Dear Editors of World Journal of Gastroenterology,

Please find enclosed our revised paper entitled Metronomic Capecitabine Inhibits Liver Transplant Rejection in Rats by Triggering Recipients' T Cell Ferroptosis (Manuscript NO.: 83404, Basic Study) and our response report to the editor. We have revised the manuscript based on the reviewer's comments and suggestions. We thank you, the editor, and the reviewers for your constructive comments, and hope that it meets the high standards of quality and excellence that your magazine is known for.
The following are point-by-point replies to the reviewer's concerns regarding our submitted paper revision.

Reviewer 1

Comment 1: The authors confirmed the effect of CAP on $CD3^+$ T cells. Among T cells, which of $CD4^+$ or $CD8^+$ cells are affected by CAP?

Response: We sincerely thank the reviewer's comments, and all comments are well taken. Our study was based on previous clinical studies that suggested metronomic capecitabine (CAP) might have immunosuppressive effects [1], but no studies focused on the CAP-mediated effects on immune cells. CAP can be metabolized to 5-fluroracil (5-FU) under a series of enzymes and transferred to fluorouracil deoxynucleotide to inhibit thymidine synthase so as to affect cell cycle and cell function [3], of which the most important target enzyme is thymidine phosphorylase (TP). Previous studies have demonstrated that lymphocyte express a high level of TP than other types [2]. Therefore, we thought that CAP might target lymphocytes and investigated its relationship to CAP's immunosuppressive effects. In this study, we investigated the effect of CAP on T cells, which are the most important effector cells in acute rejection after transplantation. Once rejection occurs, activated $CD4^+$ T cells can quickly differentiate into different subtypes such as Th1, Th2, Th17, and Treg, while $CD8^+$ T cells mainly differentiate into cytotoxic T cells, which directly cause graft injury [4]. Our ongoing research demonstrates that metronomic CAP could

regulate $CD4^+$ T cells activation and differentiation for those allograft recipients (heterotopic heart transplantation from Balb/c to C57). We use collected spleens from different groups (CAP-treated allograft vs. untreated allograft) to perform RNA-sequence and then analyze gene expressions, as shown in Fig 1A&B, GO and KEGG analysis reveals that metronomic CAP acts primarily on T cells especially $CD4^+$ T cells. In Fig 1C, metronomic CAP can significantly inhibit Th1 differentiation post transplantation. Taken together, metronomic CAP affects T cell especially $CD4^+$ T cell death and differentiation. Related research is still continuing, and we will further explore the effect of CAP on the differentiation and function of $CD4^+$ and $CD8^+$ T cells.

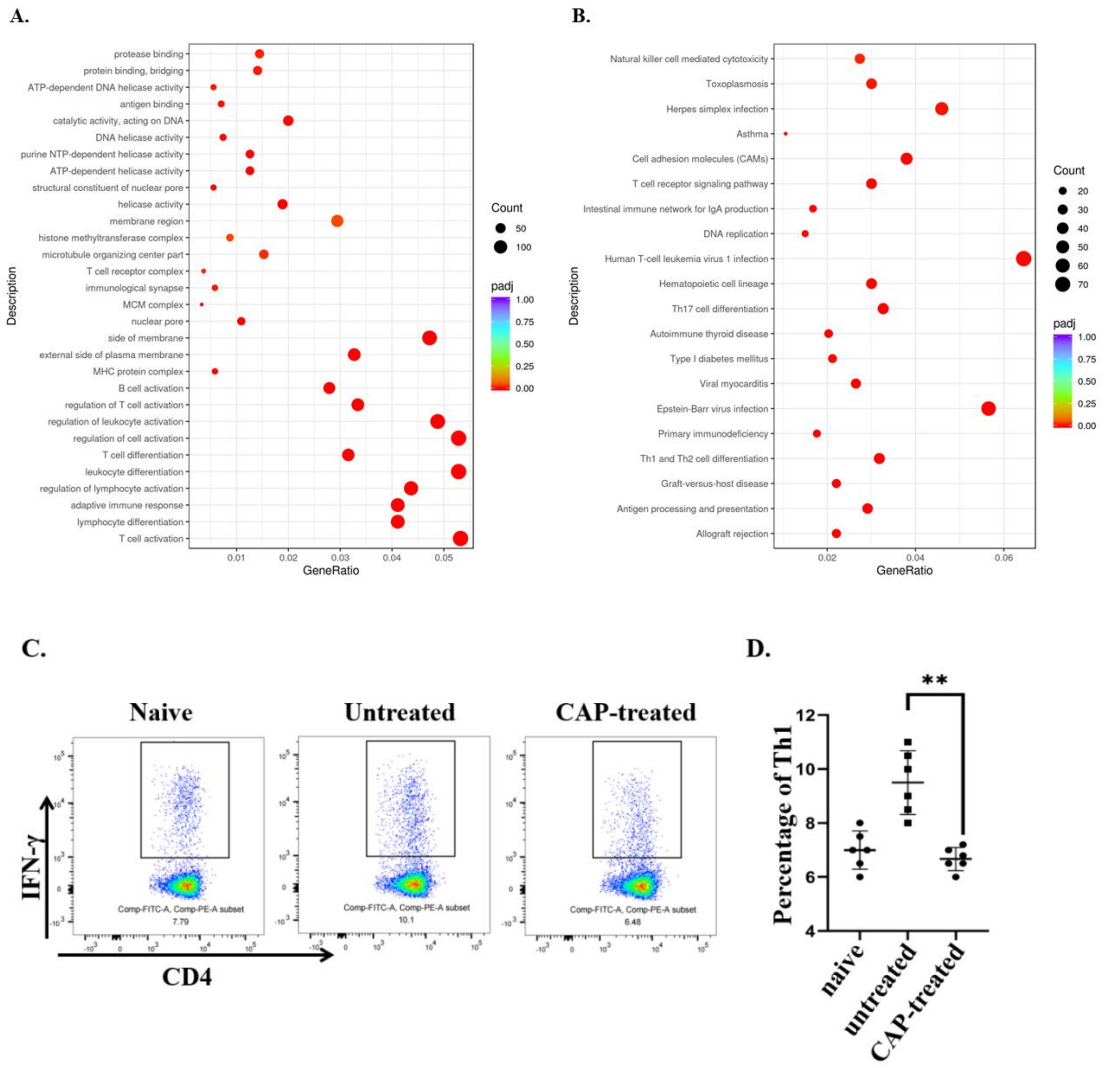

Figure 1. Metronomic capecitabine regulate $CD4^+$T cell differentiation after heterotrophic heart transplantation. (A) GO analysis. (B) KEGG analysis. (C)&(D)

Percentage of Th1 in the spleen.

(These data will be present in our another paper, here just for explanations to the reviewer's comments.)

Comment 2: It is recommended to change the expression from '5-Fu' to '5-FU'.

Response: We sincerely thank the reviewer's comment, which was amended accordingly. We have revised all expressions of '5-Fu' to '5-FU' in the manuscript. We hope this can meet the editor's and the reviewer's demands.

Reviewer2

Comment 1: The major concern in the study is related to the study design. More specifically, how it translates or reproduces clinical practice; this is because, apparently, there was no initial immunosuppression to the transplanted rats from the control group until 7 days. This is not a routine practice of any centre. Therefore, I wonder if the effects seen were just a consequence and what would be this effect on the administration of the gold standard (tacrolimus).

Response: We sincerely thank the reviewer's comment. The aim of this study was to determine whether CAP can inhibit rejection after liver transplantation. We will focus on its clinical practice and wish to provide a better immunosuppressive regimen in the coming future. Currently, single immunosuppressant is rarely used in transplant centres after liver transplantation. Combined immunosuppressants can achieve better immunosuppressive effects and avoid the common side effects of immunosuppressive agents. For example, mycophenolate mofetil (MMF) was initially shown to be insufficiently immunosuppressive at safe doses alone, whereas MMF, when synergizing with tacrolimus, was immunosuppressive enough to prevent allograft rejection and was well tolerated [5, 6]. We suggest that CAP can be incorporated into multi-agents combination immunosuppressive regimens and may benefit patients undergoing liver transplantation for hepatocellular carcinoma. Although CAP alone

cannot replace tacrolimus, it may provide a novel immunosuppressive regimen for transplant patients by combining it with tacrolimus or rapamycin.

In our ongoing experiment, we combined CAP with rapamycin to prevent rejection in rats, of which results are amazing. As shown in Fig2, metronomic CAP combined with rapamycin significantly reduces acute rejection and alleviates graft injury.

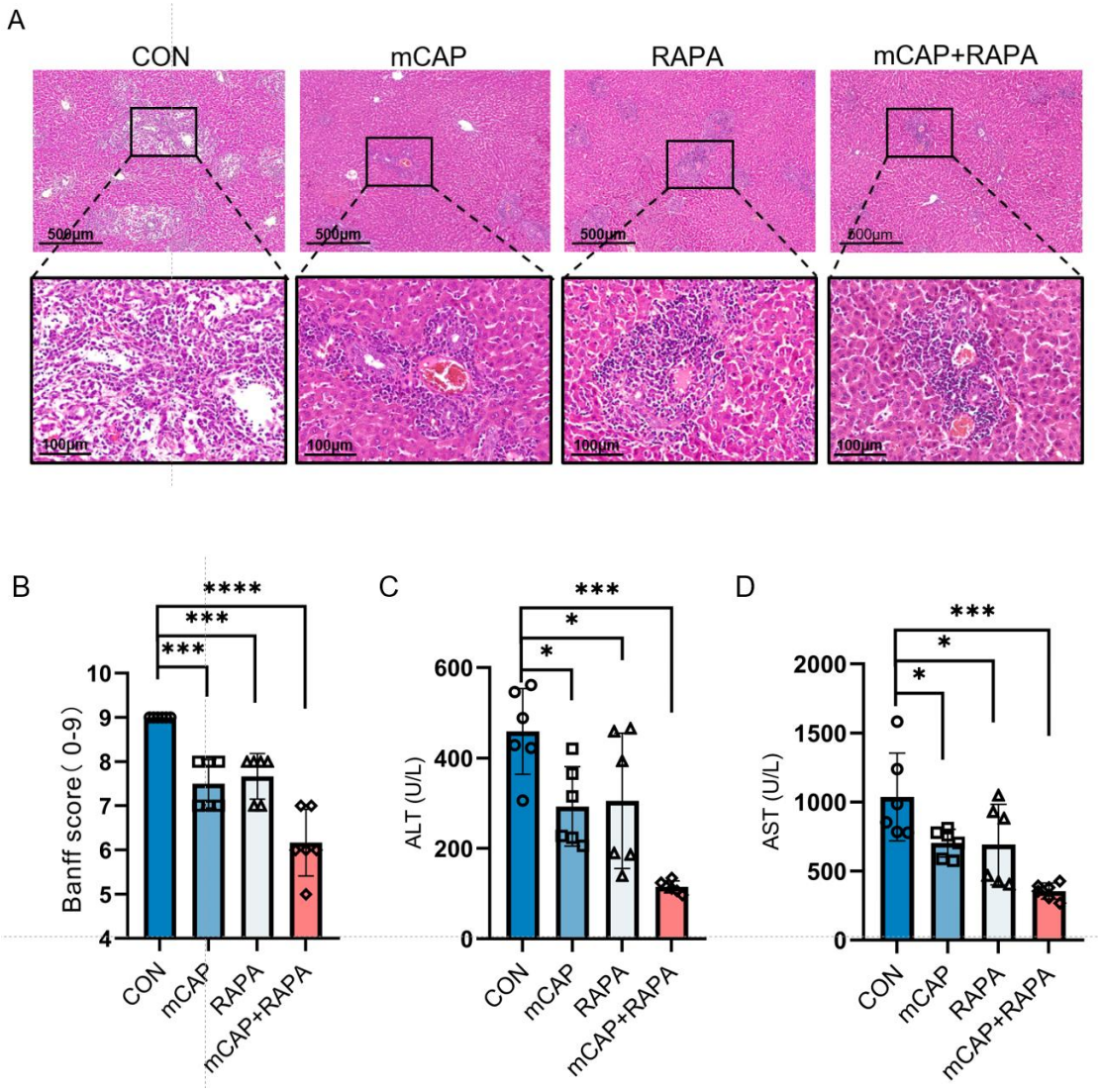

Figure 2. Metronomic CAP combined with rapamycin reduces acute rejection and alleviates graft injury. (A) Alanine transaminase (ALT). (B) Aspartate transaminase (AST). (C) Liver allograft tissue was stained with H&E (50× and 200×). Liver architecture was significantly injured in CON group, with a large number of

inflammatory cells infiltrated. In the metronomic capecitabine (mCAP) combined with rapamycin (RAPA) group, liver tissue destruction was significantly mild. (D) The severity of acute rejection was graded according to the Banff liver rejection criteria.

(These data will be present on our subsequent paper, here just for explanations to the reviewer's comments.)

Comment 2: The Conclusion section in the abstract and the main text must be amended. It must state that all the findings were verified experimentally in a rat model. The same applies to the core tip. The discussion must emphasise how feasible the utilisation of the medication is after liver transplantation, considering the adverse effects.

Response: The reviewer's suggestions were valuable. We declare that all findings were validated in the rat model and add the relevant description in the conclusion section of the abstract, the main text, and the core tip.

Metronomic CAP is a chemotherapy regimen in which low doses of CAP are given on a continuous, frequent schedule without long breaks [7, 8]. This treatment approach has shown promise in several types of cancer and is being increasingly used in clinical practice [9-11]. Overall, metronomic capecitabine is considered safe, with fewer side effects than traditional chemotherapy regimens. Many clinical and basic experiments have verified the safety of capecitabine, also including for patients with hepatocellular carcinoma or patients after liver transplantation [1, 12-14]. The discussion of the utilization possibility of the medication after liver transplantation has been added to the discussion section of this article.

Comment 3: Do the authors propose its use for the recurrence of HCC after liver transplantation? Or in cases of HCC transplants?

Response: We sincerely thank the reviewer's comment. Firstly, we did this trial based

on our previous paper review and we found that metronomic CAP can benefit these patients who suffered tumor recurrence and underwent liver transplantation. With no significant differences with the best supportive treatment for the tumor process, no rejection was seen during the whole therapy [1]. The phenomenon reminded us that metronomic CAP can benefit those patients. Secondly, a number of clinical trials in different centers have confirmed the safety and efficacy of metronomic CAP in patients with hepatocellular carcinoma, and it can be used in patients with hepatocellular carcinoma who are ineffective or intolerant to sorafenib [14-17]. Finally, more studies are in progress, and we will also construct a tumor-bearing mouse model to further investigate the effect and mechanism of capecitabine on hepatocellular carcinoma after liver transplantation.

REFERENCE

[1] M. Ravaioli, A. Cucchetti, A.D. Pinna, V. De Pace, F. Neri, M.A. Barbera, L. Maroni, G. Frega, A. Palloni, S. De Lorenzo, M.C. Ripoli, M.A. Pantaleo, M. Cescon, M. Del Gaudio, G. Brandi, The role of metronomic capecitabine for treatment of recurrent hepatocellular carcinoma after liver transplantation, Scientific reports 7(1) (2017) 11305.

[2] A. Yoshimura, Y. Kuwazuru, T. Furukawa, H. Yoshida, K. Yamada, S. Akiyama, Purification and tissue distribution of human thymidine phosphorylase; high expression in lymphocytes, reticulocytes and tumors, Biochimica et biophysica acta 1034(1) (1990) 107-13.

[3] N. Safinia, T. Vaikunthanathan, R.I. Lechler, A. Sanchez-Fueyo, G. Lombardi, Advances in Liver Transplantation: where are we in the pursuit of transplantation tolerance? European journal of immunology 51(10) (2021) 2373-2386.

[4] V. Ronca, G. Wootton, C. Milani, O. Cain, The Immunological Basis of Liver Allograft Rejection, Frontiers in immunology 11 (2020) 2155.

[5] H. Shimmura, K. Tanabe, K. Habiro, R. Abe, H. Toma, Combination effect of mycophenolate mofetil with mizoribine on cell proliferation assays and in a mouse heart transplantation model, Transplantation 82(2) (2006) 175-9.

[6] M.D. Vu, S. Qi, D. Xu, J. Wu, J. Peng, P. Daloze, S. Sehgal, B. Leduc, H. Chen, Synergistic effects of mycophenolate mofetil and sirolimus in prevention of acute heart, pancreas, and kidney allograft rejection and in reversal of ongoing heart allograft rejection in the rat, Transplantation 66(12) (1998) 1575-80.

[7] E. Pasquier, M. Kavallaris, N. André, Metronomic chemotherapy: new rationale for new directions, Nature reviews. Clinical oncology 7(8) (2010) 455-65.

[8] G. Bocci, R.S. Kerbel, Pharmacokinetics of metronomic chemotherapy: a neglected but crucial aspect, Nature reviews. Clinical oncology 13(11) (2016) 659-673.

[9] X. Wang, S.S. Wang, H. Huang, L. Cai, L. Zhao, R.J. Peng, Y. Lin, J. Tang, J. Zeng, L.H. Zhang, Y.L. Ke, X.M. Wang, X.M. Liu, Q.J. Chen, A.Q. Zhang, F. Xu, X.W. Bi, J.J. Huang, J.B. Li, D.M. Pang, C. Xue, Y.X. Shi, Z.Y. He, H.X. Lin, X. An, W. Xia, Y. Cao, Y. Guo, Y.H. Su, X. Hua, X.Y. Wang, R.X. Hong, K.K. Jiang, C.G. Song, Z.Z. Huang, W. Shi, Y.Y. Zhong, Z.Y. Yuan, Effect of Capecitabine Maintenance Therapy Using Lower Dosage and Higher Frequency vs Observation on Disease-Free Survival Among Patients With Early-Stage Triple-Negative Breast Cancer Who Had Received Standard Treatment: The SYSUCC-001 Randomized Clinical Trial, Jama 325(1) (2021) 50-58.

[10] L.H. Simkens, H. van Tinteren, A. May, A.J. ten Tije, G.J. Creemers, O.J. Loosveld, F.E. de Jongh, F.L. Erdkamp, Z. Erjavec, A.M. van der Torren, J. Tol, H.J. Braun, P. Nieboer, J.J. van der Hoeven, J.G. Haasjes, R.L. Jansen, J. Wals, A. Cats, V.A. Derleyn, A.H. Honkoop, L. Mol, C.J. Punt, M. Koopman, Maintenance treatment with capecitabine and bevacizumab in metastatic colorectal cancer (CAIRO3): a phase 3 randomised controlled trial of the Dutch Colorectal Cancer Group, Lancet (London, England) 385(9980) (2015) 1843-52.

[11] V.M. Patil, V. Noronha, A. Joshi, S. Dhumal, M. Mahimkar, A. Bhattacharjee, V. Gota, M. Pandey, N. Menon, A. Mahajan, N. Sable, S. Kumar, K. Nawale, S. Mukadam, B. Solanki, S. Das, V. Simha, G. Abraham, A. Chandrasekharan, V. Talreja, D.S. H, S. Srinivas, L. Kashyap, S. Banavali, K. Prabhash, Phase I/II Study of Palliative Triple Metronomic Chemotherapy in Platinum-Refractory/Early-Failure

Oral Cancer, Journal of clinical oncology : official journal of the American Society of Clinical Oncology 37(32) (2019) 3032-3041.

[12] G. Brandi, F. de Rosa, V. Agostini, S. di Girolamo, P. Andreone, L. Bolondi, C. Serra, C. Sama, R. Golfieri, A. Gramenzi, A. Cucchetti, A.D. Pinna, F. Trevisani, G. Biasco, Metronomic capecitabine in advanced hepatocellular carcinoma patients: a phase II study, The oncologist 18(12) (2013) 1256-7.

[13] A. Granito, S. Marinelli, E. Terzi, F. Piscaglia, M. Renzulli, L. Venerandi, F. Benevento, L. Bolondi, Metronomic capecitabine as second-line treatment in hepatocellular carcinoma after sorafenib failure, Digestive and liver disease: official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver 47(6) (2015) 518-22.

[14] A. Casadei Gardini, F. Foca, M. Scartozzi, N. Silvestris, E. Tamburini, L. Faloppi, O. Brunetti, B. Rudnas, S. Pisconti, M. Valgiusti, G. Marisi, F.G. Foschi, G. Ercolani, D. Tassinari, S. Cascinu, G.L. Frassineti, Metronomic capecitabine versus best supportive care as second-line treatment in hepatocellular carcinoma: a retrospective study, Scientific reports 7 (2017) 42499.

[15] Y.Z. Patt, M.M. Hassan, A. Aguayo, A.K. Nooka, R.D. Lozano, S.A. Curley, J.N. Vauthey, L.M. Ellis, Schnirer, II, R.A. Wolff, C. Charnsangavej, T.D. Brown, Oral capecitabine for the treatment of hepatocellular carcinoma, cholangiocarcinoma, and gallbladder carcinoma, Cancer 101(3) (2004) 578-86.

[16] Y. Xia, Y. Qiu, J. Li, L. Shi, K. Wang, T. Xi, F. Shen, Z. Yan, M. Wu, Adjuvant therapy with capecitabine postpones recurrence of hepatocellular carcinoma after curative resection: a randomized controlled trial, Annals of surgical oncology 17(12) (2010) 3137-44.

[17] F. Trevisani, G. Brandi, F. Garuti, M.A. Barbera, R. Tortora, A. Casadei Gardini, A. Granito, F. Tovoli, S. De Lorenzo, A.L. Inghilesi, F.G. Foschi, M. Bernardi, F. Marra, R. Sacco, G.G. Di Costanzo, Metronomic capecitabine as second-line treatment for hepatocellular carcinoma after sorafenib discontinuation, Journal of cancer research and clinical oncology 144(2) (2018) 403-414.