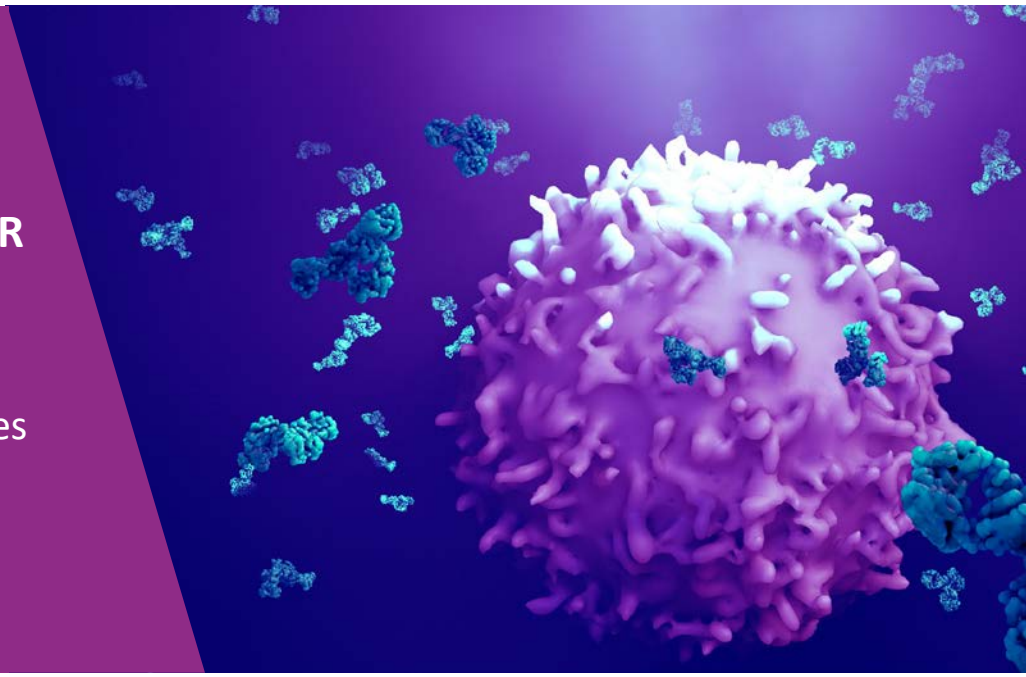
imSAVAR

IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 3.4
1st iteration in vivo and in vitro models IL-2 MoA

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 (WP) 3 of imSAVAR focusses on innovative models for safety assessment of immuno-inflammatory disease therapeutics. Immunotherapy with interleukin (IL)-2 was chosen as a first concrete use case for the work in WP3. In a first step, a preliminary immune-related adverse outcome pathway (irAOP) has been generated and published in deliverable D3.1. This irAOP of IL-2-mediated toxicities was used as a tool for the progress of the WP on a theoretical and practical level. The information gathered by intensive literature research was structured with the help of the irAOP. Its refinement resulted in the identification of irAOP-branches leading to organ-specific toxicities mediated by IL-2 immunotherapy. According "sub-irAOPs" were used as a central orientation for the development of experimental models. This provided the basis for the planned lab work analysing IL-2-mediated immunological effects on different levels of complexity and with respect to different organs. Within this deliverable report, we describe these models and the cooperation of imSAVAR partners initiated to work on the identified critical topics.

Document Information

Deliverable Report	**D3.4: 1st iteration in vivo and in vitro models IL-2 MoA**
Date	**31.05.2022**
Report prepared by	**Paul-Ehrlich-Institute** Fraunhofer ITEM, Fraunhofer IZI, Fraunhofer ITMP, Lund University, Jena University Hospital
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**

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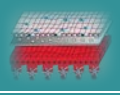
1. Methods

irAOPs as theoretical structuring tool are the central method for the progress of the imSAVAR project. Preliminary irAOPs for IL-2-mediated toxicities were generated based on the OECD Guidance Document and published in the imSAVAR deliverable D3.1. In the last months, we continued to structure the information gained for IL-2 immunotherapy. Intensive literature research and cooperative work of all WP3 subtask-groups (table 1) resulted in the refinement of these irAOPs (figure 1). These irAOPs served as a valuable orientation for the design of experimental assays. These will be performed with different levels of cellular complexity that range from single cell cultures to complex organ-on-a-chip and in vivo models. Furthermore, with the experimental work we will fill remaining scientific knowledge gaps, analyse potential biomarkers, and approach future predictive pre-clinical assay systems.

Table 1: Partners of WP3 working on IL-2. The methods used by the partners cover different levels of cellular complexity ranging from single cell cultures to complex cell culture systems to in vivo models.

*Table 1: **Partners of WP3 working on IL-2.** The methods used by the partners cover different levels of cellular complexity ranging from single cell cultures to complex cell culture systems to in vivo models.*

WP subgroup	Focus (team)	Cellular complexity
WP 3.1/3.2	Hepatotoxicity (ITMP)	
WP 3.1/3.2	moDCs (University Lund)	
WP 3.1/3.2	Vascular leakage (PEI)	
WP 3.1/3.2	Pulmonary toxicity/ Dermatoxicity(ITEM)	
WP 3.3	Gut on a chip (Uniklinikum Jena)	
WP 3.4	*In vivo* SLE-Model (Fraunhofer IZI)	

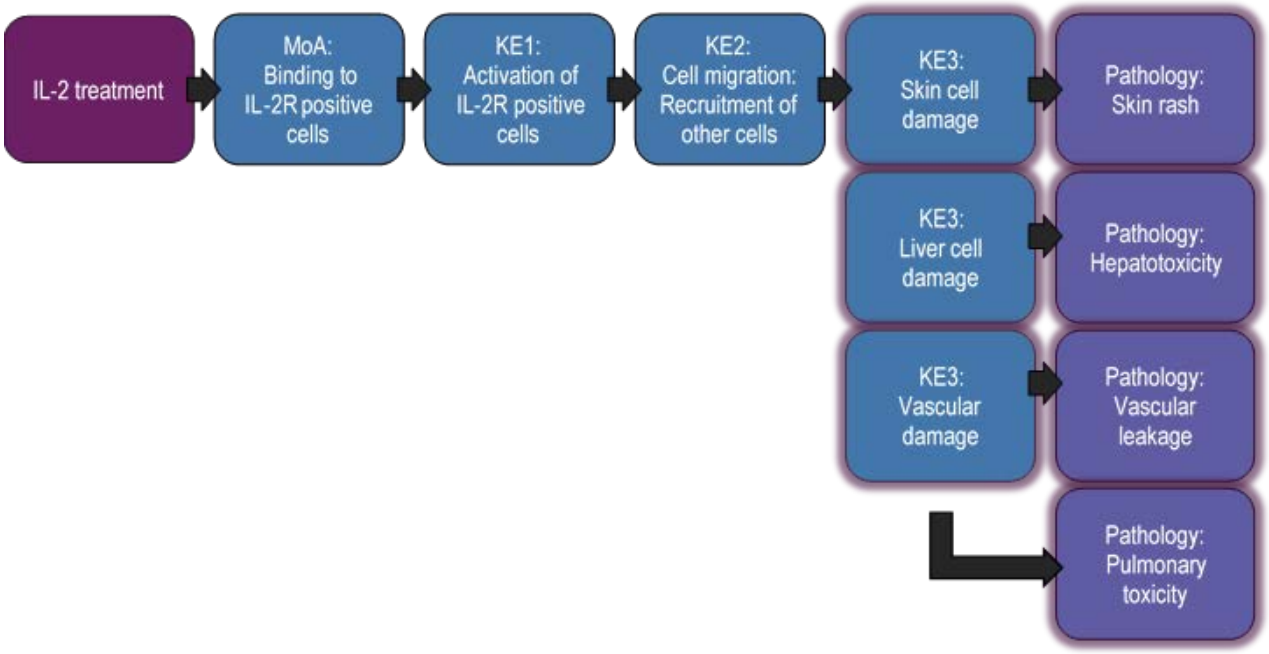


Figure 1: The irAOP of IL-2-mediated toxicities diverges into different branches. The mode of action (MoA) and key events (KE) 1 and 2 are similar to the initial irAOP while in KE3, branches of organ-specific damage are defined. Accordingly, different organ-specific damage are defined. Accordingly, different organ-specific model systems were chosen to approach the experimental analyses of these toxicities.

The assay systems deviated from the irAOPs are described as results in this deliverable. The developed readouts are described in the according imSAVAR deliverable D3.3.

2. Results

Using the irAOPs as a tool gave rise to results on both a theoretical and a practical level. The theoretical structuring of the information around IL-2-mediated pathologies resulted in the identification of different organ-specific toxicological effects (figure 1). Integration of the different pathology-branches will generate a complete picture regarding adverse outcomes induced by IL-2 immunotherapy. To that point, refined irAOPs for IL-2-induced skin rash, hepatotoxicity, and vascular leakage were developed. They will be published in separate scientific publications as part of a joint imSAVAR issue (in preparation). Within this issue, the efforts made so far within imSAVAR on current case studies will be disseminated in form of classical review articles, AOP-focussed review articles, and first original data manuscripts. In the future, we intend to further interconnect the work of the imSAVAR partners within and between work packages.

Practically, the refined irAOPs were used for the identification of potential assay systems (see imSAVAR deliverable D3.1). Wet lab experiments were planned in close cooperation of the WP3 members in bi-weekly meetings to design protocols as harmonized as possible (as concrete example: using the same IL-2 product from the same distributor in the same concentrations in vitro). Meetings and discussions on a regular basis continue to enable to refine the used methods and to evaluate the results in a broad picture. In vitro models to analyse the mode of action (MoA) and respective toxicities of low- and high- dose recombinant IL-2 comprise organ-specific immunologic cell cultures. Additionally, cross-cutting meetings and discussions were initiated and continued in order to connect and bundle the expertise of suitable imSAVAR-partners. The experimental models used to analyse IL-2-mediated toxicities are summarized in

table 2. In vitro approaches will be complemented with respective microphysiological Organ-on-Chip (OoC) systems and in vivo models.

For the in vitro analysis of IL-2-mediated dermal toxicities, ex vivo skin cell cultures derived from human skin explants, and 3D skin models (ITEM, Hannover, the latter in cooperation with Fraunhofer IGB) are used as assay systems. Analyses will be focussed on human mast cell and ILC2 cultures, since these cell types were identified as potential key players for IL-2-mediated skin rash in the irAOP.

IL-2-mediated lung-toxicities will be analysed using cultivated human precision-cut lung slices as an ex vivo lung-model (ITEM, Hannover).

The IL-2-induced hepatotoxic effects are analysed within single and co-cultures.Primary human immune cells (T helper cells, cytotoxic T cells, regulatory T cells, NK cells) and cell lines such as Jurkat cells (T cell-line) or HepaRG (hepatic cell line) are used (ITMP, Frankfurt am Main). Cross-cutting cooperation with Dynamic42 will enable complementary analyses with a Liver-on-Chip model.

To analyse mechanisms of IL-2-mediated vascular leakage, human umbilical vein endothelial cells (HUVECs) and primary immune cells (peripheral blood mononuclear cells, PBMCs) are analysed in separate and co-cultures. Additionally, an in vitro assay on a transwell basis is under establishment (PEI, Langen). Communication with other partners working with e.g. vessel-on-a-chip models were initiated to complement the analyses of vascular leakage in the future.

As illustrated in the preliminary irAOP (figure 1), a connection of IL-2-induced vascular leakage and pulmonary toxicity has been identified. To approach that experimentally, concerted experiments with the vascular leakage assay and precision-cut lung slice cultures are currently planned.

Additionally, also the role of distinct immune cells, such as dendritic cells, monocyte-derived dendritic cells, or in vitro-differentiated Langerhans cells during IL-2 therapy will be analysed (LUND).

Currently planned in vivo studies will on one side analyse IL-2-mediated skin toxicities. On second side, the therapeutic efficacy and safety of long-term low-dose IL-2 will be investigated within subtask 3.4 with a murine model of induced systemic lupus erythematosus (SLE). In addition, interference of long-term IL-2 treatment with modulated immune response against viral infection will be analysed. At this stage, the animal experiment application has been approved and preliminary experiments are planned.

Finally, the effect of high-dose IL-2 therapy will be analysed using a Gut-on-Chip model (Uniklinikum Jena).

Table 2: Methods conducted by the different partners

Partner	Pathology	Methods/Model
Fraunhofer ITEM	Skin rash/pulmonary toxicity	• *Ex vivo* culture of human skin • Differentiation and culture of primary human blood cells • 3D skin models (Fraunhofer IGB)* • Culture of *ex vivo* precision-cut lung slices

Fraunhofer ITMP	Hepatotoxicity	• Hepatic and T cell lines (Jurkat/HepG2/HepaRG) • Primary human cells isolated from PBMCs ($CD8^+$ T cells, NK cells, Treg cells, Th1 cells, Th2 cells) • Analyses with liver-on-a-chip models (Dynamic42)*
Paul-Ehrlich-Institut	Vascular leakage	• Single and co-cultures with HUVECs and PBMCs (as crude culture or with isolated cell types) • *In vitro* vascular leakage assay
University Lund	Diverse	• *In vitro* differentiated monocyte-derived dendritic cells from PBMCs • *In vitro* differentiated Langerhans cells from PBMCs
University Jena*	Gut toxicity	• Gut-on-a-chip
Fraunhofer IZI	Lupus and infections	• Murine *in vivo* model for lupus

3. Discussion

The different assay systems described briefly in this deliverable are currently under final establishment. Furthermore, they are being used to generate original data, which are intended to be published in peer review journals. After publication, these data will be incorporated in the following iteration deliverables. Additionally, these data will be the basis for further refinement of the generated irAOPs.

We could identify that IL-2-induced (side) effects have a broad impact affecting different cell types, tissues, and organs resulting in a variety of (adverse) outcomes. Hence, we proposed to exclude the term “Treg” from the respective deliverables, to be able to broaden our analyses and display the range of IL-2-mediated toxicities and the underlying mechanisms.

The theoretical work conducted so far with the help of irAOPs so far was successfully used for the progress of the project. Theoretical scientific background and the developed irAOPs are currently in a publication process. Thus, this achieved progress will be disseminated in near future. It is the result of an intensive introduction phase into the topics including the direct application of the irAOPs as a tool.

Experimental setups were designed to fill to the “black boxes” identified using the irAOP for knowledge structuring. Respective wet lab analyses are currently ongoing. Resulting data will be applied to fill these knowledge gaps, the development of suitable pre-clinical safety assessment models, and/or biomarker identification. In addition, the gained insights will be used for the optimization of existing assay systems. Taken together, the usage of irAOPs is a suitable tool in the scope of imSAVAR. Structuring the theoretical background helped to focus the available expertise of the different partners on the relevant knowledge gaps. The information from experimental work as well as the theoretical background from the irAOPs within the reviews will be an important orientation for further concrete sub-projects, such as the development of the irAOP disease maps (imSAVAR D4.5). Together, this reflects the cooperative work between the partners leading to the progress of the project as a whole.

4. Abbreviations

HUVEC	Human umbilical vein endothelial cells
IL-2	Interleukin-2
ILC2	Type 2 innate lymphoid cells
irAOP	Immune-related adverse outcome pathways
MoA	Mode of action
NK cells	Natural killer cells
OECD	Organisation for Economic Co-operation and Development
PBMC	Peripheral blood mononuclear cell
SLE	Systemic lupus erythematosus
Tregs	Regulatory T cells

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.