Responses to reviewers' comments (Manuscript ID: 80205, Minireviews)

Dear editor,

Thank you for your rapid response to our manuscript entitled "**Nanomedicine-based multimodal therapies: recent progress and perspectives in colon cancer" (Manuscript ID: 80205).** We are particularly grateful to you and the reviewers for your detailed and constructive comments, which are all valuable and very helpful for revising our paper and the important guiding significance to our research. We have studied revision comments carefully and tried to address all concerns. The relevant changes have been marked with red word in the manuscript. The detailed revisions and explanations to each point raised by the reviewers are demonstrated as following.

We hope this manuscript has improved satisfactorily. Thank you very much for your effort in evaluation of our manuscript.

Sincerely yours

Prof. Dr. Dawei Gao

Applying Chemistry Key Lab of Hebei Province, Department of Bioengineer, Yanshan University

No.438 Hebei Street, Qinhuangdao, 066004, P. R. China

Tel: (+86)3358387553

Email: dwgao@ysu.edu.cn

Reviewer #1

Scientific Quality: Grade B (Very good)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The article reviewed narratively about applying nano-medicine to colonic cancer. It is an exciting report. Although several other nano-medicines for cancers have been reported, there have been no reports focusing on colonic cancer previously. Therefore, the contents of the article are fit for the journal. However, there are some concerns about this article.

Q1: *Please describe the literature search methods. The authors could make the flowchart as a figure.*

Answer to Q1: We sincerely appreciate the valuable comments of reviewer. Firstly, we analyze the research question and clarify the search topic, e.g., colon cancer. Then, we search the major databases and enter the keywords and filter the relevant literature to obtain the original literature. We analyze the characteristics of each literature and summarize them to write the article. The flowchart of literature search methods was drawn as follows:

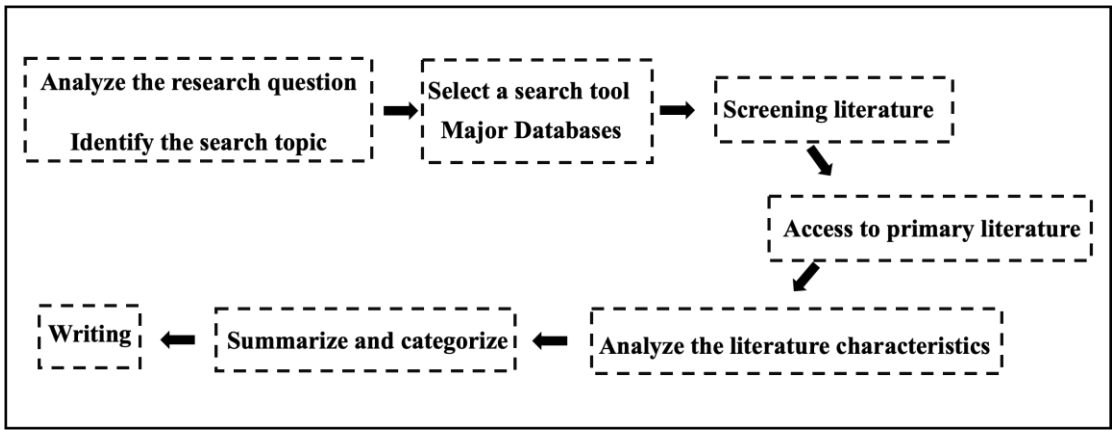

Figure R1. The flowchart of literature search methods

Q2: *The number of references is small for a full review article.*

Answer to Q2: We express our honest thanks for your useful comments. The positioning of this review is minireview. Following your valuable suggestion, we have added the references of each part respectively. There are 99 references for this minireview after addition. The additional references of the manuscript were highlighted with red words.

Q3: *Is there applying artificial intelligence for nano-medicine for colonic cancer?*

Answer to Q3: We appreciated the reviewer's professional comment. The applications of artificial intelligence in colon cancer treatments mainly includes two aspects. The first is screening, localization and differential diagnosis of colon cancer [1-4]; The second is the use of clinicopathological data to determine the comprehensive treatment effect of colon cancer patients and to predict recurrence [5-7]. The contribution of artificial intelligence to nanomedicines for colon cancer treatment has not been reported yet, but artificial intelligence has a wide prospect in the field of nanomedicine design. Many literatures have reported the optimization of nanodrug combinations and prediction of nanoparticle delivery to tumors for better therapeutic effects through artificial intelligence [8-12], and we predict that the future direction of synthesizing anti-colon cancer nanomedicines through artificial intelligence-assisted synthesis will also have great application significance.

References

[1] Zacharakis G et al. World Journal of Gastroenterology, 2022, 24: 028.

[2] Fang Y et al. Computational Intelligence and Neuroscience, 2022: 4217573.

[3] Troyanskaya O et al. Nature Cancer. 2020, 1(2): 149-152.

[4] Wang X et al. European Journal of Surgical Oncology 2022.

[5] Duan X et al. Computational Intelligence and Neuroscience, 2022: 2180788.

[6] Reichling C et al. Journal of Clinical Oncology, 2019: 3574-3574.

[7] Kamei Y et al. Oncology, 2021, 99(5): 318-326.

[8] Ho D et al. Nanoscale Horizons, 2019, 4(2): 365-377.

[9] Serov N et al. Advanced Drug Delivery Reviews, 2022, 184: 114194.

[10] Villa Nova M et al. Frontiers in Digital Health, 2022.

[11] Liu L et al. Nanoscale, 2021; 13(46): 19352-19366.

[12] Lin Z et al. Int J Nanomedicine 2022; 17: 1365-1379.

Q4: *Please state the primary and secondary outcomes at the end of the introduction.*

Answer to Q4: We appreciated the reviewer's professional comment. We described the primary and secondary results at the end of the introduction. The relative contents have added into the Introduction with red words and listed as follows.

Among them, NDDSs was widely used to improve the therapeutic effect due to its characteristics of improving the water solubility of chemotherapy drugs, prolonging the blood circulation time, targeted drug delivery, small side effects, reversing multi-drug resistance, etc.; PDT was a new treatment for colon cancer that uses specific wavelengths of light to excite photosensitizers. In the excited state, the photosensitizers transfer energy or electrons to the surrounding oxygen, thus producing singlet oxygen and killing cancer cells; Radiation therapy could cause DNA strand break of tumor cells under X-ray irradiation, and produce high cytotoxic free radicals to damage colon tumor cells; Compared with other ROS therapies, CDT had the advantages of stronger in situ catalytic ROS generation, tumor specificity and deep tissue penetration, and does not require additional stimulation, providing a new idea for the future treatment of colon cancer; Gas therapy had the advantages of enhancing drug release, chemotherapy, and synergistic therapy with other therapies to increase therapeutic effect, but its application in colon cancer needs to be explored more widely; Immunotherapy had been widely used in the treatment of colon cancer. The immunogenicity of tumor cells was activated by means of photothermal and ROS, and immunoadjuvant was used to reduce the

immunosuppression in the tumor microenvironment and enhance the immune effect. These strategies provide new ideas for the clinical treatment of colon cancer.

Reviewer #2

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The authors wrote a review article about the nanomedicine-based multimodal therapies in colon cancer. As colon cancer is an important GI diseaes and also an important malignant disesae. The development of new treatment is promising and benefitial for the patients. The article is well written and educational. There are several points that may need further clarification.

Q1: *There's a generalized question, how could these nano-medicine been distributed into specific colon cancer cell, by IV, PO or colonoscopy topical treatment? Nanoparticles may also elicit toxicity by disrupting membranes within the cell, how about the safety profile and potential adverse events of Nano-medicine in colon cancer treatment.*

Answer to Q1: We sincerely appreciate the valuable comments of reviewer. Nanomedicine is usually injected intravenously into the body. Due to the abnormal vascular structure of solid tumors and the lack of lymphatic reflux in the tumor area, nanomedicine can reach and stagnate in the tumor tissue, which is called enhanced permeability and retention (EPR) effect[1]. In addition, the nanomedicine modified by the tumor target groups (hyaluronic acid[2], folic

acid[3], and tumor homologous cell membrane[4]) can also accumulate highly in the tumor region. The reason is that these target groups are able to combine with the tumor surface receptors. A number of animal experimental studies have proved that the nanomedicine has little side effect on the heart, liver, spleen, lung, and kidney of mice. Therefore, the nanomedicine show great biosafety and prospects[5-7].

References

[1] Rui Sun, et al. Advanced Drug Delivery Reviews, 2022, 191: 114614.

[2] Huiyuan Zhang, et al. Advanced Functional Materials, 2018, 1802830.

[3] Xuan Wang, et al. Small, 2021, 2100130.

[4] Xue-Feng Bai, et al. ACS Nano, 2022, 16: 18555.

[5] Ying Chen, et al. Advanced Functional Materials, 2022, 2209927.

[6] Jingjing Wang, et al. Journal of the American Chemical Society, 2022, 144: 19884.

[7] Xin Chang, et al. Nano Letters, 2022, 22: 8321.

Q2: *The authors talked about anti-inflmmatory drugs, natural product's role in colon cancer, however, are they nano-medicine? Please describe more specifically and may delete them if they're not nanomedicine. Similiar question also exist in the role of metronidazole in chemotherapy.*

Answer to Q2: We appreciated the reviewer's professional comment. Drugs such as dexamethasone, curcumin and metronidazole have been shown to be effective in the treatment of colon cancer. In order to improve their efficacy, researchers have designed different nanodrug delivery systems to load these drugs. The drug-loaded nanodrug delivery systems are treated as nanomedicine.

Q3: *In phototherapy part, H2S was also frequently noted in patients with irritable bowel syndrome and inflammatory bowel syndrome, is this treatment toward H2S*

focus only on colon cancer treatment or also other GI disease.

Answer to Q3: Thank you for your nice question. Treatment of H_2S is not just focused on colon cancer. H_2S is involved in the occurrence and development of breast cancer, liver cancer, stomach cancer and other malignant tumors. Lower concentration of endogenous H_2S can promote the growth of cancer cells, while higher concentration of H_2S can inhibit the development of tumors[1]. Chen et al. designed multifunctional iron sulfide nanoparticles to produce H_2S in situ, inhibit the activity of cytochrome c oxidase in pancreatic cancer cells, and inhibit tumor growth[2]. Therefore, H_2S mediated tumor therapy is universal.

References

[1] Haonan Li, et al. Redox Biology, 2020, 34: 101564.

[2] Zebin Yang, et al. Advanced Functional Materials, 2020, 31: 2007991.

Q4: *The authors mentioned many nanomedicine, is there any therapy which had been available in clinical practice or completed phase III clinical trial, the authors may can make a summary table of the treatments currently on phase I to phase III clinical trial, especially those on phase III clinical trial which is predicted to be on market soon.*

Answer to Q4: Thank you for your patient suggestion. Concerning colorectal cancer, the number of clinical trials involving nanomedicine was shown in Table 1.

Table 1 Nanoparticles in a colorectal cancer clinical trial

Intervention/Treatment	Condition or Disease	Official Title	ClinicalTrials.gov Identifier
SN-38 liposome	Colorectal Cancer	Phase II Trial of LE SN38 in Patients with Metastatic Colorectal Cancer After	NCT00311610

		Progression on Oxaliplatin	
CPX-1 (Irinotecan HCl: Floxuridine) Liposome Injection	Colorectal Neoplasms	Multicenter, Open-Label, Phase 2 Study Of CPX-1 (Irinotecan HCl: Floxuridine) Liposome Injection in Patients With Advanced Colorectal Carcinoma	NCT00361842
Bupivacaine liposome suspension	Colon Cancer	The Effect of Exparel on Post-Operative Pain and Narcotic Use After Colon Surgery	NCT02052557
Liposomal bupivacaine Bupivacaine/epinephrine /dexamethasone	Colorectal Cancer	A Prospective Randomized Trial of Transversus Abdominis Plane (TAP) INtraoperative Block with Bupivacaine/Dexamethasone against Liposomal Bupivacaine (Exparel®): the TINGLE Trial	NCT03723447
Pegylated Liposomal Mitomycin-C Lipid-based Prodrug	Metastatic Colorect	A Phase I, Dose-Escalating, Safety Study of an	NCT01705002

	al Cancer	Intravenously Administered Pegylated Liposomal Mitomycin-C Lipid-based Prodrug (PL-MLP, PROMITIL) in Cancer Patients with Solid Tumors.	
Liposome Irinotecan	Metastatic Colorectal Cancer	Phase I and Pharmacokinetic Study of Biweekly PEP02 (Liposome Irinotecan) in Patients with Metastatic Colorectal Cancer Refractory to First-line Oxaliplatin-based Chemotherapy	NCT00940758
TKM-080301	Colorectal Cancer with Hepatic Metastases	A Phase 1 Dose Escalation Study of Hepatic Intra-Arterial Administration of TKM 080301 (Lipid Nanoparticles Containing siRNA Against the PLK1 Gene Product) in Patients With Colorectal, Pancreas, Gastric,	NCT01437007

Q5: *Metastatic colon cancer is the main cause of colon cancer related mortality, the authors mentinoned about PDL-L1 blocking ability nanoparticles, what is its diffrence comparing with current anti-PD1 treatment? Is there any OTHER nanomedicne focusing on cancer cells which easily metastate or metastatic cancer? Will the metastatic site influence the application of nanomedicine?*

Answer to Q5: Thank you for your nice question. The current anti PD-1 therapy is to block the PD-1/PD-L1 pathway by combining the PD-1 antibody specifically with the PD-1 expressed on T lymphocytes, thereby relieving the immunosuppression of T lymphocytes and promoting T lymphocytes to kill tumor cells. Nanoparticles with PD-L1 blocking ability combine with PD-L1 expressed on tumor cells to block PD-1/PD-L1 pathway and achieve cancer treatment. The current anti PD-1 therapy has some limitations: anti PD-1 therapy may destroy the balance of immune tolerance and cause a series of immune related adverse reactions. Meanwhile, the current anti PD-1 treatment is generally combined with systemic chemotherapy drugs, and the adverse reaction caused by systemic administration is also a major reason limiting its clinical application. Nanoparticles with PD-L1 blocking ability can cause less autoimmune side effects, significantly reducing the costs related to transportation and storage. Nanoparticles can also be combined with chemotherapy, phototherapy and other treatment methods to achieve precise targeted treatment, enhance efficacy and reduce systemic toxicity. Nanodrugs have broad application prospects in inhibiting tumor invasion and metastasis. By modifying the nano drugs targeting ligands or cell membranes, the circulation time of drugs in the blood can be prolonged, and more drugs can accumulate in the primary and metastatic tumor sites[1]. Meanwhile, nano drug

delivery system can load gene drugs, regulate tumor cell proliferation and apoptosis related genes, and inhibit tumor metastasis[2]. Functionalized nanoparticles can also interfere with epithelial mesenchymal transformation and reduce tumor metastasis[3]. Nanodrugs can activate immune pathways, trigger systemic anti-tumor immune response, and overcome tumor metastasis and recurrence[4]. The metastasis and recurrence of cancer will promote the continuous development of nanomedicine. Concerning different cancers, more reasonable nano drugs will be designed to achieve precise treatment of cancer.

References

[1] Debarshi Banerjee, et al. Trends in Pharmacological Sciences, 2019, 40: 403.

[2] Peng Wang, et al. Advanced Science, 2017, 4: 1700175.

[3] Jiamao Luo, et al. Bioactive Materials, 2022, 13: 179.

[4] Xuan Wang, et al. Nature Communications, 2022, 13: 5685.

Q6: *The authors stated that "Nanomedicine-based immunotherapy has been widely used in the treatment of colon cancer." However, the reference above are most cell and animal model. Please specify it.*

Answer to Q6: We express our honest thanks for your useful comments. We are very sorry for the mistakes in this manuscript. The relevant changes have been marked with red words in manuscript and listed as follows.

Immunotherapy based on nanomedicine has been widely used in cell and animal models, and has good anti-tumor efficacy. It will certainly become one of the most potential therapeutic means in clinical treatment.