Peer Review File

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Reviewer A

The authors provide a manuscript which attempts to review the growing number of DNA repair targeted therapies in clinical trials (alone or in combination) for NSCLC. The topic is interesting, but ultimately, it remains to be seen if any of these therapies will make any difference in NSCLC, which limits the impact. Further limitations of the manuscript include very superficial and inconsistent discussion of the mechanistic rationale for targeting the DNA damage repair (DDR) pathways in NSCLC, particularly in the background section, but additionally throughout the manuscript. There are numerous missing references for claims the authors make. Much of the manuscript is difficult to read, as one "fact" will be listed after another "fact," with no context or reason for these to be discussed together or in that order. Additionally, the review lacks focus, with tables listing therapies used for other cancers (besides NSCLC) and text including targeted therapies from pre-clinical to clinical trials. Also limiting the impact, many novel targets/small molecular inhibitors are missed, while attention is spent discussing some therapies (specifically old DNA-PK, ATR and ATM inhibitors) that have already been shown to not be of clinical utility. Some specifics are included below:

Example of lack of context: The phrase, “Smoking is the main cause of LC, accounting for 90% of cases, suggesting a genetic predisposition.” does not make sense. The fact that cancer arises with cigarette smoking does not suggest a genetic predisposition. The authors need to provide an appropriate rationale for a genetic predisposition or remove this comment. (This is just one example of many similar statements.)

Reply: I revised following advice as follows: “Smoking is the main cause of LC, accounting for 90% of cases, however, approximately 20% of newly-diagnosed lung adenocarcinoma cases are never- or light-smokers in developed countries now. Smokers have higher somatic mutational burden than non-smokers and smokers show additional genomic instability process likely contributes to tumor progression.” See page2 line 47~52.

Example of two "facts" put together which do not have a common or clear message: the sentence “NSCLC primary tumors exhibit high genomic diversity with heterogenous tumor driver mutations present in some clones and absent in others, and a molecular approach to LUAD diagnosis and treatment in the era of precision medicine is necessary due to LUAD's actionable mutations.” does not fit the point the authors are trying to make. These are simply 2 facts put together in one sentence, and the fact that clones may not all carry the same mutation makes it possible the patient will not benefit from targeted therapies. (This is an example of many more similar errors throughout the manuscript).

Reply: We revised as advised “NSCLC primary tumors exhibit high genomic diversity with heterogenous tumor driver mutations present that clones may not all carry the same mutation makes it possible the patient will not benefit from targeted therapies.” See page 2, line 53-56.

There are numerous grammatical errors: lines 66, 67, 68, 200… too many others to count.

Reply:

1) We revised as follows: “DNA damage is repaired by specific cellular pathways during normal cell cycle. When DNA damage fails to be repaired or excised, the mutations will eventually trigger carcinogenesis.” See page 2, line 57~58.

2)We deleted “There are several ATR inhibitors under development. deficiency increase sensitivity to ATR pathway targeted drugs in vitro.” See page7, line 210.

Example of unnecessary components written out of context: The discussion of surgery and SBRT (lines 80-85) is out of place in this section dealing with DNA damage and genomic instability.

Reply: We deleted the description about surgery and SBRT. (See page3, line 84~87)

Example of missing references: There are statements made throughout the manuscript without a supporting reference – too many to count. For instance, in lines 96-97, the authors state "The activity of the DNA repair enzyme 8-
97 oxoguanine DNA N-glycosylase (OGG), is associated with lung cancer." However, there is not reference.

Reply: We added some references as follows,

1) DNA damage caused by endogenous (for example free radicals), or exogenous (for example ionizing radiation) factors can lead to genome instability and diseases such as cancer. See page2, line 43~45, reference 5-6. reference 7~9.
2) DNA double strand breaks (DSBs) are the most severe type of damage, as accumulation of incorrectly repaired or unrepaired DSBs can cause mutation, genomic instability, or induce cell death. See page 2, line 45~47, reference 10.
3) The mechanism of DRR is involved in reversing the O-alkylated DNA damage caused by methylguanine methyl transferase (MGMT). See page 3, line 93~96, reference 34.
4) DRR also removes photolesions caused by UV radiation with DNA-photolyase. See page 3, line 96~97, reference 35~36.
5) The activity of the DNA repair enzyme 8-oxoguanine DNA N-glycosylase (OGG), is associated with lung cancer. See page 3, line 97~98, reference 37.
6) BER can repair small base lesion damage such as the damage on 8-oxo-7,8-dihydro-2’-deoxyguanosine (8-OHdG) by radicals with DNA glycosylase, AP endonuclease, DNA polymerase and DNA ligase. See page 3~4, line 98~110, reference 38~39.
7) The nucleotide excision repair (NER) mechanism is responsible for repairing Cyclobutane pyrimidine dimers (CPD) and pyrimidine-pyrimidone (6-4) photoproducts ((6-4)PP) caused by UV radiation. See page 4, line 112~114, reference 38.
8) There is a group of proteins (XPA-XPG, RPA, CSA, CSB, ERCC6 and RAD23B, etc.) that are involved in NER process. See page 4, line 114~115, reference 40.
9) MMR does it with MSH2, MSH6, MLH1, PCNA and RPA proteins by identify the site of the insertion-deletion loop, remove the lesion site and replace it with newly synthesized DNA. See page 4, line 118~120, reference 43.
10) The development of Veliparib is not very successful, which may as a result of Veliparib is less PARP trapping and is inactive. See page 5, line 165~167, reference 50.
11) Tanaka K et discovered AURKB inhibitors as potent enhancers of Osimertinib-induced

apoptosis and combined EGFR-TKI and AURKB inhibitor could overcome EGFR-TKI resistance. See page 8, line 256~258, reference 67.

Example- lack of mechanistic/rationale focus: This review needs focus. It deals with anything associated in any way with the DNA, but not necessarily DNA repair proteins. For instance, TP53 and ATM are not DNA repair genes (and ultimately proteins), but the proteins that these encode are involved in the DNA damage response and ultimately DNA repair. The AURK family is involved in mitosis and chromosomal segregation. Certainly abnormalities in AURK proteins can be associated with genomic instability, but how and why these should be targeted in NSCLC is discussed little or not at all by the authors.

Reply:

1) As we had described the role of TP53 in DSB repair section (page 4, line 130~133), we deleted "Cancers often present mutations of DNA repair genes, such as TP53 mutation in majority of the NSCLC patients and ATM loss in 40% of LUAD patients (page 2, line 64)."

2)We added Figure 1 which present overview on DDR the mechanistic/rationale including PARP, ATR, AURK, APE1, DNA-PK, CHK1, CHK2.

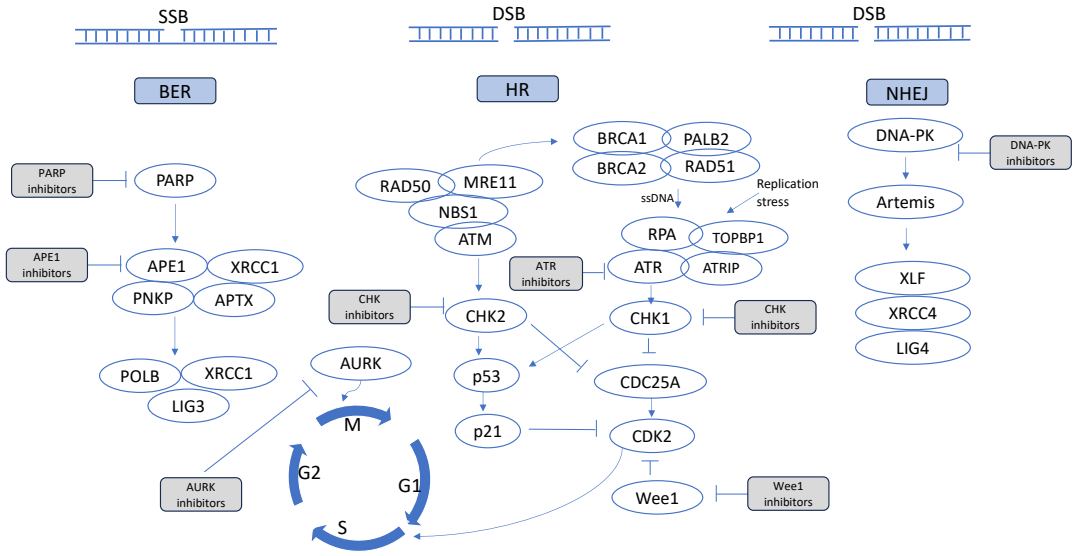

Figure 1.

Targeting DNA damage and response in NSCLC.

DNA damage types have been reported as single-strand DNA breaks (SSBs) or double-strand DNA breaks (DSBs). While base excision repair (BER) fix SSBs repair, DSBs repair requires homologous recombination repair (HRR), non-homologous end joining (NHEJ) and alternative end joining (alt-EJ) pathways. Poly(ADP-ribose) polymerase (PARP) enzymes and apurinic/apyrimidinic endonuclease 1 (APE1) are key proteins involved in the base excision repair (BER) pathway of DNA lesion. Ataxia telangiectasia-mutated (ATM) checkpoint kinase 2 (CHK2) and ataxia telangiectasia and Rad3-related (ATR) checkpoint kinase 1 (CHK1) signals are two key pathways in HRR. CHK1 and CHK2 regulate cell cycle control checkpoints. WEE1 is a distinct nuclear kinase that regulates G2/M checkpoint transition in coordination with DDR.

Aurora kinases plays a regulatory role of G2/M phase being involved in mitotic chromosomal segregation. The key role of DNA-PK kinase is in NEHJ repair. polynucleotide kinase 3 '-phosphatase (PNKP) ;XRCC1, X-ray repair cross complementing 1; POLB, DNA polymerase-β; LIG3, DNA ligase 3; ssDNA, single-strand DNA; MRN complex, MRE11-RAD50-NBS1; ATRIP, ATR-interacting protein; RPA, replication protein A; TOPBP1, DNA topoisomerase 2-binding protein; XRCC4,X-ray cross complementing protein 4; XLF, XRCC4-like factor; LIG4, DNA ligase 4.

3) We added as advised "The AURK family is involved in mitosis and chromosomal segregation. Abnormalities in AURK proteins can be associated with genomic instability. Overexpression of AURKA or AURKB was corrected with poor prognosis of overall survival in NSCLC. Some researches revealed that AURKA and AURAB was associated with resistance of EGFR-TKI, chemotherapy, and/or radiotherapy in lung cancer in pre-clinical models." See page 3, line 70~76.

Example of unfocused review- choice of compounds discussed: It is not clear how the authors chose the specific DDR inhibitors to include this review. For instance, the initial PARP inhibitors discussed were those already evaluated in clinical trials (although there is very little detail on different PARP inhibitor mechanistic functions and their impact on response to therapy). Some inhibitors include those that have been investigated only in pre-clinical models, including some in which the authors discuss results only in common cancer cell lines. Given the inclusion of DDR inhibitors with only pre-clinical evaluations, there are several novel DDR targeting drugs that have not been included in this review. Finally, many older DDR targeting drugs are included which have repeatedly failed clinical trials, some decades ago. These should not be a major focus of the review except to point out a lack of clinical usefulness.

Reply:

1) We deleted the DDR inhibitors which was only in pre-clinical in table 1 including,
    a. ATR inhibitors: VE821, Torin 2 and CGK733,
    b. AURKB inhibitors: Quercetin , ZM447439 and GSK1070916,
    c. APE1 inhibitors: CRT0044876 and Hycanthone,
    d. DNA-PK inhibitors: NU7026, NU7441 (KU-57788), LY294002, NU5455, KU0060648, IC87361, SU11752, LY294002 (SF1126) and KU0060648.
2) We deleted some older DDR targeting drugs in table 1 including,
    a. CHK1/2 inhibitors: UCN-01, SNS-032, AZD7762, PF0047736 and XL-844,
    b. AURKA inhibitors: MLN8054, SNS314, AS703569 (R763),
    c. pan-AURK inhibitors: VX680, PF03814735, CYC116
3) We added some drugs in table1 including,
    a. ATR inhibitors: ATRN-119, RP-3500 (Camonsertib), ART-0380, ATG-018, IMP9064. See the last 5 ATR inhibitors in table 1.
    b. CHK1/2 inhibitors: BBI-355, LY2880070, PHI-101, PEP07, SRA737, LY2603618 (Rabusertib); See the last 6 lines in table 1.
    c. AURKA inhibitor: VIC1911, LY3295668, TAS-119; See the last 3 AURKA inhibitors in table 1.
4) We added 3 ongoing clinical trials (See the last 3 lines in table 2; See page 8, line 264~269

in text)

a. Phase I Clinical Study of VIC-1911 Combined With Osimertinib in the Treatment of Advanced Non-small Cell Lung Cancer With EGRF- Mutation (NCT05489731)
b. A Phase 1a/1b Study of Aurora Kinase A Inhibitor VIC-1911 Monotherapy and in Combination With Sotorasib for the Treatment of KRAS G12C-Mutant Non-Small Cell Lung Cancer (NCT05374538)
c. A Phase Ib/II Trial to Evaluate Safety, Tolerability and Efficacy of Aurora Kinase Inhibitor LY3295668 in Combination With Osimertinib for Patients With EGFR-Mutant Non-Small Cell Lung Cancer (NCT05017025)

Reviewer B

This review article emphasizes on clinical update of DNA damage repair (DDR)targeting agent in non-small cell lung cancer (NSCLC) treatments. There are specific considerations that, if addressed, will significantly promote the quality of this manuscript. These recommendations are as follows:

1. Fundamental Background overview

The manuscript should incorporate a comprehensive overview on the fundamental background of each DDR machineries discussed, including PARP, ATR, AURK, APE1, DNA-PK, CHK1, CHK2. This inclusion will enable readers to better understand the landscape of DNA damage response and comprehend the rationale behind the developments of drugs within each class. The authors are suggested to draw the figures demonstrate the connection between each system.

Reply: We added figure 1 to demonstrate the overview about DDR machineries as advised. See figure 1.

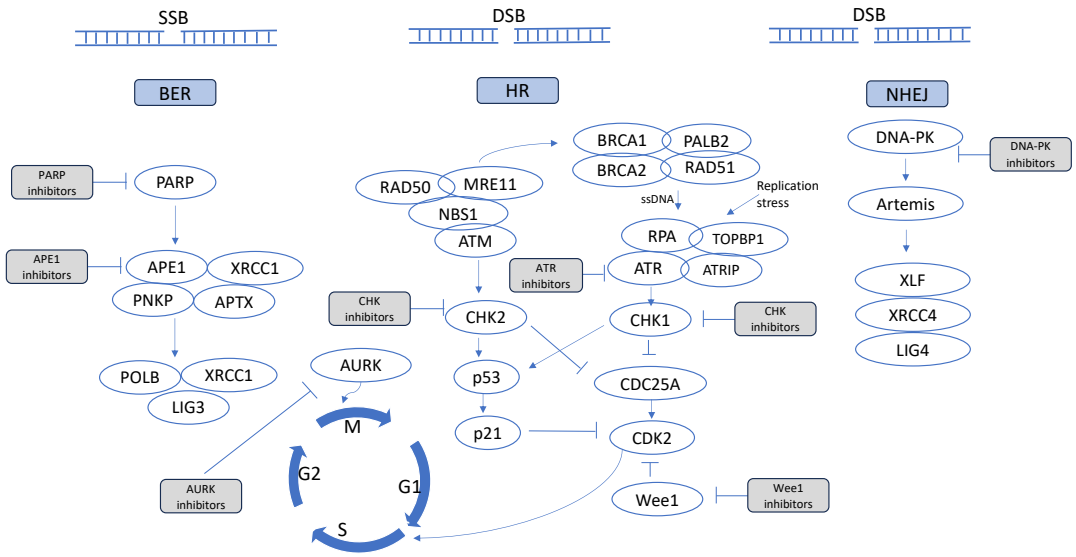

Figure 1.

Targeting DNA damage and response in NSCLC.

DNA damage types have been reported as single-strand DNA breaks (SSBs) or double-strand DNA breaks (DSBs). While base excision repair (BER) fix SSBs repair, DSBs repair requires homologous recombination repair (HRR), non-homologous end joining (NHEJ) and

alternative end joining (alt-EJ) pathways. Poly(ADP-ribose) polymerase (PARP) enzymes and apurinic/apyrimidinic endonuclease 1 (APE1) are key proteins involved in the base excision repair (BER) pathway of DNA lesion. Ataxia telangiectasia-mutated (ATM) checkpoint kinase 2 (CHK2) and ataxia telangiectasia and Rad3-related (ATR) checkpoint kinase 1 (CHK1) signals are two key pathways in HRR. CHK1 and CHK2 regulate cell cycle control checkpoints. WEE1 is a distinct nuclear kinase that regulates G2/M checkpoint transition in coordination with DDR. Aurora kinases plays a regulatory role of G2/M phase being involved in mitotic chromosomal segregation. The key role of DNA-PK kinase is in NEHJ repair. polynucleotide kinase 3 '-phosphatase (PNKP) ;XRCC1, X-ray repair cross complementing 1; POLB, DNA polymerase-β; LIG3, DNA ligase 3; ssDNA, single-strand DNA; MRN complex, MRE11-RAD50-NBS1; ATRIP, ATR-interacting protein; RPA, replication protein A; TOPBP1, DNA topoisomerase 2-binding protein; XRCC4,X-ray cross complementing protein 4; XLF, XRCC4-like factor; LIG4, DNA ligase 4.

2. Discussion on selectivity and specificity

The authors are encouraged discussed on selectivity and specificity of each class of compounds. It is important to elucidate how these agents exhibit higher selectivity toward cancer cells, rather than normal cells. This information is vital to support the safety of use of each agent in clinical practices.

Reply: We added selectivity and specificity in table 1 column 3.

Reviewer C

Specific points:

1) Throughout the article, there is significant plagiarism and improper citations. One is advised to look into these issues carefully.

Reply: There are many reviews to disclose the DNA repair, it may cause similar statements. We are not plagiarizing.

2) There are plenty of grammatical errors; I suggest authors opt for a professional editor to help ensure that grammatical mistakes are handled in the revised manuscript.

Reply:

1) We revised as follows: "DNA damage is repaired by specific cellular pathways during normal cell cycle. When DNA damage fails to be repaired or excised, the mutations will eventually trigger carcinogenesis." See page 2, line 57~58.

2) We deleted "There are several ATR inhibitors under development. deficiency increase sensitivity to ATR pathway targeted drugs in vitro." See page 7, line 210.

3) Using single-letter amino acid codes is advised while discussing specific gene mutations.

Reply: As other reviewer advised, we deleted "Cancers often present mutations of DNA repair genes, such as TP53 mutation in majority of the NSCLC patients and ATM loss in 40% of LUAD patients (page 2, line 64)." No specific gene mutation discusses for single-letter amino acid codes.

4) line 110: new DNA: this is an incorrect term; please use newly synthesized DNA.

Reply: We revised as “newly synthesized DNA”. See page 4, line 120.

5) Lines 126-134: Ideal to include the specific cell cycle stage where these pathways operate.
Reply: We revised as advised: “Homologous recombinant repair (HRR) is a complicated process pathway to repair DSB in S and G2 phases of the cell cycle.” See page 4, line 135~137.

6) 153: It's an incorrect statement. Revise it with a clear message.
Reply: We revised as follows: “Inhibition of PARP impair repair of SSBs that leads to synthetic lethality in HR-deficient (such as deleterious of BRCA1/2) cells which unable accurately repair DSBs.” See page 5, line 161~163.

7) Lines 156, 157: citations are missing
Reply: We added citations.
1) There are several PARP inhibitors have been approved by FDA in different cancers with or without HR-deficient such as Olaparib, Niraparib, Rucaparib, Talazoparib. See page 5, line 163~165, reference 49.
2) The development of Veliparib is not very successful, which may as a result of Veliparib is less PARP trapping and is inactive. See page 5, line 165~167, reference 50.

8) Throughout the article, the flow of language is not smooth; authors jump from one paragraph to another without presenting any interlinks. Honestly, a thorough proofreading by a professional editor would be helpful. In its current form, this review article is unsuitable for TLCR publication.
Reply: We reorganized the section 1.1.
LC generally exhibits a distinct genomic profile compared with other tumors, with high somatic mutational burden. Smoking is the main cause of LC, accounting for 90% of cases, however, approximately 20% of newly-diagnosed lung adenocarcinoma cases are never- or light-smokers in developed countries now. Smokers have higher somatic mutational burden than non-smokers and smokers show additional genomic instability process likely contributes to tumor progression. NSCLC primary tumors exhibit high genomic diversity with heterogenous tumor driver mutations present that clones may not all carry the same mutation makes it possible the patient will not benefit from targeted therapies.

See page 2, line 48~56.

Reviewer D:
I only checked the section "1.1. DNA Damage and genomic instability", however, too many errors are observed. I show the errors below. In fact, I cannot point out all the mistakes in this manuscript.

In Line 45-47 of page 2: “LC remains the leading cause of cancer death, with an estimated 2.2 million new cases of death and 1.8 million deaths in 2020.” I referred to Paper 3 and confirmed that 22,000 is the number of new patients, not the number of deaths. So please describe as follows: “LC remains the leading cause of cancer death, with an estimated 2.2

million new cases and 1.8 million deaths in 2020."
Reply: We revised as follows: "LC remains the leading cause of cancer death, with an estimated 2.2 million new cases and 1.8 million deaths in 2020." See page 2, line 35~36.

In Line 54 of page 2: "Smoking is the main cause of LC, accounting for 90% of cases, suggesting a genetic predisposition." I am unable to understand why the authors think that genetic predisposition explains the occurrence of the majority of lung cancer cases. As the authors mentioned in this sentence, smoking behavior accounts for most lung cancer cases worldwide, however, approximately 20% of newly-diagnosed lung adenocarcinoma cases are never- or light-smokers in developed countries now. I am not sure whether the authors are trying to describe this situation or not.
Reply: We revised following advice as follows: "Smoking is the main cause of LC, accounting for 90% of cases, however, approximately 20% of newly-diagnosed lung adenocarcinoma cases are never- or light-smokers in developed countries now. Smokers have higher somatic mutational burden than non-smokers and smokers show additional genomic instability process likely contributes to tumor progression" See page 2, line 49~53.

In Line 66 of page 2: "DNA damage is repair by specific cellular pathways during normal cell cycle." The authors should describe as follows: "DNA damage is repaired by specific cellular pathways during the normal cell cycle."
Reply: We revised as follows: "DNA damage is repaired by specific cellular pathways during the normal cell cycle." See page 2, line 57.

In Line 68-70 of page 2: "For instance, EGFR exon 19 deletion corrected with decreased expression of ERCC1 but also impact ERCC1 foci formation in response to DNA cross-link damage, contributing to DNA damage and repair (DDR) deficiency." I referred to Paper 6. The authors of Paper 6 found EGFR exon 19 deletion downstream signals not only inhibited ERCC1 expression but also influenced ERCC1 function in response to DNA damage. I am unable to understand what the authors of this review are trying to say in this sentence.
Reply: We want to describe DNA damage and fail to repair will trigger genome instability and carcinogenesis. In NSCLC, EGFR mutation are common to find, which impact in response to DNA damage and trigger carcinogenesis.

In Line 71 of page 2: The role of TP53 in DNA repair is complex and multifaceted. While TP53 is involved in DNA repair, its role in this process is complex and may be influenced by various factors. I do not think that TP53 should be involved as a DNA repair gene.
Reply: As we had described the role of TP53 in DSB repair section (page 4, line 145~147), we deleted "Cancers often present mutations of DNA repair genes, such as TP53 mutation in majority of the NSCLC patients and ATM loss in 40% of LUAD patients (see page 2, line 71)."

In Line 71 of page 2: The authors should replace "Mendelian syndromes" with "Mendelian disorders". A syndrome is a medical condition that is characterized by a particular group of signs and symptoms.
Reply: We revised as "Mendelian disorders" See page 3, line 73.

In Line 84 of page 3: KRAS is not a tyrosine kinase, but is a GTPase.

Reply: We revised as follows: “Despite a number of inhibitors had been developed for targeting EGFR, ALK, ROS1, KRAS, MET, NTRK, HER2 and RET”. See page 3, line 85~87.

In Line 85 of page 3: KRAS mutations are more prevalent in smokers than never-smokers with non-small cell lung cancer.

Reply: This section describes the current target therapies of NSCLC. So, we didn’t add the KRAS mutation are more prevalent in smokers here.