Meeting Report

# Report of the 1st and 2nd Mystery of Reactive Oxygen Species Conferences

doi:10.14573/altex.2203011

## Introduction

To reduce or replace animal experimentation, approaches are needed where computational networking of tissue and cellular biological events addresses disease mechanisms. One important tool for this is the adverse outcome pathway (AOP) framework, which describes how molecular and cellular events cause adverse health effects. The AOP framework is compiled by the scientific community in the Adverse Outcome Pathway Knowledge Base (AOP-KB) as part of Organisation for Economic Co-operation and Development (OECD) projects[1].

AOPs begin with a molecular initiating event (MIE), which is the starting point describing how stressors interact with specific molecules in cells. AOPs then progress through a series of key events (KEs) to an adverse outcome (AO), via key event relationships (KERs). KERs describe the causal linkages between each KE using biological knowledge, empirical evidence, and quantitative understanding. This modular framework increases efficiency in AOP development and facilitates the development of AOP networks, the ultimate tool for AOP application. A central tenet of AOP development is sharing KEs across pathways. This enables an enhanced understanding of cross-talk across pathways to represent the complexity of biology.

Reactive oxygen species (ROS) play crucial roles in a variety of diseases and physiological conditions. ROS are produced by various stressors such as chemicals, particles or radiation energy, and endogenous peptides or the enzymatic machinery. Transient ROS play an important role in the defense mechanism of immune cells and redox signaling, whereas prolonged ROS elevation is involved in disease development and progression.

With global progress in the AOP development field, multiple, slightly nuanced KEs related to ROS have been created in the AOP-Wiki[2]. Many of these KEs are highly similar and largely redundant, which has catalyzed a need to create harmonized consensus KEs on ROS that can be shared in a modular fashion between closely related pathways and networks.

To address this need, a consortium of ROS and AOP experts has been formed to discuss the "Mystery of ROS" and develop consensus KEs for this field. This meeting report summarizes initial efforts of the "Mystery of ROS" consortium to harmonize the ROS-related KEs currently available in the AOP-Wiki. The two new modular KEs reflect discussion from the group relating to the effects of ROS presenting a "double-edged sword" by describing the concepts of "up-regulation of ROS" and "diminished protective response."

## Summary of the discussions in the consortium

The international online conferences on the Mystery of ROS took place on May 31, 2021 and October 8, 2021. At the first conference on Mystery of ROS (I), a brief introduction of the ROS collaboration was followed by eight presentations, which are listed in Table 1.

**Tab. 1: Outline of Mystery of ROS (I)**

| | Content | Presenter |
|---|---|---|
| 1 | Introduction | Dr Shihori Tanabe |
| 2 | Overall purpose of the collaboration | Dr Jason O'Brien |
| 3 | KE1632 (Increase in RONS) in AOP293&294 | Dr Jessica Helm / Dr Rudel's group |
| 4 | KE257 (Increase, ROS production) in AOP299, 327-330, 386, 387, and NEA etc. | Dr Knut Erik Tollefsen |
| 5 | KE1115 (Increased, ROS) in AOP382-384 | Dr Young-Jun Kim |
| 6 | KE1753 (chronic ROS) in AOP298 & KE1869 (oxidative stress response) in AOP379 | Dr Shihori Tanabe |
| 7 | DNA damage and ROS | Dr Carole Yauk |
| 8 | Nanomaterial-related oxidative stress | Dr Sabina Halappanavar |
| 9 | Radiation-related ROS | Dr Vinita Chauhan and Dr Danielle Beaton |

[1] https://aopkb.oecd.org/

[2] https://aopwiki.org/

**Tab. 2: Outline of Mystery of ROS (II)**

| | Content | Presenter |
|---|---|---|
| 1 | Introduction | Dr Shihori Tanabe |
| 2 | Overall purpose of the collaboration | Dr Jason O'Brien |
| 3 | Inflammation KE coordination | Dr Dan Villeneuve |
| 4 | ROS as mediators in disease | Prof. Pietro Ghezzi |
| 5 | Response to ROS and coagulation | Dr Shihori Tanabe |
| 6 | DNA damage and ROS | Dr Carole Yauk |
| 7 | Nanomaterial-related oxidative stress | Dr Sabina Halappanavar |

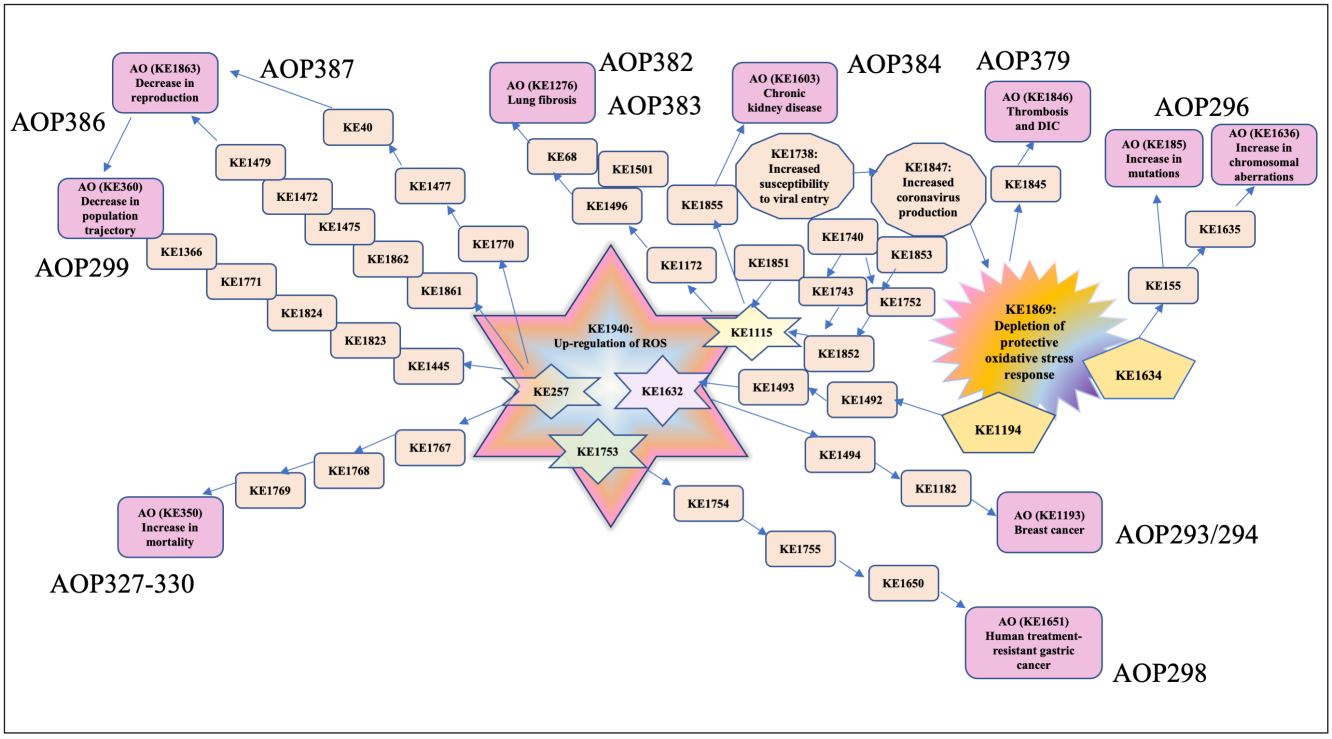


**Fig. 1: Adverse outcome pathway (AOP) story coordination with a group of harmonized key events (KEs)**
AOPs discussed by the Mystery of ROS consortium are linked with ROS-related KEs. Hexagram star shapes indicate the ROS-related KEs; pentagon shapes indicate DNA damage-related KEs; dodecagon shapes indicate coronavirus-related KEs. Adverse outcomes (AOs) are colored in pink. DIC, disseminated intravascular coagulation

The Mystery of ROS consortium carefully considered many critical aspects of the creation of ROS-related KEs. It was identified that ROS are needed for critical cellular functions at optimal levels; however, excessively high levels of ROS production over prolonged durations lead to adverse effects. Thus, the cellular balance between ROS production and scavenging to maintain homeostasis needs to be represented within the KEs.

The second conference on the Mystery of ROS (II) included four presentations and panel discussions on DNA damage and ROS and on nanomaterial-related oxidative stress (Tab. 2). The initial concept for a ROS-related KE group was introduced, which led to the creation of harmonized KE1940[3] "Up-regulation of ROS" in a collaborative effort of conference attendees (Fig. 1). The Mystery of ROS collaboration discussed the harmonization of the existing ROS-related KEs and associated AOPs in the AOP-Wiki (Fig. 1; Tab. S1[4]).

A similar discussion on inflammation-related KEs has resulted in a consensus around a hub of three modular events, which was presented at the Mystery of ROS (II) as a model (Villeneuve et al., 2018).

[3] https://aopwiki.org/events/1940
[4] doi:10.14573/altex.2203011s

## Plans of the consortium

Members of the consortium agreed to work toward harmonization of the molecular-level KEs focused on up-regulation of ROS production under the umbrella of KE1940[3] "Up-regulation of ROS". KE1940 focuses on the production of ROS *per se*, while the reduction of protective enzyme activity that also results in ROS increase will be the focus of the KE1869[5] "Depletion of protective oxidative stress response" (Short name "Depleted Protective Response to ROS") at "molecular" and "cellular" level KEs for ROS.

## Conclusion

The consortium had fruitful discussions on harmonizing ROS-related AOPs. Although no definitive consensus on the KEs was reached, there was agreement that further conversations are needed to elucidate how best to represent the complexity of oxidative stress. Since oxidative stress is caused by excessive ROS production and depletion of scavenging machinery, all aspects of ROS should be considered for harmonizing AOPs. These discussions of the Mystery of ROS consortium will strengthen AOPs and facilitate the development of AOP networks to support the design and justification of new approach methodologies.

## Reference

Villeneuve, D. L., Landesmann, B., Allavena, P. et al. (2018). Representing the process of inflammation as key events in adverse outcome pathways. *Toxicol Sci 163*, 346-352. doi:10.1093/toxsci/kfy047

## Conflict of interest

Karsta Luettich and Hasmik Yepiskoposyan are employed by Philip Morris International (PMI).

## Acknowledgments

The authors would like to acknowledge Dr Sabina Halappanavar, Dr Dan Villeneuve, Prof. Pietro Ghezzi, Dr Ed Perkins, Dr Penny Nymark, Dr Maria João Amorim, all participants in Mystery of ROS, OECD AOP Coach Team members, and EAGMST members. The author would like to thank members of the National Institute of Health Sciences (NIHS), Japan. This work was funded by Japan Agency for Medical Research and Development (AMED) Grant Number JP21mk0101216 (S.T.), Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 21K12133 (S.T.), Research Council of Norway (RCN) through its Centre of Excellence (CoE) funding scheme (Project No. 223268), and NIVAs Computational Toxicology Program, NCTP (www.niva.no/nctp) (K.E.T.). This work was supported by the project CERST (Center for Alternatives to Animal Testing) of the Ministry for culture and science of the State of North-Rhine Westphalia, Germany (file number 233-1.08.03.03- 121972/131 – 1.08.03.03 – 121972) and the European Union's Horizon 2020 Research and Innovation Program, under the Grant Agreement number 963845 of the ONTOX project (E.F.). This work was supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No 857560 (I.S.). This work was supported by the Ministry of Health, Labour, and Welfare (MHLW), Japan (S.T.). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the authors' organization, the European Commission in European Union (EU), JSPS, Ministry of Education, Culture, Sports, Science and Technology-Japan (MEXT), or MHLW in Japan.

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[5] https://aopwiki.org/events/1869