Using interactive platforms to encode, manage and explore immune-related adverse outcome pathways

Alexander Mazein[1,#], Muhammad Shoaib[1,#], Miriam Alb[2], Christina Sakellariou[3], Charline Sommer[4], Katherina Sewald[4], Kristin Reiche[5], Patricia Gogesch[6], Luise A Roser[7], Samira Ortega Iannazzo[6], Sapna Sheth[8], Susanne Schiffmann[7], Zoe Waibler[6], Vanessa Neuhaus[4], Susann Dehmel[4], Venkata Satagopam[1,9], Reinhard Schneider[1,9], the imSAVAR Consortium, Marek Ostaszewski[1,9]*, Wei Gu[1,9]*

[1] Luxembourg Centre for Systems Biomedicine, University of Luxembourg, 6 Avenue du Swing, L-4367 Belvaux, Luxembourg

[2] Universitätsklinikum Würzburg, Medizinische Klinik und Poliklinik II, Oberdürrbacher Straße 6, 97080 Würzburg, Germany

[3] Department of Immunotechnology, University of Lund, Medicon Village, Scheelevägen 2, Box 117, 221 00 Lund, Sweden

[4] Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Preclinical Pharmacology and In-Vitro Toxicology, Nikolai-Fuchs-Str. 1, 30625 Hannover, Germany; Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Member of the German Center for Lung Research (DZL), Hannover, Germany

[5] Fraunhofer Institute for Cell Therapy and Immunology IZI, Department of Diagnostics, Perlickstraße 1, 04103 Leipzig, Germany

[6] Paul-Ehrlich-Institut, Division of Immunology, Section 3/1 "Product Testing of Immunological Biomedicines", Paul-Ehrlich-Str. 51-59, 63225 Langen, Germany

[7] Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Theodor-Stern-Kai 7, 60596 Frankfurt am Main, Germany

[8] BioSci Consulting Rijksweg 328, 3630 Maasmechelen, Belgium

[9] ELIXIR Luxembourg, 6 Avenue du Swing, L-4367 Belvaux, Luxembourg

[#] Authors contributed equally to the manuscript.

* Correspondence: Marek Ostaszewski (marek.ostaszewski@uni.lu) or Wei Gu (wei.gu@uni.lu)

ORCID:

Alexander Mazein (0000-0001-7137-4171)
Muhammad Shoaib (0000-0002-4854-4635)
Miriam Alb (0000-0001-6454-9906)
Christina Sakellariou (0000-0002-2376-7808)
Charline Sommer (0000-0002-6390-3878)
Katherina Sewald (0000-0002-7804-4527)
Kristin Reiche (0000-0002-4452-4872)
Patricia Gogesch (0000-0003-0025-0782)
Vanessa Neuhaus (0000-0002-0947-4929)
Susann Dehmel (0000-0003-4860-2767)
Luise A Roser (0000-0001-9080-8342)
Samira Ortega Iannazzo (0000-0001-8942-3357)
Sapna Sheth (0000-0003-3155-907X)
Susanne Schiffmann (0000-0001-5035-2504)
Zoe Waibler (0000-0001-5758-2652)
Venkata Satagopam (0000-0002-6532-5880)

Reinhard Schneider (0000-0002-8278-1618)
Marek Ostaszewski (0000-0003-1473-370X)
Wei Gu (0000-0003-3951-6680)

Abstract

We address the need for modelling and predicting adverse outcomes in immunotoxicology to improve non-clinical assessments of immunomodulatory therapy safety and efficacy. The integrated approach includes, first, the adverse outcome pathway concept established in the toxicology field, and, second, the systems medicine disease map approach for describing molecular mechanisms involved in a particular pathology. The proposed systems immunotoxicology workflow is demonstrated with CAR T cell treatment as a use case. To this end, the linear adverse outcome pathway (AOP) is expanded into a molecular interaction model in standard systems biology formats. Then it is shown how knowledge related to immunotoxic events can be integrated, encoded, managed and explored to benefit the research community. The map is accessible online via the MINERVA Platform for browsing, commenting and data visualisation (https://minerva.pages.uni.lu). Our work transforms a graphical illustration of an AOP into a digitally structured and standardised form, featuring precise and controlled vocabulary and supporting reproducible computational analyses. Because of annotations to source literature and databases, the map can be further expanded to match the evolving knowledge and research questions.

Keywords: AOP, adverse outcome pathway, systems biology, immunomodulatory therapies, CAR T cells, chimeric antigen receptor, disease mechanisms, cytokine release syndrome, CRS

Introduction

Developing efficient tools for assessing the risks of immunomodulatory therapeutic modalities is a key step in improving predictivity of drug development during the non-clinical stage and offering innovative immunobiology models and biomarkers. The project imSAVAR (Immune Safety Avatar: nonclinical mimicking of the immune system effects of immunomodulatory therapies) aims at a better understanding of immunotoxic mechanisms and improving models (https://imsavar.eu). The adverse outcome pathway (AOP) concept is one of such tools that allows knowledge-based evaluation of the involved molecular mechanisms[1].

In immunotoxicology, immune-related adverse outcome pathways (irAOPs) are used to visualise and study adverse effects of treatments. They allow highlighting a molecular initiating event (MIE), the key events (KEs), key event relationships (KERs) and an adverse outcome (AO), representing their order and also aligning these KEs to test systems and values of measured clinical parameters. These irAOPs can be used to help clinicians to assess the safety of a given treatment and propose new biomarkers and new treatment strategies[1–3]. Modelling strategies of AOPs are described in the OECD "Users' Handbook supplement to the Guidance Document for developing and assessing Adverse Outcome Pathways"[4]. Specifically, the AOP graphical

representation is discussed in the handbook in Development Tips 1 and 2[4]. A classical AOP is initially structured in a several-step linear diagram and stored as an image. While these graphical representations are informative and useful, there are limitations that make it difficult to interactively explore, share and use them for further modelling and prediction. We propose to address these limitations by employing advanced tools in systems biomedicine and network biology, e.g. the MINERVA platform[5].

In systems biomedicine, the standardised representation and machine readability needed for interactive exploration, annotation and modelling of pathways are offered by approaches such as the disease maps. A disease map is a conceptual model of relevant mechanisms represented as a collection of interconnected signalling and metabolic pathways[6,7]. Examples of such maps are resources for cancer[8], Parkinson's disease[9], rheumatoid arthritis[10], asthma[11] and, the most recent development, the COVID-19 Disease Map for capturing virus-host interaction mechanisms of the SARS-CoV-2 infection[12]. These maps can include multiple layers from molecular to intercellular and intertissue/interorgan interactions to reflect the physiological level of complexity[11]. Disease maps are designed for integrating prior knowledge, making sense of newly-generated data, modelling and predictions. The primary purpose of building a disease map is to structure knowledge about disease mechanisms in a single repository. The repository is usually integrated with a web-server-based user interface to enable interactive visualisation and exploration of this knowledge. State-of-the-art disease maps also utilise the systems biology standards to ensure interoperability with other biological resources.

In both approaches, irAOPs and disease maps, the basis is a pathway representing key events that result in a particular outcome. The approaches differ and complement each other in the level of details and in their main focus. The irAOP focuses on the higher level of molecular and physiological events, multiple levels of entities (key molecules, cells, tissues, organs, organism), a variety of functional relationships (switch on, switch off, branching), as well as quantitative clinical parameters and assays. They have a common structure consisting of a MIE, a series of KE connected by KERs and an AO. They usually do not provide a comprehensive molecular description of every aspect of a biological process *per se.* The disease map, on the other hand, concentrates on detailed molecular mechanisms relevant to induce a particular condition and has the ability to visualise the higher-level relationships required in immunotoxicology. Integrating the concepts of immunotoxicology irAOP with the systems biomedicine disease map offers a promising improvement to the classical irAOP.

By combining the two approaches, we aim to build a solution that combines their advantages while addressing anticipated knowledge gaps. It also paves the way to tackle the challenges of multi-scale representation including molecular, cellular and immune system levels, with a perspective of creating an executable computational model for making predictions.

Major advantages of the enhanced pathway-based AOP approach compared to the standard modelling and visualisation frameworks as recommended by the OECD AOP concept[4] and the AOP Wiki (https://aopwiki.org/aops): 1) focus on relevant molecular pathways with an ability to represent intercellular and physiological relationships; 2) applying well-established standards and editors for the reconstruction of the underlying biology; 3) identifying knowledge gaps during

the reconstruction process and clarifying the mechanisms of the MIE, KEs, KERs and AO; 4) using the MINERVA platform for online visualisation and exploration, with such entities as proteins, genes and metabolites identified and linked to appropriate external databases, and with multiple plugins available. In more details the approach and the MINERVA platform are discussed in the Methods section.

In this work, we propose an integrated systems toxicology framework and create a proof-of-concept knowledge-based and irAOP-based map of molecular interactions for the cytokine release syndrome mediated by CAR T cells.

Methods

The representation of the underlying biology for adverse outcome pathways

The irAOP concept connects the MIE and the corresponding AO via a series of KEs and KERs known to be involved at different levels of biological organisation[1,4]. In one of the following works, a linear adverse outcome pathway was expanded into a complex network of molecular events, with feedback and feedforward loops and inter-relationships between individual key events presented[2,3]. By building on this example, we applied the state-of-the-art advances in the standard systems biology field and used such formats as the Systems Biology Graphical Notation (SBGN)[13] and Systems Biology Markup Language (SBML)[14] to formalise and visualise our knowledge.

The systems biology approach for adverse outcome pathways

In systems medicine, a disease map is a conceptual model of disease mechanisms, with events depicted on the level of molecular interactions[6,7]. Figure 1 describes the systems immunotoxicology framework integrated from the adverse outcome pathways concept and the adapted systems medicine disease map methodology[15].
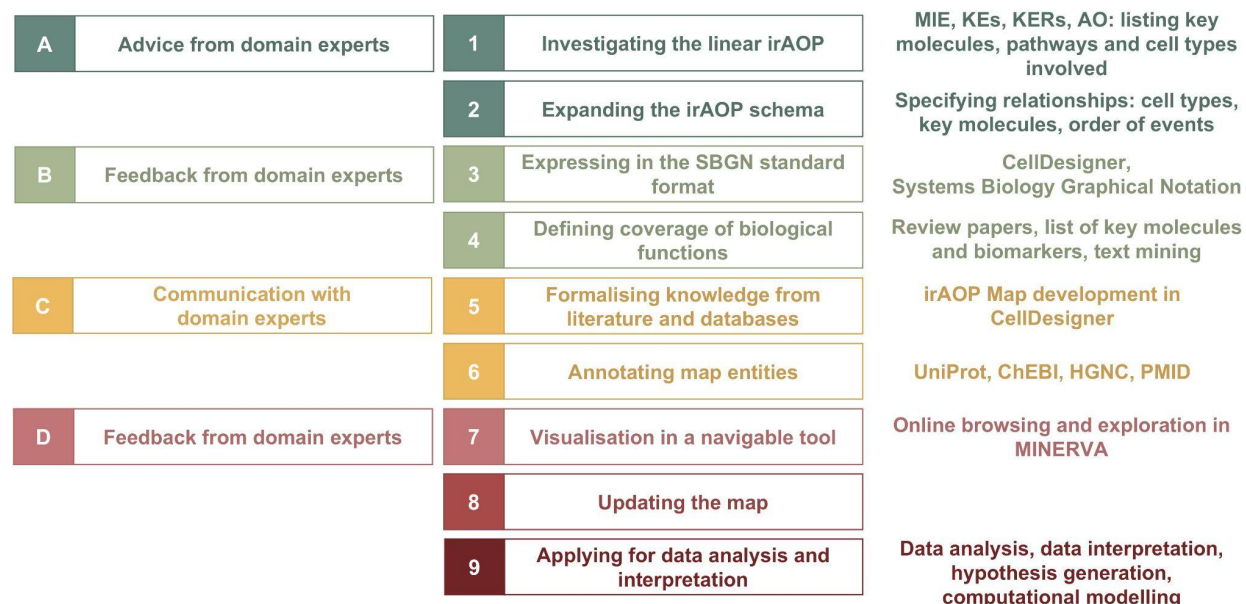
A	Advice from domain experts	**1**	Investigating the linear irAOP			MIE, KEs, KERs, AO: listing key molecules, pathways and cell types involved
		2	Expanding the irAOP schema			Specifying relationships: cell types, key molecules, order of events
B	Feedback from domain experts	**3**	Expressing in the SBGN standard format			CellDesigner, Systems Biology Graphical Notation
		4	Defining coverage of biological functions			Review papers, list of key molecules and biomarkers, text mining
C	Communication with domain experts	**5**	Formalising knowledge from literature and databases			irAOP Map development in CellDesigner
		6	Annotating map entities			UniProt, ChEBI, HGNC, PMID
D	Feedback from domain experts	**7**	Visualisation in a navigable tool			Online browsing and exploration in MINERVA
		8	Updating the map			
		9	Applying for data analysis and interpretation			Data analysis, data interpretation, hypothesis generation, computational modelling

Figure 1. 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). Descriptions of the steps are provided in the text.

Steps to construct the irAOP maps:

Step 1. Investigating the linear irAOP. Key molecules, related pathways and cell types involved are hypothesised. The relevant publications are reviewed. The information is organised in the form of a weight-of-evidence-table for all the key events and discussed with the experts (Alb et al., in preparation).

Step 2. Expanding the irAOP schema. A top-level view is built by representing key biological mechanisms and connecting the previously identified key molecules into a network.

Step 3. Expressing knowledge in the standard SBGN format. When the network components are identified and connected, the biological mechanisms are expressed in the standard graphical systems biology languages[13] to have both a human- and computer-readable top-level view diagram, thus ensuring shareability and enabling computational modelling.

Step 4. Defining coverage of biological functions. The boundaries of the conceptual model are assessed and relevant key pathway modules are listed as the main components of the map.

Step 5. Formalising knowledge from literature and databases. Based on the top-level view (Steps 2 and 3) and our assessment of the complexity (Step 4), a pathway-level detailed network of molecular interactions is built. Systems biology editors are employed for the network construction, e.g. CellDesigner (http://www.celldesigner.org), Newt Editor[16] (http://newteditor.org), SBGN-ED[17] (http://sbgn-ed.org) or the yEd Graph editor

(https://www.yworks.com/products/yed) in combination with the ySBGN converter (https://github.com/sbgn/ySBGN).

Step 6. Annotating the map entities. The map objects have to be identified by linking them to external databases. For example, proteins are identified via UniProt IDs (https://www.uniprot.org) and molecular interactions are confirmed with PubMed IDs (https://pubmed.ncbi.nlm.nih.gov). For that, we follow stable identifiers using the Identifiers.org[18] or MIRIAM annotation[19].

Step 7. Visualisation in a navigable tool. For making the results easily accessible and explorable we use the MINERVA platform[5,20,21] (see the next Section for details).

Step 8. Updating the map. With newly published information or feedback from the research community, the map can be further refined and updated. Modularised structure makes it easier to manage and update the map components[22].

Step 9. Applying for data analysis and interpretation. The complete map can be used for 'omics data visualisation, analysis and interpretation[12]. The map can also be transformed into a simulatable computational model, for example, a Boolean model, for predictions and advanced hypothesis generation[10].

Communication with domain experts (A, B, C and D in Figure 1) is one of the most important components ensuring adequate representation of knowledge from published research on the topic, prioritising key aspects and making the map a practically applicable resource.

The MINERVA Platform

The MINERVA Platform is a standalone web-service for user-friendly online visualisation and exploration of extensive systems biology networks[5]. The MINERVA documentation is available at https://minerva.pages.uni.lu.

The MINERVA Platform allows interactive browsing of biological networks, provides access to their annotation and connects to external databases for more information about the molecules involved. Evidence for interactions is stored as PubMed IDs with links to PubMed entries. Custom data can be uploaded and visualised in colours of different intensities according to the values provided. MINERVA enables search and exploration of drug targets via online queries to DrugBank[23] (https://www.drugbank.com) and ChEMBL[24] (https://www.ebi.ac.uk/chembl).

MINERVA works with CellDesigner XML format, SBGN and SBML with layout information.

Video demo tutorials are available for asthma as a use case[11] and cover such functionalities as 1) navigating and searching in MINERVA, 2) adding comments directly on the map, 3) exploring the drug target search functionality, and 4) visualising 'omics data (https://asthma-map.org/tutorials).

Results

Immune-related adverse outcome pathway of CAR T cell treatment

Cytokine release syndrome (CRS) is the most common type of toxicity caused by CAR T cell therapy and, in general, is one of the common types of toxicity caused by advanced therapy medicinal products (ATMPs)[25–28]. Figure 2a presents the proposed respective irAOP. The MIE, the KE and the AO are described below.

Molecular initiating event (MIE): specific recognition of antigen-expressing cells. Expression of a chimeric antigen receptor (CAR) enables T cells to recognise and bind to antigen-expressing cells such as tumour cells in a non-MHC restricted manner. As a result, CAR T cells are activated[29–32].

Key event 1 (KE1): activation of CAR T cells. The activation of CD8$^+$ CAR T cells leads to the release of cytolytic enzymes such as granzyme B (GZMB), and both activated CD8$^+$ and CD4$^+$ CAR T cells produce cytokines including IL-2 and IFN-γ[32–35].

Key event 2 (KE2): increased proinflammatory mediators. The released cytokines can be measured by using cytokine release assays and multiplex analysis tools. The production of IFNG by CAR T cells triggers IL-6 production by monocytes and endothelial cells and IL-1, IL-6 and nitric oxide by macrophages[36–40].

Key event 3 (KE3): tissue-resident and endothelial cell activation. The increased levels of proinflammatory mediators then lead to inflammation and its amplification, as well as to activation of other immune cells. This means enhanced IL-6 and IL-1 production by monocytes and activation of endothelial cells with production of von Willebrand factor (VWF) and angiopoietin-2 (ANG2)[28]. VWF plays a key role in coagulation[41], and ANG2 promotes capillary leak[42].

Key event 4 (KE4): systemic inflammation. Consequently, systemic inflammation develops. It includes an increase of pro-inflammatory cytokines and activation of the innate immune system. The levels of IL-6 and C-reactive protein are inflammatory markers of systemic inflammation.

Adverse outcome (AO): cytokine release syndrome. CRS is linked to macrophage activation syndrome which is associated with activated lymphocytes that produce IFNG and activated macrophages that produce IL-6 and TNF-α[37,39,40,43].

This knowledge was formalised into a top-level overview diagram with the key molecules mapped and interconnected (see below).

The extended representation of the underlying biology

Based on the linear AOP and the relevant textual information, we built the top-level view of the CAR T cell irAOP-Map. For that, we analysed the available information. While in the textual description to the linear irAOP the information was sufficient, it needed to be extended for the requirements of the biological network construction. When reviewing known molecules involved,

we identified cases of non-specific proteins: IL-1 means two different proteins IL1A and IL1B, and NFAT can potentially mean five different proteins in UniProt (Table 1, columns "Symbol" and "Identifier"). The analysis of the source and target cell types helped to find connectivity in the network and identify the gaps in the current model (Table 1, columns "Source" and "Target"). The first version of the top-level view of the CAR T cell irAOP-Map is shown in Figure 2b.
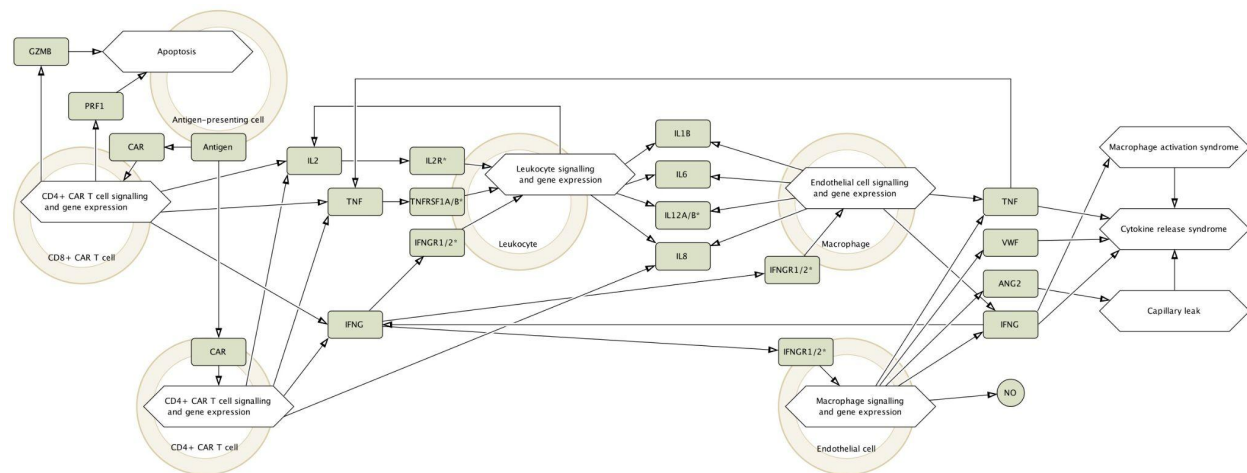
a

b

Figure 2. CAR-T-cell-induced cytokine release syndrome (CRS): a comparison of a linear irAOP and a more detailed representation of the underlying biology. **a.** The linear irAOP for the CAR-T-cell-induced cytokine release syndrome. MIE, molecular initiating event; KE, key event; AO, adverse outcome. **b.** The top-level view of the CAR T cell irAOP-Map - the underlying biology. The map is available in MINERVA for commenting and exploring at https://imsavar.elixir-luxembourg.org/minerva/index.xhtml?id=cart14.

Table 1. CAR T treatment: key molecules involved, with HGNC symbols and UniProt identifiers. n/a, not applicable.

Name	Symbol	Identifier	Source	Target	Receptors (candidates)
IL-2	IL2	UniProt:P60568	CAR T cell, Leukocyte	Leukocyte	IL2RA, IL2RB, IL2RG
IFN-γ	IFNG	UniProt:P01579	CAR T cell, Macrophage, Endothelial cell	Leukocyte, Macrophage, Endothelial cell	IFNGR1, IFNGR2
TNF-α	TNF	UniProt:P01375	CAR T cell, Macrophage, Endothelial cell	Leukocyte	TNFRSF1A, TNFRSF1B
CD69	CD69	UniProt:Q07108	CAR T cell	Antigen-expressing cell (via ligand)	n/a

CD25	IL2RA	UniProt:P01589	CAR T cell	n/a	n/a
NFAT	NFATC1, NFATC2, NFATC4, NFATC3, NFAT5	omitted	n/a (transcription factors)	n/a (transcription factors)	n/a (transcription factors)
Perforin	PRF1	UniProt:P14222	CAR T cell	Antigen-expressing cell	n/a
Granzyme B	GZMB	UniProt:P10144	CAR T cell	Antigen-expressing cell	n/a
IL-1	IL1A, IL1B	UniProt:P01583; UniProt:P01584	Leukocyte, Macrophage	T cell, Neutrophil, B cell	IL1R1, IL1R2
IL-6	IL6	UniProt:P05231	Leukocyte, Macrophage	T cell	IL6R, IL6ST
NO	nitric oxide	CHEBI:16480	Macrophage	Tumor cell	n/a
IL-8	CXCL8	UniProt:P10145	CAR T cell	T cell	CXCR1, CXCR2
IL-12	IL12A; IL12B	UniProt:P29459; UniProt:P29460	CAR T cell, Leukocyte, Macrophage	T cell	IL12RB1, IL12RB2
C-reactive protein	CRP	UniProt:P02741	Hepatocytes, macrophages, endothelial cells, lymphocytes	n/a (clinical parameter)	n/a (clinical parameter)
Ferritin	FTH1; FTL; FTMT	UniProt:P02794; UniProt:P02792; UniProt:Q8N4E7	Macrophage	n/a (clinical parameter)	n/a (clinical parameter)
MCP1	CCL2	UniProt:P13500	Macrophage	Endothelial cell	CCR2
IL-8	CXCL8	UniProt:P10145	Leukocyte	Neutrophil	CXCR2
VWF	VWF	UniProt:P04275	Endothelial cell	Platelets	P1BA, GP1BB
ANG2	VPS51	UniProt:Q9UID3	Endothelial cell	Endothelial cell	TEK

Table 1 demonstrates how network reconstruction helps to ask questions and identify gaps in our understanding of the biological processes involved. We clarify and formalise available knowledge by identifying molecules and specifying the listed cell types. In such a map development protocol, by necessity, for ensuring the connectivity between the map objects, we have to actively investigate the connections, the receptors and cell types involved. For example, knowing that IL-2 is produced by CAR T cells and affects leukocytes, as the next step, we focus on IL-2 receptor proteins IL2RA, IL2RB and IL2RG, the corresponding signalling cascade and the activated transcription factors (Figure 3).
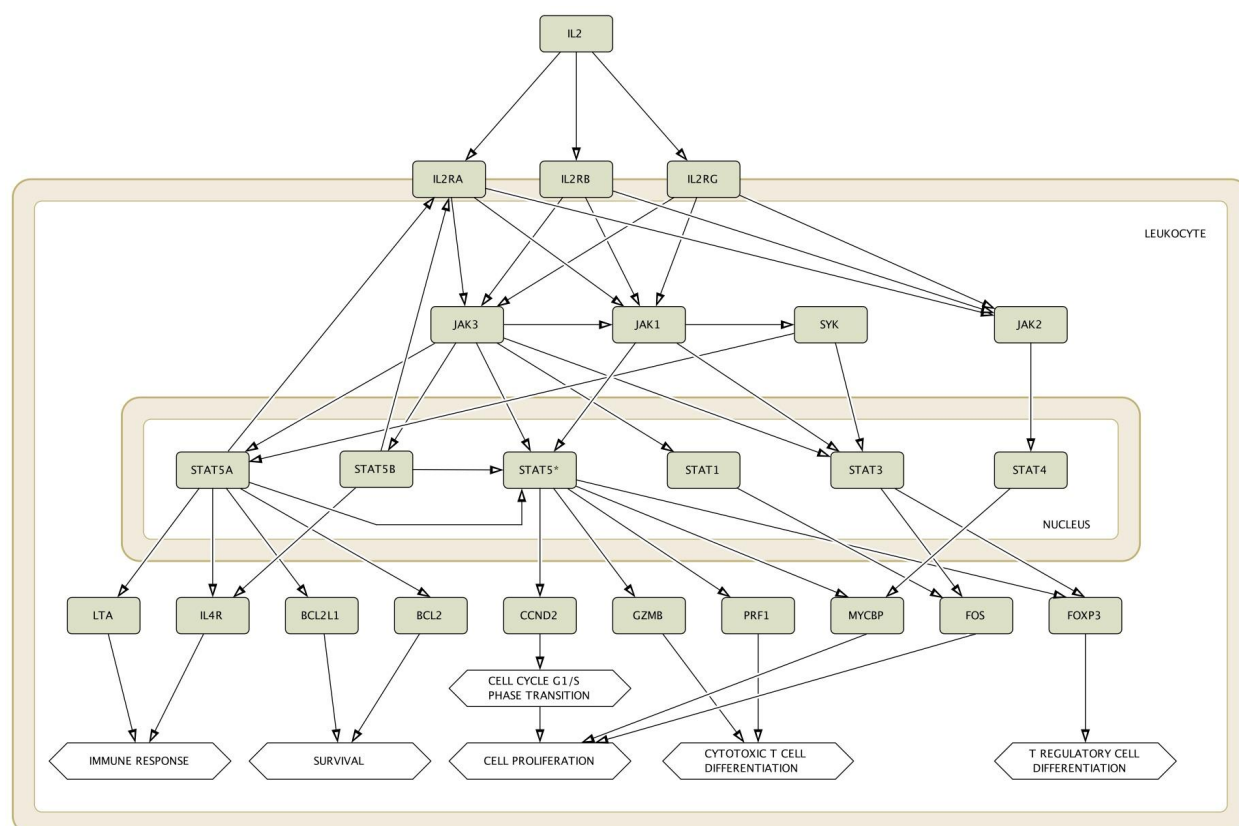
Figure 3. IL-2 signalling. This pathway is manually converted to the SBGN Activity Flow format from the MetaCore pathway map ID 2770 Immune response IL-2 signalling via JAK-STAT (https://portal.genego.com). The proteins are presented with the use of HGNC names (https://www.genenames.org). The map is available in MINERVA for commenting and exploring at https://pathwaylab.elixir-luxembourg.org/minerva/index.xhtml?id=il2v09.

Future development

Within the imSAVAR project (https://imsavar.eu), we plan to extend the CAR T cell irAOP-Map, refine the described systems immunotoxicology protocol and demonstrate the applicability of such knowledge databases resources. One of the promising applications is developing executable computational models for predictions and hypothesis generation[44,45], potentially with the use of the CaSQ tool[46] and the Cell Collective Boolean modelling pipeline[47–49].

Conclusion

In the proposed framework, we bring together the recent advances in the immunotoxicology and systems biomedicine fields. We bridge the experience of developing conceptual descriptions of disease mechanisms, disease maps, to the practice of describing immunotoxic effects in the form of AOPs. With this, the AOP concept is enhanced with detailed description of intracellular and intercellular molecular interactions captured in the standard systems biology format. Focusing on the CAR-T-cell-treatment-induced CRS, we demonstrate how a molecular

interaction map can be developed from a linear AOP and offer the results in an easily accessible and explorable way in the web-based MINERVA platform. On the other hand, this framework can improve the development of disease-specific AOPs, identify knowledge gaps in existing AOPs and lead to the design of new experiments. Because we apply a transparent and consistent methodology with the use of standards, this framework can be reused and applied to other immunomodulatory treatment scenarios. Continuing our work in this direction we aim to build explorable knowledge resources that could benefit immunotoxicology research and contribute to improving non-clinical assessment of immunomodulatory therapies.

Key points

- In immunotoxicology, an adverse outcome pathway shows a sequence of molecular and cellular events that result in a toxic outcome upon treatment with a specific drug.
- In systems biomedicine, a disease map is a description of disease mechanisms on the levels of molecular interactions and intercellular communication for integrating prior knowledge, making sense of newly-generated data, modelling and predictions.
- We are applying the disease map approach to the area of immunotoxicology and offer an interactive web-based platform for expanding immune-related adverse outcome pathways to detailed representations of the underlying biology.
- The objective is to model adverse outcomes as a non-clinical assessment strategy by integrating our understanding of the disease complexity and knowledge on the mechanisms of the adverse outcomes of the treatment.
- We focus on the adverse outcome pathway of CAR T cell treatment and from a simplified linear pathway build a detailed representation of the underlying biology.

Abbreviations

AO, adverse outcome
ATMPs, advanced therapy medicinal products
CAR, chimeric antigen receptor
CRS, cytokine release syndrome
irAOP, immune-related adverse outcome pathway
KE, key event
KERs, key event relationships
MIE, molecular initiating event
SBGN, Systems Biology Graphical Notation
SBML, Systems Biology Markup Language

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Disclosure statement

The authors report there are no competing interests to declare.

Data availability statement

All the maps are freely accessible online via the MINERVA platform.

References

1. Ankley, G. T. *et al.* Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. *Environ. Toxicol. Chem.* **29**, 730–741 (2010).
2. Horvat, T. *et al.* Adverse outcome pathway development from protein alkylation to liver fibrosis. *Arch. Toxicol.* **91**, 1523–1543 (2017).
3. Leist, M. *et al.* Adverse outcome pathways: opportunities, limitations and open questions. *Arch. Toxicol.* **91**, 3477–3505 (2017).
4. OECD. *Users' Handbook supplement to the Guidance Document for developing and assessing Adverse Outcome Pathways*. https://www.oecd-ilibrary.org/environment/users-handbook-supplement-to-the-guidance-document-for-developing-and-assessing-adverse-outcome-pathways_5jlv1m9d1g32-en (2018) doi:10.1787/5jlv1m9d1g32-en.
5. Gawron, P. *et al.* MINERVA-a platform for visualization and curation of molecular interaction networks. *NPJ Syst. Biol. Appl.* **2**, 16020 (2016).
6. Mazein, A. *et al.* Systems medicine disease maps: community-driven comprehensive representation of disease mechanisms. *NPJ Syst. Biol. Appl.* **4**, 21 (2018).
7. Ostaszewski, M. *et al.* Community-driven roadmap for integrated disease maps. *Brief. Bioinform.* **20**, 659–670 (2019).
8. Kuperstein, I. *et al.* Atlas of Cancer Signalling Network: a systems biology resource for integrative analysis of cancer data with Google Maps. *Oncogenesis* **4**, e160 (2015).
9. Fujita, K. A. *et al.* Integrating pathways of Parkinson's disease in a molecular interaction map. *Mol. Neurobiol.* **49**, 88–102 (2014).
10. Singh, V. *et al.* RA-map: building a state-of-the-art interactive knowledge base for rheumatoid arthritis. *Database J. Biol. Databases Curation* **2020**, baaa017 (2020).
11. Mazein, A. *et al.* AsthmaMap: An interactive knowledge repository for mechanisms of asthma. *J. Allergy Clin. Immunol.* **147**, 853–856 (2021).
12. Ostaszewski, M. *et al.* COVID19 Disease Map, a computational knowledge repository of virus-host interaction mechanisms. *Mol. Syst. Biol.* **17**, e10387 (2021).
13. Le Novère, N. *et al.* The Systems Biology Graphical Notation. *Nat. Biotechnol.* **27**, 735–741 (2009).

14. Hucka, M. *et al.* The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. *Bioinforma. Oxf. Engl.* **19**, 524–531 (2003).

15. Kondratova, M., Sompairac, N., Barillot, E., Zinovyev, A. & Kuperstein, I. Signalling maps in cancer research: construction and data analysis. *Database J. Biol. Databases Curation* **2018**, (2018).

16. Balci, H. *et al.* Newt: a comprehensive web-based tool for viewing, constructing and analyzing biological maps. *Bioinforma. Oxf. Engl.* **37**, 1475–1477 (2021).

17. Czauderna, T., Klukas, C. & Schreiber, F. Editing, validating and translating of SBGN maps. *Bioinforma. Oxf. Engl.* **26**, 2340–2341 (2010).

18. Wimalaratne, S. M. *et al.* Uniform resolution of compact identifiers for biomedical data. *Sci. Data* **5**, 180029 (2018).

19. Le Novère, N. *et al.* Minimum information requested in the annotation of biochemical models (MIRIAM). *Nat. Biotechnol.* **23**, 1509–1515 (2005).

20. Hoksza, D., Gawron, P., Ostaszewski, M., Hasenauer, J. & Schneider, R. Closing the gap between formats for storing layout information in systems biology. *Brief. Bioinform.* **21**, 1249–1260 (2020).

21. Hoksza, D., Gawron, P., Ostaszewski, M., Smula, E. & Schneider, R. MINERVA API and plugins: opening molecular network analysis and visualization to the community. *Bioinforma. Oxf. Engl.* **35**, 4496–4498 (2019).

22. Mazein, A. *et al.* Reusability and composability in process description maps: RAS-RAF-MEK-ERK signalling. *Brief. Bioinform.* **22**, bbab103 (2021).

23. Wishart, D. S. *et al.* DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res.* **46**, D1074–D1082 (2018).

24. Gaulton, A. *et al.* ChEMBL: a large-scale bioactivity database for drug discovery. *Nucleic Acids Res.* **40**, D1100–D1107 (2012).

25. Maude, S. L. *et al.* Chimeric antigen receptor T cells for sustained remissions in leukemia. *N. Engl. J. Med.* **371**, 1507–1517 (2014).

26. Maude, S. L., Barrett, D., Teachey, D. T. & Grupp, S. A. Managing cytokine release syndrome associated with novel T cell-engaging therapies. *Cancer J. Sudbury Mass* **20**, 119–122 (2014).

27. Brentjens, R., Yeh, R., Bernal, Y., Riviere, I. & Sadelain, M. Treatment of chronic lymphocytic leukemia with genetically targeted autologous T cells: case report of an unforeseen adverse event in a phase I clinical trial. *Mol. Ther. J. Am. Soc. Gene Ther.* **18**, 666–668 (2010).

28. Hay, K. A. *et al.* Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor-modified T-cell therapy. *Blood* **130**, 2295–2306 (2017).

29. Locke, F. L. *et al.* Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1-2 trial. *Lancet Oncol.* **20**, 31–42 (2019).

30. Freyer, C. W. Tisagenlecleucel: The First CAR on the Highway to Remission for Acute Lymphoblastic Leukemia. *J. Adv. Pract. Oncol.* **9**, 537–544 (2018).

31. Ali, S. *et al.* The European Medicines Agency Review of Kymriah (Tisagenlecleucel) for the Treatment of Acute Lymphoblastic Leukemia and Diffuse Large B-Cell Lymphoma. *The*

Oncologist **25**, e321–e327 (2020).

32. Davila, M. L., Brentjens, R., Wang, X., Rivière, I. & Sadelain, M. How do CARs work?: Early insights from recent clinical studies targeting CD19. *Oncoimmunology* **1**, 1577–1583 (2012).

33. Sadelain, M., Brentjens, R. & Rivière, I. The basic principles of chimeric antigen receptor design. *Cancer Discov.* **3**, 388–398 (2013).

34. Hudecek, M. *et al.* The nonsignaling extracellular spacer domain of chimeric antigen receptors is decisive for in vivo antitumor activity. *Cancer Immunol. Res.* **3**, 125–135 (2015).

35. Majzner, R. G. *et al.* Tuning the Antigen Density Requirement for CAR T-cell Activity. *Cancer Discov.* **10**, 702–723 (2020).

36. Singh, N. *et al.* Monocyte lineage-derived IL-6 does not affect chimeric antigen receptor T-cell function. *Cytotherapy* **19**, 867–880 (2017).

37. Giavridis, T. *et al.* CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. *Nat. Med.* **24**, 731–738 (2018).

38. Mai, J., Virtue, A., Shen, J., Wang, H. & Yang, X.-F. An evolving new paradigm: endothelial cells – conditional innate immune cells. *J. Hematol. Oncol.J Hematol Oncol* **6**, 61 (2013).

39. Obstfeld, A. E. *et al.* Cytokine release syndrome associated with chimeric-antigen receptor T-cell therapy: clinicopathological insights. *Blood* **130**, 2569–2572 (2017).

40. Teachey, D. T. *et al.* Identification of Predictive Biomarkers for Cytokine Release Syndrome after Chimeric Antigen Receptor T-cell Therapy for Acute Lymphoblastic Leukemia. *Cancer Discov.* **6**, 664–679 (2016).

41. Kiouptsi, K. & Reinhardt, C. Physiological Roles of the von Willebrand Factor-Factor VIII Interaction. *Subcell. Biochem.* **94**, 437–464 (2020).

42. Mikacenic, C. *et al.* Biomarkers of Endothelial Activation Are Associated with Poor Outcome in Critical Illness. *PloS One* **10**, e0141251 (2015).

43. Billiau, A. D., Roskams, T., Van Damme-Lombaerts, R., Matthys, P. & Wouters, C. Macrophage activation syndrome: characteristic findings on liver biopsy illustrating the key role of activated, IFN-gamma-producing lymphocytes and IL-6- and TNF-alpha-producing macrophages. *Blood* **105**, 1648–1651 (2005).

44. Monraz Gomez, L. C. *et al.* Application of Atlas of Cancer Signalling Network in preclinical studies. *Brief. Bioinform.* **20**, 701–716 (2019).

45. Parton, A., McGilligan, V., Chemaly, M., O'Kane, M. & Watterson, S. New models of atherosclerosis and multi-drug therapeutic interventions. *Bioinforma. Oxf. Engl.* **35**, 2449–2457 (2019).

46. Aghamiri, S. S. *et al.* Automated inference of Boolean models from molecular interaction maps using CaSQ. *Bioinforma. Oxf. Engl.* **36**, 4473–4482 (2020).

47. Helikar, T. *et al.* The Cell Collective: toward an open and collaborative approach to systems biology. *BMC Syst. Biol.* **6**, 96 (2012).

48. Helikar, T., Kowal, B. & Rogers, J. A. A cell simulator platform: the cell collective. *Clin. Pharmacol. Ther.* **93**, 393–395 (2013).

49. Abou-Jaoudé, W. *et al.* Logical Modeling and Dynamical Analysis of Cellular Networks. *Front. Genet.* **7**, 94 (2016).