CENTRO DE INVESTIGACIÓN Y DE ESTUDIOS AVANZADOS
DEL INSTITUTO POLITÉCNICO NACIONAL

Prof. Dr. Lian-Sheng Ma
Company Editor in Chief
Baishideng Publishing Group Inc
Founder and CEO
California, USA

Department of Pharmacology
January 18, 2022.

Dear Prof. Dr. Lian-Sheng Ma,

We appreciate the comments you made to our manuscript titled "*Current and novel approaches in the pharmacological treatment of hepatocellular carcinoma*" by Villarruel-Melquiades *et al*. and we think they are a great contribution to improve our review.

Following the comments from the reviewers:

1. We checked the manuscript format to identify any errors.
2. We modified the Table 2 and 3 order.
3. We gave examples of useful drugs combinations when response to current treatment is poor.
4. We included more information about the upgrades between the new drugs and the existing drugs according to their pharmacological effects and its importance for identify novel drug targets.
5. The new corresponding references were added.

Please find below the response to the reviewers point by point. We believe that our manuscript has been improved. We hope you find our work suitable for publication in *World Journal of Gastroenterology*.

We will be looking forward for your comments.

Sincerely,

Dr. Javier Camacho

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Response to the reviewers.

The original comment from the reviewer is in bold letters. The response is in normal letters. The new or modified text included in the manuscript is in red letter and is red-tracked in the text file.

REVIEWER 1.

The current potential and new pharmacological methods, including immunotherapy, drug combination and drug repositioning, should be helpful to improve the prognosis of HCC patients. This review shows that combination therapy has more significant benefits for HCC patients than monotherapy. In addition, evidence has been provided that several non neoplastic drugs may be useful in the treatment of this cancer. In addition, compared with the current systemic treatment, immunotherapy has significant effects in some cases, and is promising for drug treatment of advanced liver cancer.

R. We thank the reviewer for his motivating comment.

REVIEWER 2.

At present, hepatocellular carcinoma (HCC) remains a malignant tumor with high morbidity and mortality in many countries. Unfortunately, systemic medication is not effective in most cases due to late diagnosis and tumor resistance. This review reviews the major pharmacological approaches, preclinical studies, and approved and ongoing liver clinical trials against HCC, which are expected to help improve the efficacy of HCC.

R. We thank the reviewer for his interesting comment. We believe it contributes substantially to improve our manuscript.

1. **Considering the time and cost of new drug research and development, the reuse of old drugs and drug combination therapy is a wise idea. Can the authors further refine the combination of specific drugs and the order of selection that can provide clinical options in situations where treatment response is poor?**

R. We gave examples of useful drugs combinations when response to current treatment is poor.

Currently, monotherapies are ineffective in fighting cancer mainly due to the development of resistance of cancer cells to available drugs [72], and preclinical findings may not be replicated in patients. For instance, the monoclonal antibody

bortezomib demonstrated promising antineoplastic activity in preclinical assays, but in humans, it did not show notable single-agent activity compared to sorafenib (Kim et al., 2012). Thus, more and better treatment options are needed for these patients, and drug combinations are one promising option. This strategy consists of simultaneously administering two or more drugs aimed at different cancer-specific drug targets and has shown significant benefits compared to monotherapy [73]. However, combining drugs must become a rational strategy that guarantees significant pharmacological responses, especially for those patients who either did not respond to current therapy or developed resistance.

Several tools have been developed for the identification of potentially useful drug combinations; these tools include dose–response matrices, RNA interference technology (RNAi), and the wide adaptation of clustered regularly interspaced short palindromic repeats (CRISPR) systems, as well as more novel techniques such as patient-derived xenograft (PDX) models and ex vivo primary cell and organoid models (Pemovska et al., 2018). For instance, Lim et al. used PDXs of HCC, PDX-derived organoids (PDXO) and a hybrid experimental-computational approach – namely, the quadratic phenotypic optimization platform (QPOP) – and found that the combination of the second-generation proteasome inhibitor ixazomib (Ixa) and the CDK inhibitor dinaciclib (Dina), which they tested in vitro and in vivo, is effective against HCC (Lim et al., 2022). Another example of the usefulness of these strategies is CRISPR–Cas9 combinatorial screening, a technique that accelerated the discovery of combination treatment with the approved drug ifenprodil (an NMDA receptor antagonist) and sorafenib as a new therapeutic alternative for advanced HCC (Xu et al., 2021).

Therefore, implementing the abovementioned strategies should aid the discovery of potentially useful drug combinations. In this manner, medical staff may have different choices and establish a selection order more suitable for each HCC patient in a personalized manner (Canzoneri et al., 2019; Suwinski et al., 2019).

New references:

Lim JJ, Hooi L, Dan YY, Bonney GK, Zhou L, Chow PKH, Chee CE, Toh TB, & Chow EKH. (2022). Rational drug combination design in patient-derived avatars reveals effective inhibition of hepatocellular carcinoma with proteasome and CDK inhibitors. Journal of Experimental & Clinical Cancer Research, 41(1), 249. https://doi.org/10.1186/s13046-022-02436-9

Kim GP, Mahoney MR, Szydlo D, Mok TSK, Marshke R, Holen K, Picus J, Boyer M, Pitot HC, Rubin J, Philip PA, Nowak A, Wright JJ, & Erlichman C. (2012). An international, multicenter phase II trial of bortezomib in patients with

hepatocellular carcinoma. Investigational New Drugs, 30(1), 387–394. https://doi.org/10.1007/s10637-010-9532-1

Xu F, Tong M, Tong CSW, Chan BKC, Chu HY, Wong TL, Fong JHC, Cheung MSH, Mak KHM, Pardeshi L, Huang Y, Wong KH, Choi GCG, Ma S, & Wong ASL. (2021). A Combinatorial CRISPR–Cas9 Screen Identifies Ifenprodil as an Adjunct to Sorafenib for Liver Cancer Treatment. Cancer Research, 81(24), 6219–6232. https://doi.org/10.1158/0008-5472.CAN-21-1017

Canzoneri R, Lacunza E, & Abba MC, (2019). Genomics and bioinformatics as pillars of precision medicine in oncology. Medicina, 79(Spec 6/1), 587–592.

Suwinski P, Ong C, Ling MHT, Poh YM, Khan AM, & Ong HS, (2019). Advancing Personalized Medicine Through the Application of Whole Exome Sequencing and Big Data Analytics. Frontiers in Genetics, 10, 49. https://doi.org/10.3389/fgene.2019.00049

2. **What are the upgrades between the new drugs and the existing drugs according to their pharmacological effects? Or is it just a different cellular pathway? If it is the latter, how do the authors view other possible unknown cellular pathways that contribute to the development and progression of HCC?**

R. We included more information about the upgrades between the new drugs and the existing drugs according to their pharmacological effects and its importance for identify novel drug targets.

One of the differences between repositioned drugs and existing drugs approved to treat HCC is that the latter target relatively few signalling pathways; in contrast, repositioned drugs have the enormous advantage of targeting a surprisingly wide variety of signalling pathways involved in liver carcinogenesis (Table 3). For instance, Nair et al. identified CDC20 as a marker of poor prognosis during the development of early and advanced HCC. Through molecular docking studies, it was determined that labetalol, a beta blocker, binds with high affinity to CDC20 (Nair, Saraswathy, et al., 2021), suggesting that the effect of labetalol against the development of HCC should be tested. The same research group further investigated this possibility by in vitro cytotoxicity studies, in which labetalol significantly inhibited the growth of the HepG2 cell line (Nair, Hema Sree, et al., 2021). Subsequent application of bioinformatics analysis tools to repositioned drugs provides an incredible advantage for the identification of unknown drug targets and signalling pathways potentially involved in liver carcinogenesis.

New references:

Nair G, Saraswathy GR, & Hema Sree, GNS, (2021). 48P Target mining and drug repurposing for hepatocellular carcinoma via bioinformatic and computational approaches. Annals of Oncology, 32, S19. https://doi.org/10.1016/j.annonc.2021.01.063

Nair G, Hema Sree GNS, Saraswathy GR, Marise VLP, & Krishna Murthy TP, (2021). Application of comprehensive bioinformatics approaches to reconnoiter crucial genes and pathways underpinning hepatocellular carcinoma: A drug repurposing endeavor. Medical Oncology, 38(12), 145. https://doi.org/10.1007/s12032-021-01576-w

REVIEWER 3.

This study reviews the new pharmacological approaches currently available against liver cancer, such as immune checkpoint inhibitors (ICIS), monoclonal antibodies against programmed cell death 1 (PD-1) and drug repurposing. The authors' arguments are sound, well-documented and well-documented, but there are still the following problems that suggest corrections.

R. We thank the reviewer for his valuable comment. The corrections are listed below.

1. **The full stop after the abstract should be corrected to a colon.**

R. We changed the full stop to a colon.

Abstract: Hepatocellular carcinoma (HCC) is one of the most lethal malignant…

2. **The use of abbreviations in the abstract should be minimized.**

R. We eliminated ICIs and PD-1 abbreviations from the original abstract.

3. **Table 2 and Table 3 should be placed after the first cited paragraph in the text. The current position of Tables is not clear enough.**

R. We modified the position of Table 2 and Table 3, this time we placed them immediately after they are mentioned in the main text.