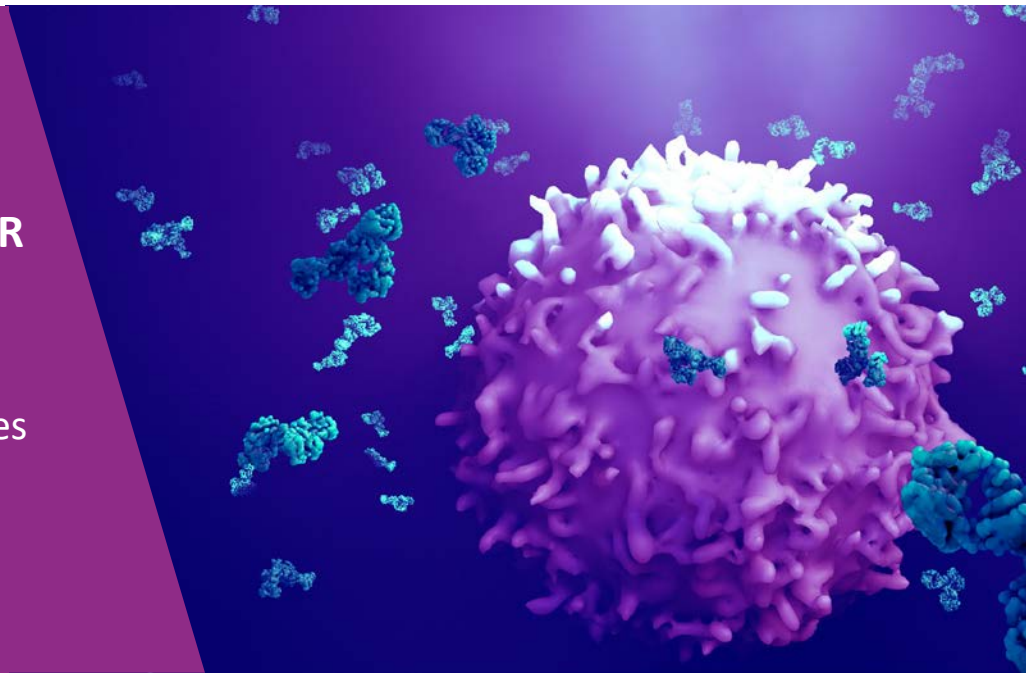
imSAVAR

IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 4.6

Second iteration to characterize molecular mechanisms leading to immune-related adverse events

DELIVERABLE REPORT

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 853988.
The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and JDRF INTERNATIONAL.

imi innovative medicines initiative

efpia

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.

Abstract

Work package 4 (WP4) focuses on the development of biomarkers for predicting the risk of observing harmful adverse outcomes in first-in-human (FIH) studies of immunomodulatory therapeutics. Current preclinical models to assess safety of immunotherapies are often species-dependent and incomplete, since they only reflect the human immune system in a limited fashion, which often leads to misleading predictions of human immune-related adverse events (irAEs). Hence, WP4 aims at establishing biological characteristics (biomarkers) that are measurable and evaluable and can be integrated into safety models in order to (i) assess if the model mimics the underlying human biological processes leading to an immune-related adverse outcome as closely as possible, to (ii) assess if the biomarker is reliably predicting the risk of harmful adverse outcomes in FIH studies, and to (iii) support safe starting dose selection for FIH studies.

In imSAVAR four firstly defined mode of actions (MoAs) of immunomodulatory therapeutics will be addressed that require development and confirmation of biomarkers: (i) CAR (chimeric antigen receptor) T-cells, (ii) BiTEs (bispecific T-cell engagers), (iii) CPI (checkpoint inhibitors) for cancer immunotherapy, and (iv) recombinant human interleukin-2 (rhIL-2) as a MoA for inflammatory disease therapeutics. We align biomarker development with immune-related adverse outcome pathways (irAOPs) to foster a common understanding of the processes triggered through a molecular initiating event and eventually leading to adverse outcomes.

With Deliverable D4.6, we describe updated versions of machine-readable, interactive, and expandable models for molecular mechanisms leading to recombinant human IL-2 (rhIL-2) mediated skin rash, vascular leakage, and hepatotoxicity as well as to CAR T mediated cytokine release syndrome (CRS). First versions of first models were described in D4.5. The extended models are public demonstrators and are continuously being developed.

Document Information

Deliverable Report	**D4.6: Second iteration to characterize molecular mechanisms leading to immune-related adverse events**
Date	**28.04.2023**
Report prepared by	**University of Luxembourg** Fraunhofer ITEM, Fraunhofer IZI, Fraunhofer ITMP, Paul-Ehrlich-Institute, Lund University, University Hospital of Würzburg
Project	**imSAVAR - Immune Safety Avatar: nonclinical mimicking of the immune system effects of immunomodulatory therapies** Grant Agreement No.: 853988 (IMI2-2018-15-04)
Project Coordinator	**Fraunhofer-Gesellschaft zur Foerderung der angewandten Forschung e.V.** Prof. Dr. Dr. Ulrike Köhl Dr. Kristin Reiche **Novartis Pharma AG** Dr. Jonathan Moggs Hannah Morgan, PhD
Type	**Deliverable Report | Public**

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 853988. The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and JDRF INTERNATIONAL.

Table of Contents

1. Background

Immune-related AOPs: The development of biomarkers is guided by irAOPs. This are conceptional frameworks describing the current knowledge of biological effects triggered by an initiating event leading to adverse health effects. WP3 conducted a comprehensive literature search to reveal detailed descriptions of key events and key event relationships for rhIL-2-mediated reactions. Based on this knowledge WP3 developed irAOPs for rhIL-2-mediated skin rash, vascular leakage and hepatotoxicity as described in deliverables D3.1, D3.3, and D3.4. The same approach was applied by WP2 to reveal irAOPs and biomarkers for CAR T-cell - mediated CRS. For details see deliverable report D2.1.

Machine readable representations for irAOPs: Most AOPs are represented using static formats such as e.g. graphics outlining the sequence of key events leading to an adverse event. Details of the content and relations between key events are described using a textual format. However, a static and pure textual representation of irAOPs hinders sharing of knowledge and cooperative development. Hence, as outlined in D4.5 the imSAVAR consortium decided to implement machine-readable representations for irAOPs such that interactive modelling of key events and key event relationships is possible. Digital representations of irAOPs facilitate shared development, evaluation and refinement of mechanisms leading to immune-related adverse events. As a proof-of-concept study and a first iteration to characterize molecular mechanisms leading to immune-related adverse events we selected the irAOP describing rhIL-2-mediated skin rash and a first version was implemented in MINERVA (see D4.5). This initial version was extended and MINERVA networks for additional irAOPs implemented.

2. Methods – The MINERVA platform

MINERVA (Molecular Interaction NEtwoRk VisuAlization) Platform [1-3] is a standalone webserver for visual exploration, analysis, and management of molecular networks encoded in systems biology formats, including CellDesigner, SBML, and SBGN. MINERVA is a webservice using the Java Server Faces 2 technology. The server side, including data parsing, integration, annotation, and verification, is implemented in Java 8. The platform uses the Postgres SQL database for data storage and the Hibernate framework as a middle layer between web server and database. The user web-interface is generated in JavaScript and content is visualized by OpenLayers/Google Maps API. Visualization of uploaded networks generated by the platform is accessible via a web browser to all viewers with the weblink to the resource.

3. Results – MINERVA networks for rhIL-2- and CAR T-cell - mediated side effects

In 03/2021 WP4 established an irAOP map working group that meets bi-weekly to have interactive sessions to discuss a molecular interaction network describing key events of rhIL-2-mediated skin rash, vascular leakage, and hepatotoxicity. The working group is led by UNILU and includes participants from WP2-4. Based on the irAOP for rhIL-2-mediated skin rash, vascular leakage, and hepatotoxicity and the knowledge base resulting from the literature search conducted by WP3, we transferred and extended the static irAOP descriptions to machine-readable representations. In detail, we used the MINERVA tool for a machine-readable, interactive, and expandable representation of molecular mechanisms triggering rhIL-2-mediated skin rash, vascular leakage, and hepatotoxicity. Furthermore, maps representing the

biological knowledge of irAOP of CAR T-cell treatment have also been prepared. These four MINERVA networks are continuously refined and are available as public demonstrator at:

URLs of public demonstrators depicting irAOPs for rhIL-2-mediated skin rash, vascular leakage and hepatotoxicity as well as CAR T-cell – mediated CRS

A. rhIL-2-mediated skin rash: https://imsavar.elixir-luxembourg.org/minerva/index.xhtml?id=IL2skinrash

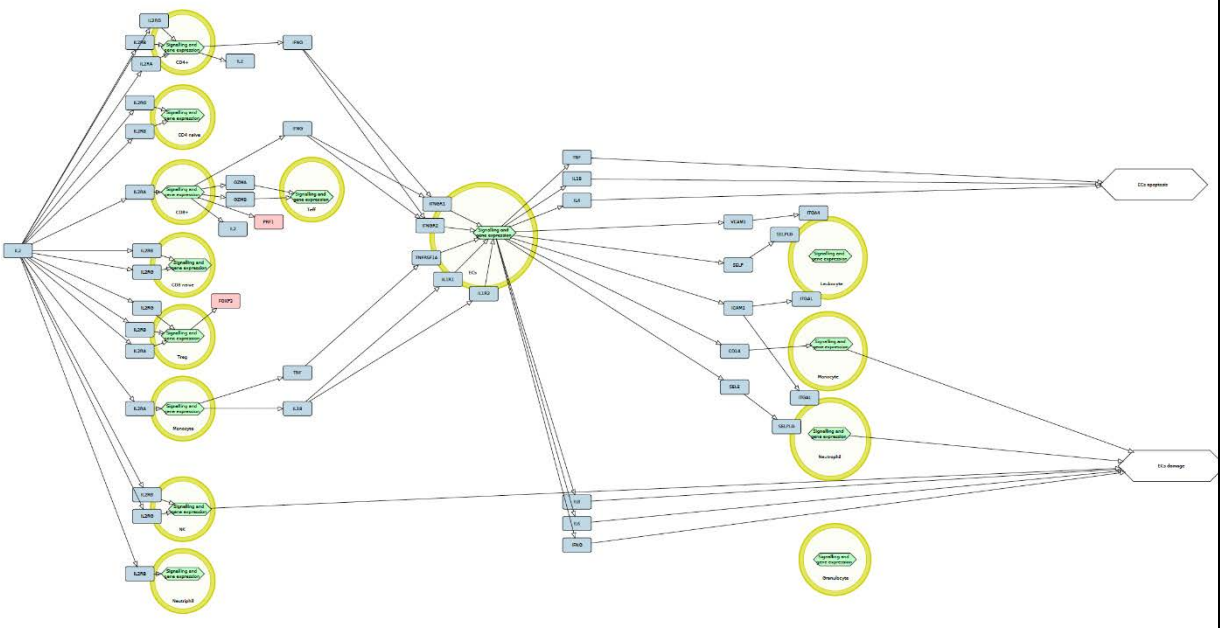
B. rhIL-2-mediated vascular leakage: https://imsavar.elixir-luxembourg.org/minerva/index.xhtml?id=IL2vascularleakage

C. rhIL-2-mediated hepatotoxicity https://imsavar.elixir-luxembourg.org/minerva/index.xhtml?id=IL2hepatotoxicity

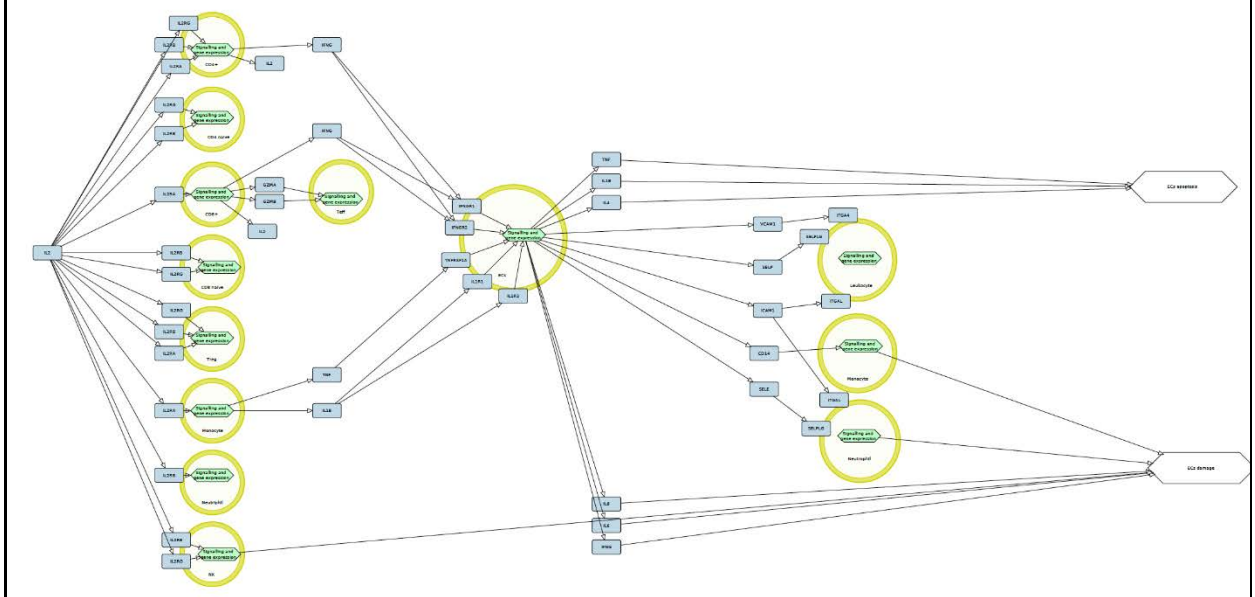
D. CAR T-cell - mediated CRS: https://imsavar.elixir-luxembourg.org/minerva/index.xhtml?id=cart14

We identified the major compartments involved in induced side effects. For each of the compartments we added a network of molecules and cells possibly interacting during induced side effects, as well as associations to signalling pathways and phenotypes, as exemplarily shown for these extracts of the four networks:

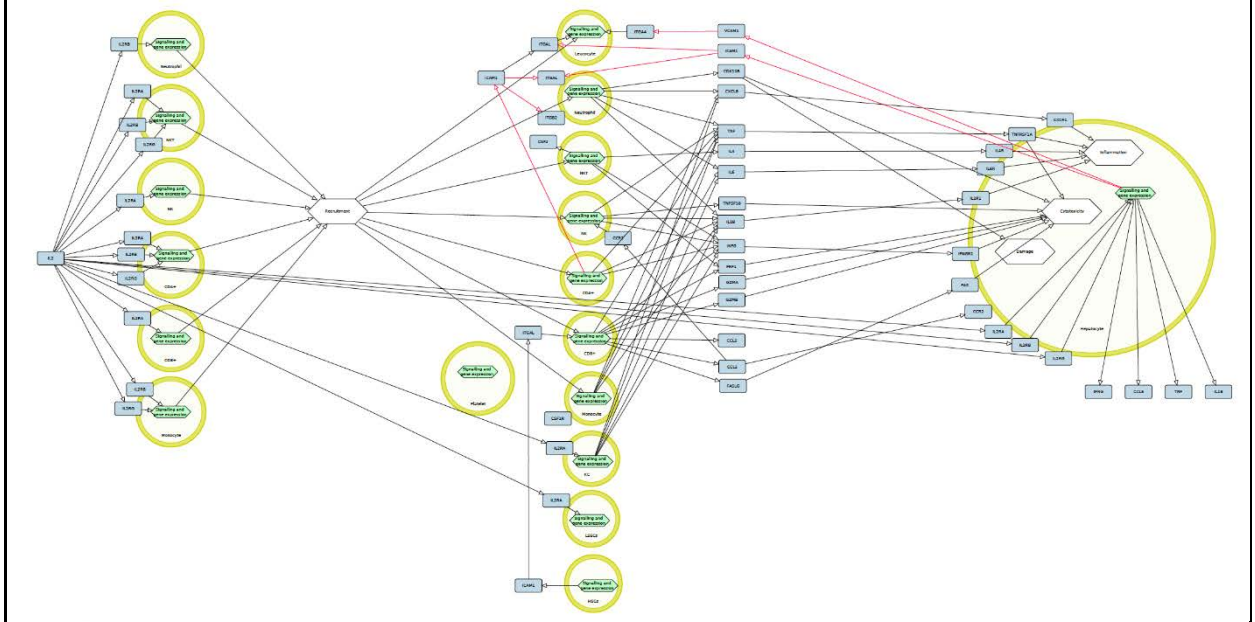
Extract 1 of the MINERVA network for rhIL-2-mediated skin rash https://imsavar.elixir-luxembourg.org/minerva/index.xhtml?id=IL2skinrash



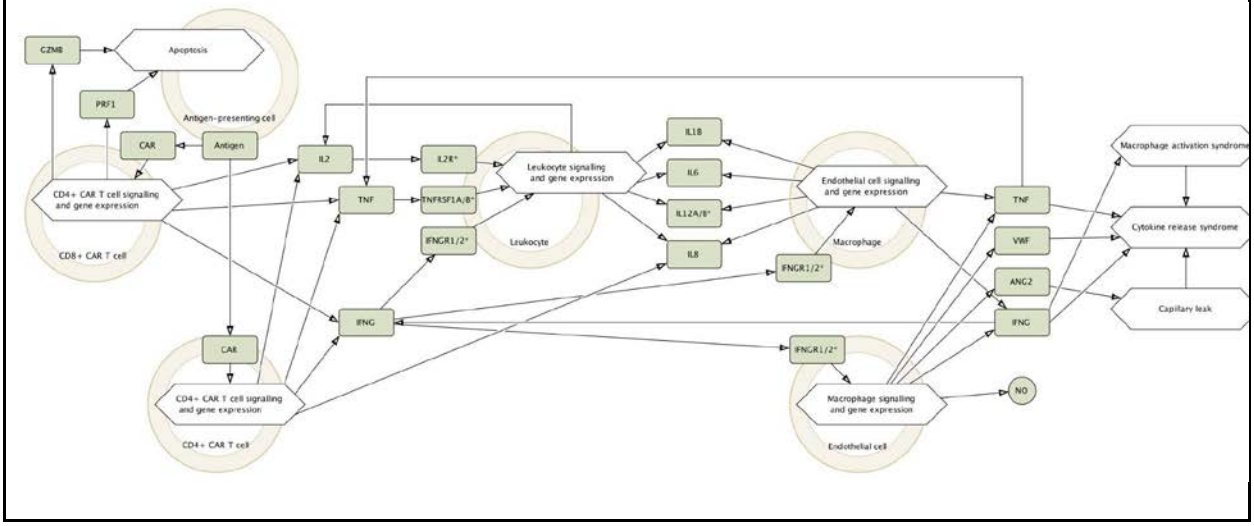
Extract 2 of the MINERVA network for rhIL-2-mediated vascular leakage
https://imsavar.elixir-luxembourg.org/minerva/index.xhtml?id=IL2vascularleakage



Extract 3 of the MINERVA network for rhIL-2-mediated hepatotoxicity
https://imsavar.elixir-luxembourg.org/minerva/index.xhtml?id=IL2hepatotoxicity



Extract 4 of the MINERVA network for CAR T cell-mediated CRS
https://imsavar.elixir-luxembourg.org/minerva/index.xhtml?id=cart14



The MINERVA networks for these four irAOPs are built using the integrated approach we developed in imSAVAR for machine-readable knowledge representation (see Figure 1). Details of the imSAVAR systems biology approach for adverse outcome pathways is described in a preprint manuscript Mazein et al. [4].

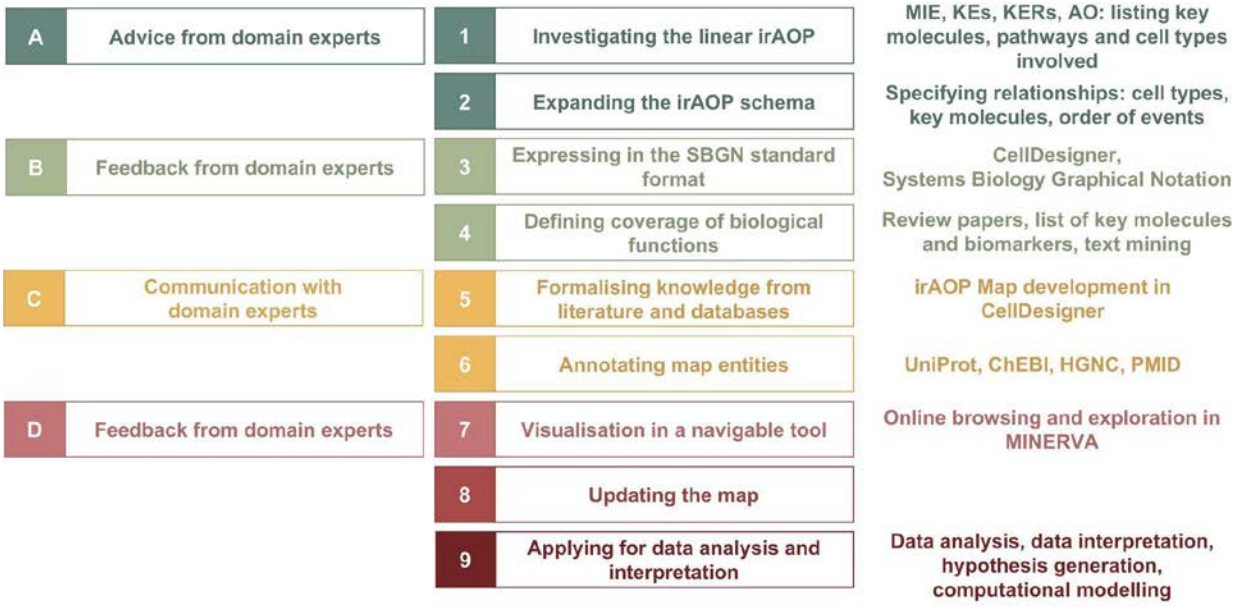


Figure 1: irAOP map development workflow. The systems framework for knowledge management and exploration in immunotoxicology. irAOP, immune-related adverse outcome pathway; MIE, molecular initiating event; KEs, key events; KERs, key event relationships; AO, adverse outcome; UniProt, a database of protein sequence and functional information (https://www.uniprot.org); ChEBI, Chemical Entities of Biological Interest (https://www.ebi.ac.uk/chebi); HGCN, HUGO Gene Nomenclature Committee names (https://www.genenames.org); PMID, references in the form of PubMed IDs (https://pubmed.ncbi.nlm.nih.gov).

In summary, we used CellDesigner editor and the MINERVA platform as tools of choice to establish machine-readable and sharable representations of irAOP mechanisms. With that, MINERVA networks were implemented to characterize molecular mechanisms leading to rhIL2-mediated skin rash, vascular

leakage, and hepatotoxicity as well as to CAR T-cell-mediated CRS. We used the knowledgebase as compiled in the literature searches of WPs 2&3 as the basis to define interactions between molecules, signalling pathways and adverse pathology. The MINERVA networks (see URLs in the table above) will continuously be developed within the irAOP working group and will also serve as a guide to characterize and evaluate molecular mechanisms for other irAOPs addressed in imSAVAR. Lastly, we describe the imSAVAR approach using MINERVA as an interactive platform to encode, manage and explore irAOPs in a preprint manuscript available on bioRxiv [4].

4. Abbreviations

AOP -- adverse outcome pathways

BiTE – bispecific T-cell engagers

CAR – chimeric antigen receptor

CRS – cytokine release syndrome

CPI – checkpoint inhibitor

ICANS – immune effector cell associated neurotoxicity

IL-2 – interleukin-2

irAEs – immune-related adverse events

irAOPs – immune-related adverse outcome pathways

KE – key event

KER – key event relationships

MIE – molecular initiating event

MoA – mode of action

rhIL-2 – recombinant human IL-2

5. References

1. P. Gawron, M. Ostaszewski, V. Satagopam, S. Gebel, A. Mazein, M. Kuzma, S. Zorzan, F. McGee, B. Otjacques, R. Balling, and R. Schneider, “MINERVA—a platform for visualization and curation of molecular interaction networks.” npj Systems Biology and Applications, vol. 2, p. 16020, Sep. 2016, doi:10.1038/npjsba.2016.20.

2. D. Hoksza, P. Gawron, M. Ostaszewski, E. Smula, R. Schneider, “MINERVA API and plugins: opening molecular network analysis and visualization to the community.” Bioinformatics, 25 April 2019,

3. D. Hoksza, P. Gawron, M. Ostaszewski, J. Hausenauer, R. Schneider, “Closing the gap between formats for storing layout information in systems biology.” Briefings in Bioinformatics, 05 July 2019,

4. A. Mazein, M. Shoaib, M. Alb, C. Sakellariou, C. Sommer, K. Sewald, K. Reiche, P. Gogesch, L. A Roser, S. Ortega Iannazzo, S. Sheth, S. Schiffmann, Z. Waibler, V. Neuhaus, S. Dehmel, V. Satagopam, R. Schneider, imSAVAR Consortium, M. Ostaszewski, W. Gu, “Using interactive platforms to encode, manage and explore immune-related adverse outcome pathways.” bioRxiv https://www.biorxiv.org/content/10.1101/2023.03.21.533620v1

Acknowledgement

The authors would like to express their gratitude to the Innovative Medicines Initiative 2 Joint Undertaking (JU) for the financial support of this research under grant agreement No 853988. The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and JDRF INTERNATIONAL.