Peer Review File

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Reviewer A

Comment 1: Lines 5/6 of the abstract: the statement is wrong since telomerase activation is only responsible for telomere maintenance and ongoing cell proliferation. The molecular basis of tumour development are molecular events such as the activation of oncogenes and the inactivation of tumour suppressor genes by mutation and generation of genomic instability. Moreover, the term "cancerization" does not exist in English. Please correct the wrong statement.

Reply 1: We have modified our text as advised (see Page 1, line 6).

Changes in the text: "is the necessary molecular basis for cell cancerization" => "maintains the length of telomere"

Comment 2: Line 15: remove "and" in front of "etc." Also, add "the" in front of FDA and explain abbreviation.

Reply 2: We have modified our text as advised (see Page 1, line 14-15).

Changes in the text: "…antibodies, etc. Some of them have been approved for undergoing clinical trials by the Food and Drug Administration(FDA) for the treatment of…"

Comment 3: Line 18: Please add "phase" in front of "III", otherwise content and grammar are wrong.

Reply 3: We have modified our text as advised (see Page 1, line 18).

Changes in the text: "…even entered into phase III clinical trials.…"

Comment 4: Line 20: the term "unition" does not exist, replace with "combination". Also, replace "targeted with "targeting", otherwise grammar is wrong.

Reply 4: We have modified our text as advised (see Page 1, line 20).

Changes in the text: "…including combination with other vaccines targeting human telomerase reverse transcriptase(hTERT), traditional chemotherapy .…"

Comment 5: Line 29: it should be "FIELD"

Reply 5: We have modified our text as advised (see Page 1, line 29).

Changes in the text: "…in this field"

Comment 6: The statement in line 34 contradicts that in line 36 which states that cancer cells are not directly targeted while the former claims that the immune system is strengthened by drugs that target cancer cells. Please correct the first wrong statement and reconcile the contradiction.

Reply 6: We have modified our text as advised (see Page 2, line33-34).

Changes in the text: “strengthens the patient's immune system by utilizing drugs to eliminate cancer cells” => “aims to arm patients with cancer-fighting immunity ”

Comment 7: Line 47: Please describe in more detail how “specific killing of cancer cells” works, what mechanisms are involved etc. Perhaps include an additional figure.

Reply 7: For the comprehensive consideration of comments 123 and word count, we have made major revisions to this paragraph. Please see Page 2, line39-55.

Changes in the text: We write the paragraph 2 from the following order: classification of anticancer immunity, innate and adaptive immunity, cellular immunity and humoral immunity and their simple principles (see Figure 1 for details), and finally the importance of TAAs for immune response.

Comment 8: Line 51: “with THE help…”

Reply 8: Because we have made major revisions to the paragraph 2, please refer to comment 7 or the revised paragraph 2.

Changes in the text: Please refer to comment 7 or the revised paragraph 2.

Comment 9: Line 53:replace “in” with “AND”

Reply 9: Because we have made major revisions to the paragraph 2, please refer to comment 7 or the revised paragraph 2.

Changes in the text: Please refer to comment 7 or the revised paragraph 2.

Comment 10: Lines 61/62: see number 1 above, same mistake.

Reply 10: We have modified our text as advised (see Page 2, line61).

Changes in the text: “…which is the necessary for cellular carcinogenesis.”

Comment 11: Line 66: please remove the Chinese symbols, it should presumable be figure 1?!

Reply 11: Maybe the format changes after converting pdf, here it should be Figure 1.We have modified our text as advised (see Page 3, line66).

Changes in the text: “…cancer immunotherapy[10] (Figure 1) .”

Comment 12: Line 67: When you write about many studies you need to cite at least a few of them.

Reply 12: We have modified our text as advised (see Page 3, line67).

Changes in the text: “…many preclinical and clinical studies[11],[12].”

Comment 13: Line 69: better replace 1st “have” with “include”.

Reply 13: We have modified our text as advised (see Page 3, line69).

Changes in the text: “…approaches include some agents…”

Comment 14: Line 73: the statement requires a reference.

Reply 14: We have modified our text as advised (see Page 3, line79).

Changes in the text: “…are currently recruiting[3],[14].”

Comment 15: Please briefly explain the differences and criteria for phase I, II and III trials since not every reader might be aware of it.

Reply 15: We have modified our text as advised (see Page 3, line70-76).

Changes in the text: “…Phase I clinical trials include preliminary clinical pharmacology and human safety evaluation tests to provide a basis for the formulation of a dosing regimen. Phase II clinical trials assessing the safety and tolerability of the drug, are usually larger than phase I studies. Phase III clinical trials are conducted in a larger and often more diverse target population in order to demonstrate efficacy and to estimate the incidence of common adverse reactions[13].…”

Comment 16: Line 79: Table 3: Why do you have table 3 before table 1? Please structure and number properly. The table looks messy and is not informative. Rows 1 and 4, right column: What do the numbers mean here? Row 3, left: there are notes for supplemental material which should not be included here and, row 3, right refers to “supplemental materials” which are not included in the review, thus please provide them here or attach supplemental material. Row 5 right: “literature” should only be used in singular, thus, remove “s” at the end. Last row, right: It should be “NOT applicable”. Perhaps you should rather give the information as text than as a table.

Reply 16: Yes, we took a look again, and found it was really a little messy here. Since we saw “…The Methods section should include a completed table…” in the “Guidelines for Authors-2.2.3 Narrative Review” of the journal system, we added this table. Now we have browsed published narrative reviews basically does not have this table, so we decided to remove it. If there is anything wrong, please contact us again.

Changes in the text: We removed the table.

Comment 17: Line 80: Numbering should start at the beginning with “introduction” and not here in the middle. Please amend.

Reply 17: We have modified our text as advised (see Page 3, line83).

Changes in the text: We put “1 Therapeutic vaccines” at the beginning of the line.

Comment 18: Table 1 should not be after table 3. Please amend and re-number accordingly.

Reply 18: Because we removed the original "Table 3", this is still "Table 1" (see Page 4 , line 93).

Changes in the text:"…vaccines to date (Table 1)."

Comment 19: Line 83: Before describing any “application principle” the principles of design, generation of vaccines, types of vaccines etc should be described in some details for the reader.

Reply 19: We added a paragraph between "1 Therapeutic vaccines" and "1.1 Peptide vaccines" to introduce the definition, types of therapeutic vaccines, and the main types of vaccines targeting hTERT(see Page 3-4, line84-90). And we removed the "The application principle". Please see Comment 20 for more details.

Changes in the text: “Therapeutic vaccines，as an active immunotherapy, aims to using TAAs to induce durable and specific anti-tumor immune responses, which are well tolerated and almost no dose-

related toxicity[15]. Current therapeutic vaccines include peptide vaccine, mRNA vaccine, cell vaccine, virus vector vaccine and so on. For hTERT, the main types of therapeutic cancer vaccines are peptide vaccines and DNA vaccines, which are steadily entering clinical trials and have achieved good clinical therapeutic effects[16]. ”

Comment 20: Lines 83/84: I don’t think that you describe the process correctly: Before a vaccine can be applied to humans (volunteers) there need to be other steps such as in vitro (cell-based) as well as animal experiments as preclinical steps) to be successfully performed in order to obtain any ethical approval. You correctly describe this below in lines 98/9. So perhaps you should rather mention the application into mice or other animal models prior to going into humans, first. Also, the passage “designed by researchers” seems superficial, see remarks above about the many steps required prior to any use in humans.

Reply 20: We have modified our text as advised (see Page 3, line69).

Changes in the text:"After investigated in vitro (cell-based) as well as in vivo (animal experiment), the hTERT-derived peptide vaccine is inoculated into humans…"

Comment 21: Line 87: Please explain for the reader what “adaptive immune response” is since in the introduction you have only mentioned the innate one. Please add either here or in the intro and describe the differences between adaptive and innate immune responses.

Reply 21: We added to the introduction the adaptive immune response and its distinction from innate immune responses (see Page 2, line40-45).

Changes in the text:"…The innate immune response, where TAAs can directly or indirectly activate macrophages and NK cells and induce their anticancer functions, emerges earlier than the adaptive immune response after the stimulation of TAAs[3],[4]. However, the adaptive immune response including cellular immunity and humoral immunity, plays a more important role in specific anticancer responses and can be capable of producing an immunological memory effect…"

Comment 22: Line 91: Please explain what a “cancer-immune cycle” is.

Reply 22: We have modified our text as advised (see Page 4, line102-104).

Changes in the text:"…in the entire cancer-immune cycle including seven steps (T cells neither respond nor work on their own, but exist in the context of a series of steps, some of which are even extrinsic to the immune system and the cancer)[14], making…"

Comment 23: Line 103: the statement requires a reference.

Reply 23: We have modified our text as advised (see Page 4, line116).

Changes in the text:"GV1001 is an MHC class II-restricted peptide vaccine containing 16 amino acids from the active site of hTERT (hTERT611-626, EARPALLTSRLRFIPK)[22]."

Comment 24: Line 111: It is impossible to analyse any “blood supply” in vitro. Instead, the study from reference 19 analysed angiogenesis and found it reduced. While this might indirectly be related to blood supply, please rather mention the angiogenesis feature from the paper in order to avoid misunderstanding and mis-interpretation of the paper and study.

Reply 24: We have modified our text as advised (see Page 5, line125-126).

Changes in the text: We added "by significantly reduced angiogenesis through regulation of hypoxia-inducible factor" after "The results of a study on renal cell carcinoma patients showed that GV1001 could induce apoptosis by significantly reduced angiogenesis through regulation of hypoxia-inducible factor in vitro and in vivo". The modified whole sentence is "The results of a study on renal cell carcinoma patients showed that GV1001 could induce apoptosis by significantly reduced angiogenesis through regulation of hypoxia-inducible factor in vitro and in vivo by significantly reduced angiogenesis through regulation of hypoxia-inducible factor, indicating that GV1001 may be an effective therapeutic target for renal cell carcinoma."

Comment 25: Line 115: please better describe what role an “elimination of MHC restriction” might have on vaccine efficiency.

Reply 25: Here we don't say it accurately, so we correct it. We would like to illustrate that GV1001 possessing multiple HLA-class I/II epitopes can initiate a combined response of CTL and Th cells (see Page 5, line127-130).

Changes in the text: "In addition, GV1001 was found to harbour various MHC-class I or II epitopes, which means inoculation of GV1001 into lung cancer patients can potentially elicit combined CD8+ CTL and CD4+ Th cell immune responses[29]."

Comment 26: Line 129: please add “the benefit of” between “supporting” and “ the combination” .

Reply 26: We have modified our text as advised (see Page 5, line143).

Changes in the text:"…supporting the benefit of the combination of…"

Comment 27: Line 132: please add “a” in front of “two … regimen”.

Reply 27: We have modified our text as advised (see Page 5, line146).

Changes in the text:"…using a two treatment regimen…"

Comment 28: Line 135: “A …memory”.

Reply 28: We have modified our text as advised (see Page 6, line150).

Changes in the text:"…establish a durable T-cell memory…"

Comment 29: Line 144: replace “in” with “OF…cells”.

Reply 29: We have modified our text as advised (see Page 6, line159).

Changes in the text:"…death of pancreatic…"

Comment 30: Line 149: Please do not write the author’s name in capital letters.

Reply 30: We have modified our text as advised (see Page 6, line164).

Changes in the text:"Minev et al. showed…"

Comment 31: Lines 151 and 154 do not match in content: The former states that the authors used melanoma cells in vitro while the second talks about patient’s PBMCs. There is some information missing regarding these in vivo data on patients. Also, please provide information how the vaccine was applied, both in vitro and in vivo.

Reply 31: We have modified our text as advised (see Page 6, line166-172).

Changes in the text:"Ayyoub et al. stimulated highly enriched CD8+ cells from melanoma patients PBMC (peripheral blood mononuclear cells) with synthetic hTERT540 peptide (the hTERT-derived peptide 540-548aa) in the presence of autologous APCs and cytokines. While the hTERT540 peptide was recognized by HLA-A0201-restricted T cell lines derived from healthy donors, these specific CD8+ T cells failed to recognize HLA-A0201+ target cells expressing hTERT in melanoma patients, which may be related to improper handling of antigenic peptides[39]."

Comment 32: Line 152: wrong language and thus distorted content: You cannot "analyse results… with methods". You can only OBTAIN results using …methods. Please rephrase.

Reply 32: We have modified this part, please see Page 6, line166-172.

Changes in the text:"Ayyoub et al. stimulated highly enriched CD8+ cells from melanoma patients PBMC (peripheral blood mononuclear cells) with synthetic hTERT540 peptide (the hTERT-derived peptide 540-548aa) in the presence of autologous APCs and cytokines. While the hTERT540 peptide was recognized by HLA-A0201-restricted T cell lines derived from healthy donors, these specific CD8+ T cells failed to recognize HLA-A0201+ target cells expressing hTERT in melanoma patients, which may be related to improper handling of antigenic peptides[39]."

Comment 33: Likewise, in lines 160/1 there is a contradiction since PBMCs from patients are not in vitro, but in vivo samples, even when these cells are analysed after isolating them from the patients, they are still in vivo-related cell material. Please correct.

Reply 33: We have modified our text as advised (see Page 6, line174).

Changes in the text: We removed "in vitro".

Comment 34: Line 172: What do you mean with "basically treated"? Why not just "treated"? Please explain or remove.

Reply 34: We have modified our text as advised (see Page 7, line187).

Changes in the text: We removed "basically".

Comment 35: Line 175: please explain the abbreviations CTLA and PD-1.

Reply 35: We have modified our text as advised (see Page 7, line190-191).

Changes in the text:"… ipilimumab (Cytotoxic T lymphocyte-associated antigen-4 blocker, CTLA-4 blocker) and nivolumab (Programmed death 1 blocker, PD-1 blocker)…"

Comment 36: Line 176: What "significant clinical results" were found? Please provide this important information.

Reply 36: We removed "significant clinical results" and added "UV1 combined with ipilimumab induced more rapid and frequent immune responses" (see Page 7, line192-193).

Changes in the text:"…found that UV1 combined with ipilimumab induced more rapid and frequent immune responses[44]."

Comment 37: Line 184: what you describe, are not "methods" but APPROACHES. Please amend.

Reply 37: We have modified our text as advised (see Page 7, line201).

Changes in the text:“methods” => “approaches”

Comment 38: Line 187: When describing the results for the patients you have to say which of the 3 approaches and tumour types it was related to those. Please specify and add.

Reply 38: We have modified our text as advised (see Page 7, line204-206).

Changes in the text:"…UV1 vaccination. Patients have been followed for survival for a median of 28.2 months (range, 4.7 – 87.3), 61.8 months (range, 11.7 – 96.3), and 55.7 months (range, 3.5 – 79.5), respectively.During 8 years…"

Comment 39: Line 205: Were these experiments done in vitro with cultured tumour cells? Please specify.

Reply 39: We added “GX301 was administered by intradermally injecting 500 μg of each peptide (dissolved in Montanide ISA-51) in the skin of the abdomen. And patients were clinically and immunologically monitored up to 6 months from the first immunization. ”. (see Page 8, line222-225).

Changes in the text: "In phase I/II clinical trials in patients with prostate or kidney cancer, GX301 was administered by intradermally injecting 500 μg of each peptide (dissolved in Montanide ISA-51) in the skin of the abdomen. And patients were clinically and immunologically monitored up to 6 months from the first immunization. Researchers isolated…"

Comment 40: Line 215: Please add “characteristics” after “immunogenicity” since otherwise grammar of the sentence is wrong since a vaccine cannot have safety, but either be safe or have “safety characteristics” or something along these lines.

Reply 40: We have modified our text as advised (see Page 8, line237).

Changes in the text:"…immunogenicity characteristics[49]."

Comment 41: Lines 215-218: Please split into 2 sentences since the number of administrations and the prognostic value of the vaccine seem not to be directly related and the structure of the sentence is too complex and thus the content partially unclear.

Reply 41: We have modified our text as advised (see Page 8, line238).

Changes in the text:"Based on clinical results, increasing the number of administrations improves the immune response and this vaccine may have certain prognostic value. Furthermore, combination therapy with immune checkpoint inhibitors may produce further improvements."

Comment 42: Line 222: what do you mean with being “inaccessible in the protein structure”? Accessible to what or whom? Please explain.

Reply 42: We have not said this sentence very accurately. Therefore, we removed "that is normally inaccessible in the protein structure", and added “whose functional peptide is hidden inside the protein”. (see Page 9, line244).

Changes in the text:"…one is a wild-type low-affinity cryptic hTERT peptide (RLFFYRKSV) whose functional peptide is hidden inside the protein, and the other…"

Comment 43: Line 223: the statement requires a reference.

Reply 43: We have modified our text as advised (see Page 9, line246).

Changes in the text:"…peptide[50]."

Comment 44: Line 254: Please do not use the term “expression” for proteins or RNA since only genes are expressed. Please use “levels/amounts” instead.

Reply 44: We removed "expression", and added “amounts”.(see Page 10, line276).

Changes in the text:"…achieve stable long-term amounts of proteins…"

Comment 45: Line 255, please explain “humoral” immune response, perhaps already in the introduction.

Reply 45: We have explained the humoral immunity in the introduction and the Figure 1, please see Page 2, line46, and Page 36.

Changes in the text: Here, we have not revised it. If you think it is better to add something about humoral immunity in here, please contact us.

Comment 46: Lines 256/7 please remove Chinese characters here and add the table number. As mentioned before, please number tables in the order the occur in the text.

Reply 46: We have modified our text as advised (see Page 10, line279).

Changes in the text:"… (Table 2)."

Comment 47: Line 258: What do you mean with “re-used”? and why is it different from other vaccine types such as peptides which are also synthetically manufactured.

Reply 47: What we mean here is that "DNA vaccines can continuously express TAA protein and stimulate the body to produce extensive humoral and cellular immune responses". We removed "can be reused to provide long-term protection and", and added “DNA vaccines can continuously express TAA protein and stimulate the body to produce extensive humoral and cellular immune responses. Moreover, DNA vaccines”.(see Page 10, line279-281).

Changes in the text:"Compared with other vaccines, DNA vaccines can continuously express TAA protein and stimulate the body to produce extensive humoral and cellular immune responses. Moreover, DNA vaccines are well tolerated by patients without causing serious adverse reactions[62],[63]."

Comment 48: Line 264 it should be “integrate INTO”, not “with”. Please replace.

Reply 48: We have modified our text as advised (see Page 10, line287).

Changes in the text:"…integrate into the…"

Comment 49: Line 266, while you here mention electroporation (please provide a reference for it’s in vivo use!), in line 253 you stated that the vaccine was injected IM. Please reconcile this apparent contradiction.

Reply 49: Electroporation is usually used for cells in vitro, but we checked the references again, and there is indeed a clinical test method that first intramuscular injection and then electroporation, as described in reference 64. For a further illustration, we have added some content after the “electroporation” (see Page 10, line289).

Changes in the text:"electroporation to increase in vivo delivery of pDNA to tissues, primarily skin and skeletal muscle, following a direct SC or IM injection[64]."

Comment 50: Line 271: Vectors do not express proteins, but contain DNA which then is transcribed and translated within the cell. Please correct.

Reply 50: We removed "can express the full-length hTERT protein", and added “contain the full-length hTERT gene incorporating two mutations (R589Y and D1005Y) which was subcloned into pGX0001 and named as phTERT. ”(see Page 10, line296-298).

Changes in the text:"Recombinant DNA technology has been used to construct DNA vectors that contain the full-length hTERT gene incorporating two mutations (R589Y and D1005Y) which was subcloned into pGX0001 and named as phTERT. The sequence of phTERT, a DNA vaccine that is highly optimized to produce a more efficient antigenic epitope, can improve the recognition and binding rate of T cells."

Comment 51: Lines 271-3: You just stated about the full-length hTERT protein, what do you now mean with “highly optimized”? By whom? Please provide appropriate references!

Reply 51: We checked the references again, and found that it was true to say so, as described in reference 65. To avoid confusing readers, we have modified this section, please see page(see Page 10, line296-298).

Changes in the text: "Recombinant DNA technology has been used to construct DNA vectors that contain the full-length hTERT gene incorporating two mutations (R589Y and D1005Y) which was subcloned into pGX0001 and named as phTERT. The sequence of phTERT, a DNA vaccine that is highly optimized to produce a more efficient antigenic epitope, can improve the recognition and binding rate of T cells."

Comment 52: Line 274: As mentioned above, how does injection work together with electroporation? This requires an explanation since in my view the 2 methods exclude each other.

Reply 52: Electroporation is usually used for cells in vitro, but we checked the references again, and there is indeed a clinical test method that first intramuscular injection and then electroporation, as described in reference 64.

Changes in the text: Therefore, we did not modify it. If you think that the modifications are still needed, please contact us.

Comment 53: Which cells secreted these molecules?

Reply 53: We added “ by T cells” .(see Page 11, line303).

Changes in the text:"…were secreted by T cells."

Comment 54: Lines 282/3 only tumour cells can proliferate, not a tumour which grows.

Reply 54: We have modified our text as advised (see Page 10, line309).

Changes in the text:"…reduced the rate of tumor cell proliferation…"

Comment 55: Lines 286 and 288: The statements require references.

Reply 55: The reference of this statement is the reference 68.

Changes in the text:"…with solid tumors[68]…"

Comment 56: Line 311: What do you mean with “Telomerase expression”? htert, htr or what? Please specify. The term “telomerase” is mainly used related to its activity and that cannot be expressed.

Reply 56: We removed "specifically targeting telomerase expression", and added “making it target to specific indications with high telomerase activity”(see Page 12, line338).

Changes in the text:"…in the early stages of disease or making it target to specific indications with high telomerase activity may yield…"

Comment 57: Line 315: What do you mean with “self-specific”? Please explain.

Reply 57: What I want to mean is “autospecific”. We have modified our text as advised and added some contents (see Page 12, line342).

Changes in the text:"Cell-based immunotherapy is on the basis of the administration of living immune cells to patients, aiming to boost the immune system[72]. Researchers collect human autospecific immune cells…"

Comment 58: Line 316: what do you mean them “expand them by thousand”? Do you mean “thousand-fold”?

Reply 58: What I want to mean is “in thousands of times”.We removed "expand them by thousand", and added “amplify them in thousands of times”. (see Page12, line345).

Changes in the text:"…amplify them in thousands of times…"

Comment 59: Line 317: How would you enhance “targeted killing function”? Please describe the molecular manipulations performed here in detail.

Reply 59: What I want to mean is that these cells could kill cancer cells because they could recognize TAA marks which cancer cells contained. However, considering the concise language and the word limit, we revised this sentence into “Researchers collect human autospecific immune cells (such as DCs and T cells), culturing them in vitro to amplify them in thousands of times. And then, the amplified cells will be infused into the human body to enhance the immune activity of the body to target kill tumor cells [73].” (see Page 12, line343-347).

Changes in the text:“Researchers collect human autospecific immune cells (such as DCs and T cells), culturing them in vitro to amplify them in thousands of times. And then, the amplified cells will be infused into the human body to enhance the immune activity of the body to target kill tumor cells [73].”

Comment 60: Line 318: Mutations occur in DNA, not in cells, thus the term “mutant cell” is wrong. Please correct.

Reply 60: Yes, it should be “other cells with mutated genes”. However, likewise, we removed this part. If necessary to rejoin, please contact us.

Changes in the text:“Researchers collect human autospecific immune cells (such as DCs and T cells), culturing them in vitro to amplify them in thousands of times. And then, the amplified cells will be infused into the human body to enhance the immune activity of the body to target kill tumor cells [73].”

Comment 61: The sentence in lines 315-320 is too long and thus loses focus. Please split into more.

Reply 61: We have modified our text as advised (see Page 12, line342-347).

Changes in the text:"Cell-based immunotherapy is on the basis of the administration of living immune cells to patients, aiming to boost the immune system[72].Researchers collect human autospecific immune cells (such as DCs and T cells), culturing them in vitro to amplify them in thousands of times. And then, the amplified cells will be infused into the human body to enhance the immune activity of the body to target kill tumor cells [73]. "

Comment 62: Line 319: Please describe how you circumvent (not break) immune tolerance and enhance (remove "activating" since it duplicates) immune activity. This also refers to point 60.

Reply 62: We realized that immune tolerance could not be completely avoided and could only alleviated. Likewise, we removed this part. If necessary to rejoin, please contact us.

Changes in the text:"Researchers collect human autospecific immune cells (such as DCs and T cells), culturing them in vitro to amplify them in thousands of times. And then, the amplified cells will be infused into the human body to enhance the immune activity of the body to target kill tumor cells [73]. "

Comment 63: Please provide references for this paragraph since you have not cited a single paper.

Reply 63: We have modified our text as advised (see Page 12, line344, 347, 354).

Changes in the text: We added three references to this paragraph 72,73,74.

Comment 64: Likewise, the statement in lines 329/30 requires a reference.

Reply 64: We have modified our text as advised (see Page 12, line357).

Changes in the text:"…adaptive immunity[75]."

Comment 65: Line 331: please remove Chinese letters/words and provide table number.

Reply 65: We have modified our text as advised (see Page 13, line358).

Changes in the text:"…(Table 2)…"

Comment 66: Line 334: Again you just say "many manipulation methods" without providing any specific molecular description of those which means it lacks any required scientific content and thus, the statements in lines 333-337 are devoid of content and highly superficial. Please add meaningful content.

Reply 66: We think what you say makes sense. The the statements in lines 333-337 is really too superficial. So we decided to delete this part and revise it along with comment 67,68,77. If there is something wrong to write, please tell us (see Page 13, line358-364).

Changes in the text:"…The basic application principle of DC-based therapies (Table 2) is that the patient's own DCs were isolated in vitro and loaded on TAA by various means, such as co-incubation, cell fusion, transfection, after which the DCs containing TAA antigenic specificity would be reinfused into hosts to induce specific anticancer immune responses. Current loading hTERT on DC vaccines have been mainly applied in two ways: one is to stimulate DCs with hTERT protein in vitro to sensitize the DCs[78], the other is to acquire and present hTERT-derived antigens by overexpressing the hTERT protein[79]."

Comment 67: Importantly, the entire paragraph 2.1 does not provide any specific data regarding hTERT since all statements are true also just for dendritic cells. Please either describe any content which would justify this paragraph or otherwise remove it or combine with 2.1.1

Reply 67: We have modified our text as advised and modified along with the article 66 comments (see Page 13, line358-364).

Changes in the text: Same as comment 66.

Comment 68: Line 341: What is "rate of access to a vaccine" and how does this relate to the price? Remove "rate" for it to make any sense.

Reply 68: We have removed this part (see Page 13, line358-364).

Changes in the text: Same as comment 66.

Comment 69: Line 342: Your heading just includes GRNVac1, but you also descrive GRNVac2. Please make sure that your headings correspond to the content.

Reply 69: We have modified our text as advised (see Page 13, line365).

Changes in the text: "2.1.1 GRNVAC1 and GRNVAC2"

Comment 70: Line 345: The statement requires a reference.

Reply 70: We have modified our text as advised (see Page 13, line369).

Changes in the text: "…(LAMP1)[50]."

Comment 71: Please describe how hTERT peptides generated in lysosomes get onto the surface of DCs? Also, please provide references, so that one can get any further reading on these methods.

Reply 71: We have modified our text as advised (see Page 13, line369-372).

Changes in the text: "The cytoplasmic domain of LAMP-1 contains the aminoacid sequence Tyr-Gln-Thr-lle, whose structure conforms to Tyr-Xaa-Xaa-hydrophobic amino acid motif that mediates cellmembrane internalization and possibly lysosomal targeting of several cell surface receptors[77]."

Comment 72: Line 351: requires a reference after "vaccines". Also, you have not mentioned a study before, so it is unclear where any results come from.

Reply 72: The reference for this sentence and results is 78. (see Page 13, line375-380).

Changes in the text: "… In addition, the results of a study showed that chimeric vaccines containing LAMP1 have been shown to induce stronger T-cell responses in patients with metastatic prostate cancer than nonchimeric vaccines. And the vaccine was clinically well tolerated for 3 or 6 weeks after injection into patients with metastatic prostate cancer, eliciting strong CD8+ and CD4+ T-cell responses but no significant autoimmune symptoms[78]…."

Comment 73: Line 352: "injection inTO…"

Reply 73: We have modified our text as advised (see Page 13, line379).

Changes in the text: "…injection into…"

Comment 74: Line 359: What delivery method you refer to? You have not described any in order to get a difference between Vac1 and Vac2. Please make sure that your statement contain any content and are not just empty phrases with no evidence at all.

Reply 74: We were trying to talk about vaccine delivery systems or technology. Now, considering the scientific statements and word limit, we removed "The production methods are the same, but in terms of the delivery method, GRNVAC2 may be the better choice." and added some new contents. (see Page 13, line384-387).

Changes in the text: "Different from GRNVAC1, GRNVAC2 is an allogenic dendritic cell product derived from human embryonic stem cells[80]. Because it is not limited by MHC type, GRNVAC2 may have great potential for the treatment of tumors with unknown T-cell epitopes[50]."

Comment 75: Reference 64 is a general review, please provide a primary reference on GNRVac2.

Reply 75: We have modified our text as advised (please see Page 13, line385,387).

Changes in the text: Same as comment 74.

Comment 76: Line 360: "potential for development"-of/into what? Your statement seems incomplete, please specify/clarify.

Reply 76: We have removed this part (please see Page 13, line384-387).

Changes in the text: Same as comment 74.

Comment 77: Lines 364/5: Please explain the difference between htERT-stimulation of DC cells and overexpression of hTERT in DCs and also provide reference for each approach/method.

Reply 77: We have illustrated and provided references in the "2.1 hTERT-targeting DCs" (see Page 13, line361-364).

Changes in the text: "Current loading hTERT on DC vaccines have been mainly applied in two ways: one is to stimulate DCs with hTERT protein in vitro to sensitize the DCs[76], the other is to acquire and present hTERT-derived antigens by overexpressing the hTERT protein[79]."

Comment 78: Line 366, please explain that ipilimumab is an anti-CTLA-4 blocking antibody.

Reply 78: It has already been described above. (see Page 7, line190).

Changes in the text: None.

Comment 79: Lines 366, 372, 373 and 374: the study from ref. 65 vaccinated just one single patient, not "patients" as you write. Please correct everywhere make sure that you describe the content of referenced studies correctly!

Reply 79: We have modified our text as advised (see Page 14, line389,396,397).

Changes in the text: "… a metastatic melanoma patient…" and "…the patient…"

Comment 80: You provide reference 65 as an example-for what? From your description of the study is not obvious which method they used in their approach. Please clarify!

Reply 80: Likewise, we have removed "In addition to stimulating DC cells with the hTERT protein, DCs can acquire and present hTERT-derived antigens by overexpressing the hTERT protein. For example, ". We have clarified the method of study in reference 81. (see Page 14, line389-394).

Changes in the text: "Moldy et al. vaccinated a metastatic melanoma patient who has treated with ipilimumab, with indoleamine 2,3-dioxygenase (IDO)-silenced DC vaccines comprised DCs cotransfected with mRNA for survivin (left arm) or hTERT tumor antigen (right arm). During the vaccination period, T cells generated immune responses to survivin and the hTERT tumor antigens, significantly reducing lung, liver, and skin cancer metastasis and resulting in certain clinical benefits[81]."

Comment 81: Line 368: you also wrongly describe their method (ref. 65) since the study used either surviving OR hTERT, not both as you described when you said "and". Please correct.

Reply 81: We have modified our text as advised (see Page 14, line391).

Changes in the text: "… or hTERT…"

Comment 82: Line 377: Please specify that the CTL immune response was against a variety of tumour cell types as described in ref. 66.

Reply 82: We have modified our text as advised (see Page 14, line400-401).

Changes in the text: "… CTL immune response against a variety of tumour cell types"

Comment 83: Line 385: If you refer to "other tumours" you need to provide references for that, otherwise remove as an unsubstantiated statement.

Reply 83: We have modified our text as advised (see Page14, line408).

Changes in the text: We removed "and other tumours".

Comment 84: Line 389: What do you mean with "relatively efficient" and what was it compered to?

Reply 84: We removed "relatively efficient" and added "highly active". (see Page 14, line412).

Changes in the text: "…obtain highly active DCs…"

Comment 85: While in line 389 you call them DCs, you in the next line call them DC-like and TAPC? Can you please clarify this here and what cell type TAPCs usually are?

Reply 85: We have modified our text as advised (see Page 14, line413).

Changes in the text: "…This production of therapeutic DC cells, named tumor antigen presenting cells(TAPCells)…"

Comment 86: Line 394: What is a "PSA doubling time"? PSA is an antigen and not a cell that can double! Please make sure you explain each abbreviation properly and that your statements are scientifically correct.

Reply 86: We have modified our text as advised (see Page 14, line417-418).

Changes in the text: "… prolonged the serum prostate specific antigen doubling time (PSADT) …"

Comment 87: Similarly: in line 397: What is ACT? Please give full name of abbreviations in headings before using the abbreviation, even if it is in the abbreviation list.

Reply 87: We have given full name of ACT in "2 Cell-based immunotherapy". (see Page 12, line350).

Changes in the text: None.

Comment 88: Line 400 and throughout the manuscript: When you talk about "cancer cell killing", what exact mechanism you refer to? Apoptosis, necrosis etc? Please specify.

Reply 88: The mechanisms by which immune cells kill cancer cells are mainly presented in Figure 1. Here, we find it more appropriate to write "restraining the growth and survival of tumor cells".(see Page 15, line424).

Changes in the text: "… which resulted in restraining the growth and survival of tumor cells."

Comment 89: Line 401: What is "TLI"? Transgenic lymphocyte immunization! Please provide this in the text and add to your list of abbreviations.

Reply 89: We have given full name of TLI in "2 Cell-based immunotherapy". (see Page 12, line352).

Changes in the text: None.

Comment 90: Line 406: You probably mean "TLI" here while "TILs" are cells-you seem to get confused yourself by your abbreviations.

Reply 90: In fact, both statements exist. Given that these two statements may confuse readers, we changed the order of the two parts, please see Page 15, line425-437.

Changes in the text: None.

Comment 91: Line 412: please explain TCR as T- cell receptors as well as all the other abbreviations in lines 412 and 413.

Reply 91: We have given full name of these abbreviations in "2 Cell-based immunotherapy". (see Page 12, line351-354).

Changes in the text: None.

Comment 92: Your text in lines 406-413 requires appropriate references!

Reply 92: We have modified our text as advised (see Page 15, line429,433).

Changes in the text: "… time consuming[91]." and "… have emerged[92]."

Comment 93: Line 414: Why Do TCR-T-cells target hTERT by expressing specific TCR receptors? I cannot see any such specificity! Please clarify and provide references, so that the reader can get more information about the underlying details.

Reply 93: We have modified our text as advised (see Page 15, line438-439).

Changes in the text: "TCR-engineered T cell therapy aims to screening for TCR α β chain sequences highly specific for hTERT…"

Comment 94: Line 419: Please remove the Chinese symbols, whatever they mean here (figure, table?).

Reply 94: Here We have updated two articles and removed the original last article (see Page 15, line443).

Changes in the text: "… expression[95],[96],[97],[98]."

Comment 95: Line 419: reference 77 does not seem to be about telomerase/hTERT and the other 2 references (78, 79) show specific results, yet, you just state a very general method of isolating and re-introducing T-cells. They also do not seem to use TCR-T-cell, since the "T" in "TCR" already contains "T-cell". Since all T0cells have TCRs you should better explain what exactly your term "TCR-T-cell" means.

Reply 95: Reference 77 is relevent to hTERT. We have updated two articles and removed the original last article considering some of the references may be outdated. TCR-T cell means TCR-engineered T cell. In order not to puzzle readers, we changed it to "TCR-engineered T cell"(see Page 15, line438,440,441,446,451).

Changes in the text: "… TCR-engineered T cell…"

Comment 96: Line 420: The use of "i.e." is not indicated since the authors named the specific hTERT-specific TCR sequence "Radium-4" while it is not something that had already been known what "i.e." would mean.

Reply 96: We have modified our text as advised (see Page 15, line445).

Changes in the text: "… which was called Radium-4…"

Comment 97: Line 421: Again, it was exactly ONE patient this TCR was obtained from, not many! Please correct!

Reply 97: We have modified our text as advised (see Page 15, line445).

Changes in the text: "… from the pancreatic cancer patient…"

Comment 98: Line 425 requires a reference-is it still number 80 or another one?

Reply 98: Yes, they are the same reference. We moved this reference to the end of the latter sentence.(see Page 16, line450).

Changes in the text: "… solid tumors[99]."

Comment 99: Line 426: What do you mean with "high technical barriers"? Please specify.

Reply 99: Given that "high technical barrier" is not easy to scientifically explain, we removed "high technical barriers" and added "persistence of T cells in vivo" (see Page 16, line451).

Changes in the text: " There are still challenges regarding TCR-T-cell therapy, for example, persistence of T cells in vivo[100]…"

Comment 100: Line 427: also "tumour escape" needs to be better explained in a molecular, scientific fashion without giving just sloppy buzz words.

Reply 100: We have modified our text as advised (see Page 16, line452-453).

Changes in the text: "… tumor escape due to the expression of cancer-related antigens varying in different cells within tumor cells[101]…"

Comment 101: Lines 429/30: Could you call this an "epitope" which seems to be the more scientific term? Also "antigen fragment gene" does not seem to be correct since a gene does not code for "antibody fragments". Please consider rephrasing.

Reply 101: We replaced "antigen fragment gene" with "single-chain antibody" and revise the whole sentence. Please see Page 16, line456-457.

Changes in the text: "… combining an single-chain antibody (ScFv) that can recognize a TAA of interest with signaling molecules of the T cells (CD3, CD28, etc.) …"

Comment 102: Line 433: please explain the abbreviation "BiTES".

Reply 102: We have given full name of these abbreviations in "2 Cell-based immunotherapy". (see Page 12, line353).

Changes in the text: None.

Comment 103: Line 434: Please replace "in series" which is incorrect English, with "next to" or "in conjunction with" or something else in correct grammar.

Reply 103: We have modified our text as advised (see Page 16, line462).

Changes in the text: "… antigen in conjunction with…"

Comment 104: Lines 433-438 require references.

Reply 104: We have modified our text as advised (see Page 16, line459,461,465).

Changes in the text: "…CAR-T cells with the ability to recognize the TAA without MHC restriction[102]. These cells then kill cancer cells through the perforin and granzyme axis, the Fas and Fas ligand axis, as well as the cytokines[103]." and "… thus, BiTEs can not only effectively activate T cells but also recognize tumor cells[104]."

Comment 105: Line 460: What do you mean with "immediate protection"? Please clarify.

Reply 105: We mean that antibodies can immediately neutralize the antigen, which is faster in providing protection relative to cellular immunity (see Page 17, line489-490).

Changes in the text: "… After all, the generation of memory immune responses after antigen vaccination usually requires two to six weeks, but antibody therapy allows the body to directly neutralize antigens, resulting in immediate passive immunity…"

Comment 106: Line 463: since you do not just summarize but also give an outlook, I would suggest to add "outlook" or "future directions" to the heading.

Reply 106: We have modified our text as advised (see Page 17, line494).

Changes in the text:"**Summary and outlook**"

Comment 107: Line 466: Please remove Chinese symbols and add Figure number.

Reply 107: We have modified our text as advised (see Page 17, line497).

Changes in the text:"…(Figure 2)"

Comment 108: Line 467: The FDA is only the agency for the USA, but there are surely also other countries involved in clinical trials, for example, Samsung Pharmaceuticals is from Korea etc, thus, please correct.

Reply 108: Considering the scientific statements, we revised the sentence, please see Page 17, line 497-503.

Changes in the text: "Many of these approaches have been approved by the FDA and other countries to enter clinical trials, and some of these therapies, such as "RIAVAX™ (GV1001)" (Samsung Pharmaceutical Co., Ltd., NCT02854072), GV1001 (GemVax & Kael, NCT04032067), for the treatment of pancreatic cancer[110] and BPH, respectively, have entered phase III clinical trials. And RZ-001 (Rznomics, Inc., NCT05595473) for the treatment of HCC have entered phase I/II clinical trials. "

Comment 109: Line 470: Please explain abbreviations BPH and HCC.

Reply 109: We have modified our text as advised (see Page 17, line501). And we have given full name of HCC in "1.1.1 GV1001". (see Page 5, line123).

Changes in the text:"Many of these approaches have been approved by the FDA and other countries to enter clinical trials, and some of these therapies, such as "RIAVAX™ (GV1001)" (Samsung Pharmaceutical Co., Ltd., NCT02854072), GV1001 (GemVax & Kael, NCT04032067), for the treatment of pancreatic cancer[110] and BPH, respectively, have entered phase III clinical trials. And RZ-001 (Rznomics, Inc., NCT05595473) for the treatment of HCC have entered phase I/II clinical trials."

Comment 110: Line 472: Please use small "h" in hTERT.

Reply 110: We have modified our text as advised (see Page 17, line504).

Changes in the text:"…hTERT…"

Comment 111: Line 478: please provide a reference for the statement.

Reply 111: We have modified our text as advised (see Page 18, line510).

Changes in the text:"…targeting hTERT[8]."

Comment 112: Line 480: Remove "the" in "The clinical trial results".

Reply 112: We have modified our text as advised (see Page 18, line512).

Changes in the text:"Clinical trial results…"

Comment 113: Line 486: Please remove "Increasing" since evidence cannot be increasing. Perhaps use "Accumulating" instead.

Reply 113: We have modified our text as advised (see Page 18, line518).

Changes in the text:"Accumulating clinical trial…"

Comment 114: Line 500: AS above-only by the US FDA? Or also other countries' agencies?

Reply 114: We addded "first" between "were" and "approved" (see Page 18, line532).

Changes in the text:"…were first approved by the FDA…"

Comment 115: Line 506: Viral mutations are not relevant for telomerase-targeted therapies or mRNA vaccines. Please correct or remove. Also, do not use the term "expression" for proteins as only genes are expressed.

Reply 115: We removed "to accommodate viral mutations", and replaced "expressed" with "translated"(see Page 18, line539).

Changes in the text:"…their sequences can be precisely designed and modified. The translated proteins have…"

Comment 116: Line 509: Since mRNA vaccines deliver RNA, these are NOT antigens, but code for them! Please correct.

Reply 116: We have modified our text as advised (see Page 19, line542).

Changes in the text:"…delivering the code of antigens…"

Comment 117: Line 512: Since you suggest new outlooks which have not been mentioned before, you cannot say "In summary". Please rephrase.

Reply 117: We have modified our text as advised (see Page 19, line544).

Changes in the text:"Future research on hTERT-targeted immunotherapeutic approaches may be devoted to the following four aspects:…"

Comment 118: Line 514: hTERT is a protein which cannot have a" biological mechanism". Likewise, "interaction with the human body" is too superficial. Please rephrase and specify with more molecular details.

Reply 118: We have modified our text as advised (see Page 19, line545).

Changes in the text:"…(1) further exploration of the regulation mechanism of hTERT and its application in anti-tumor research…"

Comment 119: Line 516: Number 3 is already been suggested and widely practiced, so this is not any new approach or direction.

Reply 119: We removed this statement (see Page19, line548).

Changes in the text: We removed this statement (see Page19, line548).

Comment 120: Line 524: The term "survival expectancy" is incorrect (check also previous use in the text). It should be either "survival RATE" or "LIFE expectancy". Please correct.

Reply 120: We have modified our text as advised (see Page 19, line553).

Changes in the text:"…survival rate…"

Comment 121: Line 544: Please order your abbreviations by the alphabet, add more used abbreviations and structure properly, so that it fits into complete lines.

Reply 121: We have modified our text as advised (see Page 20, line575).

Changes in the text: We order abbreviations by the alphabet, please see it at abbreviations.

Comment 122: References: 93/95 references are not sufficient for such a comprehensive review. Please add more from the places where indicated above.

Reply 122: We have modified our text as advised (see Page 20, line575).

Changes in the text: We added several literature articles, totaling 116 articles.

Comment 123: Tables 1 and 2: Please order in a way that all columns are readable, since Year numbers are not visible, don't use brackets for references and remove Chinese letters from table 1. For table 2: Add references 94 and 95 not here, but into the general reference list.

Reply 123: We have modified our text as advised (see Page 31-35).

Changes in the text: Please see Page 31-35.

Comment 124: Figure legend 1, line 6: it should be "peptideS".

Reply 124: We have modified our text as advised (see Page 36, line6).

Changes in the text:"…antigenic peptides…"

Comment 125: Please rename the 2nd Figure 1 into Figure 2!

Reply 125: We have modified our text as advised (see Page 37).

Changes in the text:"Figure 2.…"

Comment 126: Figure 2, line 3: perhaps electroporation is just one of several possible methods, so rather keep it more general. Line 7: please explain "leukapheresis".

Reply 126: We have modified our text as advised. We illustrate that electroporation is commonly used, and explain the leukapheresis (see Page 37, line3,7-9).

Changes in the text:"…are usually electroporated to…" and "…leukapheresis, performing using apheresis equipment to separate leukocytes from peripheral blood, at the same time returns autologous plasma, platelets and erythrocytes to the patient, is utilized to…"

Comment 127: Most importantly, your suggestion under point 6 in figure 2: Please consider any risks for injecting hTERT RNA to be translated into hTERT protein-in which cells? hTERT can also be harmful to patients!

Reply 127: We have modified our text as advised (see Page 38, point 6).

Changes in the text:"(6) hTERT mRNA may be introduced into the human body and after being engulfed by the APC, it directly translated into the hTERT protein to induce an immune response. But we should pay some attention to underlying side effects."

Reviewer B

1. Regarding the abbreviations in the article, please use their full name when first appearing. And please ensure the accuracy of all the abbreviations.

   Reply: We have checked the full name of each abbreviation when first appearing and the accuracy of all the abbreviations.

2. ***Ref 12*** and ***Ref 34*** are duplicated. Please check and revise.

   Reply: We have checked and removed the ***Ref 34***.

3. Please check all your figures and tables to make sure **all abbreviations** have been defined in their **legends or footnotes**.

   Reply: We have checked and modified as requested.

4. For the Narrative Review, the Methods section should include a completed table as follows:

   Table X. The search strategy summary

Items	**Specification**
Date of search (specified to date, month and year)	
Databases and other sources searched	
Search terms used (including MeSH and free text search terms and filters) Note: please use an independent supplement table to present detailed search strategy of one database as an example	
Timeframe	
Inclusion and exclusion criteria (study type, language restrictions etc.)	
Selection process (who conducted the selection, whether it was conducted independently, how consensus was obtained, etc.)	
Any additional considerations, if applicable	

   Note: Please note that a narrative review is less methodologically demanding than a systematic review, as it does not require a search of all literature in a field. Therefore, the search strategy summary of a narrative review is mainly used for more transparent reporting.

   Reply: Table2 The search strategy summary

Items	**Specification**
Date of search (specified to date, month and year)	9/4/2024
Databases and other sources searched	PubMed，CNKI，ClinicalTrials.gov
Search terms used (including MeSH and free text search terms and filters) Note: please use an independent supplement table to present detailed search strategy of one database as an example	“telomerase”, “hTERT”, “cancer or tumor”, “immunotherapy”, “peptide vaccine”, “DNA vaccine”, “cellular therapy”, “Adoptive Cell Transfer Therapy”, ect.
Timeframe	27/9/1994-9/4/2024

Inclusion and exclusion criteria (study type, language restrictions etc.)	In terms of content, we first selected the relevant review literature in the past five years, and then searched the relevant clinical research results according to each therapy. In languages, we read English and Chinese research literatures.
Selection process (who conducted the selection, whether it was conducted independently, how consensus was obtained, etc.)	Four authors selected studies together.
Any additional considerations, if applicable	Not applicable.