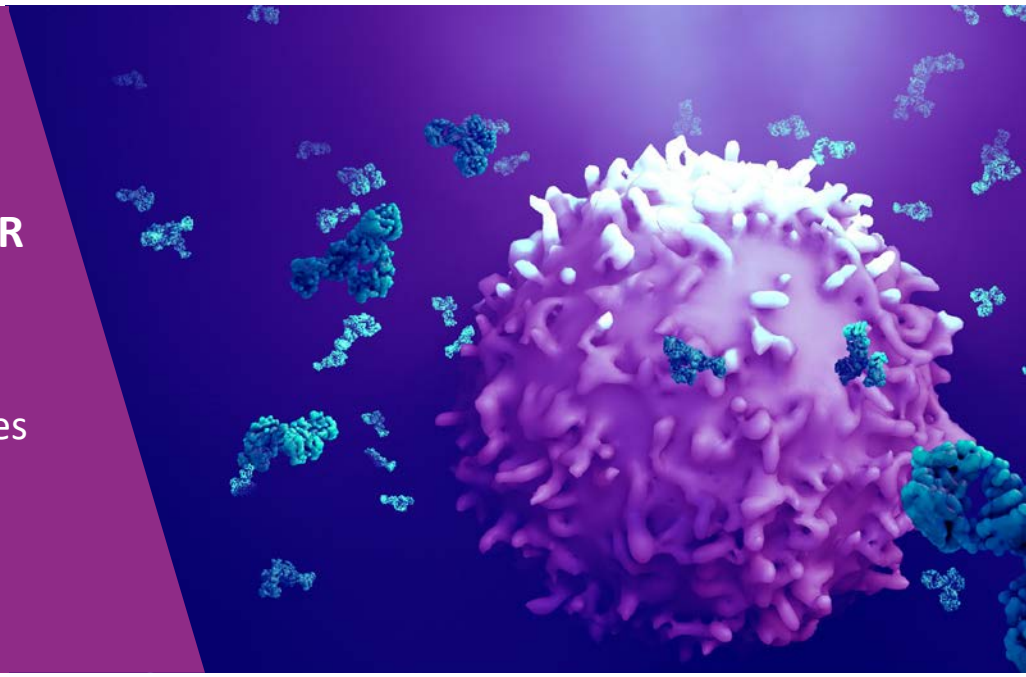
imSAVAR

IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 1.3

1st imSAVAR Workshop

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

The first imSAVAR Stakeholder workshop took place on 29 June 2020 and was conducted as an online event. There were 87 attendees during the meeting. The workshop introduced the concept of imSAVAR and immune-related Adverse Outcome Pathways (irAOPs) with emphasis on cytokine release syndrome (CRS).

Since the risks of therapeutics for immune-oncology and immune-inflammatory diseases (e.g. CRS) are not sufficiently understood yet, imSAVAR aims at the nonclinical mimicking of the immune system effects of immunomodulatory therapies.

There was a robust discussion throughout the workshop. The irAOPs could be further detailed with additional knowledge from the attendees and unsolved issues were identified. In addition, the dialogue revealed strengths and weaknesses and helped posing important questions regarding the different models and their benchmarking.

The workshop was the first of three workshops described in the project plan.

Document Information

Deliverable Report	**D1.3: 1st imSAVAR Workshop**
Date	**11.09.2020**
Report prepared by	**Fraunhofer-Gesellschaft zur Foerderung der angewandten Forschung e.V** BioSci Consulting BVBA, Universitaetsklinikum Wuerzburg – Klinikum der Bayerischen Julius-Maximilians-Universitaet, Paul-Ehrlich-Institute
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 **Novartis Pharma AG** Dr. Jonathan Moggs
Type	**Deliverable Report | Public**

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1. Introduction

Current nonclinical in vitro and in vivo models do not adequately represent the complexity of the human immune system and therefore we are not able to assess the safety of new therapies based on biologics or cellular products that modulate the immune system.

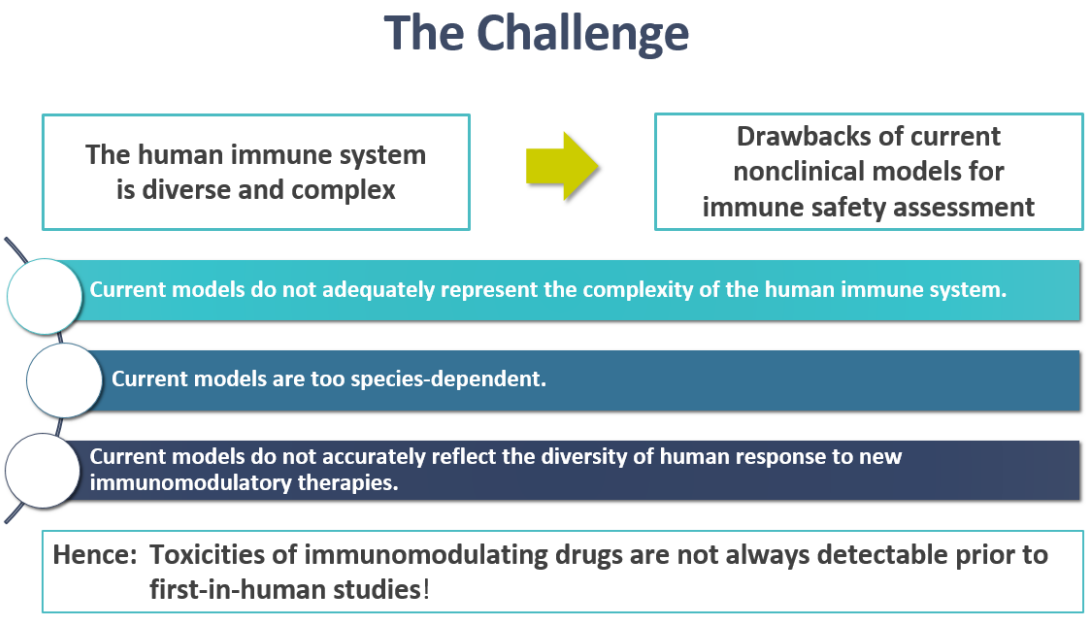

Figure 1: The challenge of imSAVAR

There is currently an urgent need to better understand the risks of therapeutics for immune-oncology and immune-inflammatory diseases including infections, cytokine release syndrome, malignancies, and autoimmunity. Within imSAVAR we want to improve predictivity of non-clinical safety testing. The aims of the imSAVAR project are for specific immune modulatory modes of action to:

1. Refine and standardize existing models, and develop new models, techniques and technologies,
2. Identify biomarkers for non-clinical assessment of safety,
3. Establish a platform that combines models with a network of bio samples and,
4. Create a sustainable immune safety stakeholder community.

The intended impact that researchers will be able to readily gain early insights into the safety of new therapies before they enter the clinical phase of development.

2. Adverse outcome pathways

Adverse outcome pathways, AOPs, are modular networks that connect different levels of biologic organization, which can be molecular level, cellular level, or organ level, to a certain adverse outcome. They have a common structure consisting of a molecular initiating event, a series of key events connected by key event relationships and an adverse outcome. Within ImSAVAR we will use the AOP concept to describe severe clinical adverse effects as observed in patients after treatment with new immunomodulatory drugs. We will therefore define changes in biological state that are measurable in. Biomarkers will be used as indicators of the underlying immunological processes and to assess the suitability of non-clinical safety models.

The first steps is to collect and review existing knowledge that build a weight of evidence for key events and connections within a draft AOP, determine existing gaps in knowledge and models to guide experiments and develop models to fill those gaps.

3. AOP for CAR-T cell induced cytokine release syndrome

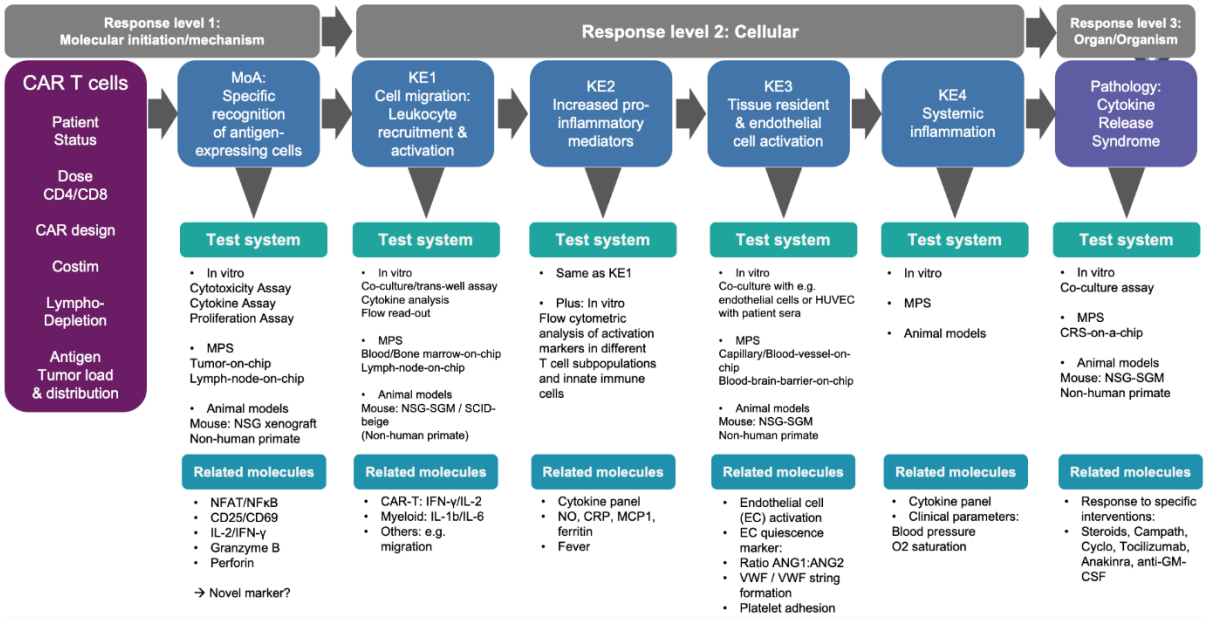

Figure 2: CAR T cells and response levels

Cytokine Release Syndrome (CRS) is the most common type of toxicity caused by chimeric antigen receptor (CAR) T cells. Within ImSAVAR we started to develop an AOP for CAR T cell induced cytokine release syndrome describing a sequence of events leading to the severe clinical adverse outcome. The molecular initiating event is the binding of the CAR to the target molecule which may results in an undesired cascade of molecular signaling that has activating effects on the T-cells, CAR-T cells, and other components of the immune system and surrounding environment. The outcome might be a systemic inflammatory response, including involvement of the endothelium. The relationship between dose levels, the preconditioning regimen as well as the composition of the CAR T cell product and the onset of adverse reactions is still unclear.

Current in vitro assays to assess CRS, such as the cytokine release assays, do not provide insight into how many T-cells are releasing cytokines, nor the kinetics of cytokine release. It is also difficult to measure how contributing cells amplify each other, such as the production of inflammatory mediators by endogenous immune cells and the involvement of endothelial cells. This is where lymph node and bone marrow on a chip systems can be useful allowing for the modelling of the interactions of different components that would typically be modeled in isolation in individual in vitro assays.

4. irAOP for bispecific antibody induced cytokine release syndrome

CRS is also a common type of toxicity caused by bispecific antibodies. The bispecific antibody irAOP, which will be the subject of a subsequent workshop, has also been developed and drafted. One of the key differences is in the initiating key event. When you look in the literature and see what that synapse

formation looks like, it looks much more similar to the classic immunological synapse for the CD3 bispecifics than for the CAR T cells. The binding of the bispecific antibody to CD3 on the T cell and the antigen expressed by the tumor would then be the first key event in the AOP. This then leads to T-cell activation and the lysis of the antigen-specific target cell by release of the cytolytic granules and further to T-cell proliferation activation and release of several cytokines, such as interferon gamma, IL-2. Eventually this also leads to further recruitment of immune cells and additional release of pro-inflammatory cytokines, and potentially to activation of monocytes and macrophages, and the endothelium. The incidence of CRS with bispecific antibody treatments is much lower than what we see with CAR-T cell therapy.

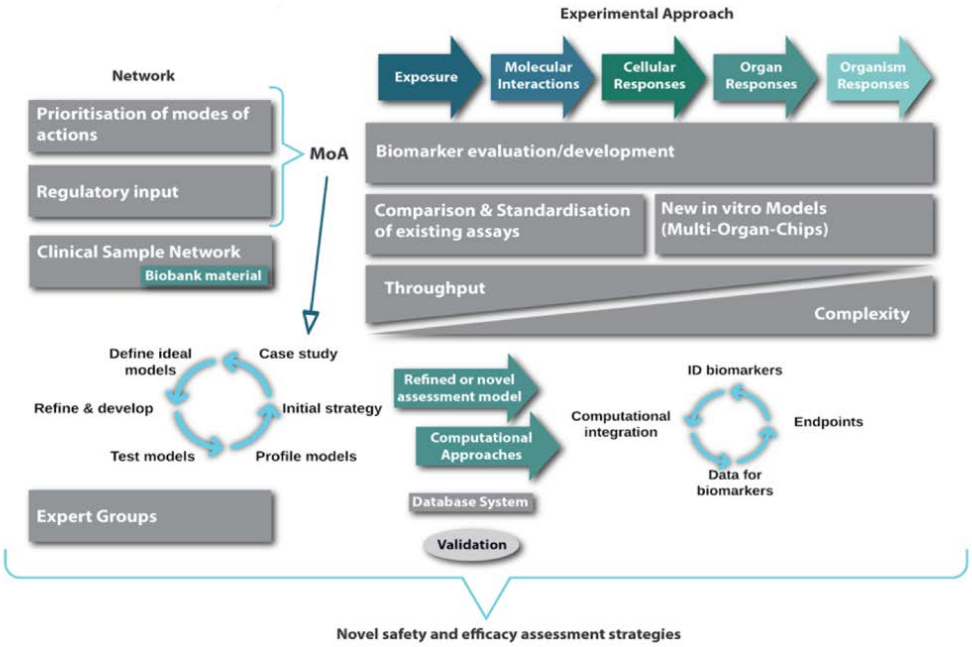

Figure 3: imSAVAR strategy

5. Dialogue

During the online workshop a dialogue between the 87 attendees was conducted around a set of questions and topics. This dialogue is briefly summarized below.

Highlighting the strengths and weaknesses of each model

For all the proposed in vitro and in-vivo models it would be useful to have information on what they contain, what they are useful for, and what the major immunological safety assessment gaps are. This would include specifying which parts of mechanistic pathways are represented or missing. For example, are there species-related differences in the way that cytokines from human immune cells interact with the mouse endothelial cells?

To what level of detail do the AOP's need to be developed?

The FDA has pointed out that it is difficult to extrapolate simple models to patients. Thus, adding complexity to the current set of models is a good way to proceed. This should be in a stepwise manner and link that effort with good biomarker screening and focus on outcomes that can be related to what is

observed in a clinical setting. There should be some consideration of tumor burden as it is related to the risk of CRS. Transgene is developing some organ-on-a-chip models that reflect tumor burden.

How should we view micro-physiologic/organ on a chip (MPS/OOAC) models?

Benchmarking models against animal models and even clinical data is not always straightforward. One question is how well CRS in a model relates to patients. This is where the approach of the AOPs can be helpful particularly when the models that are developed are then compared to the real-world experience of an existing compound in the context of the AOP. We need to have ongoing cross-linking of activities on mode of action and nonclinical safety assessment with real clinical data. For example, comparing multiplex cytokine assays. In nonclinical models you do not know if an increase in certain cytokines will or will not be a problem in the clinical setting. The best way to have a regulatory impact will be the ability to link nonclinical models to clinical data. This should be discussed with regulatory experts.

The biggest challenge is getting access to relevant clinical data. The approach in the end should be two efforts in parallel - one focused on the adverse outcome pathways as a framework to develop and another looking for clinical data for benchmarking the models. Another consideration is looking at the modular nature of the AOPs and determining how well the models under a key event predict or relate the subsequent key event. It means that there needs to be consensus about the key events. Although, this does not mean you have to wait for consensus to proceed as the models could be used under different key events.

One more layer of complexity is the specific therapeutic entities. It is not just for the point of action, it is for the specific entity within a mode of action that we need to consider. It could be that we end up with a toolbox of representative compounds for testing models. There are ongoing discussions about collating a set of compounds for which we can use to test in the models and compare to established clinical safety outcomes.

One other consideration is the novelty of the biomarkers. Many of the current models use simple endpoints and it could be that different molecular profiling techniques might be able to provide translational biomarkers that can be better coupled to clinical measurements and outcomes.

How can we harmonize different MPS/OOAC (micro-physiological systems/organ on chip models)?

This could be done by looking at a common set of compounds or a set panel of readouts. There are a lot of current efforts to develop a comprehensive list of MPS systems and standards. Use of MPS to assess immune toxicology is a new concept.

How to aid safety/efficacy testing for solid tumor treatments?

It is not known if there is a similar cytokine release syndrome when targeting solid tumors. The challenge is more about getting the therapy to the tumor. The question may be more focused on whether or not dosing is adequate. There are a lot of gaps in knowledge about solid tumors. For example, they recruit different cells in response to immune cell therapy. Models should be adaptable to allow for the study of other mechanisms of action. It is likely that the initiating key events would be the same but the other parts of the AOP will like to be different.

Do you think that there will be a biomarker that predicts the risk of CRS?

This is about the difference of being quantitative in prediction or more about hazard identification. There are a lot of factors that likely have to come together for the development of CRS. It may be that the best you get is an assessment that a compound is no worse than other compounds currently on the market.

Position of imSAVAR relative to other initiatives

Beyond imSAVAR, there are no current initiatives that plan for such in-depth mechanism-based assessments of enhanced immune safety models and biomarkers. The current position of imSAVAR compared to other initiatives needs to be continually assessed and is a question we should address at each workshop. The insight that it is very novel is helpful for developing our plans further.

Engaging a stakeholder community

One of the objectives of imSAVAR is to build a stakeholder community and also get input from regulators. There are a number of other initiatives and having a sense of what they are doing and getting some of their experts as advisory board members of this project would be very useful.

There will be other stakeholder workshops where we will review other AOPs. We are also planning a number of online webinars as an initial way to engage a community around imSAVAR.

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.