Organisation for Economic Co-operation and Development

Adverse Outcome Pathway Scientific Review Report

AOP 272: Direct deposition of ionizing energy leading to lung cancer.

Short name: Ionizing energy leading to lung cancer.

OECD contacts
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Report written by Erwin L Roggen (elro@3rsmc.onmicrosoft.com)

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1. Introduction and background to specific AOP

Introduction:

According to the World Cancer Research Fund, lung cancer is the most commonly diagnosed cancer with the highest incidence of occurrence on a global scale (excluding non-melanoma skin cancers). It is a multi-faceted disease exhibiting various genetic lesions and involving the accumulation of multiple molecular abnormalities over time.

Beside smoking, environmental, and indoor radiation exposure are significant contributors to lung cancer initiation and development. Risk assessment measures for defining acceptable exposure levels of radiation still remain uncertain; including the scientific research to support the justifications.

Background:

This AOP has organised the molecular and cellular based research in the area of radiation in a modular, simplistic path towards lung cancer. It has used data-rich key events to a classic targeted response onto a cell that is applicable to multiple radiation stressors and well supported thorough empirical evidence.

It has been well established that energy in the form of ionizing radiation causes DNA damage (such as DNA strand breaks, mutations, chromosomal aberrations) resulting in cancer cell differentiation/proliferation and eventually neoplastic transformation.

The AOP is also a case example of how existing evidence from radiation stressors can fortify empirical evidence surrounding key events that may be non-radiation specific and vice versa. By using a radiation centric molecular initiating event (MIE), networks can be developed for multiple adverse outcomes distinct to a radiation response. As different radiation stressors can trigger the MIE, the AOP will have wide applicability.

It was the goal of the authors to motivate radiation researchers to use this framework for bringing together research data, exchanging knowledge, identifying priority areas and better co-ordinating research in the low-dose ionizing radiation field.

2. Synthesis of main issues of the review

1. The remark was made that the MIE was not stressor independent.
2. The SRP[(1)] requested a clear motivation for why deposition of energy and not dose was selected as the 'stressor'.
3. The SRP expressed concerns with respect to the flow of the KEs, and the positioning of KE3 and KE4 with respect to KE1 and KE2. Double DNA strand breaks, mutations and chromosomal aberrations were considered equally important, with mutations and chromosomal aberrations also being the result of impaired DNA repair (KE2).
4. The SRP made the remark that important KEs were missing (e.g., ROS-mediated damage, inflammation, cell death) and that important risk modulating factors were not properly addressed (e.g., smoking).
5. The SRP was wondering why data on molecular and cellular mechanisms were missing, while such data were available from extensive (recent) studies.
6. The SRP noticed that important relevant literature was missing. A list with the most important references was provided by the SRP.

[(1)]SRP: Scientific Review panel

3. Summary record of the teleconference

3.1. TC agenda review panel meeting (May 6, 2021)

- Brief introduction to the process
- Discussion of the compilation of the individual scientific review reports which was produced and distributed by the review manager in advance of the meeting.
- Other issues.

3.2. Main issues and responses during the call

There was a great consensus with regard to the main issues related to this AOP. The following issues were considered most important:

- The remark was made that the MIE was not stressor independent in the strict sense of the word, which is in contrast to its definition.
    - It was proposed to include molecular and cellular data for other MIE's (e.g., chemical driven) that result in DNA damage.
- The selection of energy deposition in stead of dose was challenged,
    - An explanation for why deposition of energy and not dose was selected as the 'stressor' was requested.
- There were general concerns with respect to the flow of the KEs, and the positioning of KE3 and KE4 with respect to KE1 and KE2. Double DNA strand breaks, mutations and chromosomal aberrations were considered equally important, with mutations and chromosomal aberrations also being the result of impaired DNA repair (KE2).
    - The focus on double strand breaks instead of DNA damage was put on the list of issues to be discussed with the authors,
- Important KEs were missing (e.g., ROS-mediated damage, inflammation, cell death) while important risk modulating factors were not properly addressed (e.g., smoking).
    - This was considered an important weakness of the AOP.
- Data on molecular and cellular mechanisms were missing, while such data were available from extensive (recent) studies.
    - These data be included in the AOP.
- Important relevant literature was missing.
    - A list with the most important references was provided by the SRP.

3.3. Action list

The review manager will reach out to the authors of AOP272. The authors will be provided with a compilation of the comments, concerns, remarks raised by the members of the scientific review panel.

A (virtual) meeting will be organised to discuss these comments, concerns, remarks with the authors, and give them the opportunity to reply and/or explain their point of view.

The concrete outcome of this meeting is anticipated to be an agreed list of revisions to be made by the authors.

This deliverable was reproduced in section 4 below.

4. Summary of (planned) revisions.

The issues on the agenda of the meeting between the Scientific Review Panel and the authors (May 19, 2021) were synthesised in section 2.

The outcome of this meeting was as follows:

The authors are in agreement of the following proposed revisions:

1. The authors will include a clear motivation for why deposition of energy was selected as the 'MIE' in the background section.
2. The authors will include in the background section, information on relevant KEs (i.e., oxidative stress, inflammation, and cell death) that will be networked to AOP272. This information includes relevant AOPs that are under development.
3. The authors will highlight within the background section that smoking as an important risk modulating factor to lung cancer development with the inclusion of appropriate references. Also here, this information includes relevant AOPs that are under development.
4. The authors will incorporate the references provided by the reviewers within the appropriate KERs.

The Scientific Review Panel and the Authors agreed to drop the following:

1. The concern about the MIE not being stressor-free was dropped.
2. The concerns of the scientific review panel (SRP) with respect to the flow of the KEs, and the positioning of KE3 and KE4 with respect to KE1 and KE2, was dropped. It was decided that the relevant forms of DNA damage were included in the AOP and overall, the flow makes sense.
3. The remark regarding the lack of molecular and cellular mechanisms was dropped, as it appeared that an extensive amount of documentation was provided by the authors. The links to this documentation were however not part of the snapshot that was provided to the review panel.

5. Further discussion

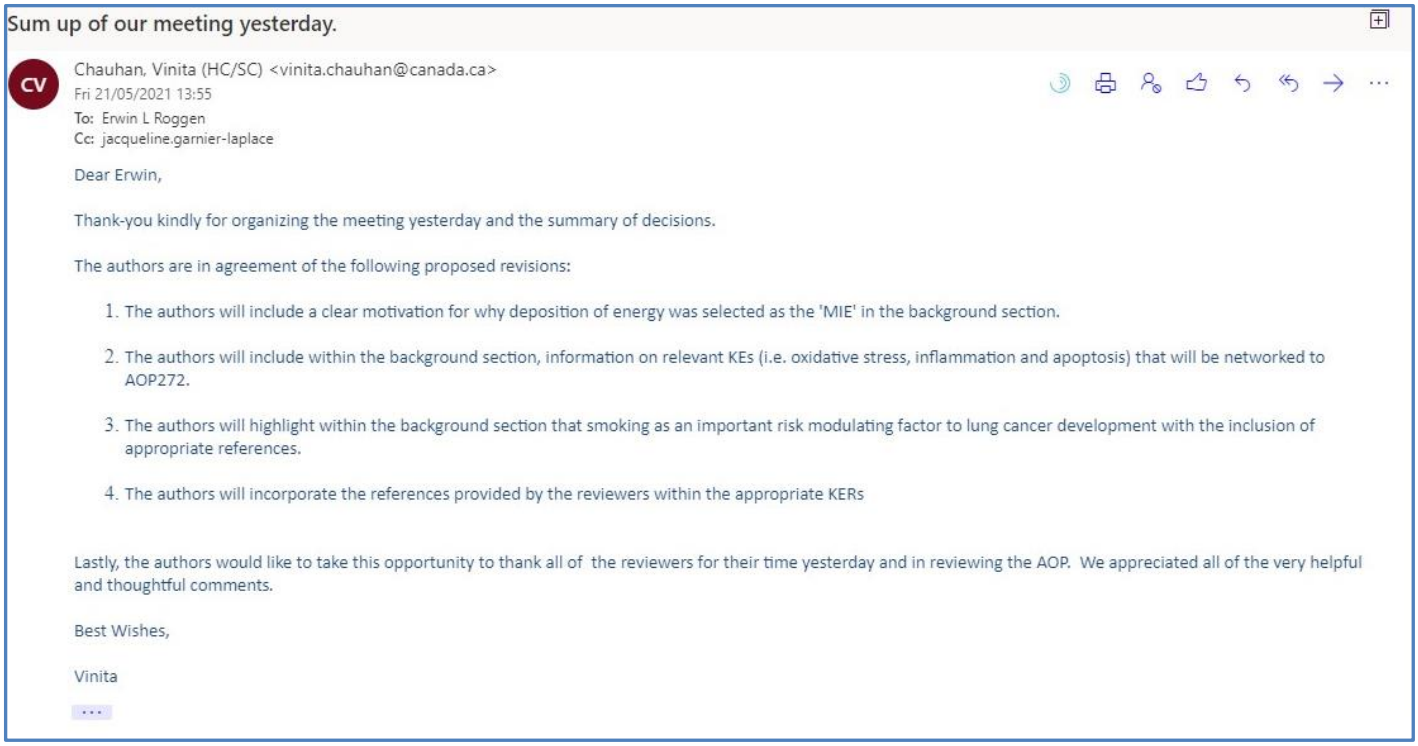
At this point no further discussion is planned or expected to be necessary.

6. Outcome of the scientific review

The authors are in agreement of the following proposed revisions:

1. The authors will include a clear motivation for why deposition of energy was selected as the 'MIE' in the background section.
2. The authors will include within the background section, information on relevant KEs (i.e. oxidative stress, inflammation and cell death) that will be networked to AOP272.
3. The authors will highlight within the background section that smoking as an important risk modulating factor to lung cancer development with the inclusion of appropriate references.
4. The authors will incorporate the references provided by the reviewers within the appropriate KERs.

Authors statement of agreement:



Sum up of our meeting yesterday.

CV

Chauhan, Vinita (HC/SC) <vinita.chauhan@canada.ca>
Fri 21/05/2021 13:55
To: Erwin L Roggen
Cc: jacqueline.garnier-laplace

Dear Erwin,

Thank-you kindly for organizing the meeting yesterday and the summary of decisions.

The authors are in agreement of the following proposed revisions:

1. The authors will include a clear motivation for why deposition of energy was selected as the 'MIE' in the background section.
2. The authors will include within the background section, information on relevant KEs (i.e. oxidative stress, inflammation and apoptosis) that will be networked to AOP272.
3. The authors will highlight within the background section that smoking as an important risk modulating factor to lung cancer development with the inclusion of appropriate references.
4. The authors will incorporate the references provided by the reviewers within the appropriate KERs

Lastly, the authors would like to take this opportunity to thank all of the reviewers for their time yesterday and in reviewing the AOP. We appreciated all of the very helpful and thoughtful comments.

Best Wishes,

Vinita

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Annex 1: Table with reviewers' name

Erwin L Roggen	elro@3rsmc.onmicrosoft.com	+45 31538020	**Review manager**	3RsMC ApS
Simon Bouffler	Simon.bouffler@phe.gov.uk	+44 0 1235 825086	**Review panel member**	Public Health England
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Based on the analysis of the declaration of interest from the review manager, the review organiser can confirm that the review manager has no potential conflict of interest (COI).
Based on the analysis of the declarations of interest made by the reviewers, the review manager can confirm that there are no potential COIs of reviewers.

Annex 2: Individual reviewers' comments

Reviewer A

1. Scientific quality:

- *Does the AOP incorporate all appropriate scientific literature and evidence?* Yes.
- *Does the scientific content of the AOP reflect current scientific knowledge on this specific topic?* Yes.
- *Other scientific issues?* I do not understand why the evidence drawn from alkylating agent studies has been included, similarly I am unclear why studies of germ-line mutation are references.

2. Weight of evidence:

- *Is the weight-of-evidence judgement/scoring well described and justified based on the evidence presented? If not please explain*. Yes.
- *Please consider weight-of-evidence for each Key Event Relationship (KER) and for the AOP as a whole.* The judgements on weight of evidence for all KERs is presented and in my view the judgments are sound in all case but one. On page 9 the evidence relating the plausibility of KER3 (increase in mutations) and the eventual AO (ling cancer) is provided, and the judgement is given as 'moderate' – I would consider the evidence to be strong here.
- *Other issues related to weight of evidence*. None.

3. Additional Observations:

- *Are there gaps that need to be filled out? If yes, specify*. Initially I was uncertain if the MIE of 'direct energy deposition' in cellular biomolecules was a correct use of the term MIE. There are other non-radiation lung carcinogens that do not act by energy deposition but rather by chemical modification of biomolecules. Thus, the given MIE is not stressor-independent; it could of course be considered stressor-independent when one considers only radiations of various types. Is there an option of re-defining the MIE so that it is more widely applicable to non-radiation agents? For example, could 'direct physical or chemical modification of biomolecules' be considered as a more generic term. In some cases where uncertainties are identified, the literature cited does not always appear to have been critically evaluated. While I can see that these sections will need to encompass and highlight what can be more 'marginal' views, is it correct to highlight such uncertainties where there is very limited or poor-quality publications used to support them? Overall, the authors have achieved a remarkable task in bringing together a very wide evidence base; the sheer length of the AOP document is impressive and it represents a very substantial piece of work. It does not however provide any genuinely novel insight into the process of lung carcinogenesis following radiation exposure, rather similar information has been captured in other formats and mathematical models. It does however provide an exemplar of the AOP approach in the radiogenic disease risk field. I was surprised to learn how much documentation is required for AOPs.
- *Other observations.* None.

Reviewer B:

1. Scientific quality:

- *Does the AOP incorporate all appropriate scientific literature and evidence?* The scientific literature of the last decades has been comprehensively reviewed. However, most recent studies of radiation-related risk might have been missed and should be discussed. The same applies to molecular studies of genomic profiles of lung cancer tissue from patients with and without radiation exposure. Cahoon et al. (2017), doi: 10.1667/RR14583.1, reports the most recent risk estimates for the life span study (LSS) of Japanese a-bomb survivors. More importantly, radiation risk is considered in conjunction with smoking as the main risk factor for lung cancer. The studies of lung cancer risk for uranium miners need to be updated with the most recent data sets. Rage et al. (2020), doi: 10.1136/oemed-2019-105981, present already details of a large pooled miners cohort. The references cited therein for the separate studies could be helpful. to update risk studies for individual cohorts. Campbell et al. (2016) doi:10.1038/ng.3564 have published a comprehensive genomic profiling of lung adenocarcinoma and squamous cell carcinoma. This study is important since profiles of radiation-induced lung cancer can be potentially judged against those of Campbell et al. (2016). Studies of genetic variations in radon-induced lung cancer should also be added (e.g. Leng et al. (2013), doi: 10.1093/carcin/bgt024). On the other hand, many cited studies are not specific to radiation-induced lung carcinogenesis but are concerned with general molecular radiation-related processes that are proposed as MIEs for carcinogenesis in any organ. It is not always clear how these general processes apply to lung carcinogenesis in particular. A comb-out of less important (non-specific)

studies and the inclusion of the most recent studies on risk and molecular findings especially dedicated to lung carcinogenesis would make the AOP more focused and relevant.

- *Does the scientific content of the AOP reflect current scientific knowledge on this specific topic?* By featuring the DNA repair pathway described in KE1-KE4 as the unique road to radiation-induced lung cancer the AOP reflects the accepted notion of radio-carcinogenesis in general but may potentially omit other relevant pathways. The induction of radiation-induced lung cancer could possibly happen without involving the DNA repair pathway. Cell-based models applied to miner cohorts (reviewed in Rühm et al. (2017) doi: 10.1080/09553002.2017.1310405) point to enhanced clonal expansion as the results of protracted radon exposure, although increased mutation rates may also play a role. For radon-induced lung cancer growth advantage by inactivation of epithelial cells has been proposed as a mechanism to explain the model results (Heidenreich & Paretzke 2008, doi: 10.1667/RR0957.1). For a mixed field of photons/neutrons from the a-bomb explosions Castelletti et al. (2019 doi:10.1093/carcin/bgz036 developed a biologically-based model of lung adenocarcinoma from smoking and radiation. They found chronic enhancement of clonal expansion the main biological effect of acute radiation exposure whereas the effect of increased mutations rates was found not be of statistical significance. Furthermore, after analysis of genomic profiles the authors suggest two separate molecular pathways to lung adenocarcinoma. The two pathways are characterised by groups of oncogenes termed as either receptor mutant (R^{mut}) or transducer mutant (T^{mut}) with respect to their functions close to the cell membrane or along the direction of the cell nucleus. In line with the model results an experimental mouse model for low LET exposure to the esophagus resulted in enhanced clonal expansion of p53-mutated cells which outcompeted wild-type cells due to the greater resistance to oxidative stress. (Fernandez-Antoran et al. (2019), doi 10.1016/j.stem.2019.06.011). Radiation action in tissue does not only lead to DSBs, mutations and CAs. It may also cause DNA methylation (reviewed in Miousse et al. 2018 doi:10.1615/CritRevOncog.2018025687). The essentiality of the DNA repair pathway should be reassessed in view of the findings discussed above.
- *Other scientific issues?* A clearer distinction between different radiation qualities (photons, alpha particles, heavy ions, space radiation) in the characterisation of the MIE would help to illustrate the variety in molecular damage. Do photons and alpha particles cause effectively the same damage albeit with different radiobiological effectiveness? Or are there qualitative differences which may trigger different paths to lung cancer. Interestingly, the MIE is related to energy deposition and not to radiation dose. Is there a reason not to start the AOP with tissue dose? There is also considerable difference in the radiation risk for different histologic types of lung cancer (Kreuzer et al. 2000, PMID: 11135223, Ramkissoon et al. 2018, Egawa et al. 2012). Whereas lung adenocarcinoma is more likely caused by photons squamous cell carcinoma are mostly related to alpha particles. Since it may be possible in the future that the present AOP splits into more end-point specific AOPs the histologic subtypes should be considered with greater depth.

2. Weight of evidence:

- *Is the weight-of-evidence judgement/scoring well described and justified based on the evidence presented? If not please explain.* The WoE judgement is well balanced with strengths and weaknesses duly highlighted.
- *Please consider weight-of-evidence for each Key Event Relationship (KER) and for the AOP as a whole.* Although the WoE for each KER is convincing the case for the AOP as a whole may be incomplete because important KEs are understood only qualitatively. For example, the authors could not identify data establishing KER5 (increase mutations leads to increase cell proliferation). Whereas experimental evidence is mostly related to single KERs biologically-based models may help for a more comprehensive understanding of the interplay between KE and the relation to risk assessment. Kaiser et al. (2021), doi: 10.1080/09553002.2020.1784490, have discussed the role of such models in the AOP framework.
- *Other issues related to weight of evidence.* Radiation-induced lung carcinogenesis is of stochastic nature. Although WoE for the KERs to lung cancer may be high for each KER separately the present AOP does not address the dynamics and synergetic effects of lung carcinogenesis and does not reflect the stochasticity of KERs. In particular, does the presented sequence of KEs inadvertently result in lung cancer or may the process die out in some cases?

3. Additional Observations:

- *Are there gaps that need to be filled out? If yes, specify.* The compilation of material for the AOP is impressive and offers to the reader a general and connected view of the present knowledge on radiation-induced lung cancer. However, an obvious research gap is related to the timeline of lung carcinogenesis. Whereas in-vitro and in-vivo studies have a typical follow-up of one month after exposure for cell-based and tissue effects (KEs 1-4), lung cancer mostly develops many years after exposure. For uranium miners

lung cancer risk peaks about 15 years after first exposure to radon (Assenmacher et al. (2019), doi: 10.1007/s00411-019-00800-6). Although atypical alveolar hyperplasia (AAH) are known as pre-neoplastic lesions for lung adenocarcinoma, their composition and timeline for development are barely known. Future research clearly needs to describe the dynamic development of pre-neoplastic lesions and the molecular processes involved.

- *Other observations.*
  p21/140
  In the "Considerations of Potential Applications of the AOP" it is emphasized that at present AOPs are not applied for regulatory decision-making in the RP community. On the other hand, in the field of toxicology AOP development is driven by the need for regulatory arrangements. AOPs are developed under the auspices of OECD-NEA but recommendations for radiation protection are issued by international bodies such as UNSCEAR or ICRP and also by national committees. In this situation there might be a potential for future conflict should OECD-NEA emerge as an additional player to issue RP recommendations. Quantitative recommendations for radiological protection are mainly based on risk estimates from radio-epidemiological cohorts with adequate personal dosimetry. Hence, epidemiology and dosimetry are the main drivers of RP regulations whereas contributions from radiobiology are mostly of qualitative nature. Formulation of radiation-related AOPs are a welcome step towards a integration of the three fields for improved quantitative risk assessment.
  p 3/140 last paragraph: Egawa et al. 2012 is not concerned with radon exposure. The lung cancer risk model from Furukawa et al. (2010), doi: 10.1667/RR2083.1, is applied to histologic types in the LSS.
  p20/140 Figure 1:
  RRs are given only for radon exposure (alpha-particles) but doses are shown on the x-axis. For uranium worker studies risk are calculated per WLM. How was the dose conversion done? Japanese a-bomb survivors have been exposed to a mixed field of photons/neutrons. Can LSS risk estimates also be shown in Figure 1? What about the indoor radon studies of Darby et al. (2005) and Krewski et al. (2005).
  p35/140
  Reference Okaysu R (2012) is duplicated

Reviewer C:

1. Scientific quality:

- *Does the AOP incorporate all appropriate scientific literature and evidence?* Following literature regarding mutational spectra of different radiation qualities, e.g., HLET radiation, such as alpha particle are missing and may result in different KE outcomes (large deletions for HLET versus point mutations for LLET radiation) should be added:
  Hande MP, Azizova TV, Geard CR, Burak LE, Mitchell CR, Khokhryakov VF, Vasilenko EK, Brenner DJ. Past exposure to densely ionizing radiation leaves a unique permanent signature in the genome. Am J Hum Genet. 2003 May;72(5):1162-70.
  Kraemer SM, Kronenberg A, Ueno A, Waldren CA. Measuring the spectrum of mutation induced by nitrogen ions and protons in the human-hamster hybrid cell line A(L)C. Radiat Res. 2000 Jun;153(6):743-51
  Wu LJ, Randers-Pehrson G, Xu A, Waldren CA, Geard CR, Yu Z, Hei TK. Targeted cytoplasmic irradiation with alpha particles induces mutations in mammalian cells. Proc Natl Acad Sci U S A. 1999 Apr 27;96(9):4959-64.
  Schmidt P, Kiefer J. Deletion-pattern analysis of alpha-particle and X-ray induced mutations at the HPRT locus of V79 Chinese hamster cells.
  Mutat Res. 1998 Nov 3;421(2):149-61.
  Tom K. Hei, Li-Jun Wu, Su-Xian Liu, Diane Vannais, Charles A. Waldren, and Gerhard Randers-Pehrson. Mutagenic effects of a single and an exact number of α particles in mammalian cells. PNAS April 15, 1997 94 (8) 3765-3770
  Bauchinger M, Schmid E. LET dependence of yield ratios of radiation-induced intra- and interchromosomal aberrations in human lymphocytes. Int J Radiat Biol. 1998 Jul;74(1):17-25. doi: 10.1080/095530098141681

  Bauchinger M, Schmid E, Braselmann H, Kulka U. Chromosome aberrations in peripheral lymphocytes from occupants of houses with elevated indoor radon concentrations. Mutat Res. 1994 Oct 1;310(1):135-42

Zölzer F, Hon Z, Skalická ZF, Havránková R, Navrátil L, Rosina J, Škopek J. Micronuclei in lymphocytes from radon spa personnel in the Czech Republic Int Arch Occup Environ Health. 2013 Aug;86(6):629-33. doi: 10.1007/s00420-012-0795-z. Epub 2012 Jul

Kreuzer M, Fenske N, Schnelzer M, Walsh L. Lung cancer risk at low radon exposure rates in German uranium miners. Br J Cancer. 2015 Nov 3;113(9):1367-9.

- *Does the scientific content of the AOP reflect current scientific knowledge on this specific topic?* The key events described are all essential. Key event relationships are mostly well described and quantitative response response relationships are given. Methods suggested for testing the different KE are state of the art. However, three aspects are not regarded: the role of ROS in respect to alteration of tumor microenvironment and radiation induced cell death. Radiation acts not only by initiating cells but also alterates normal tissue reactions to induce a tumor promoting microenvironment? (see also below) This may have an impact and the need to include further KE. Here low dose irradiation triggers other signalling pathways/cell mechanisms and therefore probably other KE than high dose irradiation. Mainly through stimulating unspecific immune reactions. HLET acts through direct energy deposition and produces low ROS formation, while LLET acts mainly through indirect (ROS) producing cellular reactions. Also, here different KE may be triggered. The described AOP fits well for HLET or high doses.
- *Other scientific issues?* It is stated in the overall assessment that with a radiation stressor, a single hit to the DNA molecule could drive a path forward to lung cancer although with low probability. This very nlikely and depends on the deposited energy. For solid cancer the theory is that more than 1 hit (in general 4-7 hits are needed). [Lawrence A. Loeb, Keith R. Loeb, and Jon P. Multiple mutations and cancer; Anderson PNAS February 4, 2003 100 (3) 776-781]. Also, it is stated that: at much higher doses, a cell will induce apoptosis and may not be driven to cancer induction. Here the cell signalling from cell death can promote the path to cancer for already initiated cells.

2. Weight of evidence:

- *Is the weight-of-evidence judgement/scoring well described and justified based on the evidence presented? If not please explain.*

  MIE Event: 1686: Direct Deposition of Energy. Weight of evidence for essentiality of MIE: weak. Scoring is not plausible. Why weak? Weak is defined if No or contradictory experimental evidence of the essentiality of any of the KEs. Modelling of energy deposition clearly supports this MIE – although it is not accepted by OECD. Energy deposition can be demonstrated through microbeam exposure and life cell imaging of reporter cells. Induction of strand breaks can be experimentally measured by filter elution or Comet-Assay experiments (neutral and alkaline) and correlates well with dose/energy deposition. Energy deposition correlates well with cell killing (ICRP60) and chromosomal breakage, e.g., dicentric induction, increasing dose correlates well with lung cancer incidence.

  KE 1 Event: 1635: Increase, DNA strand breaks**.** Double strand breaks or strand breaks? The diagram depicts: DNA double strand breaks for KE1? Evidence for essentiality: weak. The scoring is not plausible. In experiments a direct correlation between DSB and survival was already determined in the 80th (K H Chadwick 2017 J. Radiol. Prot. 37 422). Increased number of DSB and especially complex DSB have effects on all downstream events. DNA damage in general is a must event before KE2. If only DNA double strand breaks are here the most important KE leading to lung cancer is not clear. For chromosomal aberrations DSB are essential, but not for mutation induction DNA damage in general is important. Single strand breaks and base mutation events are magnitudes of order more frequent than DNA double-strand breaks.

  Methods to detect strand breaks: Filter elution techniques are missing, velocity sedimentation of DNA through neutral and alkaline sucrose gradients (mentioned in KER MIE and KE1), Gamma H2AX-Foci quantification by Western blot is not very sensitive.

  KE 2 Event: 155: N/A, Inadequate DNA repair. Description of Key event should stress especially the radiation induced damage and focus on the repair machinery used to repair this damage. Fidelity can only be measured indirectly should be included, e.g., Chromosomal aberrations. With respect to direct measurement of repair efficiency should GammaH2AX time course be included

  KE 3 Event: 185: Increase, Mutations. Mutational spectra of radiation induced mutations are missing.

  KE 4 Event: 1636: Increase, Chromosomal aberrations. Evidence for essentiality is weak. Scoring is not plausible.

  Here also, increased chromosomal aberrations result in increased cell death or inactivating mutations in proliferation control genes. This event is preceding cell proliferation KE and directly be correlated to lung cancer.

KE 5 Event: 870: Increase, Cell Proliferation. This KE is essential but not only mutations will result in accelerated proliferation but also cell death and inflammatory reactions will induce increased proliferation this is missing here as an upstream KE. The new data from Martincorena and collegues (Science 2018) should be incorporated: "Tilting cell fate balance away from differentiation toward proliferation may confer a competitive advantage on mutant cells in normal esophageal epithelium".

- *Please consider weight-of-evidence for each Key Event Relationship (KER) and for the AOP as a whole.*
  Relationship: 1977: Energy Deposition leads to Increased DNA strand breaks. Well described, ROS also depicted, Dose and incidence concordance is depicted, temporal concordance: microbeam studies demonstrate the temporal concordance well and should be mentioned, both KE are essential.
  Relationship: 1911: Increase, DNA strand breaks leads to N/A, Inadequate DNA repair. KER empirical evidence: double strand break and inadequate DNA repair: moderate. How is inadequate DNA repair measured? Not only studies describing repair efficiency should be mentioned here but also fidelity. Fidelity cannot be measured by the percentage of non-repaired double strand breaks, but by missrepair detection. Missrepair is detected by mutation analysis (deletions, insertions or chromosomal aberrations). Repair fidelity or sensitivity of the different cell cycle phases supports the different active and more or less error prone repair pathways.
  Data from Löbrich and Jeggo 2017 (Trends Biochem Sci. 2017 Sep; 42(9): 690–701) can be incorporated.
  Relationship: 164: N/A, Inadequate DNA repair leads to Increase, Mutations. OK
  Relationship: 1912: N/A, Inadequate DNA repair leads to Increase, Chromosomal aberrations. Also, here data from Löbrich and Jeggo 2017 (Trends Biochem Sci. 2017 Sep; 42(9): 690–701) can be incorporated. For AT patients there is a clear increased background of chromosomal aberrations and also a clear radiation induced increased CA frequency, e.g., Bucher M, Endesfelder D, Roessler U, Borkhardt A, Dückers G, Kirlum HJ, Lankisch P, Oommen PT, Niehues T, Rübe CE, Baumgartner I, Bunk F, Moertl S, Hornhardt S, Gomolka M. Analysis of chromosomal aberrations and γH2A.X foci to identify radiation-sensitive ataxia-telangiectasia patients. Mutat Res. 2021 Jan-Feb;861-862
  Relationship: 1978: Increase, Mutations leads to Increase, Cell Proliferation. Martincorena et al. Science 2018 should be integrated.
  Relationship: 1979: Increase, Chromosomal aberrations leads to Increase, Cell Proliferation**.** OK.
  Relationship: 1980: Increase, Cell Proliferation leads to Increase, lung cancer. Ok
  Relationship: 1981: Energy Deposition leads to Increase, Mutations. OK
  Relationship: 1982: Energy Deposition leads to Increase, Chromosomal aberrations. OK, see missing literature and data in section 1.
  Relationship: 1983: Energy Deposition leads to Increase, lung cancer. Radiation and promotion effect is missing, this is especially important in solid cancer development. Reference on lung cancer subtype and radon to be added: Taeger D, Fritsch A, Wiethege T, Johnen G, Eisenmenger A, Wesch H, Ko Y, Stier S, Michael Muller K, Bruning T, Pesch B. Role of exposure to radon and silicosis on the cell type of lung carcinoma in German uranium miners. Cancer. 2006 Feb 15;106(4):881-9.
  Relationship: 1931: Increase, DNA strand breaks leads to Increase, Mutations. OK
  Relationship: 1939: Increase, DNA strand breaks leads to Increase, Chromosomal aberrations. OK but add Löbrich and Jeggo 2017
  Relationship: 1984: Increase, Mutations leads to Increase, lung cancer. Comprehensive genomic profiles of small cell lung cancer are available, but apparently not considered important for this AOP **[**George J, Lim JS, Jang SJ, Cun Y, Ozretić L, Kong G, Leenders F, Lu X, Fernández-Cuesta L, Bosco G, Müller C, Dahmen I, Jahchan NS, Park KS, Yang D, Karnezis AN, Vaka D, Torres A, Wang MS, Korbel JO, Menon R, Chun SM, Kim D, Wilkerson M, Hayes N, Engelmann D, Pützer B, Bos M, Michels S, Vlasic I, Seidel D, Pinther B, Schaub P, Becker C, Altmüller J, Yokota J, Kohno T, Iwakawa R, Tsuta K, Noguchi M, Muley T, Hoffmann H, Schnabel PA, Petersen I, Chen Y, Soltermann A, Tischler V, Choi CM, Kim YH, Massion PP, Zou Y, Jovanovic D, Kontic M, Wright GM, Russell PA, Solomon B, Koch I, Lindner M, Muscarella LA, la Torre A, Field JK, Jakopovic M, Knezevic J, Castaños-Vélez E, Roz L, Pastorino U, Brustugun OT, Lund-Iversen M, Thunnissen E, Köhler J, Schuler M, Botling J, Sandelin M, Sanchez-Cespedes M, Salvesen HB, Achter V, Lang U, Bogus M, Schneider PM, Zander T, Ansén S, Hallek M, Wolf J, Vingron M, Yatabe Y, Travis WD, Nürnberg P, Reinhardt C, Perner S, Heukamp L, Büttner R, Haas SA, Brambilla E, Peifer M, Sage J, Thomas RK. Nature. 2015 Aug 6;524(7563):47-53].
  Relationship: 1985: Increase, Chromosomal aberrations leads to Increase, lung cancer. Studies investigating specifically chromothripsis in lung cancer are missing here.
- *Other issues related to weight of evidence*. None

3. Additional Observations:

- *Are there gaps that need to be filled out? If yes, specifiy*. Radiation induced cell death with link to complex chromosomal aberrations is missing and plays a key role in accelerated proliferation/repopulation and a higher chance that mutated cells have a selection advantage. This event is not the same for all radiation qualities and doses but especially triggered by complex damage induced by high LET radiation (e.g., via alpha particles) and is here a key element between chromosomal aberrations – proliferation. These cells will undergo cell death and may not be driven to cancer induction but will stimulate other cells bearing possibly a growth advantage by driver mutations, e.g., p53 mutated cells. Evidence and integration on inflammatory processes is missing – radiation as an tumor promoting agent is not integrated yet and may even have a larger impact than to induce an initiated cell. An initiated cell can only become tumorigenic if the surrounding allows it. Cancer is therefore not only the result of an accumulation of mutations in one cell or cell line but moreover its evolution is dependent on the dysfunction of defense mechanism of the surrounding cells or tissue (Barcellos-Hoff and Nguyen 2009) and the escape of the immunological surveillance (Smyth et al. 2006).
- *Other observations*. Spelling mistakes and literature formation need to be corrected. Correct the citation Schmid E, **Hieber** L, Heinzmann U, Roos H, Kellerer AM. Analysis of chromosome aberrations in human peripheral lymphocytes induced by in vitro alpha-particle irradiation. Radiat Environ Biophys. 1996 Aug;35(3):179-84.

Reviewer D:

1. Scientific quality:

- *Does the AOP incorporate all appropriate scientific literature and evidence?* Work by CC Harris in human tissues should be included. Egawa 2012 is cited to mean that there are different histologies from smoking and radiation. Not really what this paper says, and single paper is not definitive, and probably not correct as paper does not consider the marked changes in smoking lung cancer histologies over time.
- *Does the scientific content of the AOP reflect current scientific knowledge on this specific topic?* Shouldn't there be a clear section on research gaps as document says that this should drive research. This has mutations as later events after strand breaks – is this a correct sequence, or is the KE 1 strand breaks AND mutations. The document looks at inadequacy of or faulty DNA repair – this implies radiation effect on DNA repair, but what about saturation of DNA pathways that are redundant. Maybe this is implied but not clear. Document describes progression from hyperplasia to lung cancer, which opens door to reversibility of early lesions. Discussions non-threshold and linear models – so what is the data that supports or detracts from this model Impact of oxidative damage as part of the strand breaks and mutations?
- *Other scientific issues?* The following are gaps, but the document says that this is not meant to have all AOPs, so maybe not appropriate. Insufficient attention to dose. Do lower doses in the population have the same strength of evidence, could the pathways be different? Is the quantitative understanding really high? Insufficient attention to age at exposure. Is developing lungs in children affected differently than adults, for example from indoor radon? Interaction with smoking not really discussed – smoking impacts DNA repair mechanisms. Cell proliferation called out - why just this. What about host immunity (lots of data for tumour mutational burden, but do not know about radon). And what about tumour microenvironment effects by radiation.

2. Weight of evidence:

- *Is the weight-of-evidence judgement/scoring well described and justified based on the evidence presented? If not please explain.* Mostly agree, see below.
- *Please consider weight-of-evidence for each Key Event Relationship (KER) and for the AOP as a whole.*

Evidence supporting KE2 is scored strong, while it does not include have human data. And what is the impact of smoking, which is huge.

- *Other issues related to weight of evidence*. See other scientific issues above.

3. Additional Observations:

- *Are there gaps that need to be filled out? If yes, specifiy.* See other scientific issues above.
- *Other observations.*Seems like very classical approach. Isn't there a lot more molecular biology in this field?

Annex 3: Written responses from the authors

Besides the confirmation of the actions listed in section 6, no written responses was either requested by the scientific review panel or provided by the authors.