Organisation for Economic Co-operation and Development

DOCUMENT CODE

For Official Use **English - Or. English**

Adverse Outcome Pathway External Review Report

AOP 277: Impaired IL-1R1 signaling leading to increased susceptibility to infection

This document has been prepared by Dr. Chantra Eskes and Dr. Iwona Wilk-Zasadna, review managers of AOP 277 scientific review.
It compiles the views and comments of the reviewers and provides recommendations to the authors of the AOP for subsequent revision of the AOP.
It provides the basis to EAGMST for determining if AOP 277 has been adequately revised by their authors following the review and if it can be released to the Working group of the National Coordinators of the Test Guidelines Programme and to the Working Party on Hazard Assessment for endorsement

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Scientific review of the Adverse Outcome Pathway 277: Impaired IL-1R1 signaling leading to increased susceptibility to infection

- Report from review panel -

Date: 22 July 2021

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1 Introduction and background to AOP 277

The Adverse Outcome Pathway 277 outlines "*Impaired IL-1R1 signaling leading to increased susceptibility to infection*" (https://aopwiki.org/aops/277). The AOP developers are Yutaka Kimura and Setsuya Aiba from the Tohoku University Graduate School of Medicine, Japan.

AOP 277 proposes the following sequence:

- Molecular Initiating Event (MIE): Impaired IL-R1 signaling (event ID 1700)
- Key Event 1 (KE1): Inhibition of Nuclear factor kappa B (NF-κβ, event ID 202)
- Key Event 2 (KE2): Suppression of T cell activation (event ID 1702)
- Adverse Outcome (AO): Increased susceptibility to infection (event ID 986)

AOP 277 was created in December 2018, and underwent EAGMST internal review with a report dated from September 2019 (see "Discussion" in AOP 277 webpage). Change log dates also report the major changes occurred in May 2019 and Nov. 2019 (see "view history" in AOP 277 webpage). The review panel notes that some original key events were removed during the process of AOP review by EAGMST.

In January 2021 a call for nomination of experts for reviewing AOP 277 was made to the OECD Expert Group on Detailed Review Paper (DRP) development for *in vitro* immunotoxicity assays. A total of 11 nominations were received, out of which five experts from different backgrounds and geographical regions were selected in March 2021 (Annex 1).

A kick-off meeting took place on April 2, 2021 in which the process of the AOP review was presented by the OECD secretariat. After that the following steps and teleconferences took place:

- 23 April 2021: Scientific evaluation and written comments were received from all review panel members (Annex 2).
- 4 May 2021: AOP developers provided responses to the written comments from reviewers (Annex 3).
- 7 May 2021: Joint meeting between the AOP developers and the review panel to address the written comments from the review panel (Annex 4).
- 14 May 2021: Teleconference for the review panel only (Annex 5).

- 18 May 2021: Clarifications on comments from review panel to AOP developers (Annex 6)
- 25 May 2021: Additional responses provided by the AOP developers to the review panel (Annex 7).
- 28 May 2021: Further information provided by the OECD to the review panel (Annex 8).
- 11 June 2021: Teleconference from the review panel only to address the additional information received and started to draft the conclusions reported here.
- 25 June 2025: Teleconference from the review panel to finalize this review report.

2 Main issues of the review

The following general considerations were made by the scientific review panel regarding AOP 277 based on the scientific evaluation from the individual reviewers (Annex 2), responses from the AOP developers (Annexes 3, 4 and 7), discussions from the meetings of 7 and 14 May (Annexes 4 and 5) and the additional information provided by the OECD (Annex 8).

i) AOP 277 is a simple AOP that may not capture the complexity of events that may occur, as there are a number of other pathways that can lead to the adverse outcome 'increased susceptibility to infection', not currently described within AOP 277. Following clarifications from the OECD (see Annex 8), it is understood that some key events and relationships were modified and removed following internal EAGMST review. The scientific panel recommends making the AOP more specific. Information obtained from e.g. monoclonal antibodies clinical trials could help in better defining the specifics of the AOP with human mechanistic relevance of the pathway and the possible and increase risk of infections.

ii) Regarding the Molecular Initiating Event, examples given were based on antibodies. To increase the utility of the AOP for risk assessment and regulatory decision making, it was recommended that good examples are given on drugs and chemicals. Similarly, it is also recommended to provide examples based on drugs and chemicals for the occurring downstream events, i.e. drugs and/or chemicals, that by affecting IL-1R1 signalling, lead to increased susceptibility to infection. The review panel has concerns that there is not more information available on chemicals, for an AOP meant to be applicable to chemicals. If the AOP developers cannot find chemicals that have effects downstream of the IL-1R, but can find chemicals that, for example, reduce IL-1 levels, it is recommended that the AOP is expanded to include these chemicals.

iii) The review panel questioned whether NF-κβ is an essential part of the pathway between impaired IL-1R1 signalling and increased susceptibility to infection. Indeed, there may be other ways to increase susceptibility to infection that do not involve NF-κβ. For example, IL1-R1 may activate other signal transduction pathways than NF-κβ. The review panel suggests including additional key events in a kind of a hub of key events such as AP-1 in parallel to NF-κβ, so that both NF-κB and AP-1 converge on leading to impaired T cell activation. The review panel notes that the AOP originally included a key event on AP-1 that was removed. Such an addition would support the essentiality of the hub of key events.

iv) A number of different T cells exist (e.g., CD4 (e.g., Th1, Th2, Th17, Treg, etc.), CD8, gamma-delta, etc.). Furthermore, other cells (e.g. B cells, dendritic cells) also play a

critical role in infection. The panel recommends clarifying which types of T cells are addressed by AOP 277 and whether other cells may also be considered (e.g. B cells, dendritic cells). Information gathered from monoclonal antibodies clinical trial reports as suggested in point i) could also help in providing additional information here.

v) Infection is a very broad term, and linking T cells to all types of infection could be perceived as unrealistic and overly simplistic. Thus, the panel recommends clarifying which type(s) of infection(s) are covered by AOP 277.

3 Scientific assessment

The review panel addressed the main questions requested by OECD as described below and in more detail in Annex 2.

3.1. Scientific quality

3.1.1. Does the AOP incorporate all appropriate scientific literature and evidence?

The review panel is of the opinion that the AOP incorporates all appropriate scientific literature and evidence. However, more could have been done as described below.

- Address in more detail the complexity of the immune response towards pathogens and the different types of immune cells involved, from innate to specific immunity (see also section 2 on main issues of the review).
- Provide quantitative understanding for the KER “suppression of T-cell activation” based for example, on the scientific literature of T-cell dependent antibody response (TDAR) in experimental animals or vaccination responses (Ab titres) in humans.
- Identify/discuss knowledge gaps related to e.g. suppression of T cell activation.
- Ensure that relevant supporting evidence for stressors are provided in their dedicated webpages.

3.1.2. Does the scientific content of the AOP reflect current scientific knowledge on this specific topic?

The review panel is of the opinion that the content of the AOP generally reflects the current level of scientific knowledge. However, more information should have been considered such as compensatory mechanisms and redundancy in the immune response as discussed in section 2 of this report.

3.2. Weight of evidence

3.2.1. In your opinion, is the weight-of-evidence judgement/scoring well described and justified based on the evidence presented? If not please explain?

The review panel is of the opinion that the weight-of-evidence judgement/scoring is well described and justified. However, the essentiality of NF-κβ is questioned. If AOP developers would add additional key events in for example, a hub of key events such as AP-1 in parallel with NF-κβ, and then both NF-κB and AP-1 converging on Impaired T cell activation, it could help

address the issue of essentiality of this key event. In addition, it is recommended to provide evidence/literature for each stressor or alternatively, modify the evidence term to null or low.

3.2.2. Please consider weight-of-evidence for each KER and for the AOP as a whole.

In general, the weight-of-evidence was considered high. However, it would be helpful to clarify which functions of the T cell are compromised when NF-κβ is impaired, as well as the relationship between T cell activation and infection. Furthermore, if quantitative relationship between two events could be demonstrated, this would strengthen the relationship. The following recommendations are made regarding each key event relationship:

- KER “Impaired IL-1R1 signalling leads to Inhibition, Nuclear factor kappa B (NF-κβ)”: the information provided could be better organised i.e., a more detailed description of the sequence of events that link the initial molecular event to the inhibition of NF-κβ is recommended.

- KER “Inhibition, Nuclear factor kappa B (NF-κβ) leads to Suppression of T cell activation”: It is unclear from the literature provided, what degree of reduction in NF-κβ activation is required to have suppression of T cell activation. Furthermore, as recommended in section 2 iii) it would be useful to add additional key events in a kind of a hub of key events such as e.g., AP-1 in parallel with NF-κβ, and link both to the impaired T cell activation. Finally, as recommended in section 2 iv) the panel recommends clarifying which types of T cells are addressed by AOP 277 and whether other cells may also be considered (e.g. B cells).

- KER “Suppression of T cell activation leads to Increased susceptibility to infection”: the KER relates to a very broad relationship and would benefit from having more details given as to increase the weight of evidence. Furthermore, it would be helpful to understand the quantitative relationship on what degree of suppression in T cell activation is required to have increased risk of infection.

4 Summary of revisions

Developers of AOP 277 have addressed the written comments made by the reviewers and sent to the AOP developers on the 25 April 2021 as described in Annexes 3, 4 and 7. However, additional revisions may be deemed necessary based on the main issues reported in section 2, and main recommendations made in section 7 of this report.

5 Further discussions

No further discussions are foreseen.

6 Summary record of the teleconference(s)

The summary record of the following meetings can be found in annex:

- 7 May 2021: Joint teleconference between the AOP developers and the review panel (Annex 4).
- 14 May 2021: Teleconference of the review panel only (Annex 5).

No minutes were taken for the kick-off meeting from April 2, 2021, as it regarded a general presentation on the OECD AOP Review process and no specific discussions on AOP 277 took place. Furthermore, no minutes were taken for the teleconferences of 11 and 25 of June, since discussions were directly inserted into this draft report.

7 Main recommendations

Reviewers agree that the revised AOP could provide a basis for expanding a network of knowledge, testing methods and causal linkages for immunotoxicity. Developers of AOP 277 have addressed most of the written comments made by the reviewers as described in section 4. However, in order to address the main issues reported in section 2, the following recommendations are still made by the review panel regarding AOP 277:

- Include examples of drugs and/or chemicals that only lead to increased susceptibility to infection by affecting IL-R1 signalling. If AOP developers cannot find chemicals having effects downstream to the IL-1R, but can find chemicals that, for example, reduce IL-1 levels, it is recommended that the AOP is expanded to include these chemicals.
- Take into account the fact that there might be other ways to increase susceptibility to infection that do not involve NF-κβ, by making use of e.g., hub of key events that contains both AP-1 and NF-κβ.
- Clarify which T cells, and whether other cells (e.g. B cells) are addressed by AOP 277.
- Clarify which types of infection are covered by the AOP 277.

Annex 1: AOP 277 Review panel composition

Emanuela Corsini	Università degli Studi di Milano Milan, Italy
David M. Lehmann	Center for Public Health & Environmental Assessment, Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park (NC), USA
Rob Vandebriel	Centre for Health Protection National Institute of Public Health & Environment (RIVM) Bilthoven, The Netherlands
Ronald Wange	CDER, Office of New Drugs, Immediate Office U.S. Food and Drug Administration, USA
Yoshiro Saito	Division of Medicinal Safety Science NIHS, Japan

Coordinators of the AOP Scientific Review:

Chantra Eskes & Iwona Wilk-Zasadna, SeCAM, Switzerland.

Based on the analysis of the declaration of interest from the review manager, the OECD Secretariat, organiser of the review, 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 from the reviewers.

Annex 2: Scientific evaluation from reviewers

Compiled on 23 April 2021 and revised on 18 June 2021

1. Scientific quality

1.1. Does the AOP incorporate all appropriate scientific literature and evidence?

Reviewer 1

In my opinion, all appropriate scientific literature and evidence is incorporated, with the possible exception of the KER “suppression of T-cell activation”, more specifically the quantitative understanding which is not specified. Did the authors evaluate the scientific literature on the T-cell dependent antibody response (TDAR) in experimental animals or vaccination responses (Ab titres) in humans? This may provide quantitative information.

Reviewer 2

Yes, but some references should be (or can be) added in the list as shown in the below section (section 3).

Reviewer 3

This AOP is very simple with only two KEs, in addition to the MIE, to describe a complex phenomenon such as resistance to infections. It is unclear how the MIE (Impaired IL-1R1 signalling) differs from KI 1 (Inhibition, Nuclear factor kappa B (NF-kB)). Is the MIE only the binding of IL-1 to IL-1R1? IL1R1 signalling pathway also involves p38 MAPK, JNK.

Altered IL-1R1 binding signalling and reduced resistance to infection is plausible. IL-1 family cytokines are associated with acute and chronic inflammation and are essential for the innate response to infection. In addition, IL-1 is important for T cell activation. Thus, the MIE i*mpaired IL-1R1 signalling* may be equally important for innate immunity and response to infection, as well as for acquired immunity and response to infection, making impaired IL-1R1 signalling ambiguous.

As the AOP is currently described, it is unclear which types T cells are targeted and how this, considering the roles of T cells in the acquired and humoral immune responses, will only impair resistance to infection. In addition, the immune system has compensatory and alternative pathway that can replace IL-1-driven immunity, as demonstrated for IL-36 and candidiasis (doi:

10.4049/jimmunol.1800515.), thus impaired IL-1R1 signalling not necessarily lead to decrease response to infections.

While the AOP properly incorporate appropriate scientific literature, it is too simplistic and does not cover the complexity of the immune response towards pathogens and the different cells involved, from innate to specific immunity.

Reviewer 4

- The foundation of AOP277 is that impaired IL-1R1 signaling (molecular initiating event/MIE) leads to increased susceptibility to infection. The developers of the AOP are established scientists with appropriate background and experience in this area of immunotoxicology. The AOP they describe includes the MIE and two KEs leading to the AO. Overall, the AOP describes what is known about the pathway leading to increased risk of infection associated with impairment of IL-1R signaling, but the authors did not identify/discuss knowledge gaps, particularly related to suppression of T cell activation.
- Additional, relevant references to consider are listed below.
  - Bohrer, A.C., Tocheny, C., Assmann, M., Ganusov, V.V. Mayer–Barber, K.D. 2018. Cutting Edge: IL-1R1 Mediates Host Resistance to Mycobacterium tuberculosis by Trans-Protection of Infected Cells. J Immunol. 201 (6) 1645-1650; DOI: https://doi.org/10.4049/jimmunol.1800438
  - Labow, M., D. Shuster, M. Zetterstrom, P. Nunes, R. Terry, E. B. Cullinan, T. Bartfai, C. Solorzano, L. L. Moldawer, R. Chizzonite, and K. W. McIntyre. 1997. Absence of IL-1 signaling and reduced inflammatory response in IL-1 type I receptor-deficient mice. J. Immunol.159:2452-2461.
  - Rogers, H. W., K. C. Sheehan, L. M. Brunt, S. K. Dower, E. R. Unanue, and R. D. Schreiber. 1992. Interleukin 1 participates in the development of anti-Listeria responses in normal and SCID mice. Proc. Natl. Acad. Sci. USA89:1011-1015
  - van der Meer JWM, Barza M, Wolff SM, Dinarello CA. A low dose of recombinant interleukin 1 protects granulocytopenic mice from lethal gram-negative infection. Proc Natl Acad Sci USA. 1988; 85:1620–1623. [PubMed: 3125553]

Reviewer 5

The broadness of the adverse outcome proposed for this pathway (susceptibility to infection), as well as the key events of NFκB activation and impairment of T cell activation, make it difficult to assess whether all appropriate scientific literature and evidence is incorporated. Some identified deficits are captured below.

Some identified deficits:

1. No literature/evidence is provided for many of the identified stressors to show that they impair IL-1R1 activation (e.g., cinnamic aldehyde, dexamethasone, minocycline, etc.).
2. Notably, no literature or evidence is provided for any of the stressors, including those that are plausibly linked to impaired IL-1R1 activation (e.g., Anakinra). Much of the relevant literature does appear in other locations within the AOP document (see especially the table under “Empirical support”; however, this should be appropriately associated with each stressor.

1.2. Does the scientific content of the AOP reflect current scientific knowledge on this specific topic?

Reviewer 1: Yes.

Reviewer 2: Yes.

Reviewer 3

IL-1 family dominate in innate immunity and induction of inflammation, and in addition, the IL-1 family member also play a role in acquired immunity. IL-1 family augments antigen recognition and activate lymphocyte function, and IL-1β evolved to assist host defence against infections. The concordance table nicely summarizes the empirical support obtained from the experiments using several inhibitors or gene targeting mice. However, due to compensatory mechanisms and redundancy in the immune response, it is unlikely that impaired IL-1R1 signalling will necessary results in increased risk of infections.

Reviewer 4

It is well-known that IL-1 plays an important role in both the innate and adaptive immune systems. Importantly, IL-1beta “evolved to assist host defense against infection” (Dinarello CA. 2018. Immunol Rev. V281(1):8-27). Evidence from various laboratory-based inhibitor studies and knockout mice detailed by the authors support the conclusion that impaired IL-1R signaling has the potential to increase susceptibility to infection. So, yes, the AOP reflects current scientific knowledge on this specific topic. Still, the concordance table could be bolstered a little by incorporating a few additional references (see above).

Reviewer 5

The scientific content of the AOP is generally reflective of the current level of the scientific knowledge in this area. However, the manner in which it is applied in the AOP wiki leaves significant gaps in the usability of the proposed (AOP).

2. Weight of evidence

2.1. In your opinion, is the weight-of-evidence judgement/scoring well described and justified based on the evidence presented? If not please explain.

Reviewer 1: Yes/no. Defining inhibition of NF-κB as a KE, might suggest that this transcription factor is a single focal point in the signal transduction between impaired IL-1R1 signalling and suppression of T-cell activation, which I doubt is the case. On the other hand, when the decision is made to identify an effect on a transcription factor as a KE, NF-κB is the logical choice.

Reviewer 2: Yes.

Reviewer 3: No answer.

Reviewer 4: No answer.

Reviewer 5: No. There are multiple deficits.

Stressors: As noted above, no literature or evidence is provided to support any of the stressors. This is critical, since the KEs are quite pleiotropic, and relying on these KEs as measures of inhibition of IL-1R1 requires that it be known that the stressor does, in fact, inhibit IL-1R signalling. Evidence/literature should be provided for each stressor or the evidence term changed to null or low.

Weaknesses in the other areas of the AOP are captured elsewhere in this review.

2.2. Please consider weight-of-evidence for each KER and for the AOP as a whole.

Reviewer 1:

The weight-of-evidence of all three KERs is indeed high. Since cytokine responses are complex, the quantitative understanding can at best be moderate. Regarding signalling pathways, these are often descriptive by nature and do not show a clear dose-response relationship, so the quantitative understanding might be considered low. The quantitative understanding of the relationship between suppression of T-cell activation and susceptibility to infection should at least be moderate. The weight of evidence of the entire AOP is high, most importantly based on clinical observations.

Reviewer 2:

MIE1700 to KE202 High (because this relationship was shown by papers using antagonists (anakinra and gevokizumab), and knock-out mice)

KE202 to KE1702 High (because this relationship was shown by papers using NF-kB inhibitor DHMEQ and knock-out mice)

KE1702 to AO986 High (this step is highly plausible, but no direct evidence is presented in this draft AOP. However, evidence between MIE1700 to AO986 or KE202 to AO986 are presented by papers using antagonists (anakinra, canakinumab and rilonacept), and knock-out mice)

Reviewer 3:

In the described AOP, the subsets of T cells involved are not clearly defined. Blocking the activation of T helper lymphocytes certainly compromises the activation of the specific immune response against all T dependent antigens, but this probably has implications that go beyond resistance to infections, e.g. allergic responses, resistance to tumours, transplant rejection, etc. It is a bit restrictive to link IL1R dysregulation to infections alone.

Reviewer 4:

The processes that protect us from infection are very complex, involving both the innate and adaptive immune systems and IL-1 (alpha and beta) are known to play a role in both. Evidence provided by the authors reasonably support their conclusions. However, KEs need to play a clear causal role in the pathway and be measurable. For those reasons, additional information is required to establish the AOP and to help the reader understand the AOP. For example, the MIE is rather ambiguous. As written, the MIE is simply blocking IL-R1 signaling, which is too generic

for the purposes of an AOP because there are at least a few ways for this to happen. To be suitable, the MIE needs to be a specific measurable event. Like, for example, blocking the interaction between “IL-1” and the IL-1R? As written, I think the quantitative understanding for impaired IL-R1 signaling leading to inhibition of NFkB is low. In addition, it is not clear which types of T cells are involved in the pathway. And, for that reason, the endpoint is not suitably quantifiable. Given that the authors did not specify the strength of quantitative understanding for this KE, I suspect they recognize this data gap exists. To enhance the AOP, the authors should acknowledge the data gap which might stimulate additional research.

Reviewer 5:

KER: “Impaired IL-1R1 signaling leads to Inhibition, Nuclear factor kappa B (NF-kB)”

There is likely adequate evidence available in the literature to support this KER; however, much of the material presented as supporting this KER does not actually support the relationship between IL-1R1 signaling and NF-κB activation, but rather is focused on supporting that a given stressor affects IL-1R1 activity or events downstream of IL-1R1 activation, but not specifically the link between IL1-R1 activation and NF-κB activation. With the literature/data presented currently, the WOE should be changed to “moderate,” but can probably be considered “high” with inclusion of appropriate literature/data.

No attention is given to either the timing of the events relative to one another, or what magnitude of effect on the MIE is required to affect NF-κB activation (or vice versa), as such, quantitative understanding should probably be listed as “low.”

KER: “Inhibition, Nuclear factor kappa B (NF-kB) leads to Suppression of T cell activation”

Consideration should be given to whether the KE of “suppression of T cell activation” is too broad, given the many different phenotypic subtypes of T cells (e.g., [to name just a few] CD4 (e.g., Th1, Th2, Th17, Treg, etc.), CD8, gamma-delta, etc.).

There is adequate evidence available in the literature to support this KER; however, much of the material presented as supporting this KER does not actually support the link between inhibition of NFκB and suppression of T cell activation.

It is unclear from the literature provided what degree of reduction in NFκB activation is required to suppress T cell activation.

KER: “Suppression of T cell activation leads to Increase, Increased susceptibility to infection”

The authors do a reasonable job of capturing the basis for this KER, which can be derived from texts on basic immune system function. This KER is well-supported; however, both the KE and the AE are very broad, perhaps impairing the utility of this KER. Not all T cell types are necessary for adequate host defence against all pathogens. Indeed, some T cells types, when activated, can actually impair appropriate host response to given antigens.

It is notoriously difficult to quantitatively relate the degree of suppression of T cell activation that leads to a clinically meaningful increase in susceptibility to infection.

Annex 3: Responses from AOP developers to review panel written comments

Shared with review panel on 4 May 2021

Reviewer 1

1. It should be acknowledged that this AOP is more targeted towards pharmaceuticals than chemicals.

We would like to discuss this point in the web meeting.

2. The sentence at the top of page 3 “Although MyD88 is also known to be involved in TLR signalling pathway, several reports suggested that MyD88-dependent response was IL-1 receptor-mediated but not TLR-mediated. These data suggest to the essentiality of IL-1 MyD88 signalling pathway in host defence against infection.” This needs to be elaborated. There are clear differences in host defence between TLR-sufficient and -deficient animals, and also in humans e.g., TLR4 polymorphisms affect host defence. In addition, references should be added to support the authors’ claim.

We deleted the indicated sentence.

3. The paragraph at the bottom of page 7, starting with “Binding of LPS to TLR4 and the coreceptor MD2..” reads, at least to me, very complicated and could be better explained. Possibly, the authors could limit themselves to describe the signal transduction pathway(s) only for NF-κB.

We would like to discuss this point in the web meeting.

4. On page 14, bortezomib is mentioned as proteasome inhibitor and NF-κB inhibitor. Is it both?

Yes

Reviewer 2

5. Background (optional)
   5.1. 3rd paragraph, 1st sentence: PRPs is a typo of PRRs?

Yes, it was corrected.

   5.2. 4th paragraph: Duplication of the last sentence of the third paragraph?

Thank you for your indication. I deleted the indicated sentence.

6. Overall Assessment of the AOP
   Domain of Applicability: Is description of c-Rel/REL proto-oncogene not necessary? This is also a member of NF-kB superfamily. https://www.ncbi.nlm.nih.gov/gene/5966

According to the reviewer's comment, we deleted the description of c-Rel/REL.

7. Essentiality of the Key Event
   7.1. 2nd paragraph, 4th sentence: "increased susceptibility to ~": The reports of Kullenberg et al. 2016, Lequerre et al., 2008, and Migkos et al., 2015 were seems to be case series or case report, and not appropriate as references for frequency discussion. The same for Imagawa et al., 2013 on canakinumab.

It is true that the manuscript by Migkos et al. is a case report of serious tuberculous infection. So, I deleted that manuscript from the reference. However, the papers by Kullenberg et al, Lequerre et al and Imagawa et al suggested the possibility of increased susceptibility after the treatment with IL-1R blockade based on the observation of a substantial number of patients treated with IL-1R blockade.

   7.2. 3rd paragraph, 1st sentence: References (Fremond et al., 2004 ~ von Bernuth et al., 2008) should be included in the reference list.

These papers were included in the revised reference list.

   7.3. 3rd paragraph, 2nd sentence: Appropriate reference should be included for description "several reports suggested that MyD88-dependent response was IL-1 receptor-mediated but not TLR-mediated". Candidate: Huang et al., Infect Immun. 2014;82(5):2106-14.

Thank you for your kind suggestion. We added two references by Huang et al and Fremond CM et al.

8. Evidence Assessment
   2nd paragraph: References (Hannum et al., 1990, Seckinger et al., 1990b, Goh et al., 2014, and Seckinger et al., 1990a) should be included in the reference list.

The references were included in the reference list.

9. Biological plausibility
   9.1. 5th paragraph: References (Gerondakis et al., 2014) should be included in the reference list.
   9.2. 9th paragraph: References (Soares et al., 2017) should be included in the reference list.

The references were included in the reference list.

10. Concordance table empirical data

We deleted concordance table.

   10.2. MG-132 and bortezomib are an inhibitor of proteasome and not specific for inhibitions on NF-kB activation. The weight of evidence by these is not large for this AOP.

Reviewer 3

11. I do not know if it is the custom of an AOP, but Authors should better introduce the IL-1 family, including IL-1 receptors.

We would like to discuss this point in the web meeting.

12. It is not clear at what level, the dysregulation should happen: production of IL-1 (both □ and □)? Production of IL-1RA? Levels of IL1R1 expression?

It is true that impaired IL-1R can be induced by a variety of situations.

1) The lack or decreased of IL-1a or IL-1b caused by a variety of mechanisms.
2) The blockade of IL1RA by the exogenous administration of IL-1RA, anti-IL-1 antibody or anti-IL-1R1antibody.
3) The physiological or pathological suppression of IL-1RA production.
4) The physiological or pathological suppression of IL-1R1 expression.

In this AOP, we focused on 1) and 2) considering the purpose of this AOP, i.e., the immunotoxicity of chemicals.

13. Regarding the *Quantitative Understanding of Suppression of T cell activation leads to Increase, Increased susceptibility to infection*, Authors write Not Specified. In reality, in a pivotal paper published by Luster et al. in 1993, models to establish quantitative relationships between immune and host resistance tests, which include T cell functions, were established. Most of the immune-host resistance relationships appeared to approximate a linear model, suggesting for example that a 6.4 % decrease in ConA-induced T cell proliferation is associated with 10% increase risk of *Listeria monocytogenes* infection.

Thank you for your kind and supportive suggestion. I introduced the paper by Luster et al in the quantitative understanding of the linkage and response-response relationship.

We also added the following sentence in Empirical Evidence.

Certain pharmaceutical agents known as calcineurin inhibitors that suppress T cell function and are commonly used to prevent organ rejection of transplant recipients or to treat autoimmune disorders, also have an immunosuppressive side effect, known to lead to an increase in the following opportunistic infections: fungal/yeast (e.g., Cryptococcus neoformans); viral (esp. herpes-family viruses such as Epstein-Barr virus [EBV], cytomegalovirus [CMV]); atypical bacterial (e.g., mycoplasma, Nocardia, Listeria, mycobacteria); and parasitic (e.g., toxoplasmosis) infections (reviewed by Singh (2005)).

14. Are early life and later in life-stage equally sensitive to inhibition of IL-1 signalling? Due to maturation of the immune system (including signalling pathways) and immunosenescence, I am not sure that all age stages will be qualitatively and quantitatively equally sensitive.

We will try to search the appropriate references.

15. The section *Inhibition, Nuclear factor kappa B (NF-kB) leads to Suppression of T cell activation* is incomplete, references are missing. The specialized subsets of T cells involved should be better defined.

The specialized subsets of T cells involved were described in the Biological plausibility of We

added the reference in Biological plausibility of the KE relationship (Suppression of T cell activation to increased susceptibility to infection).

16. While it is clear that the insufficient T cell or B cell function causes impaired resistance to infection, it is not clear at what level of the immune activation the impairment of IL-1R signalling will impact T and B cells functions.

We added some comments on the mechanism by which the impaired IL-1R signaling impacts T and B cells function in the Key event relationship description (Inhibition of NF-kB leads to suppression of T cell activation).

17. Minor points:

17.1. The sentence: *'In addition to these human data, the experiments using knockout mice revealed that the lack of IL-1 signalling led to bacterial, tuberculosis or viral infection. (Guler et al., 2011; Horino et al., 2009; Juffermans et al., 2000; Tian et al., 2017; Yamada et al., 2000).'* Is reported twice.

One of them was deleted.

17.2. Many Greek letters are correctly written (the font Symbol should be used).

We tried to correctly use Greek letters, but sometimes it is not easy in the AOP WIKI.

17.3. What dose trimelic mean?

It should be trimeric

17.4. There are several typos that should be corrected.

We tried to correct them.

17.5 It is not clear the logic with which the different studies are reported in the concordance table.

We deleted the concordance table. Instead, we described the empirical evidence.

Reviewer 4

18. As a general comment, I find certain parts of the AOP to lack sufficient detail (i.e., MIE, T cell subpopulations). Along these lines, the authors typically refer to IL-1, which is actually two different cytokines (i.e., IL-1alpha and IL-1beta) as a single entity. For clarity, the authors should be clear about which specific IL-1 they are referring to whenever possible.

We added some details in this AOP.

It is not easy to discriminate IL-1a or IL-1b in some parts. Basically, this AOP focuses on IL-1b.

19. RELB gene is mentioned in the Overall Assessment of the AOP section and the concordance table. What it is and why it is important should be mentioned somewhere.

We deleted the concordance table.

We also RELB in the AOP.

20. Typos etc.:

20.1. Background; Add a space between “They are IL-1 receptor antagonist” and “(IL-R1)”.

20.2. Summary of the AOP, Sequence 4, The word “increase” only needs to be used once.

20.3. Essentiality of the Key Events –

20.3.1. Consider rewording the sentence to read “The data provide evidence that IL-1-MyD88 signaling pathway plays an essential role in host defence against infection.”.

20.3.2. Consider rewording sentence to read “Mice lacking NF-kB p50 are unable to effectively clear…”.

We modified the sentence.

20.4. Evidence assessment – Consider rewording the sentence to read “Cytokines, including those produced by macrophages or monocytes such as…”.

20.5. Concordance table

20.5.1. If possible, I think it would be helpful for the reader if you could figure out a way to briefly describe what each of the inhibitors does. For example, MG-132 (proteosome inhibitor), gevokizumab (XOMA 052; binds to IL-beta). Just something to help the reader immediately understand why the inhibitors are important and how they relate to each other.

We added a short explanation for them.

20.5.1 The word “Chmical” is misspelled at the top of the concordance table.

We corrected it.

Please define AEs and SAEs for the reader (associated with Kullenberg et al. 2016).

We deleted the word SAEs.

Reviewer 5

21. Unless the stressor is limited to known inhibitors of IL-1R1 activation it is not clear that the KEs represent “a dependent series of intermediate key events,” which, as I understand it, is fundamental requirement of an effective AOP. NFκB activation is not necessarily dependent on IL-1R1 activation, and neither is T cell activation in all instances. There are multiple other pathways by which a stressor can affect NFκB and/or T cell activation. Additionally, there are pathways to increased susceptibility to infection that do not rely necessarily rely on impaired T cell activation.

We would like to discuss this point in the web meeting.

22. It is unclear to this reviewer how this AOP can support development of a test guideline.

We are currently developing the IL-1 luciferase reporter assay.

23. The KE of “Inhibition, Nuclear factor kappa B (NF-κB)” may be problematic from the standpoint of broader AOP development. There are a myriad of MIEs and signalling events

that lead to NF-κB activation, yet only IL-1R1-mediated activation is captured in this KE.
We would like to discuss this point in the web meeting.

24. The AO of “Increase, Increased susceptibility to infection” may be problematic from the standpoint of broader AOP development. There are numerous MIEs and KEs that can lead to lead to increased susceptibility to infection, yet only IL-1R1-mediated activation is captured as being within the applicability domain. Moreover the “Regulatory Significance of the Adverse Outcome” domain is much too narrow, focusing only on impaired activation of IL-1R1.

We would like to discuss this point in the web meeting.

Annex 4: Minutes from the teleconference of 7 May 2021

Scientific review of the Adverse Outcome Pathway 277: Impaired IL-1R1 signaling leading to increased susceptibility to infection

Teleconference of the Review Panel and AOP developers

Friday 7 May 2021 – 13.30 to 16.00 CET time

Meeting minutes

Participants: Setsuya Aiba, Emanuela Corsini, Nathalie Delrue**,** Chantra Eskes (chair), Yutaka Kimura**,** Hajime Kojima, Tadashi Kosaka (observer), Kiyoshi Kushima, David Lehman, Takumi Ohishi, Yoshiro Saito, Rob Vandebriel, Roland Wange, Iwona Wilk-Zasadna (minutes)

Response from the AOP 277 developers to the questions and comments from the review panel

Reviewer 1

1. It should be acknowledged that this AOP is more targeted towards pharmaceuticals than chemicals.

Response: There was not enough clarity on what the appropriate wording should look like and where in the description of AOP it should be placed. AOP developers agreed to check this point and provide the feedback

2. The sentence at the top of page 3 “Although MyD88 is also known to be involved in TLR signalling pathway, several reports suggested that MyD88-dependent response was IL-1 receptor-mediated but not TLR-mediated. These data suggest to the essentiality of IL-1 MyD88 signalling pathway in host defence against infection.” This needs to be elaborated. There are clear differences in host defence between TLR-sufficient and -deficient animals, and also in humans e.g. TLR4 polymorphisms affect host defence. In addition, references should be added to support the authors’ claim.

Response: Comment resolved - the indicated sentence is deleted

3. The paragraph at the bottom of page 7, starting with “Binding of LPS to TLR4 and the

coreceptor MD2." reads, at least to me, very complicated and could be better explained. Possibly, the authors could limit themselves to describe the signal transduction pathway(s) only for NF-κB.

Response: Comment possibly resolved – the indicated paragraph potentially already deleted. It will be doubled -checked by Chantra and followed up off-line.

4. On page 14, bortezomib is mentioned as proteasome inhibitor and NF-κB inhibitor. Is it both?

Response: Yes, bortezomid is described in the scientific literature as having both activities. It will be clarified in the text of AOP by developers.

Reviewer 2

5. Background (optional)
   5.1. 3rd paragraph, 1st sentence: PRPs is a typo of PRRs?

Response: comment resolved - the typo is already corrected.

   5.2. 4th paragraph: Duplication of the last sentence of the third paragraph?

Response: comment resolved – the sentence is already removed.

6. Overall Assessment of the AOP
   Domain of Applicability: Is description of c-Rel/REL proto-oncogene not necessary? This is also a member of NF-kB superfamily. https://www.ncbi.nlm.nih.gov/gene/5966

Response: comment resolved – the indicated text is already deleted

7. Essentiality of the Key Event
   7.1. 2nd paragraph, 4th sentence: "increased susceptibility to ~": The reports of Kullenberg et al. 2016, Lequerre et al., 2008, and Migkos et al., 2015 were seems to be case series or case report, and not appropriate as references for frequency discussion. The same for Imagawa et al., 2013 on canakinumab.

Response: comment resolved – the paper of Migkos et al., 2015 is deleted; the remaining indicated papers suggest the possibility of increased susceptibility after the treatment with IL-1R blockade based on the observation of a substantial number of patients treated with IL-1R blockade and therefore, they will be stay in the text of the AOP.

   7.2. 3rd paragraph, 1st sentence: References (Fremond et al., 2004 ~ von Bernuth et al., 2008) should be included in the reference list.

Response: comment resolved - the papers are included into the revised version of the AOP.

   7.3. 3rd paragraph, 2nd sentence: Appropriate reference should be included for description "several reports suggested that MyD88-dependent response was IL-1 receptor-mediated but not TLR-mediated". Candidate: Huang et al., Infect Immun. 2014;82(5):2106-14.

Response: comment resolved – the indicated referenced were added.

8. Evidence Assessment

2nd paragraph: References (Hannum et al., 1990, Seckinger et al., 1990b, Goh et al., 2014, and Seckinger et al., 1990a) should be included in the reference list.

Response: comment resolved – the references were included in the reference list.

9. Biological plausibility
   9.1. 5th paragraph: References (Gerondakis et al., 2014) should be included in the reference list.

Response: comment resolved – the reference was included in the reference list.

   9.2. 9th paragraph: References (Soares et al., 2017) should be included in the reference list.

Response: comment resolved – the reference was included in the reference list.

10. Concordance table empirical data
    10.1. I believe “Sigma-Aldrich Specification Sheet” is not appropriate for an empirical data because it is not peer reviewed.

Response: comment resolved – the concordance table is removed, so the comment is not applicable anymore.

    10.2. MG-132 and bortezomib are an inhibitor of proteasome and not specific for inhibitions on NF-kB activation. The weight of evidence by these is not large for this AOP.

Response: AOP developers want to include more detailed information.

Reviewer 3

17. I do not know if it is the custom of an AOP, but Authors should better introduce the IL-1 family, including IL-1 receptors.

Response: Although it is reasonable that the IL-1 family should be better described, it is not clear which level of details the information should capture. It will be followed up off-line by Chantra.

18. It is not clear at what level, the dysregulation should happen: production of IL-1 (both □ and □)? Production of IL-1RA? Levels of IL1R1 expression?

Response: comment resolved – AoP is focusing on T-cell activation and binding of IL-1□ to the receptor. The MIE is defined as impaired IL-1R1 activation (regardless of the mechanism of such impairment). No modification to the AoP is needed.

19. Regarding the *Quantitative Understanding of Suppression of T cell activation leads to Increase, Increased susceptibility to infection*, Authors write Not Specified. In reality, in a pivotal paper published by Luster et al. in 1993, models to establish quantitative relationships between immune and host resistance tests, which include T cell functions, were established. Most of the immune-host resistance relationships appeared to approximate a linear model, suggesting for example that a 6.4 % decrease in ConA-induced T cell proliferation is associated with 10% increase risk of *Listeria monocytogenes* infection.

Response: comment resolved – the paper of Luster et al. (1993) was added in the section related

to the quantitative understanding of the linkage and response-response relationship. Further clarification was also included into Empirical Evidence.

20. Are early life and later in life-stage equally sensitive to inhibition of IL-1 signalling? Due to maturation of the immune system (including signalling pathways) and immunosenescence, I am not sure that all age stages will be qualitatively and quantitatively equally sensitive.

Response: comment appreciated – so far, no relevant/ appropriate references have been found, however, the search will continue.

21. The section *Inhibition, Nuclear factor kappa B (NF-kB) leads to Suppression of T cell activation* is incomplete, references are missing. The specialized subsets of T cells involved should be better defined.

Response: comment resolved – short comment was added on pg 2. The relevant extract will be sent by AOP developers to Chantra for confirmation if the comment of the reviewer has been addressed.

22. While it is clear that the insufficient T cell or B cell function causes impaired resistance to infection, it is not clear at what level of the immune activation the impairment of IL-1R signalling will impact T and B cells functions.

Response: comment partially resolved – some additional information was added into the AOP. The summary of additional changes included to the AOP will be provided by developers to Chantra.

23. Minor points:

17.1. The sentence: *'In addition to these human data, the experiments using knockout mice revealed that the lack of IL-1 signaling led to bacterial, tuberculosis or viral infection. (Guler et al., 2011; Horino et al., 2009; Juffermans et al., 2000; Tian et al., 2017; Yamada et al., 2000).'* Is reported twice.

Response: comment resolved – the duplication is deleted.

17.2. Many Greek letters are correctly written (the font Symbol should be used).

Response: although effort has been undertaken to correct the Greek letters, sometimes it is not easy in AOP Wiki. It will be reported to Nathalie and followed-up off-line.

17.3. What does trimelic mean?

Response: comment resolved – it should be read trimeric; the text is corrected appropriately.

17.4. There are several typos that should be corrected.

Response: comment resolved – the spotted typos were corrected.

- It is not clear the logic with which the different studies are reported in the concordance table.

Response: comment resolved – the concordance table has been removed, so the comment is not valid anymore.

Reviewer 4

24. As a general comment, I find certain parts of the AOP to lack sufficient detail (i.e., MIE, T cell subpopulations). Along these lines, the authors typically refer to IL-1, which is actually two different cytokines (i.e., IL-1alpha and IL-1beta) as a single entity. For clarity, the authors should be clear about which specific IL-1 they are referring to whenever possible.

Response: comment resolved – AOP focuses on IL-1b and additional clarification were added in the text. Developers agreed to send the relevant text for confirmation.

19. RELB gene is mentioned in the Overall Assessment of the AOP section and the concordance table. What it is and why it is important should be mentioned somewhere.

Response: comment resolved – the concordance table and all references to RELB have been deleted.

20. Typos etc.:

20.1. Background; Add a space between “They are IL-1 receptor antagonist” and “(IL-R1)”.

Response: comment resolved

20.2. Summary of the AOP, Sequence 4, The word “increase” only needs to be used once.

Response: comment resolved

20.3. Essentiality of the Key Events –

20.3.1. Consider rewording the sentence to read “The data provide evidence that IL-1-MyD88 signaling pathway plays an essential role in host defence against infection.”

Response: comment resolved – the sentence was reworded

20.3.2. Consider rewording sentence to read “Mice lacking NF-kB p50 are unable to effectively clear…”.

Response: comment resolved – the sentence was reworded

20.4. Evidence assessment – Consider rewording the sentence to read “Cytokines, including those produced by macrophages or monocytes such as…”.

Response: comment resolved – the sentence was reworded

20.5. Concordance table

20.5.1. If possible, I think it would be helpful for the reader if you could figure out a way to briefly describe what each of the inhibitors does. For example, MG-132 (proteosome inhibitor), gevokizumab (XOMA 052; binds to IL-beta). Just something to help the reader immediately understand why the inhibitors are important and how they relate to each other.

Response: comment resolved – short explanation was added

20.5.1 The word “Chmical” is misspelled at the top of the concordance table.

Please define AEs and SAEs for the reader (associated with Kullenberg et al. 2016).

Response: comment resolved – the concordance table was deleted; the word SAEs was deleted

Reviewer 5

21. Unless the stressor is limited to known inhibitors of IL-1R1 activation it is not clear that the KEs represent "a dependent series of intermediate key events," which, as I understand it, is fundamental requirement of an effective AOP. NFκB activation is not necessarily dependent on IL-1R1 activation, and neither is T cell activation in all instances. There are multiple other pathways by which a stressor can affect NFκB and/or T cell activation. Additionally, there are pathways to increased susceptibility to infection that do not rely necessarily rely on impaired T cell activation.

Response: comment resolved – no change in AOP is needed

Key event does not necessarily refer to the specific AOP only *i.e.* NFκB activation may be common for various AOPs. Moreover, AOP covers the events "from start to finish" *i.e.* from MIE and KEs and should not be read backwards. The common KEs allow for AOP networking.

22. It is unclear to this reviewer how this AOP can support development of a test guideline.

Response: comment resolved – see the general discussion

23. The KE of "Inhibition, Nuclear factor kappa B (NF-κB)" may be problematic from the standpoint of broader AOP development. There are a myriad of MIEs and signalling events that lead to NF-κB activation, yet only IL-1R1-mediated activation is captured in this KE.

Response: comment resolved – see point 21

24. The AO of "Increase, Increased susceptibility to infection" may be problematic from the standpoint of broader AOP development. There are numerous MIEs and KEs that can lead to lead to increased susceptibility to infection, yet only IL-1R1-mediated activation is captured as being within the applicability domain. Moreover the "Regulatory Significance of the Adverse Outcome" domain is much too narrow, focusing only on impaired activation of IL-1R1.

Response: comment resolved – see point 21, 23 and general discussion

General discussion

Link between AOP and OECD Test Guideline

AOP did not necessarily imply development of a test guideline. An AOP can provide valuable pieces of mechanistic information that can be used for many purposes, not only and not necessarily TG development. The GD on AOP development (link) indicates the following:

A variety of potential uses have been described for AOPs; the extent to which decisions can be supported by a given AOP depends on the level of uncertainty and quantitative understanding of the KERs. For example, by identifying and describing the KEs, AOPs can inform the work of the OECD Test Guideline Programme by describing the rationale for the use of particular methods and also by identifying potentially more predictive methods for

development (further described below). AOPs can also be used as a basis for developing an IATA or an integrated testing strategy (ITS). They can also be used for further development and application of alternative approaches, such as read-across, where categories are first formed and data gaps filled within the category, leading to potential refinement, reduction and/or replacement of conventional in vivo testing.

AOPs can also be used to contribute to a number of regulatory contexts, including but not limited to: (1) priority setting for further testing, (2) hazard identification, (3) classification and labelling, and (4) risk assessment. As such, as one proceeds from (1) to (4), the level of uncertainty that can be tolerated decreases and the level of evidence (e.g. detail, quality, and quantity of information and data) presented in supporting the AOP increases.

In addition, it should be noted that it is not mandatory for the authors of an AOP to report under the section on “Potential Applications of the AOP”. This is optional in the Wiki. However, reviewers are welcome to comment on the applications of the AOP, if they wish to. The User’s Handbook indicates that the evaluation of an AOP suitability for application in different regulatory contexts and the assimilation of the relevant characterisation of supporting biological information relies in part on (1) the confidence and precision with which the KEs can be measured, (2) the level of confidence in the relationships between the KEs linked in an AOP (KERs) based on biological plausibility, and empirical support for the KERs; and (3) WoE for the overall hypothesised pathway, taking into account a number of additional considerations, including any uncertainties and inconsistencies.

Future toxicology based on AOP concept

AOP277 is a very simple AOP trying to address very complex immune system, with many factors influencing resistance to infection.

However, if we try to make it more comprehensive, there might be very high number of AOPs potentially causing difficulties to evaluate immunotoxic compounds.

From the OECD level the AOP is accepted if the described MIE and KEs have weight of evidence.

Next steps

- The meeting of reviewers panel to discuss and make the final decision on acceptance of AOP for the OECD process
- In case the AOP is accepted for scientific validity, it may undergo through evaluation of suitability for Test Guideline. However, this is separated process and will be dealt by different team in collaboration with WNT.

Annex 5: Minutes from the teleconference of 14 May 2021

Scientific review of the Adverse Outcome Pathway 277: Impaired IL-1R1 signalling leading to increased susceptibility to infection

Teleconference of the Review Panel only

Friday 14 May 2021 – 13.30 to 15.30 CET time

Draft Minutes

Participants: Emanuela Corsini, Chantra Eskes (chair), David Lehman, Yoshiro Saito, Rob Vandebriel, Roland Wange, Iwona Wilk-Zasadna

1. Welcome of participants

Chantra Eskes welcomed the participants, who agreed to the proposed agenda of the meeting.

2. Debrief from responses from AOP 277 developers & 7 May meeting

2.1. Minutes from the meeting with AOP 277 developers from 7 May 2021

No comments were made to the minutes of the meeting from May 7.

It was noted that the AOP developers still need to send proposals to some of the points discussed during the meeting, i.e. comments 1, 14, 15, 16, 18 and 24.

Furthermore, it was noted that the Review Panel needs to clarify points 3 and 11 to the AOP developers. The following clarifications were given on that purpose:

- Comment 3: The AOP 277 paragraph (p. 7) starting with “Binding of LPS to TLR4 and the coreceptor MD2…” is complex and difficult to understand. It is suggested to focus on pathway going through NF-kB only, in order to simplify this paragraph.
- Comment 11: is no longer an issue, as AOP developers have clarified that AOP only addresses events happening after impairment of IL-1R1 signalling. Therefore, there is no need to further address this issue.

Action 1: *CES to share the above clarifications with AOP developers.*

2.2. Main issues of the review of AOP 277

The following general comments and recommendations were made by the scientific review panel regarding AOP 277.

i) AOP 277 is a simple AOP that may not capture the complexity of events that may occur. There are a number of other pathways which can lead to the adverse outcome 'increased susceptibility to infection', in addition to AOP 277. It was agreed to seek clarification from the OECD on whether such a simple AOP can be considered to be adequate.

ii) Regarding the Molecular Initiating Event (MIE), the examples given are based on antibodies. It was recommended that good examples are given also on drugs and chemicals for the MIE as well as for the downstream occurring events, i.e. drugs and/or chemicals that by affecting IL-1R1 signalling lead to increased susceptibility to infection.

iii) The review panel questioned whether the NF-κβ is an essential part of the pathway between impaired IL-1R1 signalling and increased susceptibility to infection. Indeed, there might be other ways to increase susceptibility to infection that do not involve NF-κβ. For example, IL1-R1 may activate other signal transduction pathways than NF-κβ. It was agreed to seek clarification from the OECD on whether NF-κβ meets the criteria of essentiality and can be regarded as a suitable AOP key event.

iv) There are a number of different T cells, furthermore other cells (e.g. B cells) may also play a role in infection. It was recommended to clarify which T cells are addressed by AOP 277 and whether other cells may also be considered (e.g. B cells).

v) Infection is a very broad term, and linking T cells to all types of infection may be simplistic. It was recommended to clarify which type(s) of infection(s) might be covered by AOP 277.

Action 2: *CES to approach OECD to clarify if AOP 277 can be considered to be an adequate AOP, and NF-κβ a suitable AOP key event based on the general comments made above to points i) and iii).*

3. Conclusions on scientific review (questions 1.1, 1.2, 2.1 and 2.2)

The review panel revised their written comments compiled on 23 April 2021. The following conclusions were made.

Question 1.1. Does the AOP incorporate all appropriate scientific literature and evidence?

Reviewer 1: comment still pending.

Reviewer 2: comment addressed.

Reviewer 3: see general comments described in agenda point 2.2.

Reviewer 4: text is improved but more could be done. It is suggested to share the suggested references with the AOP developers for their consideration.

Reviewer 5: reviewer to check if comment has been addressed.

Action 3: *CES to share the literature references suggested by reviewer 4 for consideration by AOP developers.*

Question 1.2. Does the scientific content of the AOP reflect current scientific knowledge on this specific topic?

Reviewers 1 and 2 : no comments to be addressed.

Reviewer 3: see general comments described in agenda point 2.2.

Reviewer 4: comment addressed.

Reviewer 5: reviewer to check if comment has been addressed i.e., if organisation of the presented information has been improved.

Question 2.1. In your opinion, is the weight-of-evidence judgement/scoring well described and justified based on the evidence presented? If not please explain?

The opinion of the review panel is ambivalent, see general comments discussed in the general agenda point 2.2.

Question 2.2. Please consider weight-of-evidence for each KER and for the AOP as a whole.

In general, the weight-of-evidence was considered high. However, it would be good to clarify which functions of the T cell are compromised when NF-kB is impaired, and to clarify the relationship between T cell activation and infection. Furthermore, if quantitative relationship between two events could be demonstrated, that would strengthen the relationship, however this quantification is not shown in this AOP. In details, the following conclusions were made regarding each key event relationship.

- KER “Impaired IL-1R1 signalling leads to Inhibition, Nuclear factor kappa B (NF-kB)”: the information provided could be better organised.
- KER “Inhibition, Nuclear factor kappa B (NF-kB) leads to Suppression of T cell activation”: see general comments discussed in agenda point 2.2.
- KER “Suppression of T cell activation leads to Increased susceptibility to infection”: the KER relates to a very broad relationship so that the usefulness of this KER is questioned.

4. Next steps & closure

The following next steps were agreed upon:

- CES to clarify comments 3 and 11 to AOP developers (Action 1).
- CES to share literature suggested by reviewer 4 for consideration by AOP developers (Action 3).
- CES to seek clarification from the OECD on what is considered to be an adequate AOP and KE (Action 2).

Action 4: *Once the missing information is received from AOP developers (comments 1, 3, 14, 15, 16, 18 and 24 and feed-back on shared literature), the scientific review panel will check if these comments were appropriately addressed.*

A meeting will be organised with the scientific review panel and with the OECD to finalise the review of the AOP 277 based on the clarifications received from the OECD (action 2) and the final review by the panel (action 4). This review panel meeting will take place on:

11 June 2021, 13.30 - 15.30 CET time / 7.30 - 9.30 Washington DC time / 20.30-22.30 Tokyo time.

Chantra Eskes acknowledged all participants for their precious contributions and adjourned the meeting at 14h50.

Annex 6: Clarifications from review panel

E-mail sent to AOP developers on 18 May 2021

From: Chantra Eskes
Date: 18 May 2021 09:08
To: 'Hajime Kojima'; 'Takao Ashikaga'; 'Setsuya Aiba'
Cc: 'Iwona Wilk-Zasadna'; 'Emanuela Corsini'; 'David Lehmann'; 'Rob Vandebriel'; 'Ronald Wange'; 'Yoshiro Saito'; 'Nathalie Delrue'
Topic: Scientific review of AOP 277 - Meeting between AOP developers and Scientific Review Panel from May 7 - FOLLOW-UP

Dear Hajime, dear all,

I hope you are doing well, and I am now coming back regarding the agreed actions from our last meeting from 7 May 2021.

In particular, it was agreed that:

1. The scientific review panel needed to clarify comments 3 and 11, and
2. The AOP developers will send proposals to comments 1, 14, 15, 16, 18 and 24.

Regarding point 1 above-mentioned, please find here below the clarifications from the review panel:

- Comment 3: The AOP 277 paragraph (p. 7) starting with "Binding of LPS to TLR4 and the co-receptor MD2…" is complex and difficult to understand. It is suggested to focus on pathway going through NF-kB only, in order to simplify this paragraph.

- Comment 11: is no longer an issue, as AOP developers have clarified that AOP only addresses events happening after impairment of IL-1R1 signaling. Therefore, there is no need to further address this issue.

In addition, the review panel would like to share with the AOP developers the following references for consideration regarding the association of IL-1R1 signaling and suppression of T cell activation:

- Bohrer, A.C., Tocheny, C., Assmann, M., Ganusov, V.V. Mayer–Barber, K.D. 2018. Cutting Edge: IL-1R1 Mediates Host Resistance to Mycobacterium tuberculosis by Trans-Protection of Infected Cells. J Immunol. 201 (6) 1645-1650; DOI: https://doi.org/10.4049/jimmunol.1800438

- Labow, M., D. Shuster, M. Zetterstrom, P. Nunes, R. Terry, E. B. Cullinan, T. Bartfai, C. Solorzano, L. L. Moldawer, R. Chizzonite, and K. W. McIntyre. 1997. Absence of IL-1 signaling and reduced inflammatory response in IL-1 type I receptor-deficient mice. J. Immunol.159:2452-2461.
- Rogers, H. W., K. C. Sheehan, L. M. Brunt, S. K. Dower, E. R. Unanue, and R. D. Schreiber. 1992. Interleukin 1 participates in the development of anti-Listeria responses in normal and SCID mice. Proc. Natl. Acad. Sci. USA89:1011-1015
- van der Meer JWM, Barza M, Wolff SM, Dinarello CA. A low dose of recombinant interleukin 1 protects granulocytopenic mice from lethal gram-negative infection. Proc Natl Acad Sci USA. 1988; 85:1620–1623. [PubMed: 3125553]

Regarding point 2 above-mentioned, ***it would be great if the AOP developers could provide us with their feed-back by Friday May 28***. Do you believe this is possible?

Furthermore, would you and/or the AOP developers have any comments and/or suggestions to the draft minutes from our meeting from May 7 (see attached for your convenience), please do not hesitate to let me know.

If no comments are received by May 28, I will assume you are ok with the minutes.

With kind regards,

Chantra

Annex 7: Additional responses from the AOP developers

Received on 25 May 2021

Dear Dr. Eskes and the scientific review members:

We appreciate you for your effort for reviewing our AOP277. We responded to your comments and underlined the revised parts.
We revised the manuscripts of AOP277 and AOP-Wiki. The manuscript of AOP277 was composed of the title page (summary page), MIE, KE1, KE2, AO, KER1, KER2, and KER3.

In particular, it was agreed that:
1. The scientific review panel needed to clarify comments 3 and 11, and
2. The AOP developers will send proposals to comments 1, 14, 15, 16, 18 and 24.

Regarding point 1 above-mentioned, please find here below the clarifications from the review panel:

- Comment 3: The AOP 277 paragraph (p. 7) starting with "Binding of LPS to TLR4 and the co-receptor MD2…" is complex and difficult to understand. It is suggested to focus on pathway going through NF-kB only, in order to simplify this paragraph.

According to the reviewers' comment, we revised the indicated paragraph in the revised Key Event Description of the MIE as follows.
Lipopolysaccharide (LPS) from the bacteria binds to TLR4 in complex with myeloid differentiation factor-2 (MD2), and this complex initiates signaling by recruiting the adaptor proteins MyD88, TIR domain containing adaptor protein (TIRAP), TIR-domain-containing adapter-inducing interferon-β (TRIF) and TIR-domain containing adaptor (TRAM). MYD88 associates with IL-1R-associated kinase 1 (IRAK1) and IRAK4 and recruits TNFR-associated factor 6 (TRAF6). This complex recruits TGF-β-activated kinase 1 (TAK1), leading to phosphorylation of NF-κB inhibitor (IκB), activation of nuclear factor-κB (NF-κB) and consequent transcription of a range of genes coding for pro-inflammatory cytokines, including tumour necrosis factor (TNF), IL-6, pto-IL-1□, and pro-IL-18 (Mills, 2011).

- Comment 11: is no longer an issue, as AOP developers have clarified that AOP only addresses events happening after impairment of IL-1R1 signaling. Therefore, there is no need to further address this issue.
In addition, the review panel would like to share with the AOP developers the following references for consideration regarding the association of IL-1R1

signaling and suppression of T cell activation:

- *Bohrer, A.C., Tocheny, C., Assmann, M., Ganusov, V.V. Mayer–Barber, K.D. 2018. Cutting Edge: IL-1R1 Mediates Host Resistance to Mycobacterium tuberculosis by Trans-Protection of Infected Cells. J Immunol. 201 (6) 1645-1650; DOI: https://doi.org/10.4049/jimmunol.1800438*

- *Labow, M., D. Shuster, M. Zetterstrom, P. Nunes, R. Terry, E. B. Cullinan, T. Bartfai, C. Solorzano, L. L. Moldawer, R. Chizzonite, and K. W. McIntyre. 1997. Absence of IL-1 signaling and reduced inflammatory response in IL-1 type I receptor-deficient mice. J. Immunol.159:2452-2461.*

- *Rogers, H. W., K. C. Sheehan, L. M. Brunt, S. K. Dower, E. R. Unanue, and R. D. Schreiber. 1992. Interleukin 1 participates in the development of anti-Listeria responses in normal and SCID mice. Proc. Natl. Acad. Sci. USA89:1011-1015*

- *van der Meer JWM, Barza M, Wolff SM, Dinarello CA. A low dose of recombinant interleukin 1 protects granulocytopenic mice from lethal gram-negative infection. Proc Natl Acad Sci USA. 1988; 85:1620–1623. [PubMed: 3125553*

Thank you for the kind introduction of important papers. Since the paper by Rogers et al did not clearly demonstrate increased susceptibility to infection in wild mice after administration of anti-IL-1 and anti-IL-1receptor antibodies, we did not refer to this paper. Although the manuscript by van der Meer et al is also an interesting paper, we thought that this paper is not essential in this AOP. We referred to other two papers in this AOP. (the revised Essentiality of the Key Events, the revised Evidence Assessment, and the revised Empirical support of the Summary page, and the revised Evidence Supporting this KER in the KER3).

Comment 1: It should be acknowledged that this AOP is more targeted towards pharmaceuticals than chemicals.

As suggested, we added the following sentence in the revised Abstract of the Summary page.

Although the purpose of this AOP is to elucidate biological pathways that lead to increased susceptibility to infections caused by impaired IL-1R signaling by chemicals, most of the stressors presented in this AOP were limited to pharmaceuticals because of the lack of information on chemicals.

Comment 14: Are early life and later in life-stage equally sensitive to inhibition of IL-1 signalling? Due to maturation of the immune system (including signalling pathways) and immunosenescence, I am not sure that all age stages will be qualitatively and quantitatively equally sensitive.

We found a couple of papers that described age-dependent difference in IL-1 signaling. We added those papers in the revised Domain of Applicability in the Summary page and the MIE.

The lower level of stress-induced IL-1□ expression is demonstrated in the aged murine keratinocytes (Pilkington et al., 2018).

The IL-1□ production by mouse oral mucosal leukocytes stimulated with candida albicans was reduced with aging (Bhaskaran et al., 2020).

The baseline IL-1 signaling of the upper respiratory tract lavage was reduced in murine newborn mice (Kuipers et al., 2018).

Comment 15: The section Inhibition, Nuclear factor kappa B (NF-kB) leads to Suppression of T cell activation is incomplete, references are missing. The specialized subsets of T cells involved should be better defined.

We have already described type 1, type 2 immunity and Th17 response in the Biological Plausibility in the KER3.

Comment 16: While it is clear that the insufficient T cell or B cell function causes impaired resistance to infection, it is not clear at what level of the immune activation the impairment of IL-1R signalling will impact T and B cells functions.

We had already added the following sentence in the Abstract
The activation of NF-κB plays a principal role in the immunological function of IL-1. Namely, it stimulates innate immunity such as activation of dendritic cells and macrophages. It also stimulates T cells via activated dendritic function or directly. The activation of T cells is crucial for B cell proliferation and their antibody production. The cooperation by T cells and B cells constitutes a main part of host defense against infection. Therefore, the impaired IL-1R1 signaling either by the decreased IL-1 production or the inhibition of IL-1β binding to IL-1R1 by IL-1 receptor antagonist（IL-1Ra）or anti-IL-1β antibody) results in the blockade of the effects of the pleiotropic cytokine IL-1β leading to increased susceptibility to infection.

In addition, we added the following explanation in the revised biological plausibility of KER3

The activation of NF-κB plays a principal role in the immunological function of IL-1R signalling. NF-□B plays a crucial role in the activation of dendritic cells as well as T cells. In dendritic cells, the activation of the canonical NF-□B pathway in response to pro-inflammatory stimuli, such as cytokines including IL-1□ or IL-1□ and TLR ligands, stimulate the maturation of dendritic cells with enhanced antigen presenting function. The inhibition of NF-□B suppress antigen presenting function of dendritic cells, resulting in suppression of T cell activation (reviewed by Reinhard et al (Reinhard et al., 2012) and van Delft et al (van Delft, Huitema and Tas, 2015).

In T cells, NF-kB can be activated by several pathways of signal transduction. Although the engagement of the TCR by major histocompatibility complex (MHC) plus antigen is a main stream of NF-□B activation in T cells, the stimulation of T cells by IL-1 activates NF-kB as already described before. Once in the nucleus, NF-□B governs the transcription of numerous genes involved in T cell survival, proliferation, and effector functions (Paul and Schaefer, 2013). Although CD4 T cells are able to commit to Th1, Th2 and Th17 lineages in the absence of IL-1R signaling at steady state, these committed CD4 T cells are unable to effectively secrete their cytokines upon TCR ligation. Namely, IL-1 is indispensable for CD4 T cell effector function (Lin et al., 2015).

Comment 18. As a general comment, I find certain parts of the AOP to lack sufficient detail (i.e.,

MIE, T cell subpopulations). Along these lines, the authors typically refer to IL-1, which is actually two different cytokines (i.e., IL-1alpha and IL-1beta) as a single entity. For clarity, the authors should be clear about which specific IL-1 they are referring to whenever possible.

We agree with the reviewer's comment. We tried to distinguish IL-1□ or IL-1□ whenever the referred manuscripts specified. However, some papers dealt with IL-1 receptor without specifying IL-1a or IL-1b. In such cases, we could not distinguish the role of IL-1□ or IL-1□ because they shared the same receptor.

Comments 24. The AO of "Increase, Increased susceptibility to infection" may be problematic from the standpoint of broader AOP development. There are numerous MIEs and KEs that can lead to lead to increased susceptibility to infection, yet only IL-1R1-mediated activation is captured as being within the applicability domain. Moreover the "Regulatory Significance of the Adverse Outcome" domain is much too narrow, focusing only on impaired activation of IL-1R1.

As discussed in the previous meeting, it is not easy to answer the comment *"The AO of "Increase, Increased susceptibility to infection" may be problematic from the standpoint of broader AOP development. There are numerous MIEs and KEs that can lead to lead to increased susceptibility to infection, yet only IL-1R1-mediated activation is captured as being within the applicability domain."* It is true that KE202 Inhibition, Nuclear factor kappaB is used in 3 different AOPs, such as AOP 277, 278, and 14. As suggested by the reviewer, KE202 were caused by different contexts. I am not sure that KE202 can be shared by different AOPs. But, so far, AOP14 or AOP278 used our description on KE202 without making their own KE202.

Regarding the comment "the "*Regulatory Significance of the Adverse Outcome" domain is much too narrow, focusing only on impaired activation of IL-1R1.*", we described the following in the Consideration for Potential Applications of the APO in the Summary page.

The impaired IL-1 signaling can lead to decreased host resistance to various infections. Therefore, the test guideline to detect chemicals that decrease IL-1 signaling is required to support regulatory decision-making. This AOP can promote the understanding of the usefulness of the test guideline.

Annex 8: Additional information from the OECD

E-mails sent to the scientific review panel on 28 May 2021

From: Chantra Eskes
Sent: 28 May 2021 13:14
To: 'Emanuela Corsini'; 'David Lehmann'; 'Rob Vandebriel'; 'Ronald Wange'; 'Yoshiro Saito';
Cc: Nathalie Delrue'; 'Iwona Wilk-Zasadna'
Topic: Scientific review of AOP 277: TC 14 May 2021 - FOLLOW-UP ACTIONS

Dear Review panel,

We had an information discussion with the OECD on last Friday, and are happy to inform you that Nathalie Delrue will be joining the beginning of our meeting on June 11 as to answer the questions you have raised during our last teleconference.

In particular, Nathalie raised our attention to the fact that:

- There are no problems if there are other MIE and KE associated with one AOP, since it can be used to be developed a broader network of AOPs.

- For a specific KE, it is important to find the right balance between being too specific vs. too simplistic.

- If the panel has suggestions to include additional KE and/or MIE, it is recommended to indicate which additional KE and MIE would be useful to further develop / include.

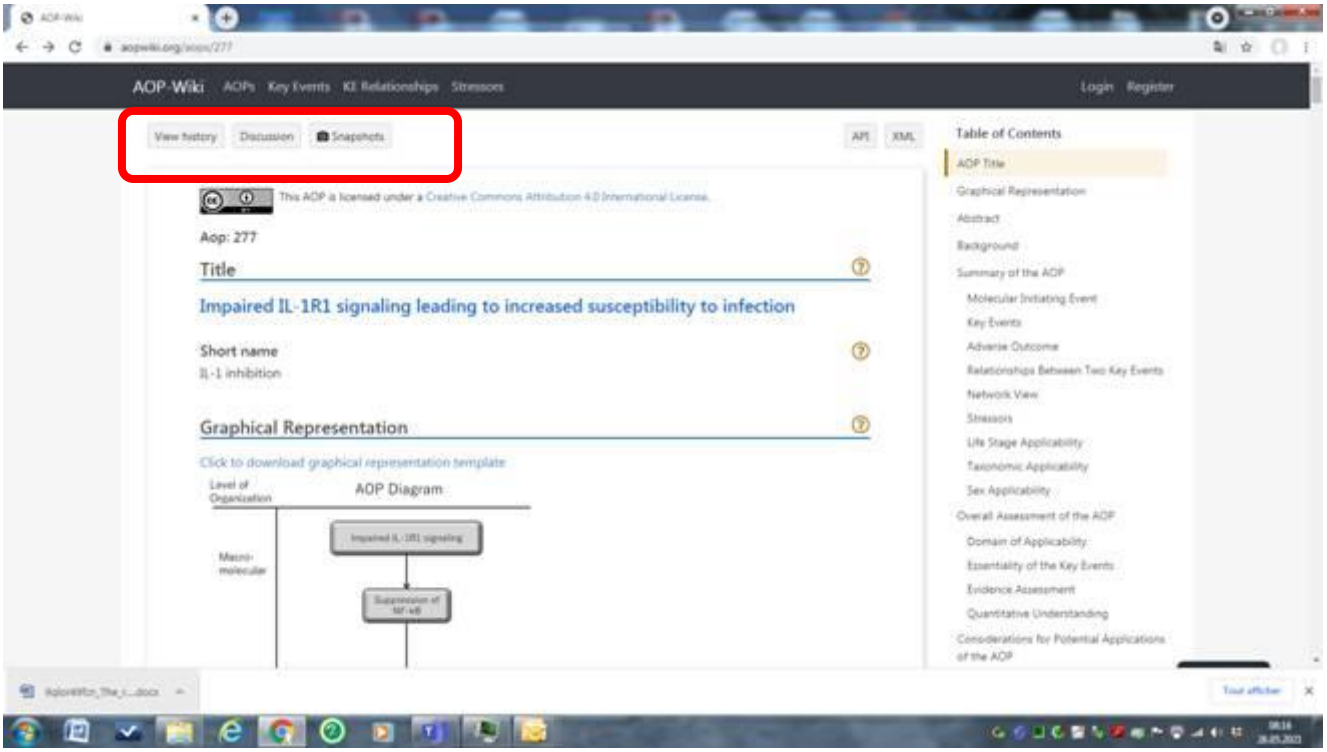
- Finally but not least, before the AOP 277 has been sent to you for review, it has already undergone an internal review by EAGMST. The outcome of this review can be found under the section “Discussion” on the top of the AOP 277 webpage (https://aopwiki.org/aops/277, see also screen print below), and I also send it here attached for your convenience.

We believe this can be an interesting additional information to take into account, as it allows to understand the requests already made to the AOP developers and how those could have impacted the development history of the AOP 277. Furthermore, in case you find it useful, additional information on the AOP 277 development history can be found under “view history” for a record of the updates in time, and under “snapshots” to check the AOP content changes over time.

I hope that such information, together with the answers provided by the test developers can be useful in making the final assessment of the AOP 277.

With best regards,

Iwona & Chantra



From: Chantra Eskes
Sent: 28 May 2021 19:52
To: 'Emanuela Corsini'; 'David Lehmann'; 'Yoshiro Saito'; ‘Rob Vandebriel'; 'Ronald Wange';
Cc: Nathalie Delrue'; 'Iwona Wilk-Zasadna'
Topic: Scientific review of AOP 277: TC 14 May 2021 - FOLLOW-UP ACTIONS

Dear all,

Please find below some further information provided by Nathalie Delrue.

With kind regards, Chantra

From: Nathalie DELRUE
To: Chantra Eskes; Iwona Wilk-Zasadna

Topic: Follow up from Friday call

Dear Chantra and Iwona,

Following our discussion on Friday, I've had a look into a few things related to AOP 277.

Based on the completeness check form available in the discussion section, it appears the main review was conducted on 26 September 2019 ("Reviewed as presented in AOP-Wiki on 09/26/2019). Unfortunately there's no snapshot generated before that date. I've noted to raise this point at the EAGMST meeting and will suggest that the completeness form includes a sentence requiring the coach to generate a snapshot of the reviewed version as we did in the past. May be you can ask the authors if they have kept the version that was available before Sept 2019.

As discussed, AOPs are a linear representation of successive key events. This doesn't prevent networks (it's the even ultimate goal), but it is then considered several AOPs.
See Dan's note on the MIE section of the completeness form: *Two MIEs are included. Because "either MIE leads to reduced IL-1 signaling", the guidance in the handbook recommends they be presented as distinct AOPs.*

I had a looked into the KE and KER that have been deleted or updated.

Based on the completeness form, the previous version of the AOP included MIE 1570 (Blocking of IL-1R), 1571 (Decreased IL-1 production) and 1572 (Impaired IL-1 signaling). They still exist but none of them is now anymore associated with an AOP in the Wiki. The authors decided to keep 1570 only but they have created a different, new KE (KE 1700).

Other KE that don't exist anymore: KE 1569 (Impaired T cell activation) – I assume it has been replaced by KE 1702
KE 1644 (Impaired Ab production)
The authors indicate: According to the reviewer's suggestion, we decided to use only 1569 and deleted 1644. Some of information described in 1644 was included in 1569. In addition, the description was shortened.

Relationship 1814 (Decreased IL-1 production leads to Impaired IL-1 signaling), 1920 (Impaired IL-1 signaling leads to Inhibition, Nuclear factor kappa B (NF-kB)) – this is now KER 2002! , 1922 (Impaired T cell activation leads to Impaired Ab production) are also not associated anymore to an AOP. There may be more; these are those listed in the internal review form.
The authors indicate: As suggested by the reviewer, by deleting MIE 1572 and KE 1564, relationship 1920 and 1922 were deleted.

We also discussed the level of detail a KE description should include. As said, it's a matter of balance between high specificity and possibility to reuse a KE in other AOPs (this is actually one question from the completeness form: *Are the KEs described in a way that allows their reuse in other AOPs).*

I had a look into the project proposals related to immunotoxicity on the AOP development workplan. And found out they were well described in the draft DRP on immunotoxicity currently under development (the extract related to AOPs is attached). The AOP 154 has recently been sent to the WNT for approval. In case it helps, the review report of this AOP is available at: [external review]

Best regards,

Nathalie

Extract from the draft Detailed Review Paper on In vitro tests addressing immunotoxicity testing with a focus on immunosuppression (May 2021)

III. Current status of AOPs on immunotoxicity testing

An Adverse Outcome Pathway (AOP) describes a logical sequence of causally linked events at different levels of biological organization, which follows exposure to a chemical and leads to an adverse health effect in humans or wildlife. AOPs are the central element of a toxicological knowledge framework, promoted by member countries through OECD, built to support chemical risk assessment based on mechanistic reasoning (OECD, 2020a). These AOPs are available in the AOP Wiki (OECD, 2020b), an interactive and virtual encyclopedia for AOP development.

All AOPs on immunosuppression currently available in the OECD work plan are on-going and shown in Table 1. Project 1.74: Inhibition of JAK3 leading to impairment of TDAR is under development and will not be discussed. However, two of them, Project 1.38 ” No. 154: Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response” and Project 1.48 “No. 277: Inhibition of IL-1 binding to IL-1 receptor leading to increased susceptibility to infection” are undergoing peer review. No. 154 shows calcineurin (CN) activity is inhibited when stressors of CN inhibitors (CNIs） bind to CN with their respective immunophilins, which interferes with the nuclear localization of nuclear factor of activated T cells (NFAT), a substrate of CN. As a result, the formation of functional NFAT complexes with activator protein-1 (AP-1) that bind at the site of IL-2, IL-4 and other T cell-derived cytokine promoters is reduced, thereby suppressing production of these cytokines. Among the affected cytokines from each of the helper T cell subsets, reduced production of IL-2 and IL-4 affects the proliferation and differentiation of B cells to suppress the TDAR. No. 277 addresses two Molecular Initiating Event (MIE)s, blocking IL-1 and decreased IL-1 production. Either MIE leads to reduced IL-1 signaling. The biological plausibility of the signaling cascade from the activation of IL-1 receptor to the activation of nuclear factor κB (NF-κB) is already confirmed. In addition, the biological plausibility that suppressed NF-κB activation leads to impaired T cell activation and antibody production leading to increased susceptibility to infection is supported by quite a few published works (OECD, 2020b). To recapitulate some aspects of the *in vivo* immunotoxic responses by using *in vitro* methods, it will be very important to more closely mimic respective *in vivo* situations based on individual AOPs, although this may be complicated and laborious.

Table 1. Ongoing AOPs for Immunosuppression in the OECD work plan

Project 1.38: The Adverse Outcome Pathway on Binding of FK506-binding protein (FKBP12) by calcineurin inhibitors leading to immunosuppression	
Lead: Inclusion in work plan: Current situation:	Japan 2015 No. 154: Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response , External review completed as presented in EAGMST meeting 2020.
Project 1.48: The Adverse Outcome Pathway on Dysregulation of IL-1 transcription leading to immunotoxicity	
Lead: Inclusion in work plan: Current situation:	Japan 2016

	No. 277: Inhibition of IL-1 binding to IL-1 receptor leading to increased susceptibility to infection, External review completed as presented in EAGMST meeting 2020.
Project 1.74: Inhibition of JAK3 leading to impairment of TDAR	
Lead: Inclusion in work plan: Current situation:	Japan 2018 No. 315: Inhibition of JAK3 leading to impairment of T-Cell Dependent Antibody Response, Under Development