i.e. beyond 65% at 5 years).[3] Unfortunately, the prognosis for patients with advanced stage HCC (Barcelona Cancer Liver Clinic [BCLC] stage C) is extremely poor, with a median overall survival (OS) of 6.6 months.[4] In advanced cases, only one systemic therapy is effective: the multikinase inhibitor sorafenib, which was approved by the U.S. Food and Drug Administration and which represented a breakthrough in the management of the disease.[5,6] However, the median life expectancy of patients with HCC on sorafenib is only 1 year, indicating the clear need to improve their outcomes. Hepatocarcinogenesis is a complex multistep process involving a number of genetic and epigenetic alterations,[7,8] our knowledge of several key molecular pathways implicated in HCC pathogenesis has revealed potential targets for therapeutic interventions, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), RAS/RAF/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) and phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathways. This review will examine our current understanding of these pathways as well as the efficacy and safety data pertaining to the most promising

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molecularly targeted agents beyond sorafenib. In this article, we first describe the pathogenesis of HCC and then provide an update on the recent data on clinical trials using molecularly targeted agents.

PATHOGENESIS OF HCC

HEPATOCELLULAR CARCINOMA IS a hypervascular tumor, and the central role of angiogenesis in its initiation, growth and subsequent dissemination to other tissues is well recognized. Angiogenesis in HCC is mediated by a complex network of growth factors, acting on both tumor cells and endothelial cells. The most widely recognized angiogenic factors are VEGF, FGF and PDGF.[9] These activate receptor tyrosine kinases (RTK) and the RAS/RAF/MEK/ERK pathway and the PI3K pathway in endothelial cells (Fig. 1).[10,11] VEGF and its receptors play a major role in tumor angiogenesis. In fact, VEGF is a potent permeability factor that promotes cell migration during invasion and acts as an endothelial growth factor that stimulates endothelial cell proliferation, inducing the budding of new blood vessels around the growing tumor masses. In human specimens and serum, increased expression of VEGF correlates with aggressive behavior of HCC and poor prognosis.[12] FGF and its family of receptors has also been implicated in HCC growth, invasion and angiogenesis.[13] While VEGF is the main driver of tumor angiogenesis, there is crosstalk between VEGF and FGF signaling in angiogenesis.[14] The upregulation of FGF has been suggested to mediate resistance to anti-VEGF receptor (VEGFR) therapy. The great majority of the HCC cases have overexpression of at least one FGF and/or FGF receptor (FGFR).[10] Hence, blocking the FGFR is another potentially important approach for the treatment of HCC. PDGF is involved in the development of immature tumor vessels,[15] while angiopoietins exert their action via stabilization of vessels by recruiting surrounding pericytes and smooth

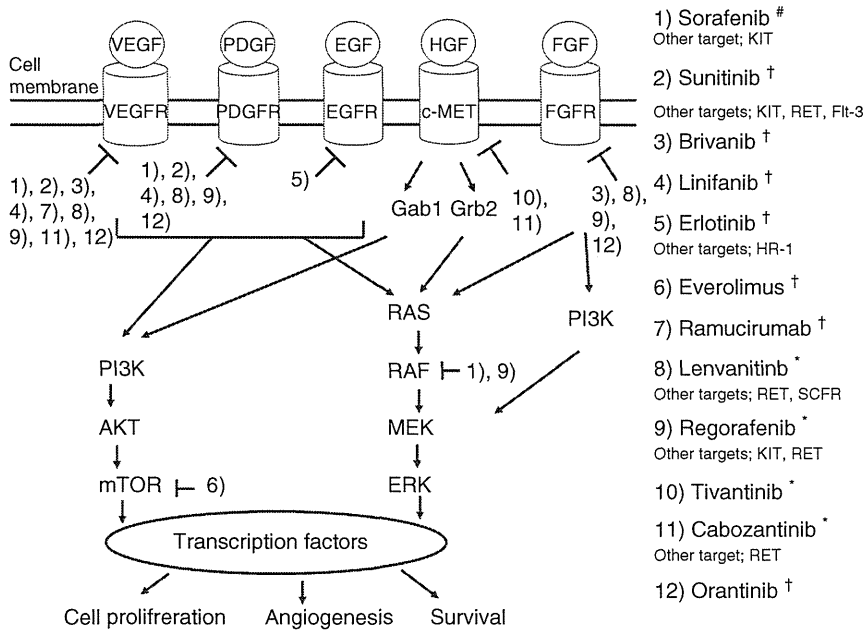

Figure 1 Schematic diagram of key molecular targets and targeted agents in hepatocellular carcinoma. #Approved globally in patients with advanced hepatocellular carcinoma (HCC). †Completed studies of phase III of molecularly targeted agents in advanced HCC. *Ongoing studies of phase III of molecularly targeted agents in advanced HCC. c-MET, c-mesenchymal-epithelial transition factor-1; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; Flt-3, Fms-like tyrosine receptor kinase-3; Gab1, GRB2-associated binding protein 1; Grb2, growth factor receptor bound protein 2; HER-1, human epidermal growth factor receptor-1; HGF, hepatocyte growth factor; MEK, mitogen-activated protein kinase/ERK kinase; mTOR, mammalian target of rapamycin; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol 3-kinase; RET, rearranged during transfection; SCFR, stem cell growth factor receptor kit; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

muscle cells.[16] PDGF is involved in fibrogenesis, angiogenesis and tumorigenesis.[17,18] PDGF expression is upregulated in the early stages of chronic hepatitis, suggesting its association with the development of fibrosis in chronic hepatitis C.[19] From a therapeutic point of view, inhibition of these targets has been shown to diminish the vascularity of tumors in preclinical studies.

Several intracellular signaling pathways are involved in HCC pathogenesis; the most studied are the PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways. The PI3K/AKT/mTOR axis is involved in multiple cellular processes, including survival and proliferation.[20] This pathway mediates its effects through activation of various tyrosine kinase receptors, such as VEGFR, EGFR and PDGFR, which in turn recruit and activate PI3K. The activation of PI3K will lead to a cascade of activation of downstream effectors, leading to activation of mTOR (Fig. 1). The activation of the mTOR pathway in HCC is associated with aggressive tumor behavior and decreased survival, which supports the efforts to target this pathway for therapeutic interventions.[21] RAS/RAF/MEK/ERK signaling regulates many important cellular processes, such as proliferation, differentiation, angiogenesis, survival and cell adhesion.[22] Importantly, the RAS/RAF/MEK/ERK pathway is constitutively activated in HCC.[23]

Apart from these major signal pathways in the pathogenesis of HCC, the hepatocyte growth factor (HGF)/c-mesenchymal-epithelial transition factor-1 (c-MET) pathway is involved in tumor growth, invasion and angiogenesis in various types of cancer.[24] c-MET is a tyrosine kinase receptor, with its ligand, HGF.[25] HGF-induced activation of c-MET ultimately leads to the activation of downstream effector molecules, including PI3K and ERK (Fig. 1).[26] Expression of the c-MET receptor protein is present in human HCC samples[27-29] and has been shown to be a poor prognostic factor in patients with HCC. Therefore, therapeutics aimed at the c-MET receptor is a rational approach for HCC.

RESULTS OF PHASE III STUDIES

STUDIES ARE INVESTIGATING various agents for HCC, most of which target the previously described VEGF axis, FGF, PDGF, RAS/RAF/ERK and mTOR signaling pathways (Fig. 1). We describe these molecularly targeted agents and completed phase III trials. We also provide information on why phase III pivotal consecutive randomized controlled trials (RCT) in HCC did not meet the primary end-points (Table 1). Seven phase III trials reported negative results for first-line therapy (e.g. with sunitinib, brivanib, linifanib or erlotinib) and second-line therapy (e.g. with brivanib, everolimus or ramucirumab). Five of these studies were designed to test for superiority (i.e. study of SUN 1170, SEARCH, BRISK-PS, EVOLVE-1, REACH), and two of these studies were designed to test for non-inferiority (i.e. study of BRISK-FL, 0100953) with a primary end-point of OS.

Sorafenib

Sorafenib is a multikinase inhibitor that inhibits serine/threonine kinases (BRaf and CRaf and VEGFR-1, -2 and -3), PDGFR-α and -β, and the stem cell factor receptor, c-kit. In the Sorafenib HCC Assessment Randomized Protocol (SHARP) study,[5] a double-blind RCT with a primary end-point of OS, sorafenib significantly increased survival times of patients with HCC from 7.9 to 10.7 months (hazard ratio [HR], 0.69; 95% confidence interval [CI], 0.55–0.87; $P = 0.001$). Among the enrolled patients, the proportion of patients with Child–Pugh liver function class A and B disease was 97% and 3%, respectively, while that with BCLC stage B and C disease was 17% and 83%, respectively. Sorafenib was the first systemic therapy to demonstrate a significant improvement in OS in patients with advanced HCC, and its subsequent approval represented a major breakthrough in the treatment of advanced HCC. A parallel phase III study was conducted in the Asia–Pacific region. Median OS was 6.5 months in the sorafenib arm and 4.2 months in the placebo arm (HR, 0.68; 95% CI, 0.50–0.93; $P = 0.014$).[6] Among the enrolled patients, the proportion of patients with Child–Pugh liver function class A and B disease was 97% and 3%, respectively, while that with BCLC stage B and C disease was 5% and 95%, respectively. Similar toxicity profiles were seen in both studies; sorafenib treatment was associated with increased rates of diarrhea, weight loss, hand–foot skin reaction and hypophosphatemia. Sorafenib is the first and only agent to demonstrate an OS benefit and to be approved by regulators globally in patients with advanced HCC.

Sunitinib

Sunitinib is another multikinase inhibitor with broad activity, inhibiting all VEGFR and PDGFR, c-kit, Fms-like tyrosine receptor kinase (Flt)3 and rearranged during transfection (RET). Sunitinib was evaluated against sorafenib in a large phase III trial.[30] All patients had Child–Pugh liver function class A disease, and the proportion of patients with BCLC stage B and C disease was 15% and 85%, respectively. Median time to progression (TTP) for sunitinib and sorafenib was 4.1 and 3.8

Table 1 Results of completed phase III trials of molecularly targeted therapies in HCC

Drug	Main target	Design (trial)	TTP/PFS (months), HR, 95% CI	OS (months), HR, 95% CI
First-line advanced HCC				
Sorafenib	RAF, VEGFR, PDGFR, c-KIT	Sorafenib vs placebo (SHARP)	4.9 vs 4.1; $P = 0.77$; HR, 0.58; 95% CI, 0.45–0.74	10.7 vs 7.9; $P < 0.001$; HR, 0.69; 95% CI, 0.55–0.87
		Sorafenib vs placebo (Asia–Pacific)	2.8 vs 1.4; $P < 0.001$; HR, 0.57; 95% CI, 0.42–0.79	6.5 vs 4.2; $P = 0.014$; HR, 0.68; 95% CI, 0.50–0.93
Sunitinib	VEGFR, PDGFR, KIT, RET, Flt-3	Sunitinib vs sorafenib (SUN 1170)	4.1 vs 3.8; $P = 0.169$; HR, 1.13; 95% CI, 0.98–1.31	7.9 vs 10.2; $P = 0.0019$; HR, 1.30; 95% CI, 1.13–1.50
Brivanib	FGFR, VEGFR	Brivanib vs sorafenib (BRISK-FL)	4.2 vs 4.1; $P = 0.853$; HR, 1.01; 95% CI, 0.88–1.16	9.5 vs 9.9; $P = 0.373$; HR, 1.06; 95% CI, 0.93–1.22
Linifanib	VEGFR, PDGFR	Linifanib vs sorafenib (0100953)	5.4 vs 4.0; $P = 0.001$; HR, 0.76; 95% CI, 0.64–0.90	9.1 vs 9.8; $P =$ NS; HR, 1.05; 95% CI, 0.90–1.22
Erlotinib	EGFR, HER-1	Erlotinib + sorafenib vs placebo + sorafenib (SEARCH)	3.2 vs 4.0; $P = 0.91$; HR, 1.13; 95% CI, 0.94–1.36	9.5 vs 8.5; $P = 0.2$; HR, 0.92; 95% CI, 0.78–1.1
Second-line advanced HCC				
Brivanib	FGFR, VEGFR	Brivanib vs placebo (BRISK-PS)	4.2 vs 2.7; $P < 0.001$ HR, 0.56; 95% CI, 0.42–0.78	9.4 vs 8.2; $P = 0.331$; HR, 0.89; 95% CI, 0.69–1.15
Everolimus	mTOR	Everolimus vs placebo (EVOLVE-1)	3.0 vs 2.6; HR, 0.93; 95% CI, 0.75–1.15	7.6 vs 7.3; $P = 0.68$; HR, 1.27; 95% CI, 0.86–1.27
Ramucirumab	VEGFR	Ramucirumab vs placebo (REACH)	2.8 vs 2.1; $P < 0.001$; HR, 0.63; 95% CI, 0.52–0.75†	9.2 vs 7.6; $P = 0.14$; HR, 0.87; 95% CI, 0.72–1.05

†Progression-free survival.

CI, confidence interval; FGFR, fibroblast growth factor receptor; HCC, hepatocellular carcinoma; HER-1, human epidermal growth factor receptor-1; HR, hazard ratio; mTOR, mammalian target of rapamycin; NS, not significant; OS, overall survival; PDGFR, platelet-derived growth factor receptor; PFS, progression-free survival; RET, rearranged during transfection, Flt-3, Fms-like tyrosine receptor kinase-3; TTP, time to progression; VEGFR, vascular endothelial growth factor receptor.

months, respectively ($P = 0.169$); however, median OS for sunitinib and sorafenib was 7.9 and 10.2 months (HR, 1.30; 95% CI, 1.13–1.50; $P = 0.0019$), respectively. The decision was based on a higher incidence of significant toxicities (including grade 3/4 thrombocytopenia [30%], neutropenia [25%] and hemorrhagic events [12%]) in the sunitinib arm and the futility of showing either superiority or non-inferiority in OS when compared with sorafenib. This trial was stopped prematurely after inferior outcomes were noted in the sunitinib arm.

Brivanib

Brivanib is a dual inhibitor of VEGFR and FGFR, both of which are implicated in the pathogenesis of HCC.[31] Two randomized phase III clinical trials were conducted to assess the use of brivanib in the first-line (BRISK-FL) and second-line (BRISK-PS) settings. BRISK-FL was a head-to-head randomized phase III clinical trial comparing brivanib with sorafenib as the first-line therapy in patients with unresectable HCC. Among the enrolled patients, the proportion of patients with Child–Pugh liver function class A and B disease was 92% and 8%, respectively, while that with BCLC stage B and C disease was 22% and 78%, respectively. The brivanib arm failed to achieve a non-inferior median OS, with 9.5 months for brivanib and 9.9 months for sorafenib (HR, 1.06; 95% CI, 0.93–1.22; $P = 0.373$). There was also no difference in TTP between brivanib and sorafenib (4.2 vs 4.1 months; HR, 1.01; 95% CI, 0.88–1.16; $P = 0.853$).[31] The study did not meet its primary OS objective based upon a non-inferiority statistical design. In the second-line setting, BRISK-PS compared brivanib with placebo in patients who were refractory or intolerant to first-line treatment with sorafenib. Although TTP was significantly longer in the brivanib arm than with placebo (4.2 vs 2.7 months; HR, 0.56; 95% CI, 0.42–0.78; $P < 0.001$), the primary end-point of the study was not met, with a median OS for brivanib and placebo of 9.4 and 8.2 months, respectively (HR, 0.89; 95% CI, 0.69–1.15; $P = 0.331$).[32] The most common grade 3/4 adverse events (AE) were hypertension (19%), hyponatremia (18%), fatigue (15%) and decreased appetite (12%).

Linifanib

Linifanib is an oral tyrosine kinase inhibitor (TKI) with selective activity against VEGFR and PDGFR. Linifanib was compared with sorafenib as first-line therapy in a non-inferiority phase III trial.[33] Enrolled patients were those with a histological and cytological diagnosis of unresectable HCC and Child–Pugh liver function class A. TTP with linifanib was significantly improved when compared with sorafenib (5.4 vs 4.0 months; HR, 0.76; 95% CI, 0.64–0.90; $P = 0.001$). However, median OS was 9.1 months with linifanib and 9.8 months with sorafenib (HR, 1.05; 95% CI, 0.90–1.22). Linifanib was less well tolerated than sorafenib, with significantly increased discontinuations and dose reductions/interruptions because of AE.

Erlotinib

Erlotinib is an orally active, potent selective inhibitor of the EGFR/human epidermal growth factor receptor-1-related tyrosine kinase enzyme. In the phase III SEARCH trial, advanced HCC patients were randomized to sorafenib plus either erlotinib or placebo.[34] Inclusion criteria were a histological and cytological diagnosis of unresectable HCC and Child–Pugh liver function class A. Median OS was 9.5 months with sorafenib plus erlotinib and 8.5 months with sorafenib (HR, 0.92; 95% CI, 0.78–1.1; $P = 0.2$). This result failed the prespecified boundaries for non-inferiority. TTP was 3.2 months with sorafenib plus erlotinib and 4.0 months with sorafenib (HR, 1.13; 95% CI, 0.94–1.36; $P = 0.91$).

Everolimus

The mTOR inhibitor, everolimus, has demonstrated antitumor activity in several malignancies. A phase III study comparing everolimus with placebo (EVOLVE-1) in patients who have failed or become intolerant to sorafenib has recently been completed. All patients had Child–Pugh liver function class A, and the proportion of patients with BCLC stage B and C disease was 14% and 86%, respectively. There were no significant difference in TTP between everolimus (3.0 months) and placebo (2.6 months) (HR, 0.93; 95% CI, 0.75–1.15). Furthermore, no significant difference in OS was seen between everolimus (7.6 months) and placebo (7.3 months) (HR, 1.05; 95% CI, 0.86–1.27; $P = 0.68$). The most common grade 3/4 AE for everolimus were anemia (7.8%), asthenia (7.8%) and decreased appetite (6.1%). No patients experienced hepatitis C viral flare. The EVOLVE-1 study failed to reach its primary end-point of extending OS with everolimus.[35]

Ramucirumab

Ramucirumab is a recombinant humanized antibody that specifically targets the extracellular domain of VEGFR-2. A phase II study of 42 patients with advanced HCC and primarily well-preserved liver function showed that first-line ramucirumab monotherapy produced a disease control rate of 69%. The median progression-free survival (PFS) was 4.0 months and

median OS was 12.0 months, respectively. Grade 3/4 toxicities included gastrointestinal bleeding (7%), hypertension (12%) and fatigue (10%). These findings prompted the initiation of the phase III RCT (REACH) comparing ramucirumab versus placebo in patients who failed or were intolerant to sorafenib (NCT01140347).[36] Eligible patients had advanced HCC, stage BCLC C or B disease that was refractory or not amenable to locoregional therapy, and Child–Pugh liver function class A. However, according to the preliminary results released at European Society for Medical Oncology Congress in 2014, ramucirumab failed to demonstrate superiority in terms of OS when compared with placebo. The OS HR was 0.866 (95% CI, 0.717–1.046; $P = 0.1391$); median OS was 9.2 months for ramucirumab versus 7.6 months for placebo. Median PFS with ramucirumab and placebo was 2.8 and 2.1 months, respectively (HR, 0.63, 95% CI, 0.52–0.75; $P < 0.0001$).[37]

ONGOING PHASE III CLINICAL TRIALS

IN ADDITION TO the antiangiogenic multi-targeted TKI, there is a growing number of biologics that target different molecular pathways, such as c-MET. Some of these treatments act on elements of intracellular signaling pathways. A number of agents have shown promising preliminary data for HCC. We also comment on ongoing phase III pivotal trials (Table 2). The inclusion criterion of all four phase III studies was Child–Pugh liver function class A disease.

Lenvatinib

Lenvatinib is an oral multi-tyrosine kinase inhibitor that targets VEGFR-1–3, FGFR-1–3, RET, mast/stem cell growth factor receptor kit and PDGFR.[38] A phase I/II trial of lenvatinib in patients with advanced HCC and Child–Pugh score A liver function status showed a median OS of 18.7 months (95% CI, 12.8–25.1) and a median TTP of 7.4 months (95% CI, 5.5–9.4). Based on these results, a phase III trial was designed to compare the safety and efficacy of lenvatinib versus sorafenib in patients with unresectable or advanced HCC and Child–Pugh A liver status (NCT01761266).[39] Subjects were categorized as stage B (not applicable for transarterial chemoembolization [TACE]) or stage C based on the BCLC staging system.

Regorafenib

Regorafenib is a multikinase inhibitor that targets kinases involved in angiogenesis (e.g. VEGFR-1–3), oncogenesis (e.g. c-kit, RET and BRAF) and the tumor microenvironment (e.g. PDGFR and FGFR).[40] Regorafenib (160 mg/day) was tested in an uncontrolled phase II study in patients with advanced HCC after failure of prior sorafenib therapy (RESORCE).[41] Median TTP was 4.3 months and median OS was 13.8 months. The most common grade 3/4 AE included fatigue (17%), hand–foot skin reaction (14%) and diarrhea (6%). Based on this data, a phase III RCT in the second-line setting is under development (NCT01774344). Inclusion criteria were BCLC stage B or C disease, and failure to receive prior treatment with sorafenib.

Tivantinib

Tivantinib is a selective inhibitor of c-MET.[42] In a randomized phase II trial comparing the use of tivantinib

Table 2 List of ongoing phase III trials of novel targeted therapy for HCC

Drug	Main target	Design (trial)	Status	NCT number
1st line				
Lenvatinib	VEGFR, PDGFR, FGFR, RET, SCFR	Lenvatinib vs sorafenib (E7080)	Recruiting	NCT01761266
2nd line				
Regorafenib	VEGFR, PDGFR, BRAF, FGFR, KIT, RET	Regorafenib vs placebo (RESORCE)	Recruiting	NCT01774344
Tivantinib	c-MET	Tivantinib vs placebo in subjects with c-MET overexpressing (JET-HCC)	Recruiting	NCT01755767
Cabozantinib	c-MET, VEGFR, RET	Cabozantinib vs placebo (CELESTIAL)	Recruiting	NCT01908426

c-MET, c-mesenchymal-epithelial transition factor-1; FGFR, fibroblast growth factor receptor; HCC, hepatocellular carcinoma; PDGFR, platelet-derived growth factor receptor; RET, rearranged during transfection; SCFR, stem cell growth factor receptor kit; VEGFR, vascular endothelial growth factor receptor.

Table 3 Results of completed phase III trials of molecularly targeted therapy in combination with TACE for HCC

Drug	Main target	Design	TTP (months, HR, 95% CI)	OS (months)
Sorafenib	RAF, VEGFR, PDGFR, c-KIT	TACE + sorafenib vs TACE + placebo	5.4 vs 3.7; $P = 0.252$; HR, 0.87; 95% CI, 0.70–1.09	29.7 vs NE; $P = 0.790$; HR, 1.06; 95% CI, 0.69–1.64
Brivanib	FGFR, VEGFR	TACE + brivanib vs TACE + placebo	12.0 vs 10.9; $P = 0.62$; HR, 0.94; 95% CI, 0.72–1.22	26.4 vs 26.1; $P = 0.53$; HR, 0.90; 95% CI, 0.66–1.23
Orantinib	VEGFR, PDGFR, FGFR	TACE + orantinib vs TACE + placebo	†	†

†Full data have not yet been reported at November 2014.
CI, confidence interval; HCC, hepatocellular carcinoma; FGFR, fibroblast growth factor receptor; HR, hazard ratio; NE, not estimable due to immaturity of data; OS, overall survival; PDGFR, platelet-derived growth factor receptor; TTP, time to progression; VEGFR, vascular endothelial growth factor receptor.

versus placebo as second-line treatment, the overall analysis showed a marginal but significant improvement in TTP in tivantinib over placebo (1.6 vs 1.4 months; HR, 0.64; 95% CI, 0.43–0.94; $P = 0.04$). A preplanned analysis of patients whose tumors demonstrated overexpression of MET by immunohistochemistry revealed a more notable improvement in TTP, with 2.7 months in the MET-high tivantinib subset versus 1.4 months in the MET-high placebo subset (HR, 0.43; 95% CI, 0.19–0.97; $P = 0.03$). Median OS was 7.2 months for patients with MET-high tumors who received tivantinib versus 3.8 months for MET-high patients who received placebo (HR, 0.38, 95% CI, 0.18–0.81; $P = 0.01$).[43] The most common grade 3/4 AE in the tivantinib group were neutropenia and anemia; severe neutropenia rates were higher prior to mandated dose reduction. Currently, a phase III study is underway to compare tivantinib versus placebo in subjects with c-MET-overexpressing HCC who have failed one prior systemic therapy (NCT01755767).

Cabozantinib

Cabozantinib, a multikinase inhibitor that inhibits MET, VEGFR-2 and RET, was studied in a phase II trial of HCC patients who had received at most one prior systemic therapy.[44] Impressive efficacy was observed; the PFS was 4.4 months while the median OS was 15.1 months in the cabozantinib arm.[45] A phase III clinical trial testing the efficacy of cabozantinib in the second-line setting is planned (NCT01908426).

Combination therapy

With regard to molecularly targeted agents combined with other treatments, surgical resection and local ablation are curative therapies for BCLC stage A, whereas TACE is used for the management of patients of BCLC stage B. Hepatic arterial infusion chemotherapy (HAIC) is used for the management of patients of BCLC stage B to C. In this article, we focused mainly BCLC stage B to C. Tables 3 and 4 summarizes data regarding the use of molecularly targeted agents combined with TACE or HAIC.

The high rate of HCC recurrence after TACE may be due to its enhancement of angiogenesis and upregulation of VEGF and PDGFR expression, resulting in the formation of rich vascular beds in residual tumors.[46] Administration of an antiangiogenic agents with TACE may block angiogenesis and may therefore lengthen time to recurrence and improve survival.

A phase III study of sorafenib in combination with TACE versus TACE alone performed in Japan and Korea likewise did not demonstrate any benefit with the combination (TTP; sorafenib vs placebo [5.4 vs 3.7 months, HR, 0.87; 95% CI, 0.70–1.09; $P = 0.252$]; OS sorafenib vs placebo; 29.7 months vs not estimable due to immaturity of data [HR, 1.06; 95% CI, 0.69–1.64;

Table 4 List of ongoing phase III trials of therapy in combination with TACE or HAIC for HCC

Drug	Design (trial)	Status	NCT number
Sorafenib	TACE + sorafenib vs TACE + placebo	Recruiting	NCT01004978
Sorafenib	TACE + sorafenib vs TACE + placebo	Recruiting	NCT01324076
Sunitinib	TACE + sunitinib vs TACE + placebo	Recruiting	NCT01164202
Sorafenib	HAIC + sorafenib vs sorafenib	Recruiting	NCT01214343

HAIC, hepatic arterial infusion chemotherapy; HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

$P = 0.79$]).[47] Two other phase III, randomized, placebo-controlled trials evaluating the efficacy of sorafenib in combination with conventional TACE are ongoing (NCT01004978 and NCT01324076).

Other phase III RCT exploring the combinations of TACE and orantinib (ORIENTAL trial, NCT01465464) and brivanib (BRISK-TA trial) have been completed, and sunitinib (TURNE trial, NCT01164202) are ongoing.

In the BRISK-TA trial, although brivanib improved time to radiographic progression (brivanib vs placebo; 8.4 vs 4.9 months; HR, 0.61; 95% CI, 0.48–0.77; $P < 0.0001$), brivanib did not improve TTP (brivanib vs placebo; 12.0 vs 10.9 months; HR, 0.94; 95% CI, 0.72–1.22; $P = 0.62$) or OS (brivanib vs placebo; 26.4 vs 26.1 months; HR, 0.90; 95% CI, 0.66–1.23; $P = 0.53$).[48]

Orantinib is an oral small molecule inhibitor of VEGFR, PDGFR and FGFR.[49] A recent press release announced that a phase III trial comparing TACE plus orantinib versus TACE plus placebo did not meet the primary end-point, but the full dataset has not yet been reported.

A phase III study of sorafenib plus low-dose cisplatin/fluorouracil HAIC versus sorafenib in patients with advanced HCC is ongoing (NCT01214343).

Biomarkers

Studies have investigated whether several biomarker can predict the response to sorafenib. Tissue markers, such as FGF3/FGF4,[50] αB-crystallin,[51] c-Jun N-terminal kinase,[52] VEGF-A[53] and pERK,[54] serum marker and angiogenesis-related cytokine have been reported.[55] Conventional tumor markers for the diagnosis of HCC, namely, des-γ-carboxyprothrombin and α-fetoprotein, have been reported to show contrasting behavior after administration of sorafenib.[56–60] However, no definitive biomarker for sorafenib has been identified. Lovelt *et al.* reported that no biomarker was significantly associated with the response to sorafenib within the SHARP study, which was the largest study of sorafenib.[61] The difficulty in identifying a specific biomarker in sorafenib therapy for HCC may be due to the presence of multiple molecular targets.

FUTURE DIRECTIONS

NINE PHASE III clinical trials (i.e. SHARP, Asia–Pacific, SUN 1170, BRISK-FL, 0100953, SEARCH, BRISK-PS, EVOLVE-1, REACH) of patients with advanced HCC have been completed, and four phase III clinical trials (i.e. E7080, RESORCE, JET-HCC, CELESTIAL) are ongoing. No targeted agent or regimens other than sorafenib significantly improve OS in patients with advanced HCC, according to phase III trials in the first- or second-line setting. Three phase III clinical trials did not demonstrate any benefit with combination therapy.

Potential reasons for negative results include heterogeneous patient population and the lack of understanding of critical drivers of tumor progression/dissemination. Other reasons include liver toxicity, flaws in trial design or marginal antitumoral efficacy of the agents. When dissecting the results of recent trials,[30–34] we can speculate that the main shortcomings for sunitinib are liver toxicity and issues with trial design.[30] Other shortcomings include lack of efficacy for erlotinib,[34] toxicity for linifanib[33] and lack of efficacy and issues with trial design for brivanib.[31,32]

Hepatocellular carcinoma is a heterogeneous disease, both in regard to its clinical manifestations with underlying liver disease, and its complex pathogenesis involving aberrant signaling in several molecular pathways. Advances in targeted therapy for HCC require a better understanding of various molecular events driving the progression of HCC as well as identification of biomarkers to predict treatment response to targeted agents. Due to the complexity of the mechanisms involved in progression of HCC, the establishment of personalized therapy will require the identification of tissue biomarkers in HCC.

Regarding patient selection, recommendations emphasized the need for standardization of inclusion criteria based on stage, such as the BCLC classification. It is evident that the population of patients with unresectable HCC consists of a highly heterogeneous group of patients with a wide spectrum of survival, ranging from a few months to longer than 2 years.[62,63] Therefore, it is difficult to precisely estimate the survival of patients during the design of clinical trials that encompass a heterogeneous population. As a result, the staging system is suboptimal in identifying a homogeneous group of patients in terms of prognosis and disease behavior.

In summary, success in the development of targeted agents for HCC relies on concerted efforts of testing of novel agents in clinical trials, advancement of knowledge of the molecular events of HCC, discovery of biomarkers to guide personalized treatment and improvements in patient selection.

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