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RESEARCH ARTICLE

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

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ABSTRACT

This work focuses on the need for modeling and predicting adverse outcomes in immunotoxicology to improve nonclinical assessments of the safety of immunomodulatory therapies. 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 illustrated with chimeric antigen receptor (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 at https://imsavar.elixir-luxembourg.org *via* the MINERVA Platform for browsing, commenting, and data visualization. Our work transforms a graphical illustration of an AOP into a digitally structured and standardized 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.

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, modeling 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 nonclinical 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.

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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 the predictivity of nonclinical safety assessments during the nonclinical stage and offering innovative immunobiology models and biomarkers. The project imSAVAR (Immune Safety Avatar:

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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 (Ankley et al. 2010).

In immunotoxicology, immune-related adverse outcome pathways (irAOPs) are used to visualize and study the 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 (Ankley et al. 2010; Horvat et al. 2017; Leist et al. 2017). Modeling strategies of AOPs are described in the OECD 'Users' Handbook supplement to the Guidance Document for developing and assessing Adverse Outcome Pathways' (OECD 2018). Specifically, the AOP graphical representation is discussed in the handbook in Development Tips 1 and 2 (OECD 2018). 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 modeling and prediction. We propose to address these limitations by employing advanced tools in systems biomedicine and network biology, e.g. the MINERVA platform (Gawron et al. 2016).

In systems biomedicine, the standardized representation and machine readability needed for interactive exploration, annotation, and modeling of pathways are offered by approaches such as disease maps. A disease map is a conceptual model of relevant mechanisms represented as a collection of interconnected signaling and metabolic pathways (Mazein et al. 2018; Ostaszewski et al. 2019). Examples of such maps are resources for cancer (Kuperstein et al. 2015), Parkinson's disease (Fujita et al. 2014), rheumatoid arthritis (Singh et al. 2020), asthma (Mazein et al. 2021), and, the most recent development, the COVID-19 Disease Map for capturing virus-host interaction mechanisms of the SARS-CoV-2 infection (Ostaszewski et al. 2021). These maps can include multiple layers from molecular to intercellular and inter-tissue/interorgan interactions to reflect the physiological level of complexity (Mazein et al. 2021). Disease maps are designed for integrating prior knowledge, making sense of newly-generated data, modeling, 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 visualization and exploration of this knowledge. State-of-the-art disease maps also utilize 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 an MIE, a series of KEs 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 visualize 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 modeling and visualization frameworks as recommended by the OECD AOP concept (OECD 2018) 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 visualization and exploration, with such entities as proteins, genes, and metabolites identified and linked to appropriate external databases, and with multiple plugins available. The approach and the MINERVA platform are discussed in the Methods section in more detail.

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 (CRS) 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 (Ankley et al. 2010; OECD 2018). In one of the following works, a linear AOP was expanded into a complex network of molecular events, with feedback and feed-forward loops and inter-relationships between individual key events presented (Horvat et al. 2017; Leist et al. 2017). 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) (Le Novère et al. 2009) and Systems Biology Markup Language (SBML) (Hucka et al. 2003) to formalize and visualize 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 (Mazein et al. 2018; Ostaszewski et al. 2019). Figure 1 describes the systems immunotoxicology framework integrated from the AOP concept and the adapted systems medicine disease map methodology (Kondratova et al. 2018).

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 this journal issue).

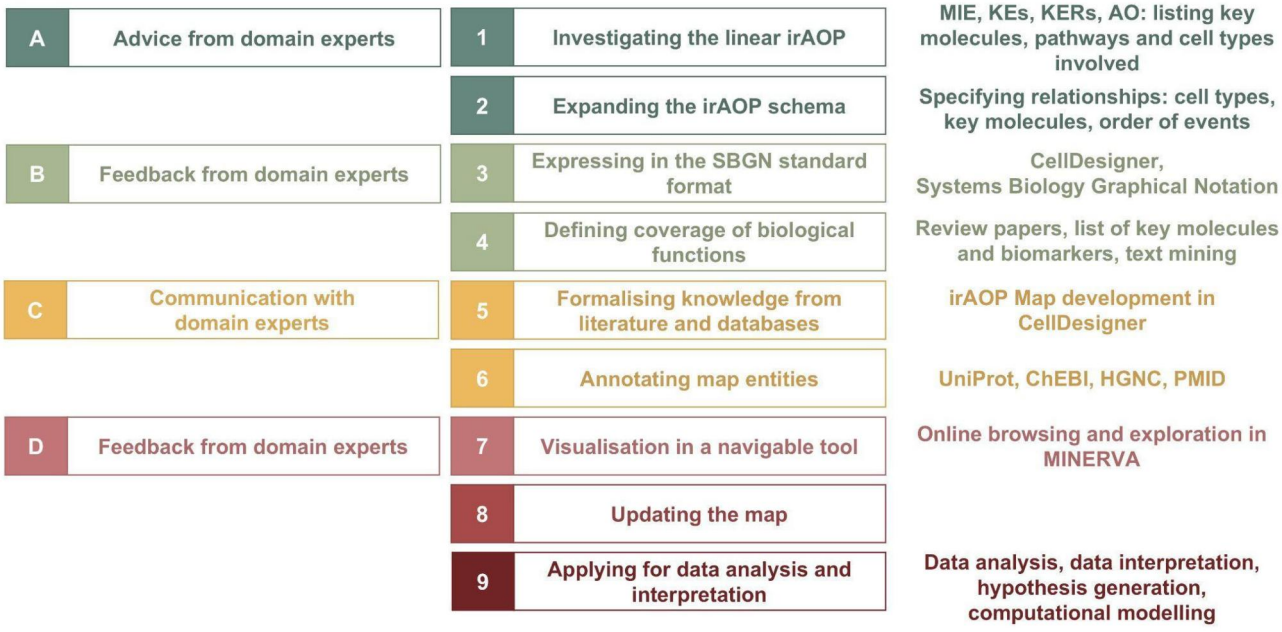

Figure 1. The systems framework for knowledge management and exploration in immunotoxicology. irAOP, immune-related adverse outcome pathway; MIE, molecular initiating event; KEs, key events; KERs, key event relationships; AO, adverse outcome; UniProt, a database of protein sequence and functional information (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.

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 (Le Novère et al. 2009) 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 (Balci et al. 2021) (http://newteditor.org), SBGN-ED (Czauderna et al. 2010) (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 (Wimalaratne et al. 2018) or MIRIAM annotation (Le Novère et al. 2005).

Step 7. Visualisation in a navigable tool. For making the results easily accessible and explorable we use the MINERVA platform (Gawron et al. 2016; Hoksza et al. 2019; Hoksza et al. 2020) (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. With continuous support, the modifications are introduced approximately once a year by the computational biology team that developed the map (Mazein et al. 2023). Modularised structure makes it easier to manage and update the map components (Mazein et al. 2021).

Step 9. Applying for data analysis and interpretation. The complete map can be used for 'omics data visualisation, analysis, and interpretation (Ostaszewski et al. 2021). The map can also be transformed into a simulatable computational model, for example, a Boolean model, for predictions and advanced hypothesis generation (Singh et al. 2020).

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, prioritizing 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 visualization and exploration of extensive systems biology networks (Gawron et al. 2016). 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 visualized in colors of different intensities according to the values provided. MINERVA enables search and exploration of drug targets *via* online queries to DrugBank (Wishart et al. 2018) (https://www.drugbank.com) and ChEMBL (Gaulton et al. 2012) (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 (Mazein et al. 2021) 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) visualizing 'omics data (https://asthma-map.org/tutorials).

Results

Immune-related adverse outcome pathway of CAR T cell treatment

CRS is one of the most common adverse events caused by CAR T cell therapy, namely the FDA/EMA approved CD19 and BCMA CAR-T cell products (Kymriah, Breyanzi, Yescarta, Tecartus, Abecma, Carvykti) and, in general, a common type of toxicity caused by immunotherapies that engage T-cells (Brentjens et al. 2010; Maude et al. 2014; Maude et al. 2014; Hay et al. 2017; Shah et al. 2023). Figure 2(a) presents the proposed respective irAOP. The MIE, the KEs, 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 recognize and bind to antigen-expressing cells such as tumor cells in a non-MHC (MHC, major histocompatibility complex) restricted manner. As a result, CAR T cells are activated (Davila et al. 2012; Freyer 2018; Locke et al. 2019; Ali et al. 2020).

Key event 1 (KE1): activation of CAR T cells

The costimulatory domains (e.g. CD28 or 4-1BB) within the CAR enable the activation of CAR T cells in a direct manner, bypassing the need of additional signaling through the endogenous CD28 or 4-1BB receptor. This leads to the recruitment of LCK (lymphocyte-specific protein tyrosine kinase) and phosphorylation of ZAP70 (zeta chain of T-cell receptor associated protein kinase 70). 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 additionally produce cytokines including interleukin (IL)-2 and interferon (IFN)-γ and start to proliferate (Davila et al. 2012; Sadelain et al. 2013; Hudecek et al. 2015; Majzner et al. 2020).

Key event 2 (KE2): increased pro-inflammatory mediators

The production of different soluble factors such as IFN-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor(TNF)-α by CAR T cells and bystander (endogenous) immune cells in the tumor microenvironment can trigger IL-6 production by monocytes (Morris et al. 2022), as well as endothelial cells. Furthermore, macrophages can produce IL-6 in addition to IL-1β and nitric oxide (Mai et al. 2013; Teachey et al. 2016; Singh et al. 2017; Obstfeld et al. 2017; Giavridis et al. 2018).

Key event 3 (KE3): tissue-resident and endothelial cell activation

The increased levels of pro-inflammatory mediators subsequently lead to inflammation and its amplification, as well as to the activation of other immune cells. This means enhanced IL-6 and IL-1β production by monocytes and activation of endothelial cells with the production of von Willebrand factor (VWF) and angiopoietin-2 (ANG2) (Hay et al. 2017). VWF plays a key role in

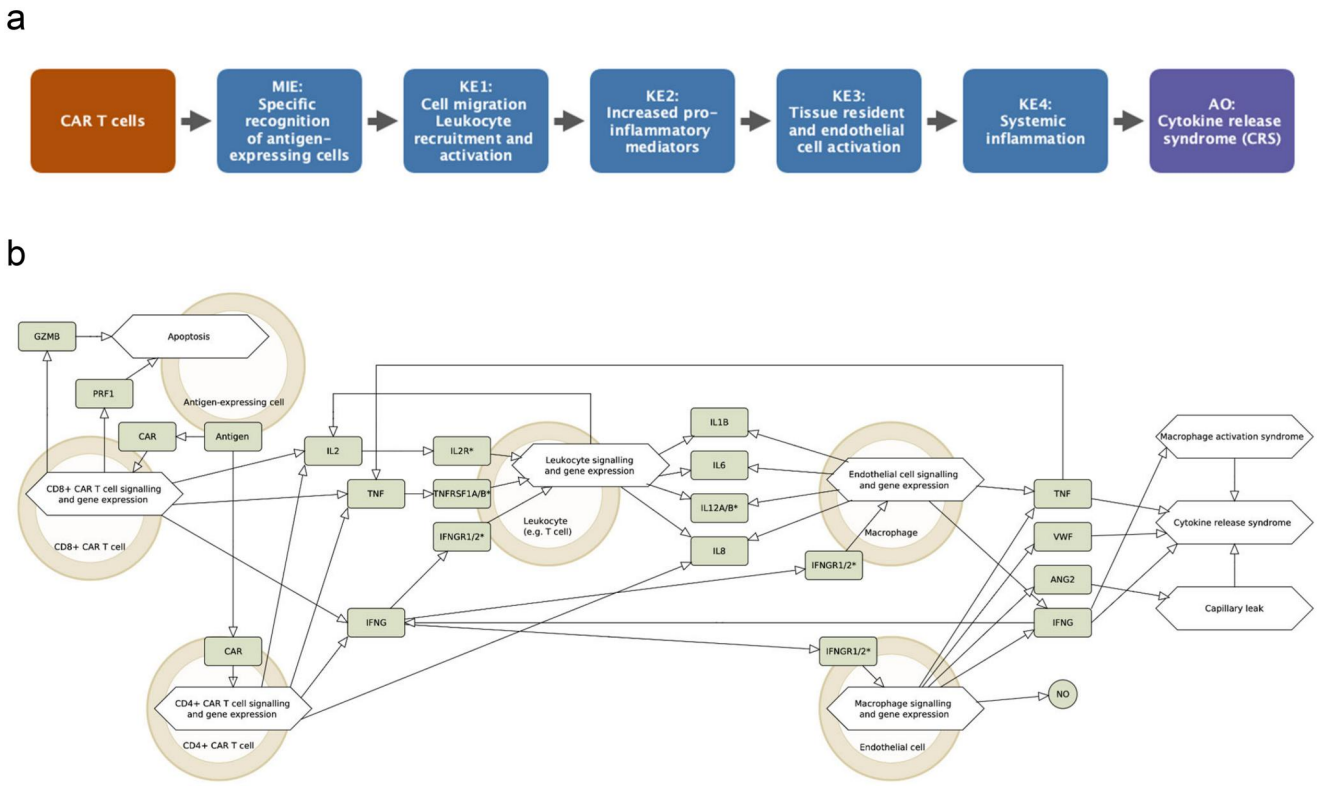

Figure 2. CAR-T-cell-induced cytokine release syndrome (CRS): a comparison of a linear irAOP and a more detailed representation of the underlying biology. (a) The linear irAOP for the CAR-T-cell-induced cytokine release syndrome. MIE: molecular initiating event; KE: key event; AO: adverse outcome. (b) 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=cart17.

Table 1. CAR T treatment: key molecules involved, with HGNC symbols and UniProt identifiers.

Name	Symbol	Identifier	Source (examples)	Target (examples)	Receptors (candidates)
IL-2	IL2	UniProt:P60568	CAR T cell, endogeneous T-cells	CAR T cell, endogenous T-cells, NK cells	IL2RA, IL2RB, IL2RG
IFN-γ	IFNG	UniProt:P01579	CAR T cell, macrophage, endothelial cell	CAR T cell, endogenous T-cells, macrophage, endothelial cell	IFNGR1, IFNGR2
TNF-α	TNF	UniProt:P01375	CAR T cell, macrophage, endothelial cell	Multiple cell types including endothelial cells	TNFRSF1A, TNFRSF1B
CD69	CD69	UniProt:Q07108	CAR T cell	T-cell activation marker, i.e. CAR T cells, endogenous T-cells	n/a
CD25	IL2RA	UniProt:P01589	CAR T cell	T-cell activation marker, part of IL-2 receptor, i.e. T-cells, CAR T-cells	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, e.g. tumor cell	n/a
Granzyme B	GZMB	UniProt:P10144	CAR T cell	Antigen-expressing cell, e.g. tumor cell	n/a
IL-1	IL1A, IL1B	UniProt:P01583; UniProt:P01584	Macrophage	T-cell, neutrophil, B cell	IL1R1, IL1R2
IL-6	IL6	UniProt:P05231	Monocytes, macrophage, T-cells, endothelial cells	T-cell	IL6R, IL6ST
NO	nitric oxide	CHEBI:16480	Macrophage	Tumor cell	n/a
IL-8	CXCL8	UniProt:P10145	Macrophages	Neutrophils	CXCR1, CXCR2
IL-12	IL12A; IL12B	UniProt:P29459; UniProt:P29460	CAR T cell, Dendritic Cells, 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	Macrophage	Neutrophil	CXCR2
VWF	VWF	UniProt:P04275	Endothelial cell	Platelets	P1BA, GP1BB
ANG2	VPS51	UniProt:Q9UID3	Endothelial cell	Endothelial cell	TEK

n/a: not applicable (Cosenza et al., 2021).

coagulation (Kiouptsi and Reinhardt 2020), and ANG2 promotes capillary leakage (Mikacenic et al. 2015).

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. Elevated serum levels of IL-6, ferritin, and C-reactive protein are inflammatory markers of systemic inflammation in CAR-T patients (Kell and Pretorius 2014; Teachey et al. 2016; Hay et al. 2017; Liu et al. 2023).

Adverse outcome (AO): cytokine release syndrome

The clinical manifestations of CRS mediated by CAR-T cell therapy are often quite similar to other syndromes that cause a massive release of proinflammatory mediators such as macrophage activation syndrome or hemophagocytic lymphohistiocytosis (Billiau et al. 2005; Grom et al. 2016; Teachey et al. 2016; Obstfeld et al. 2017; Giavridis et al. 2018; Neelapu et al. 2018; Canna and Cron 2020). Clinical features of CRS include fever, hypotension, and vascular leakage (Morris et al. 2022). In the clinic, a grading system is used as a guide for mitigation strategies such as administration of glucocorticoids (Lee et al. 2019).

This knowledge was formalized 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 analyzed 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 2(b).

Table 1 demonstrates how network reconstruction helps to ask questions and identify gaps in our understanding of the biological processes involved. We clarify and formalize available

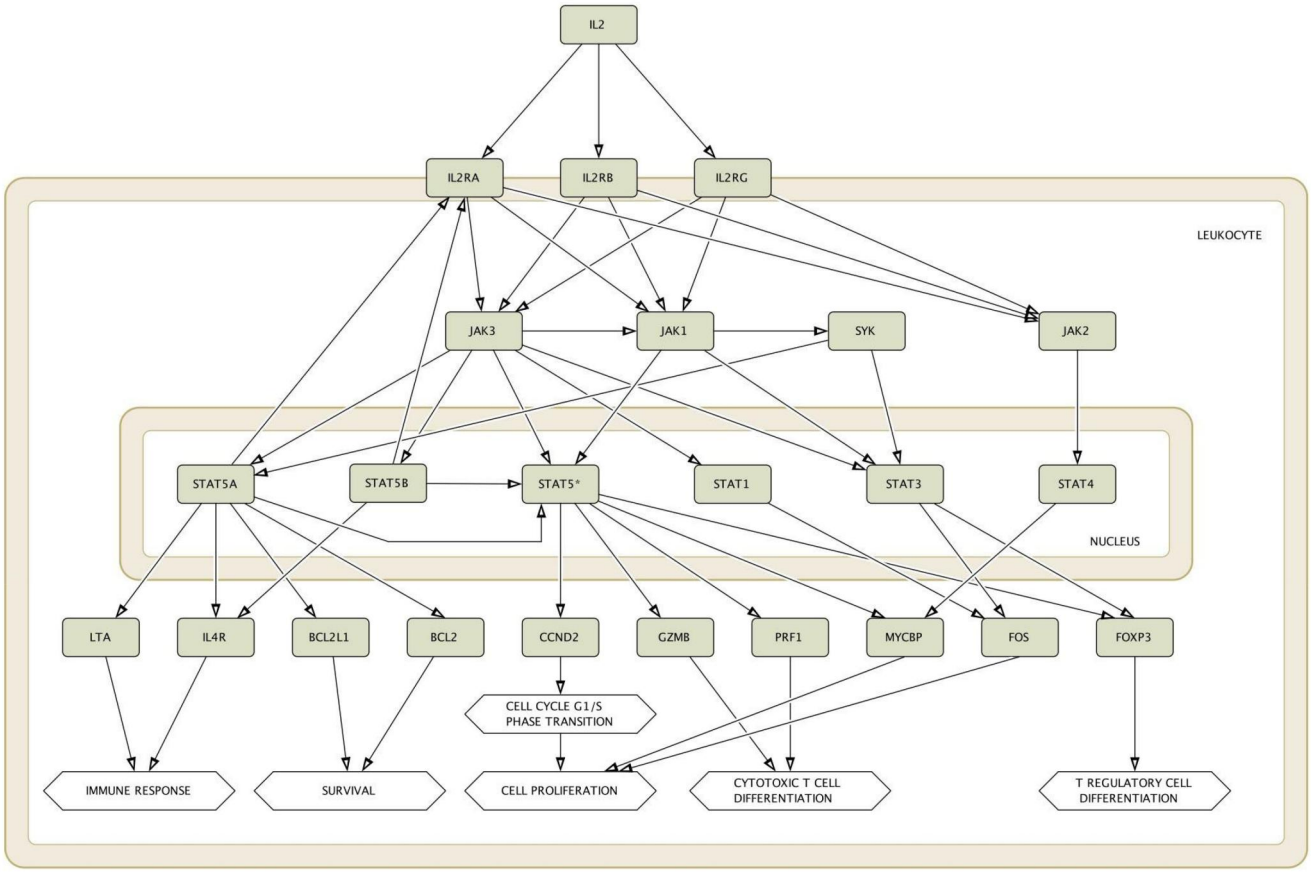

Figure 3. IL-2 signaling. This pathway is manually converted to the SBGN activity flow format from the MetaCore pathway map ID 2770 immune response IL-2 signaling *via* JAK-STAT (https://portal.genego.com). The proteins are presented with the use of HGNC names (https://www.genenames.org). The map is available in MINERVA for commenting and exploring at https://imsavar.elixir-Luxembourg.org/minerva/index.xhtml?id=il2v09.

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, receptors, and cell types involved. For example, knowing that IL-2 is produced by CAR T cells and affects leukocytes, including the CAR T cells themselves as well as bystander endogenous T cells, we next focus on IL-2 receptor proteins IL2RA, IL2RB and IL2RG, the corresponding signaling cascade and the activated transcription factors (Figure 3).

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 (Monraz Gomez et al. 2019; Parton et al. 2019), potentially with the use of the CaSQ tool (Aghamiri et al. 2020) and the Cell Collective Boolean modeling pipeline (Helikar et al. 2012; Helikar et al. 2013; Abou-Jaoudé et al. 2016).

The CAR T cell irAOP-Map resource not only describes and illustrates the mechanisms of CRS induced by CAR T cell treatment but also standardizes the description of these mechanisms, making it interoperable and useful in computational workflows. This resource can be leveraged in the context of drug development by mapping time-resolved transcriptomic or proteomic datasets from model systems or patient tissues for visual exploration and interpretation of complex data. Its primary use focuses on nonclinical validation and interpretation of readouts from model systems. A prospective next step is a translation of the map into a computational model, allowing to simulate outcomes of therapeutic interventions.

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 a 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.

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Declaration of interest

No potential conflict of interest was reported by the author(s).

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Data availability statement

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

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