Format for ANSWERING REVIEWERS

May 20, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3018-review.doc).

Title: Molecular Targeted Therapy for Hepatocellular Carcinoma: Current and Future

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 3018

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) **Recent information regarding some of the targeted agents has not always been included. For instance, the negative results of brivanib in recent studies ought to be presented and discussed. And for linifanib too even if the information we have is not more than minimal (meaningful all the same). Also, the prognostic significance of c-met over expression and details on the positive effect found for Tivantinib in c-met positive patients are worth being discussed. And the activity of IGF-targeting molecules needs to be updated.**

Answer) Thanks for the reviewer's comment. We have revised our manuscript by adding the recent data as to target agents including brivanib, linifanib, and tivantinib. Also some of comments concerning IGF-targeting molecules have been included as following;

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Temsirolimus, an mTOR inhibitor is approved for treatment of advanced renal cell carcinoma. Its efficacy and potential utility for HCC is currently being studied (NCT01079767).

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The large randomized phase III trials, BRISK (Brivanib Study in Patients at Risk) HCC program have been conducted to evaluate the role of brivanib in advanced HCC (BRISK-FL, BRISK-PS, and BRISK-APS).

The BRISK-PS trial evaluated brivanib versus placebo in patients who had failed or intolerant to sorafenib therapy (NCT00825955). This study did not meet its primary end point of improving OS, but treatment with brivanib showed improvements in response rate.[70] The BRISK-FL trial (NCT00858871) was directly comparing the clinical outcomes of brivanib versus sorafenib in 1050 patients with advanced HCC who received no prior systemic therapy. Median overall survival was 9.5 months in the brivanib arm compared with 9.9 months in the sorafenib arm, not a statistically significant difference. No significant survival differences were seen in subgroups based on geographic region, cause of HCC or disease severity. The study did not meet its primary overall survival objective based upon a non-inferiority statistical design.[71]

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Linifanib (ABT-869) is a novel receptor tyrosine kinase inhibitor with potent activity against members of the VEGFR and PDGFR families.[74] Phase II study of linifanib in advanced HCC, the estimated objective response rate was 9.1%, the median time to disease progression was 3.7 months, and the median OS was 9.7 months.[75] An open-label, randomized phase 3 study of the efficacy and tolerability of linifanib versus sorafenib in advanced HCC (NCT01009593) was conducted. The overall survival of linifanib given as monotherapy once daily was similar sorafenib given twice daily per standard of care.[76]

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The phase III placebo controlled, double-blind SEARCH (Sorafenib and Erlotinib, a Randomized Trial Protocol for the Treatment of Patients with HCC) trial, has been conducted in patients with advanced HCC.362 patients received sorafenib plus erlotinib and 358 received sorafenib plus placebo. There were no significant differences in OS or TTP between arms. Erlotinib, when added to sorafenib as standard of care in

advanced HCC, did not prolong overall survival.[83]

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The IGF/IGFR signal pathway regulates several cellular processes, including proliferation, motility and inhibition of apoptosis.[86] Ligand binding to IGF-1R triggers rapid receptor autophosphorylation, which in turn initiates downstream cellular effectors, ultimately leading to activation of PI3K, protein kinase B and the RAF/MEK/ERK pathway.[87] In HCC, dysregulation of IGF signaling occurs predominantly at the level of IGF-2. IGF-2 is overexpressed in 16–40% of human HCCs and IGF-2R (an alternative receptor for IGF-2) is underexpressed in approximately 80% of HCCs.[88,89] Their associations with disease stage, metastasis and survival and the functions of IGF and IGFR in HCC have been reported.[90,91] Several strategies targeting this system including monoclonal antibodies against the IGF-1 receptor (IGF-1R) and small molecule inhibitors of the tyrosine kinase function of IGF-1R are under active investigation.

Pre-clinical evidence obtained in HCC cells showed that IMC-A12 (cituxumumab), a human monoclonal antibody that blocks IGF-1R. A phase I study of IMC-A12 yielded a partial response in HCC.[92] however, a sub-sequent phase II study in patients with advanced HCC showed that IMC-A12 is inactive as monotherapy.[93] Up to 46% of patients developed grade 3-4 hyperglycemia in this study,
Hyperglycemia could be the dose limiting toxicity of IGF-1R monoclonal antibodies.

BIIB022 is an anti-IGF-1R monoclonal antibody that blocks binding of both IGF-1 and IGF-2 to IGF-1R. This agent does not appear to cause hyperglycemia, a common side effect of receptor specific antibodies. A planned phase I/II study comparing sorafenib with or without BIIB022 in patients with advanced HCC was terminated due to a business decision of the sponsor company.

AVE1642 is another monoclonal antibody that specifically blocks IGF-1R signaling. this agent was studied in advanced HCC patients in a phase I study in combination with sorafenib.[94] long-lasting disease stabilizations were observed in most patients with progressive disease.

OSI-906 is a novel potent dual tyrosine kinase inhibitor of both IGF-1R and insulin receptor. The unique advantage of OSI-906 over previous class of anti-IGF drugs is its ability to minimize the activity of IGF-2 where IGF-1R inhibition alone will not be sufficient. The phase II study of second-line treatment for advanced HCC patients who failed first-line treatment with sorafenib (NCT01101906) was terminated because the sponsor decided not to pursue the development of this drug.

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Tivantinib (ARQ 197) is a selective, oral MET receptor tyrosine kinase inhibitor with broad-spectrum antitumor activity as single agent. MET overexpression was shown as a negative prognostic factor in HCC after sorafenib failure. Tivantinib demonstrated a nearly doubling of progression free and overall survival in the MET high group compared to placebo in a Phase II study in patients with advanced HCC as second-line treatment.[100] The activity of tivantinib in combination with sorafenib is also promising. Adverse events include hematological toxicity, asthenia and loss of appetite. The initially high incidence of neutropenia in patients with HCC lead to dose reduction from 360 mg b.i.d. to 240 mg b.i.d. Currently, a pivotal Phase III study in advanced, MET-high HCC after sorafenib failure is planned.

(2) **Too much text makes the manuscript difficult to read. The main data from clinical trials (ORR, TTP, OS, etc) could well be presented in tables so that the text is then used only for discussion.**

Answer) Thanks for the reviewer's valuable comment. We made a new table presenting the results of clinical trials (Table 2).

Table2. Efficacy results of targeted therapies for advanced hepatocellular carcinoma

Molecular Targets /Agents	**Phase**	**Efficacy**	**Reference**

VEGF/VEGFR			
Sorafenib	Phase III SHARP sorafenib *vs* placebo	Median OS: 10.7 months *vs* 7.9 months	[58]
	Phase III (Asian)	Median OS:6.5 months *vs* 4.2 months	[59]
Sunitinib	Phase II	Median PFS: 3.9 months Median OS: 9.8 months	[65]
	Phase III sunitinib *vs* sorafenib	Median OS: 7.9months *vs* 10.2 months	
Brivanib	Phase II, first-line	Median PFS: 2.8 months Median OS: 10 months	[68]
	Phase II, second-line	Median PFS: 2.7 months Median OS: 9.8 months	[69]
	Phase III (BRISK-PS) brivanib vs placebo	Median OS: 9.4 months *vs* 8.3 months TTP: 4.2 months *vs* 2.7 months RR: 12% *vs* 2%	[70]

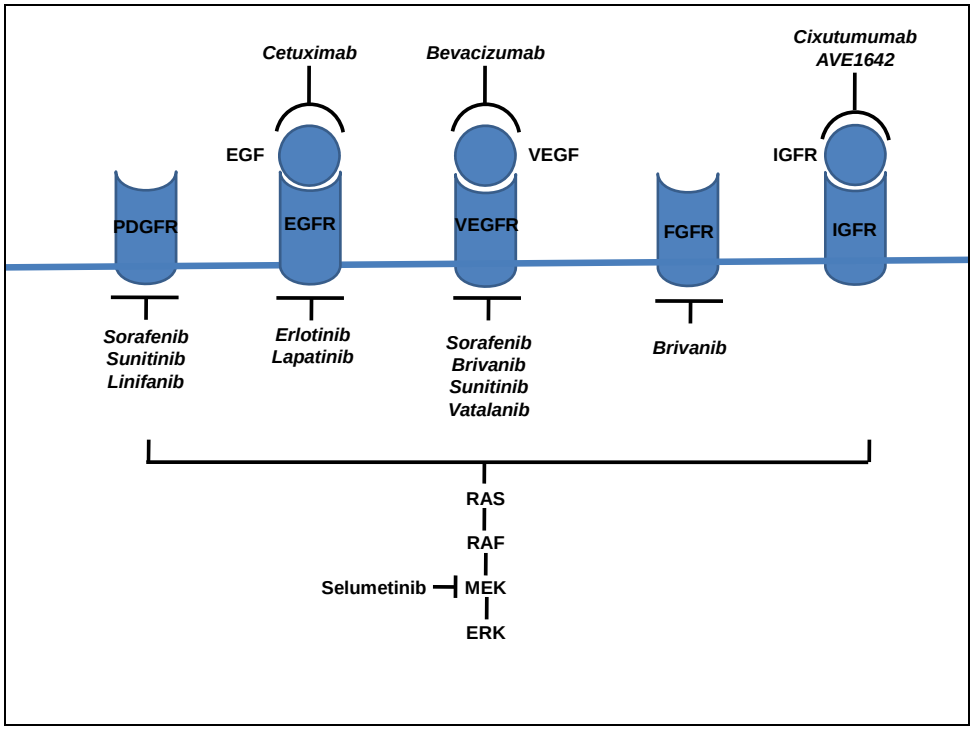
	Phase III (BRISK-FL) brivanib vs placebo	Median OS: 9.5 months *vs* 9.9 months TTP: 4.2 months *vs* 4.1 months RR: 12% *vs* 8%	[71]
Vatalanib (PTK787)	Phase I/II, combined with doxorubicin	OS: 7.3 months PFS: 5. 4months	[73]
Inifanib (ABT-869)	Phase II	TTP: 3.7 months Median OS: 9.7 months	[75]
Cediranib (AZD2171)	Phase II	Median OS: 5.8 months TTP: 2.8 months	[78]
EFGF/EGFR			
Cetuximab	Phase II	Median OS : 9.6 months Median PFS : 1.4 months	[81]
Erlotinib	Phase III (SEARCH) sorafenib/erlotinib vs	Median OS: 9.5 months *vs* 8.5 months TTP: 3.2 months *vs* 4.0 months	[83]

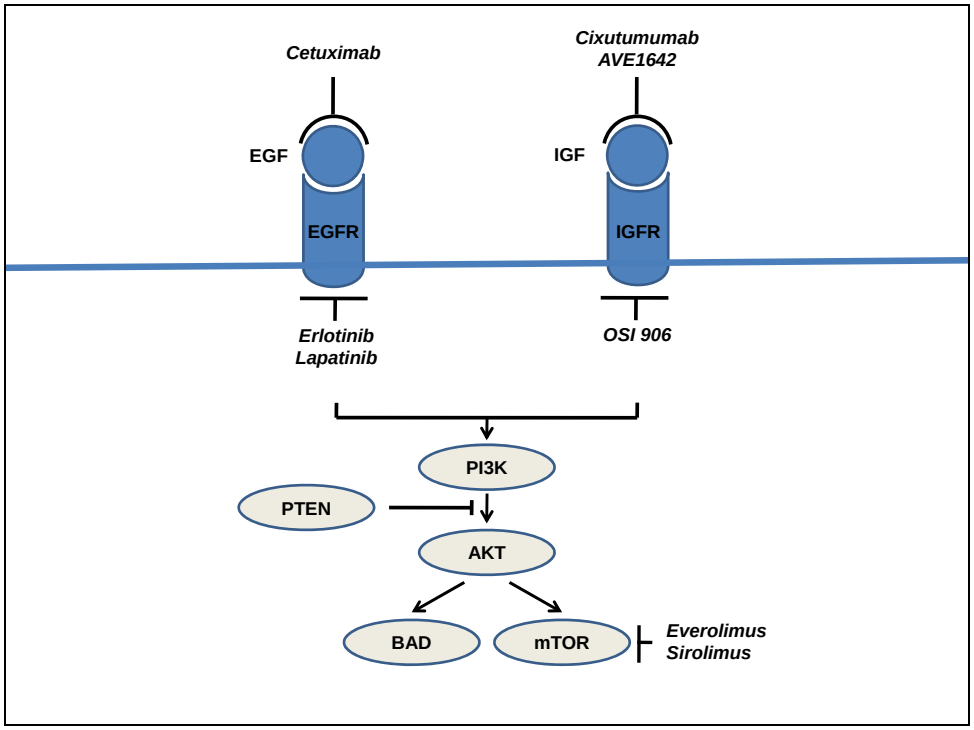
	orafenib/placebo		
Lapatinib	Phase II	Median PFS: 2.3 months	[85]
		Median OS: 6.2 months	
	Phase III	Median OS: 9.1 months vs 9.8 months	
	Lipatinib *vs* sorafenib		
IGF/IGFR			
Cituxumumab (IMC-A12)	Phase II	Median OS : 8 months	[93]
Ras/Raf/MEK/ERK			
Selumetinib (AZD6244)	Phase I/II	11 patients enrolled	[31]
		PR in 3, SD in6, PD in 2 patients	
PI3K/Akt/mTOR			
Everolimus	Phase I/II	Median PFS: 3.8 months	[37]
		Median OS: 8.4 months	
Sirolimus	Phase II	Median PFS : 15.3 weeks	[38]
		Median OS: 26.4 weeks	

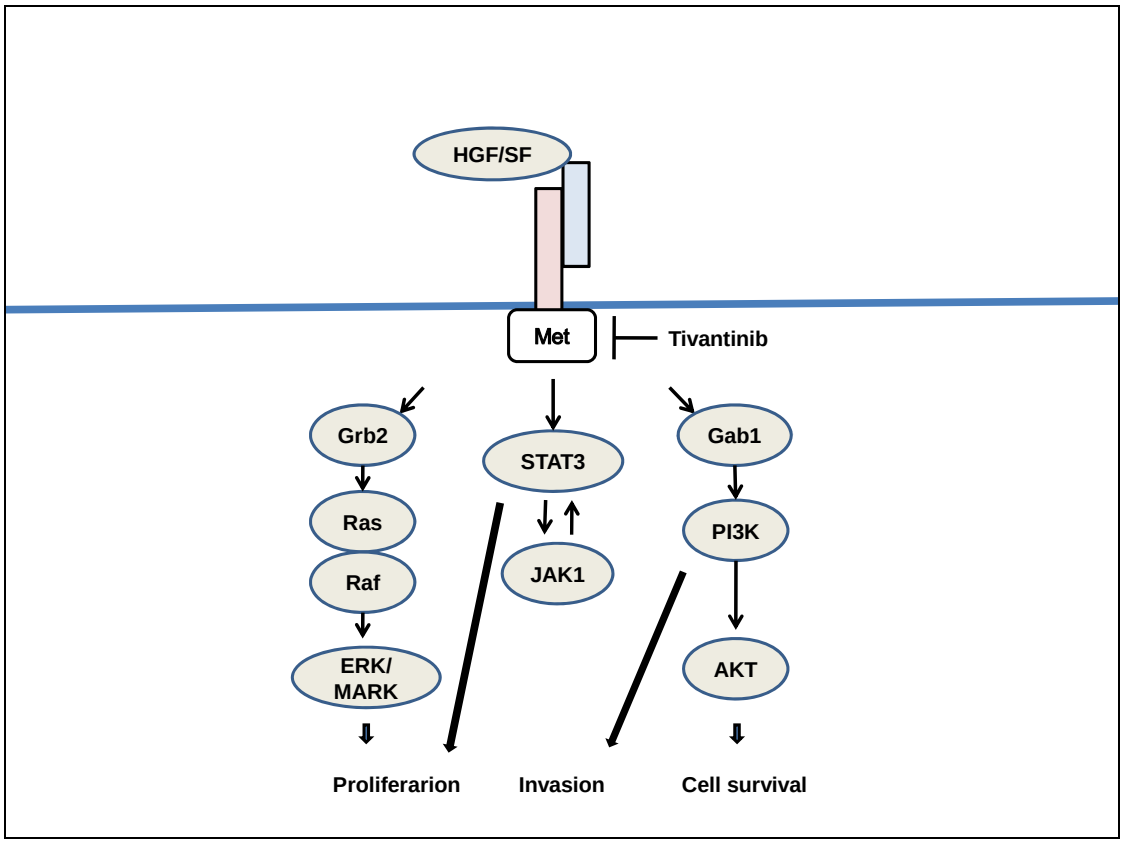
MET			
Tivantinib	Randomized Phase II		[100]
	Tivantinib vs placebo		
	ITT population	Median TTP: 6.9 weeks *vs* 6.0 weeks	
		Median OS: 6.6 months *vs* 6.2 weeks	
	c-Met high	Median TTP: 11.7 weeks vs 6.1 weeks	
		Median OS: 7.2 months vs 3.8 weeks	

(3) **The principal pathways and the precise site where the main agents exert its effect deserve individual figures rather than a single figure that results too simplistic.**

Answer) Authors thank for the reviewer's suggestion. We have made separate figures for main pathways and action site of agents as followings.


Cetuximab
Bevacizumab
Cixutumumab
AVE1642
EGF
VEGF
IGFR
PDGFR
EGFR
VEGFR
FGFR
IGFR
Sorafenib
Sunitinib
Linifanib
Erlotinib
Lapatinib
Sorafenib
Brivanib
Sunitinib
Vatalanib
Brivanib
RAS
RAF
Selumetinib
MEK
ERK


Cetuximab
Cixutumumab
AVE1642
EGF
IGF
EGFR
IGFR
Erlotinib
Lapatinib
OSI 906
PI3K
PTEN
AKT
BAD
mTOR
Everolimus
Sirolimus



(4) **Two minor specific points. NCT numbers are lacking for a number of ongoing trials. And the major role in HCV-related carcinogenesis is probably not viral proteins but the inflammation and tissue-repair program that occurs in the cirrhotic liver.**

Answer) We have inserted NCT number of ongoing trials.

As the reviewer's comment, we agree that the major role in HCV-related carcinogenesis is probably the inflammation and tissue-repair program that occurs in the cirrhotic liver. So we correct a sentence as following;

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From) The major role in hepatocarcinogenesis by HCV is played by viral proteins – core, NS3 and NS5A. HCV core protein can promote apoptosis or cell proliferation through interaction with p53 or via upregulation of Wnt-1 at the transcriptional level.

To) The contribution of HCV to hepatocarcinogenesis are mediated by viral proteins – core, NS3 and NS5A. HCV core protein can promote apoptosis or cell proliferation through interaction with p53 or via upregulation of Wnt-1 at the transcriptional level.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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