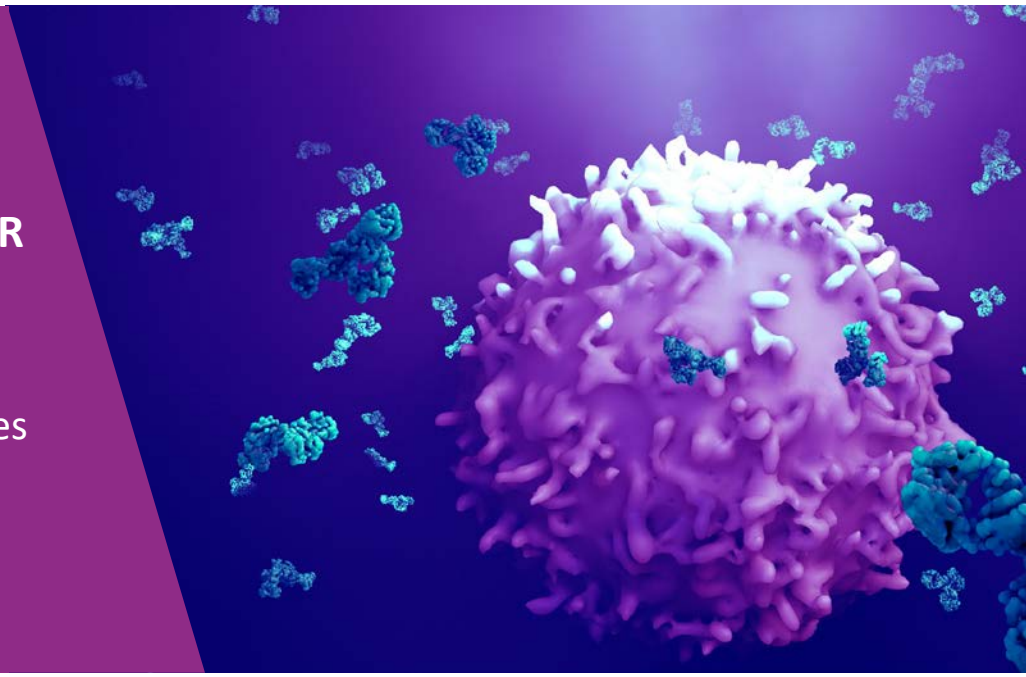
imSAVAR

IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 4.7

Third 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.

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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 nonclinical 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 D4.7, we describe an integrated version of five machine-readable, interactive, and expandable irAOPs leading to cytokine release syndrome (CRS). Initial versions of such models and related methodology were described in D4.5 and D4.6 and in Mazein et al. [1]. This extended model combining CRS irAOPs is a public demonstrator and is continuously being developed.

Document Information

Deliverable Report	**D4.7: Third iteration to characterize molecular mechanisms leading to immune-related adverse events**
Date	**29.04.2025**
Report prepared by	**University of Luxembourg** Fraunhofer ITEM, Fraunhofer IZI, Fraunhofer IZI, Fraunhofer ITMP, Paul-Ehrlich-Institute, Lund University, University Hospital of Würzburg, Labcorp, Boehringer Ingelheim International GmbH, Novartis Pharma AG
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.

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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 develop 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 (see D4.6). Here, we report establishing a resource combining five irAOPs related to CRS into a single molecular interaction map.

2. Methods

The MINERVA platform

The MINERVA Platform (minerva.uni.lu) [2-4] 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.

imSAVAR study-a-thon

Interdisciplinary work required for map design and curation was done during a dedicated study-a-thon, a hands-on workshop focused on research organised as a part of the imSAVAR project. The study-a-thon was a four-day meeting during which domain experts systematically identified cell types and molecules implicated in CRS irAOPs, which were then encoded with stable identifiers and literature references. Each day of the study-a-thon was split into domain-specific work and interdisciplinary exchanges allowing the AOP experts team and the computational biology team to establish identifier tables, create the integrated AOP network and build the base of the connectivity table of entities participating in the CRS irAOP.

3. Results

MINERVA networks for CRS irAOP

A previously established irAOP map working group worked on harmonisation of key events of five irAOPs related to CRS. This was also a topic of the imSAVAR study-a-thon, taking place in June 2023 in Leipzig, Germany, hosted by the Fraunhofer Institute. This was an intensive, hands-on exercise towards harmonisation of molecular identifiers and representation of MIEs and KEs as illustrated in Figure 1 below.

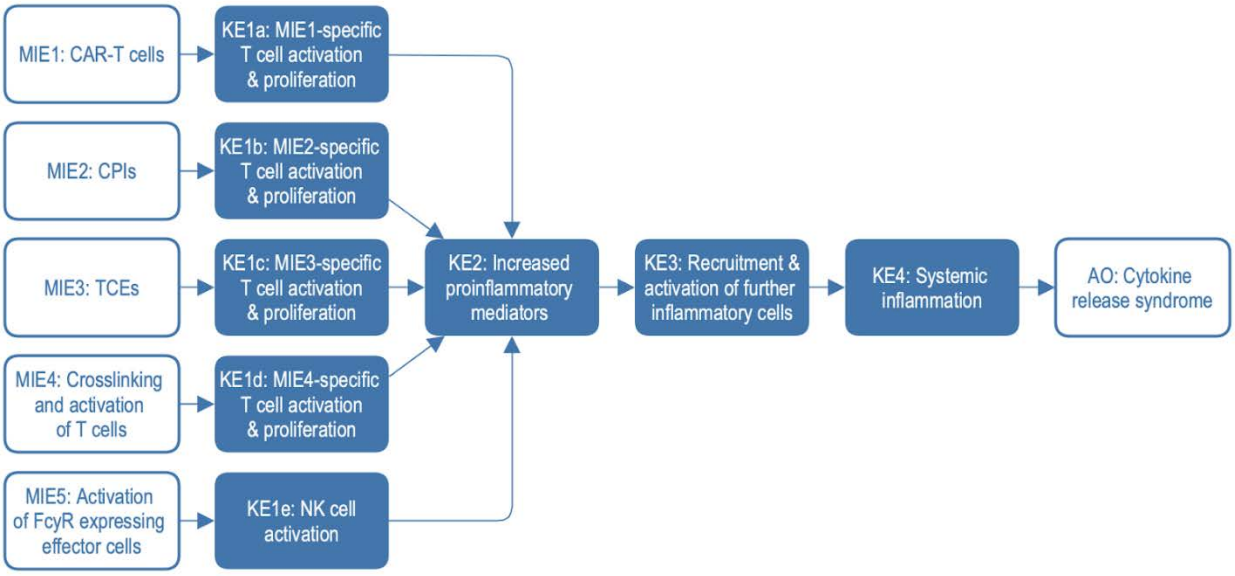


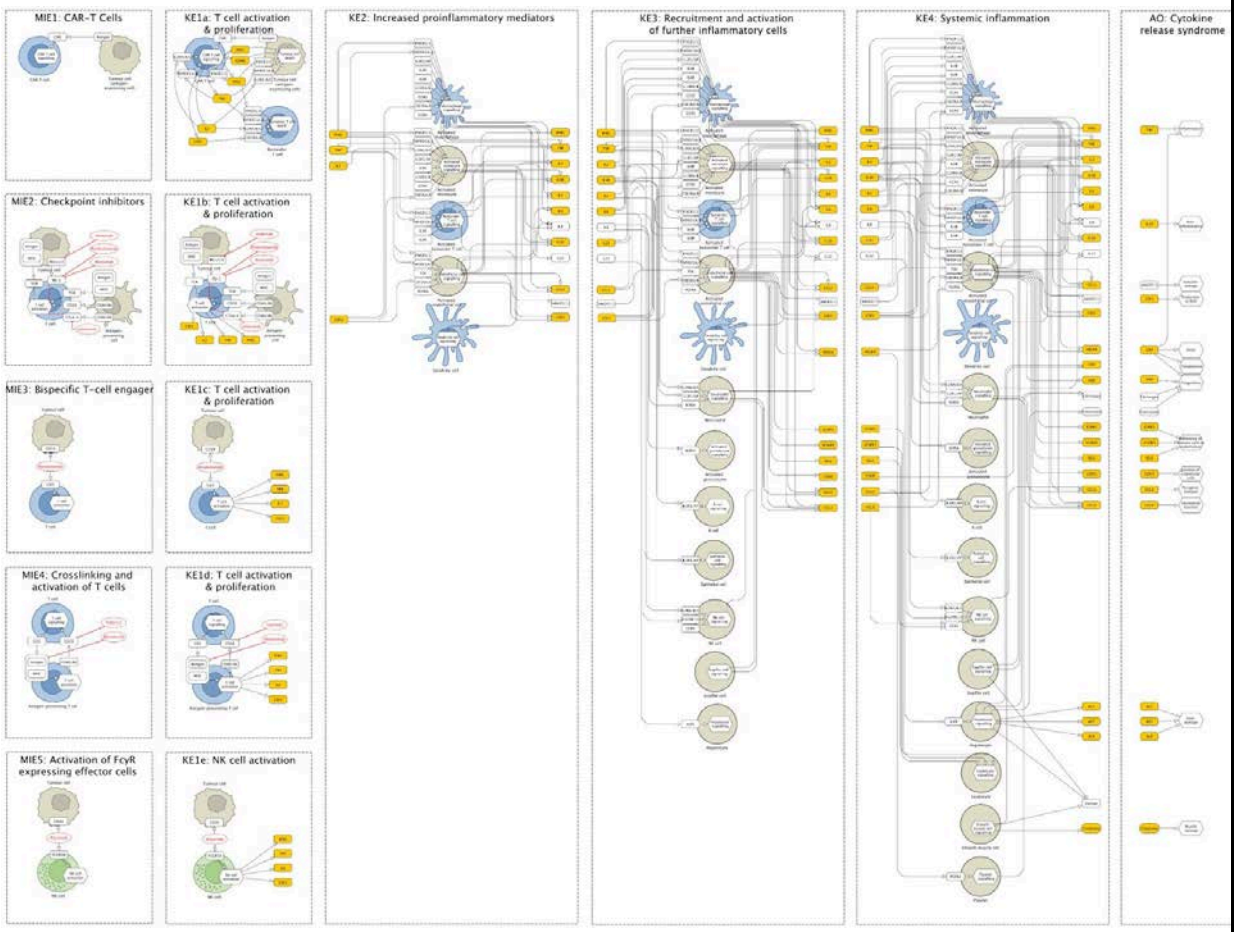
Figure 1: Immune-related adverse outcome pathway (irAOP) network with five different molecular initiating events. Clinical phenotypes of CRS include mild to severe symptoms including e.g. fever, hypotension, hypoxia, organ failure, and coagulopathy. Abbreviations: MIE, molecular initiating event; KE, key event; AO, adverse outcome.

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 abovementioned CRS irAOPs, and combine them into a single molecular interaction diagram. This diagram, and its interconnected network version are continuously refined and are available as public demonstrator at:

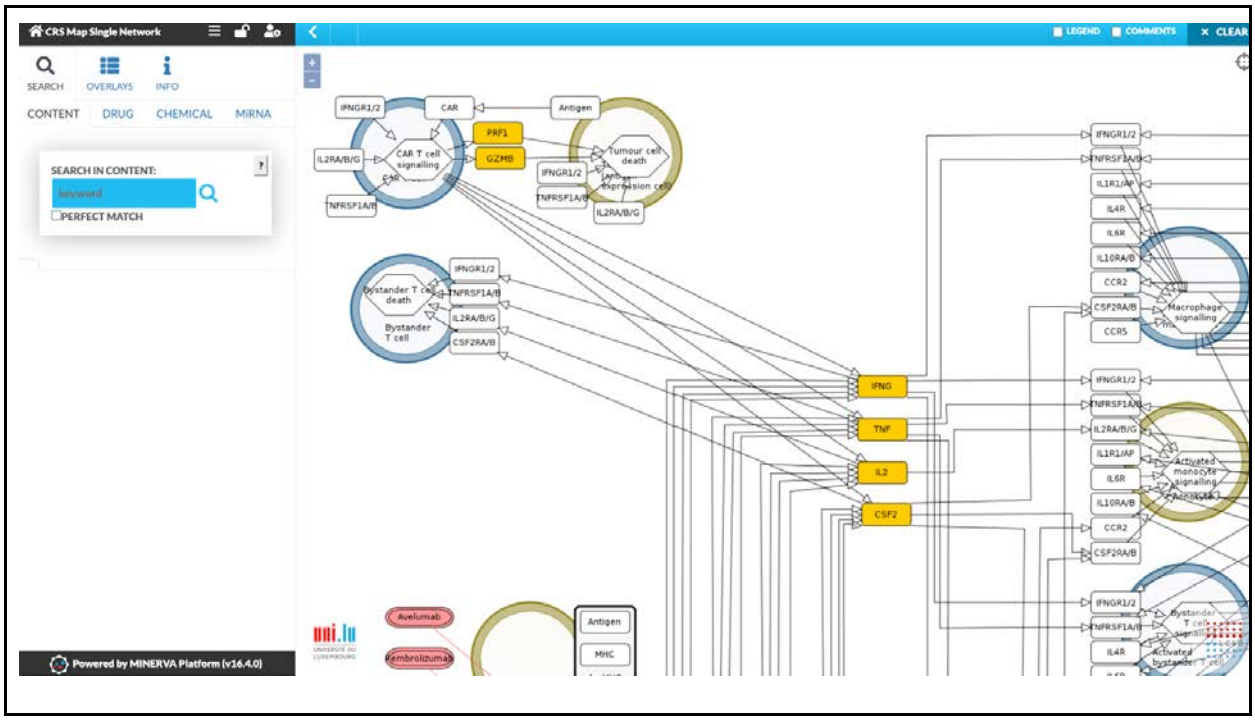
URLs of public demonstrators depicting a map and a network integrating CRS irAOPs
A. CRS irAOP Map: https://imsavar.elixir-luxembourg.org/minerva/?id=CRSmap121
B. CRS irAOP network: https://imsavar.elixir-luxembourg.org/minerva/?id=crs115

We identified the major compartments and cell types involved in induced side effects. For each of these, 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:

Extract 1 of the MINERVA CRS irAOP Map at https://imsavar.elixir-luxembourg.org/minerva/?id=CRSmap121



Extract 2 of the MINERVA CRS irAOP network at https://imsavar.elixir-luxembourg.org/minerva/?id=crs115



The MINERVA map and network combining these five irAOPs are built using the integrated approach we developed in imSAVAR for machine-readable knowledge representation (see Figure 2). Details of the imSAVAR systems biology approach for adverse outcome pathways is described in a preprint manuscript Mazein et al. [5].

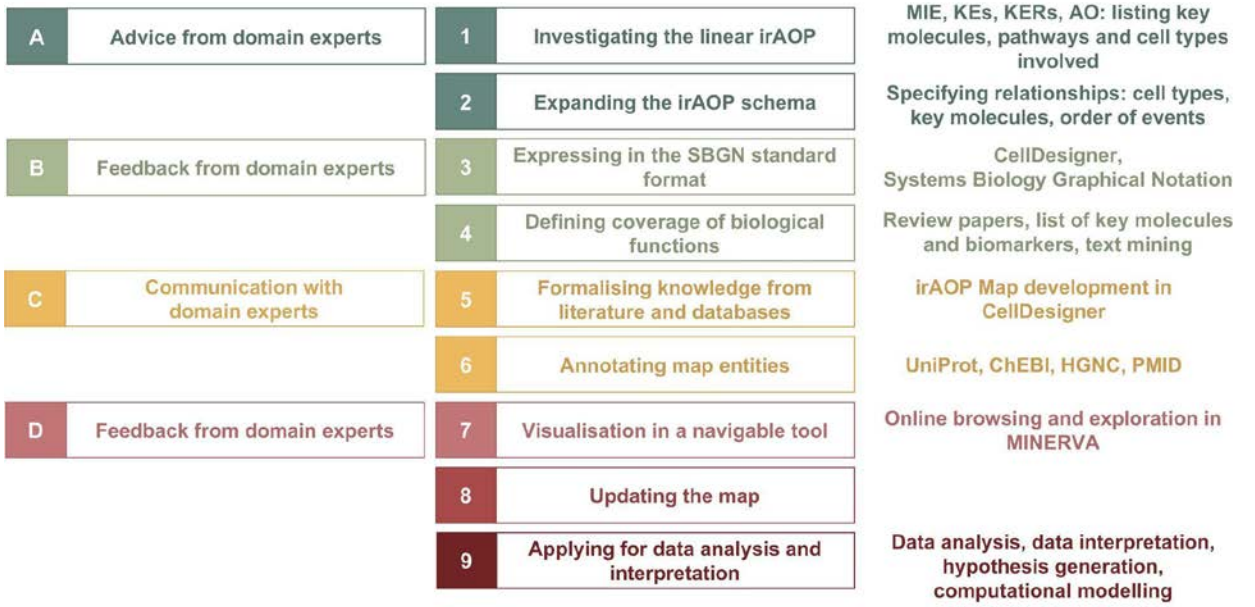


Figure 2: irAOP map development workflow. The systems framework for knowledge management and exploration in immunotoxicology. irAOP, immune-related adverse outcome pathway; MIE, molecular initiating event; KEs, key events; KERs, key event relationships; AO, AO, adverse outcome; UniProt, a database of 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).

Application of the CRS irAOP map

The CRS Map allows visual exploration of complex datasets because of its computer-readable format, stable identifiers and online access. To demonstrate the utility of the Map, we used two publicly available datasets [6, 7] to visualise differences in cytokine levels in CRS adult patients i) compared to healthy controls irrespective of the CRS grade and ii) between high (4-5) and low (0-3) CRS grades [5].

Teachey and co-authors [6] measured different soluble mediators in sera of healthy controls as well as in patients (children and adults) treated with the CTL019 CAR-T product (tisagenlecleucel), which was the first CAR-T product to be approved for market use by the U.S. FDA in 2017 and is sold under the name "Kymriah". They observed that several peak values of cytokines, including IL-6, IFN-γ, sgp130 (natural inhibitor of soluble interleukin-6 receptor trans-signalling responses), and the soluble IL-6 receptor were associated with severe CRS.

These results are represented in the CRS Map such that you can follow the levels of the different cytokines that are correlated with the respective CRS grade. Visual exploration is possible via the OVERLAYS tab in the map (https://imsavar.elixir-luxembourg.org/minerva/?id=CRSmap121), by selecting one or more of the prepared datasets visible in the left panel. Four overlays are available, for cytokine expression profiles of i) adults vs controls at baseline, ii) children with high vs low burden at baseline, iii) adults of high vs low grade CRS at day 1-3 peak values, and iv) adults of high vs low grade CRS at one-month peak values.

Further, we analysed time series data describing the expression of key CRS markers depending on grade [7]. In this study, patients were treated with a defined 1:1 ratio of CD4:CD8 CD19 CAR-Ts (clinical trial: NCT01865617). Here, the authors observed that patients, who developed high-grade CRS showed high peak values of e.g. IL-6, IFN-γ, and MCP-1 early after CAR-T cell infusion (day 1 3) but also up to day 30 after CAR-T infusion compared to patients, who only showed mild CRS symptoms. This product is now also approved for market use and is sold under the name "Breyanzi" (lisocabtagene maraleucel).

Here, the results of the study are also colour-coded in the CRS Map. Similarly, they are available in the OVERLAYS tab at https://imsavar.elixir-luxembourg.org/minerva/?id=CRSmap121, enabling the user to follow how the cytokines peak at the different time points and which cell types are involved from the CRS initiating event to the adverse outcome. Overall, eight datasets are available, four for non-severe and four for severe CRS. Time points were grouped in pairs to visualise high-level trends in the time series.

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 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, which were then corroborated during the imSAVAR study-a-thon. 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. For this purpose, we mapped two publicly available datasets to demonstrate their interpretation using the CRS Map. Lastly, we describe the imSAVAR approach using MINERVA as an interactive platform to encode, manage and explore irAOPs in a manuscript prepared for submission [5].

4. Discussion

Standardised encoding of knowledge is required for AOP construction, as they combine evidence across different studies. This allows harmonisation of stable identifiers defining participating molecules and involved cell types. This is especially important for irAOPs, as processes covered by them are particularly complex.

Graphical representation of such complex AOPs is necessary to navigate through multitude of implicated interactions. Standards of such representations, including SBGN and SBML are necessary for comprehensive encoding and downstream interoperability. Implementation of irAOPs in an online platform for visual representation is necessary to enable wide and easy access to the contents of the irAOPs, facilitating their use, review and integration with experimental data. Moreover, transparency of irAOPs represented in such a way allow their improvement, expansion and reuse. Importantly, the generated content is persisted and openly accessible to the research community.

Such a standard and accessible representation facilitates interaction with domain experts and reinforces the interdisciplinary connection with computational biomedicine. With the study-a-thon we demonstrated the capabilities and practices to perform intensive, hands-on workshops for AOP construction and revision. This can be used as a template for future similar efforts to review and curate specific AOPs.

5. Conclusion

Our work on encoding immune-related mechanisms into irAOPs led to two major outcomes. The first one is the online, standardised and persistent resource, available for the Consortium for the duration of the project, and sustained beyond the project duration as an open access repository. This resource allows interactive and transparent exploration of the irAOP content and interpretation of complex molecular data by its users.

The second outcome is a set of best practices to curate irAOPs based on domain expert review and feedback together with computational standards necessary for developing standardised and reusable irAOPs. These complex AOPs require precise definitions of participating molecules and their cellular context, and tracking the literature evidence used for their construction. Our practices, adapting already proposed workflows for disease maps construction for the needs of irAOPs, can be used for construction of future irAOPs, or refinement of already set up resources.

6. Abbreviations

AOP -- adverse outcome pathways

CAR – chimeric antigen receptor

CRS – cytokine release syndrome

CPI – checkpoint inhibitor

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

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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.