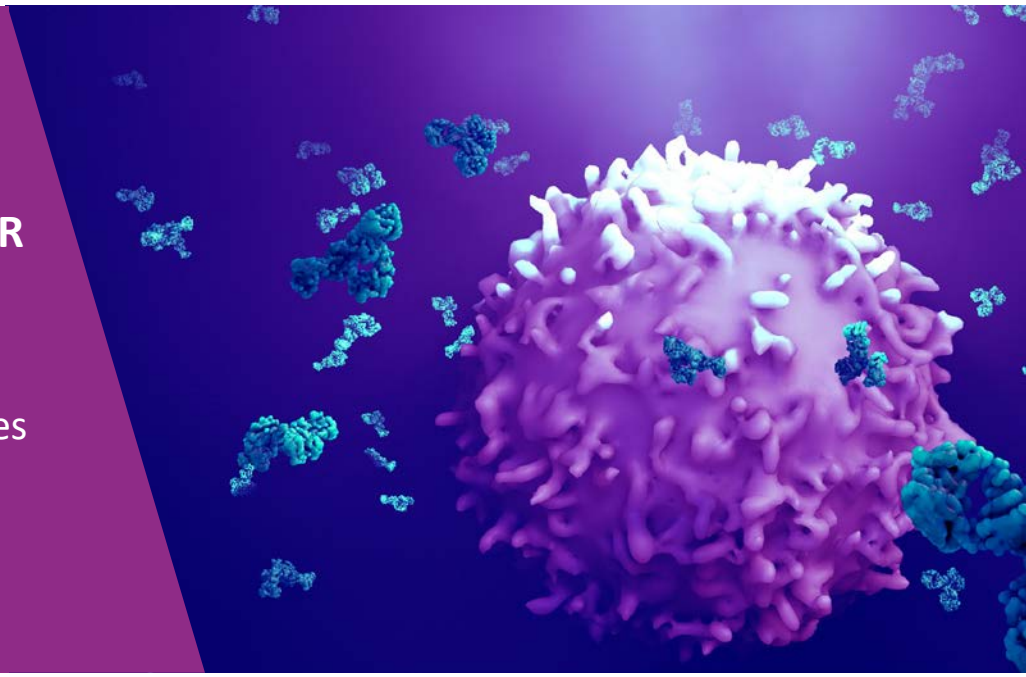
imSAVAR

IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 1.5
3rd 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.

innovative medicines initiative

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Abstract

The third and last of the series of planned imSAVAR Stakeholder Workshops took place over two half days on 12 & 13 April 2021 as an online event. Up to 72 attendees participated in this Workshop including 5 external invitees. The vision of imSAVAR and the impact the consortium anticipates, were further elucidated. imSAVAR aims to better understand toxicities of immunomodulatory therapies for immune-oncology and immune-inflammatory diseases which are insufficiently understood due to the complexity of the immune system along with inadequate existing non-clinical models.

Detailed work on interleukin 2 (IL-2) mediated immune-related adverse outcome pathways (irAOPs)—for skin rash, lung toxicity, vascular leak syndrome (VLS) and hepatoxicity—was presented and followed by feedback on additional avenues to investigate. State of the art assays and model systems used by industry and academia with regulatory acceptance were presented to showcase how these can be incorporated in the AOP framework. Multistakeholder feedback on the value of a grading concept was elaborated. Multistakeholder perspectives indicated broad support for the imSAVAR thematic concepts and helped devise the role of a stakeholder community—in essence a blueprint to maximise the impact of imSAVAR was devised.

Highlights

- AOPs are complex and not linear and creating AOP networks and connecting them will be useful
- Maximising AOPs potential: machine readable, disease maps, standardising, stakeholder education tool, beneficial outcomes pathways use to mitigate AEs and increased stakeholder specific communications
- OECD AOP guidelines are mainly suited to chemicals and imSAVAR work will generate novel insights
- Regulators urge stakeholders to communicate with them early on, especially on innovative ideas such as irAOPs which may also require awareness raising
- Regulators see value in irAOPs as a framework which consolidate available data and provide a bigger picture
- Regulators are increasingly becoming more patient-centric by moving towards patient relevant clinical endpoints
- Patient stakeholders want to be better informed, help direct research to gain best outcomes for patients with focus on opportunities to treatments which are more individualised
- There will always be trade-off between more fit-for-purpose assays versus the holistic models and both have their advantages and disadvantages
- Broad stakeholder acceptance and uptake of concepts like AOPs and grading of models helps:
    - Development of better models and their selection
    - Helps better define research and drug development process
    - Help provide detailed consolidated information for regulatory assessment
- An imSAVAR multistakeholder community can help broaden imSAVAR work to move the field forward

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1. Introduction

The human immune system is too diverse and complex for current nonclinical models to accurately assess safety and efficacy of immunomodulatory therapies for immune-oncology and immune-inflammatory diseases. Current models do not adequately mimic the human immune system, are too species dependent and are unable to reflect the diversity of human response to these novel therapies—this leads to undetected toxicities prior to first-in-human (FIH) studies. A platform of processes for non-clinical safety and efficacy assessment strategies forms the impetus for the multi-partner imSAVAR consortium. The consortium is evaluating the utility of integrating non-clinical and clinical safety experience with data derived from human *in vitro* models, engineered animal models and innovative immune-phenotyping endpoints, to go from FIH safety to clinical safety profiles. The initial focus is on four modes of action (MoA)—specifically CAR T-cells, Bi-specific T-cell engagers (BiTEs), immune checkpoint inhibitors (CPI) and interleukin 2 (IL-2)—and the respective adverse events they elicit and to develop the relevant immune-related adverse outcome pathways (irAOP). irAOPs are modular networks connecting different levels of biologic organization at molecular level, cellular level, or organ/organism level, to a certain adverse outcome. The work of the imSAVAR consortium ultimately benefits access to safer drugs for patients by improving the drug development process (see Figure 1.).

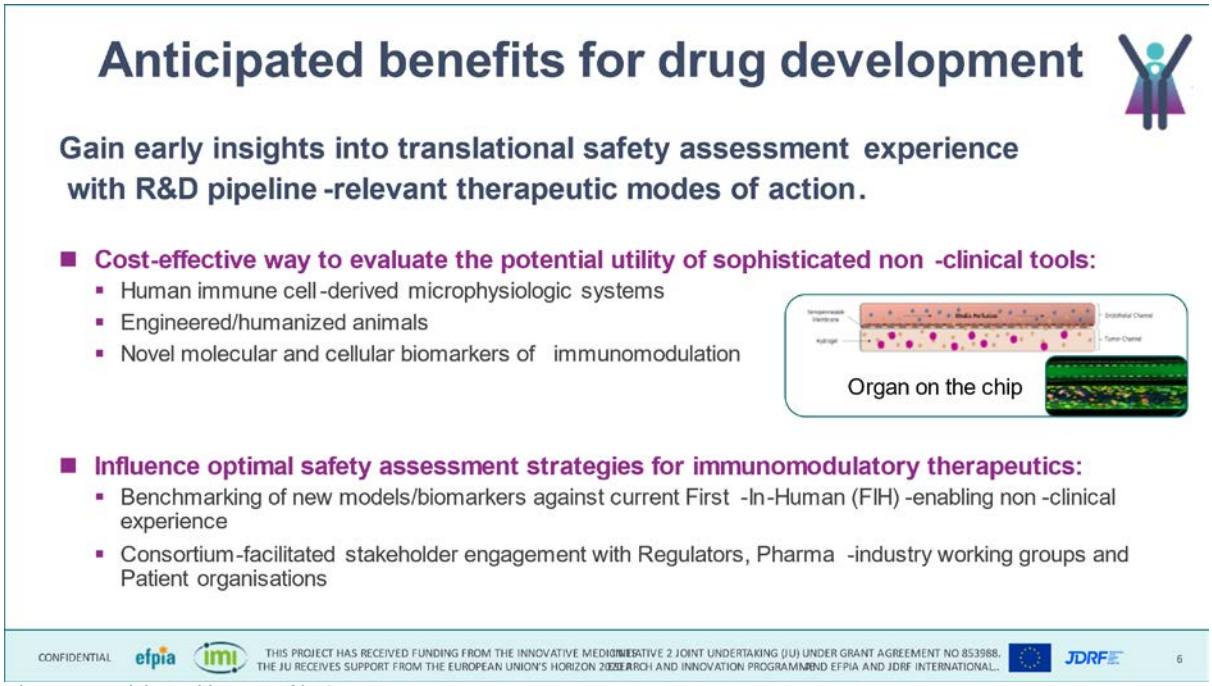

Figure 1. Anticipated impact of imSAVAR

2. irAOP for IL-2 mediated adverse events

2.1 Overview

IL-2 is a cytokine produced and released by activated T-cells with regulatory effects (Tregs) on almost all immune cell types but also on non-immune cells. The IL-2 mediated activation and proliferation of cytotoxic cells (CD8 T-cell and NK cell) as well as B-cells or monocytes/macrophages has led to its use in cancer immunotherapy with the first recombinant IL-2 (rhIL-2) drug: aldesleukin (Proleukin®). There are many adverse events (e.g. vascular leak syndrome (VLS), anaemia, lymphocytosis, eosinophilia, hepatotoxicity, and infiltration of multiple tissues with lymphocytes and eosinophils) associated with proleukin which were also observed in preclinical models, but the patho-mechanism is not well understood. New rhIL-2 drugs aim at modulation of the immune function as shown by low dose administration of proleukin. Preclinical toxicity findings associated with new rhIL-2 therapy are dose and duration dependent with skin rash highlighted as a key adverse event (AE) in comparison to lung toxicity, VLS and hepatotoxicity.

There is opportunity to leverage imSAVAR expertise and assets for enhanced safety assessment of therapeutic Treg modulators with potential to answer the following questions:

Obtain non-clinical mechanistic insight into the sequence of events leading to the major AEs (skin, liver, VLS)?

What is relationship between rhIL-2 dose level and dosing schedule/duration to the onset of skin rash and any additional toxicities (i.e. eosinophilia, hepatotoxicity VLS)?

Can dose selection be optimised by taking into account specific disease states and inter-patient variability?

Can we enhance the discovery of biomarkers for tissue/peripheral Treg subsets that could be used for clinical safety monitoring?

Further description of the IL-2 related irAOPs and roadmap can be found in the following reports: D3.1 & D3.2.

2.2 Skin rash

The clinical signs of skin rash involve cellular infiltrates in the skin. The irAOPs were developed based on the clinical signs and extensive literature research. They bring together different levels of responses from the molecular level (i.e. drug binding site) to the cellular level and ultimately to the organism response level which results in the adverse event—in this case skin rash (see Figure 2.). The next steps will involve drafting hypothetical cellular interactions and to set-up test systems to test the hypothesis.

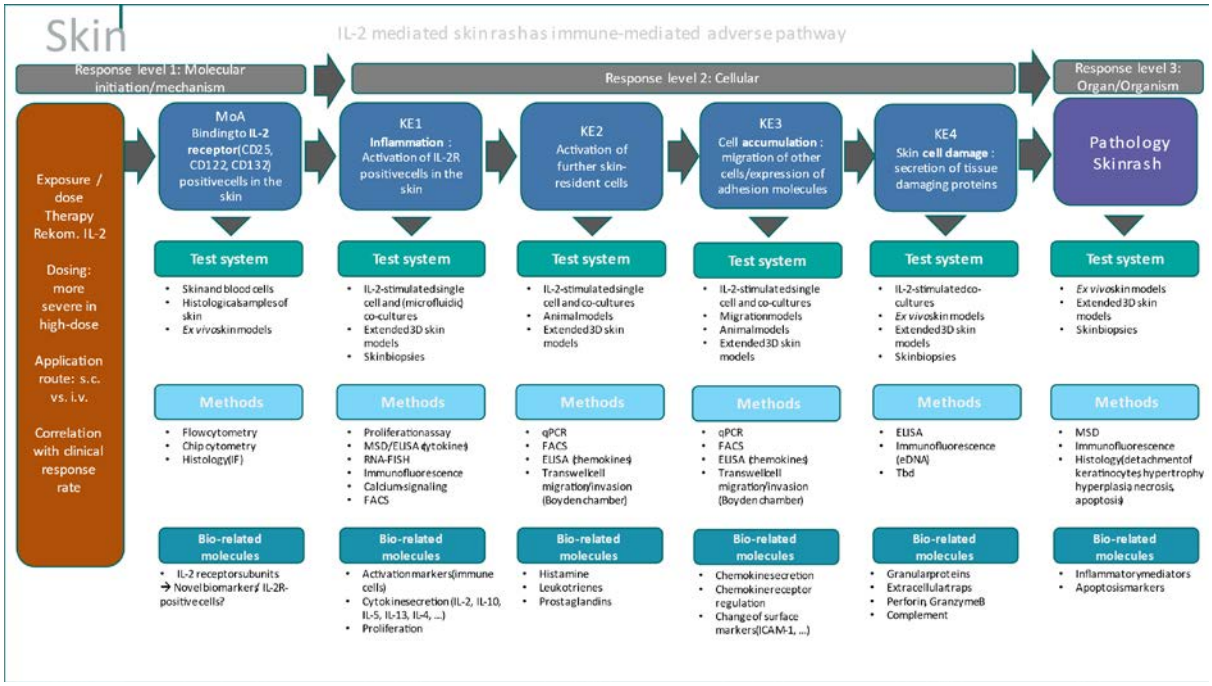

Figure 2. irAOP framework for rhIL-2 mediated skin rash

2.3 Lung toxicity

The lung toxicity AOP is somewhat different compared to the skin rash. Lung toxicity only occurs at high dose rhIL-2 therapy which is only administered intravenously. It is an AE directly resulting from VLS. Cell infiltrates and build-up of fluid in the lungs caused by VLS is supported by extensive literature. There is however a gap in the understanding of the direct effect of IL-2 on lung resident cells and cells infiltrating in the lung due to aero-vascular leak (see Figure 3.). Currently studies are being performed to understand if direct effects of IL-2 on lung tissue increases lung toxicity.

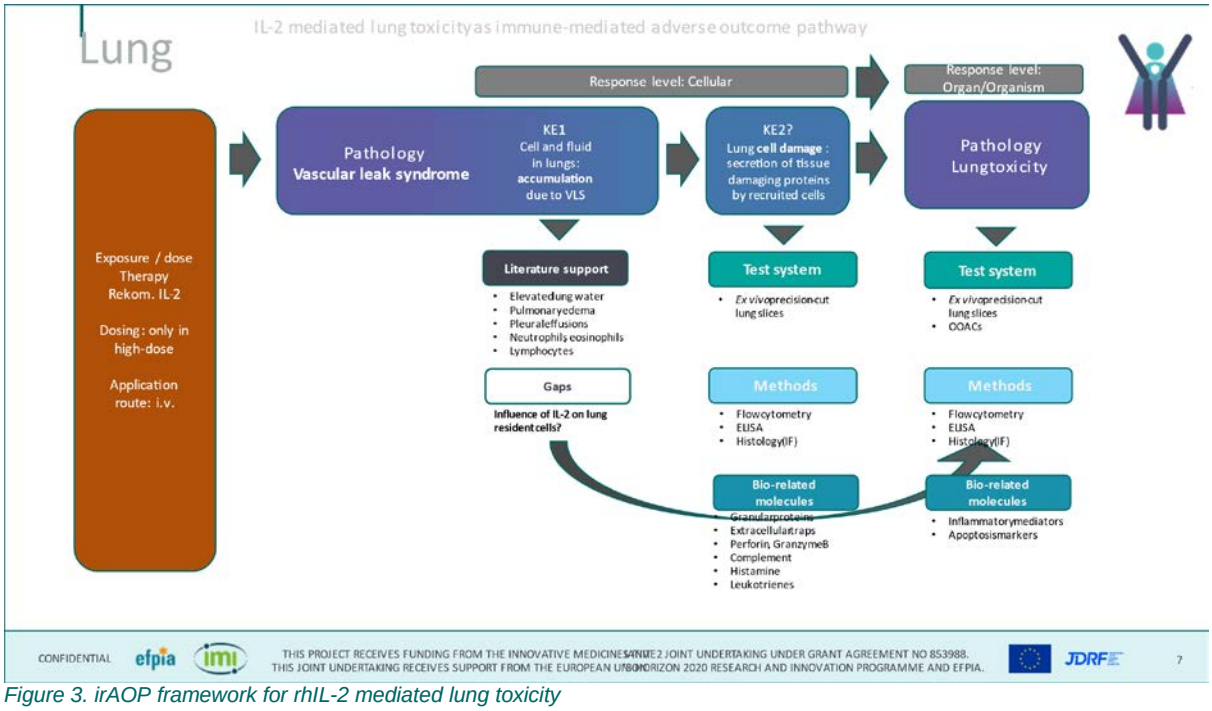

Figure 3. irAOP framework for rhIL-2 mediated lung toxicity

2.4 Vascular leak syndrome

Figure 4. shows the irAOP for IL-2 mediated VLS developed within imSAVAR. The current focus of is on the *in vitro* level with the following readouts: (1) FACS and (2) ELISA. The molecular initiation event (MIE) is the mode of action binding to IL-2R positive cells. The complexity at the organism level requires microphysiological systems (MPS). The various immune cells sense IL-2 in the blood and this is where they can also be activated. Through literature research and lab experiments, it was confirmed that endothelial cells are not directly activated by IL-2 stimulation. However, endothelial cells may play a role in the indirect activation of cytokines and chemokines. There are many contributors to the pathology of vascular leakage, and this will be further investigated.

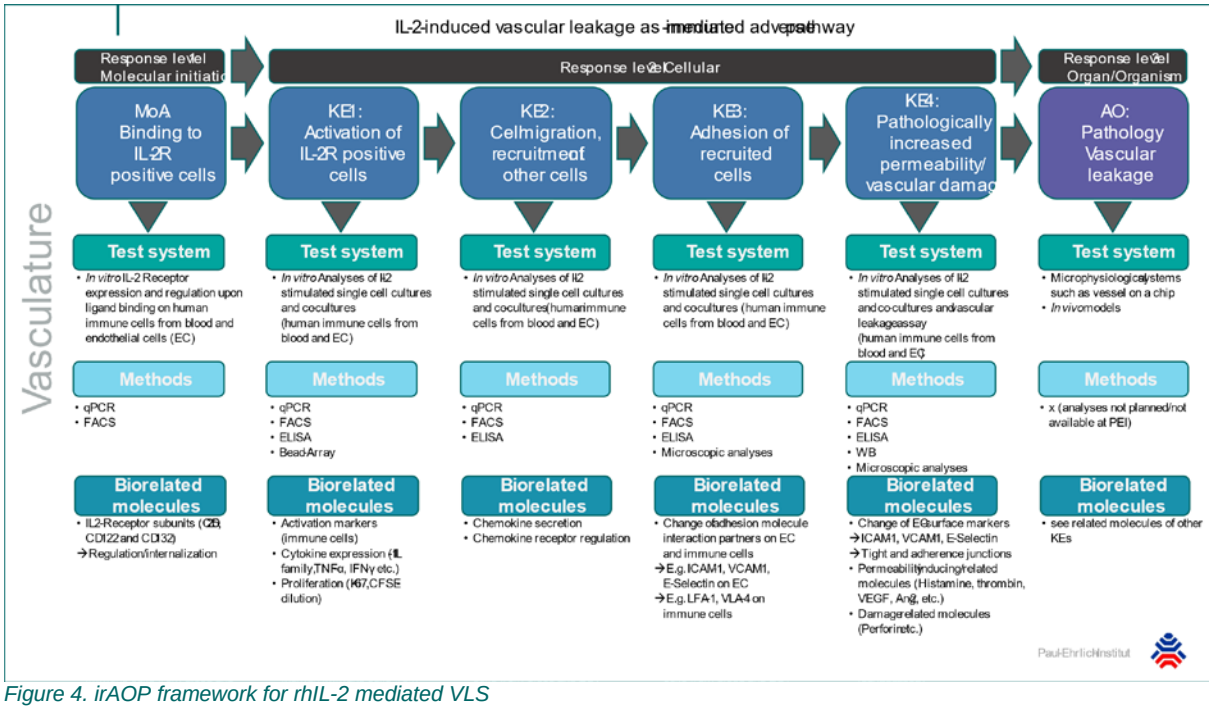

Figure 4. irAOP framework for rhIL-2 mediated VLS

2.5 Hepatotoxicity

The liver is largest bodily organ and has many different functions (e.g. protein synthesis, metabolic functions) and also includes immunological functions. Under homeostatic conditions, there is constant balance between inflammation and resolution. There is an increased macrophage population in the liver, namely the Kupffer cells. Researchers have an interest in knowing which immune cells populate the liver as liver resident immune cells determine immune properties. Isolated liver cells from liver perfusate for analysis of liver cell types, showed 60% were NK and T cells. Murine experiments also show NK and T cells are recruited to the liver under inflammatory conditions.

There will be an emphasis on NK and T cells in this AOP due to their ability to bind and react to IL-2.

It is clear that the NK and T cells that are liver resident, show different traits than those circulating in peripheral blood which is important for future experiments. Increased proleukin triggers the cascade of events from key event 1 (KE1) to the ultimate pathology—hepatotoxicity (see Figure 5.). Experiments assessing KE1 and KE2 are ongoing. CD8 mediated hepatotoxicity is depicted in the irAOP. CD8 express typical proinflammatory markers and these cytokines may already act on Kupffer cells or directly with hepatocytes through fenestrations. Impaired blood flow contribution by CD8+ does not appear in the literature as shown in KE3.

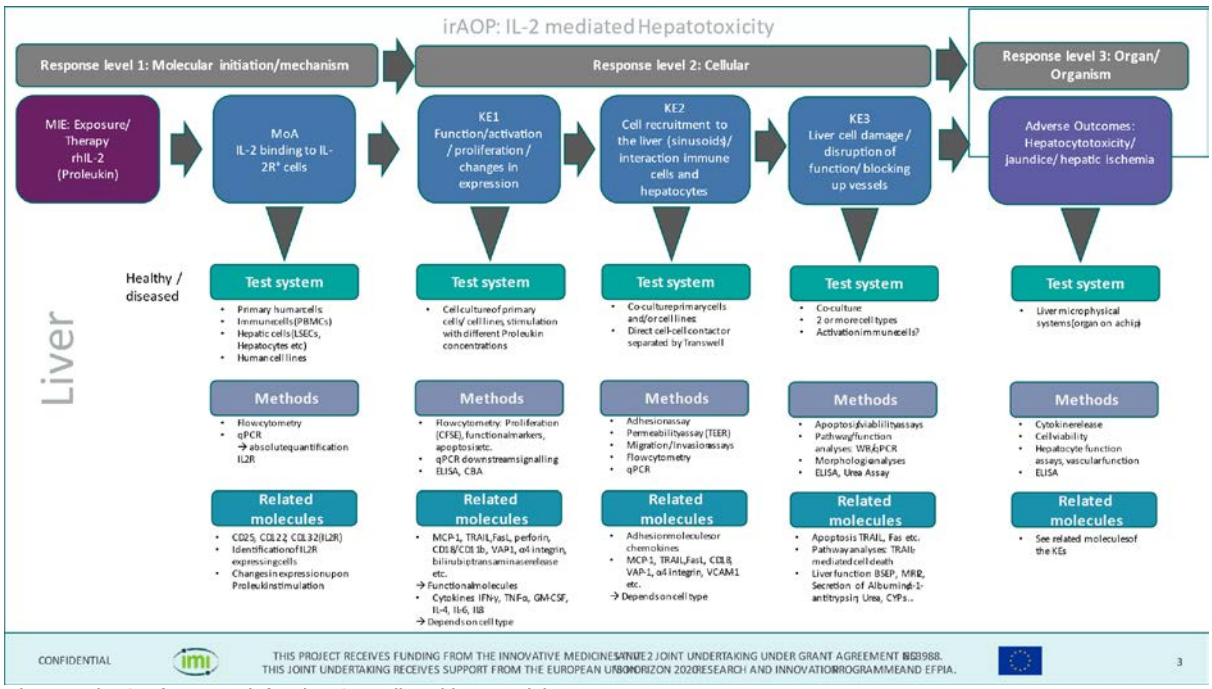

Figure 5. irAOP framework for rhIL-2 mediated hepatoxicity

2.6 Dialogue on irAOPs

The irAOPs are intricately detailed but they are in the developmental phase and require further improvements and are open to feedback.

IL-2 Therapy

Recombinant IL2 demonstrates limited observed toxicity but is efficacy maintained?

For pre-clinical models, toxicity is lower compared to proleukin but still present and there is some efficacy, but a lot of variability caused by genotype and phenotype. A better understanding from clinical phases is needed but unavailable to share as studies are ongoing. Skin rash is considered a higher risk for the recombinant proleukin compared to vascular leakage and hepatoxicity.

Differences between new recombinant IL-2 therapies and proleukin

One major difference is that the new rhIL-2 is made by an antibody with a longer half-life and therefore requires less dosing frequency as opposed to proleukin's half-life which is very short. Also, there are a few mutations on IL-2 making it less potent to trigger the trimeric and therefore needing a high dose to stimulate beta, gamma, low affinity IL-2 receptor.

Any evidence on IL-2 receptor form expressed by ILC2 in respect to other lymphoid cells and the response to IL 2 and ILC2 capacity to secrete chemokines to recruit further T cells?

ILC2 expresses all 3 receptor subunits (alpha, beta and gamma) and can form low affinity IL2 receptors and be activated during low does IL2 therapy and not only high dose therapy which induces vascular leak syndrome.
ILC2 have a THC2 type cytokine profile. They could perhaps play a role in recruitment of T cells. Due to the VLS they may appear in the biopsy but could be that they are passively transfused. Distinction between active and passive recruitment is needed.

Hepatoxicity

For the hepatotoxicity AOP will other cells shown in KE1 (e.g. Kupfer Cells, HSC, IHL) be investigated?

It would be difficult to assess Kupffer and HSC directly as these cells cannot be isolated as the liver is not very accessible but can only check literature. Macrophages could be used as substitutes but with the limitation that this is not entirely representative. In parallel other cells, T helper & NK Cells, will be screened for responses to demonstrate which has impact on hepatoxicity.

Could a liver slice be an option for a test system?

Good option but access to tissues slices and experience in working with them is at present a problem. In Hannover there is access and experience, but current work is focused on toxicity rather than immune system. However, doing this work will help understand the effect of IL 2 on these cells and this is a good way to refine the next key event (KE) better and not miss potential path-mechanisms.

Future direction of irAOP work

Will the numerous tests and assays be narrowed down and how will this be decided?

At one point they will be narrowed down but currently there is not just one good test system.

Will AOPs be shared via publications or entered in the AOP wiki?

Publishing is important for sharing knowledge as the patho-mechanisms are useful to know Detailed roadmaps will be laid out and input into the AOP wiki is envisaged as well as making them machine readable for bioinformatics use and creation of disease maps. As the AOPs are complex, and related to multiple organs, it may be useful to first network the AOPs prior to input into the AOP wiki. Deliberations are ongoing on how to best depict the AOP networks and connect them. Current basis for AOPs is on chemicals and imSAVAR can likely add a different perspective to better inform the concept.

The ambition would be that AOPs are used as a framework in the regulatory context for people to know what kind of models to develop and use. In a larger context it would be the community consensus on the mechanism of toxicity and how to test it. This assists drug developers to know that these are the preclinical models they need to perform. We also need to understand if this concept can be acceptable to regulators.

3. Grading assays

imSAVAR is interested in developing a grading system for preclinical models based on a set of criteria—potentially to better guide developers. In order to evaluate and garner feedback for this concept, understanding the current landscape of available and approved assays and models commonly used in industry and academia, how they can be incorporated within irAOPs and how to select the right assays is imperative.

3.1 Current state of the art in models and assays

In vitro assays materialized as a consequence of the test drug TGN1412 which induced multiple cytokine release syndrome (CRS) incidents in the FIH clinical study but was not predicted in the preclinical programme. Many assay formats and strategies are in use across the pharmaceutical industry and CROs and therefore regulatory & technical best practices are evolving. As current assays were optimised based on TGN1412 as a positive control, they may not be appropriate for all MoAs. There is no uniform approach in designing a cytokine release assay (CRA) strategy for new molecules, but it should focus on molecule specific scientific considerations. Commonly used assays to address CRS are quite simplistic and use whole blood or PMBC cultured with soluble or immobilized drug. Variations include co-culture of PBMCs with endothelial cell lines and different stimulation and culture conditions. Each of the assays and immunes cell matrices have their pros and cons and should be used in order to optimise or improve assay results (see Table 1. and Figure 6).

Assays
Soluble phase drug presentation with whole blood or PBMCs
Solid phase drug presentation with whole blood or PBMCs
Römer assay: High density pre-stimulation of PBMCs followed by soluble drug presentation
Endothelial cell (HUVEC/BOEC) PBMC co-culture assay with soluble drug presentation

Table 1. Common in vitro CRAs

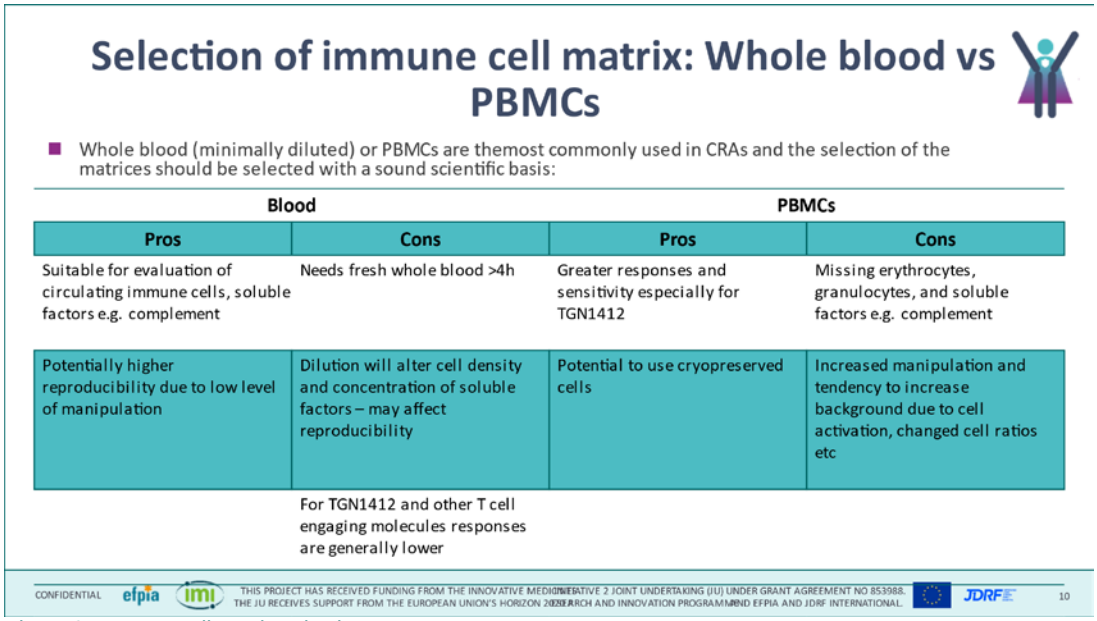

Figure 6. Immune cell matrix selection

The use and selection of CRA formats is a hot topic in the biopharma and CRO sector with many publications on cross-industry comparisons of different assay formats for MAbs and BiTEs.

Current models only take into account circulating immune cells but a complete physiological model which better mimics the existing complexity of human immune responses is needed. A shift away from individual MoA could occur via recent developments in microphysiological systems (MPS) which comprise of organ-on-chip technology and organoids. Within imSAVAR, emphasis is on organ-on-chip technology for which there are also various types and options—including targeted tissue and non-targeted tissue that are perfused with circulating immune cells and integrating tissue resident immune cells. A cytokine release assay based on an organ-on-chip system would involve the following steps:

1. Generate tissue model;
2. Perfuse drug and PBMCs;
3. Monitor recruitment;
4. Time-resolved perfusate sampling; and
5. Measure kinetics of cytokines.

By using iPSCs there is potential to create a fully autologous MPS. Although, broad adoption of MPS systems is hindered by low technology readiness levels (TRL), complexity, throughput and costs.

3.2 Integrating assays within irAOPs

The irAOP of CAR T-cell mediated CRS (further elaborated in documents D2.1, D2.3 and Figure 7.) was used as an example to demonstrate how to incorporate models and assays common to the academic and industry setting.

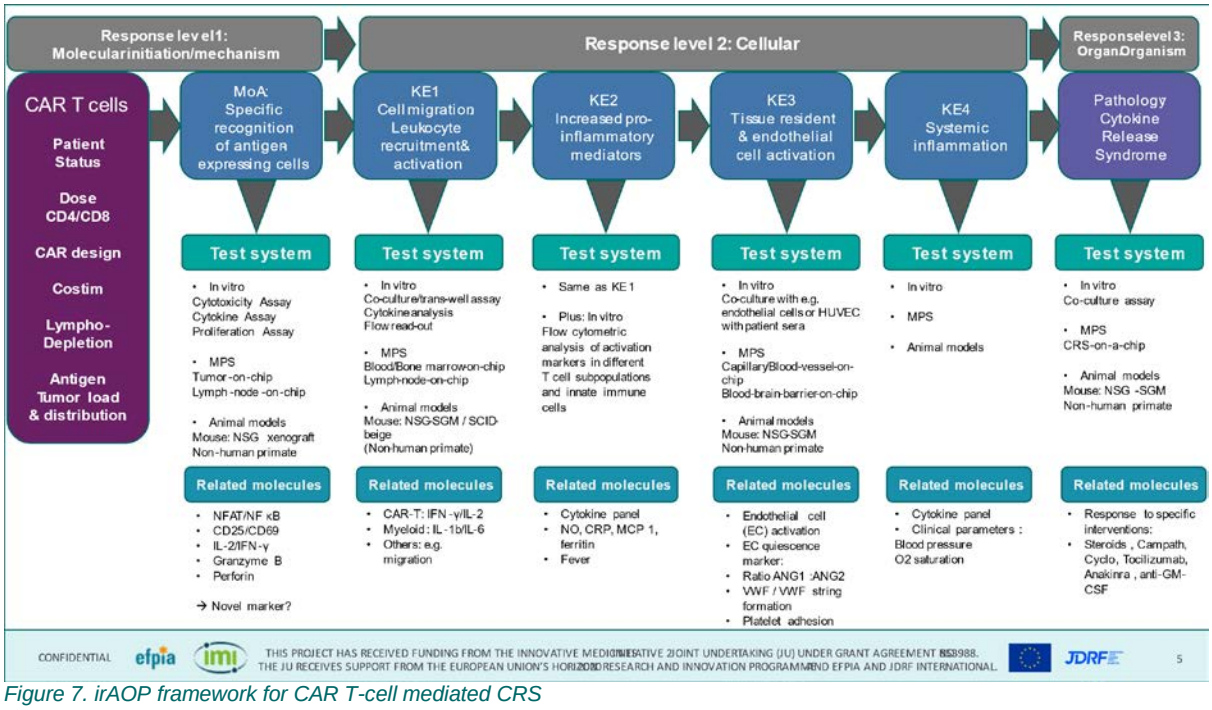

Figure 7. irAOP framework for CAR T-cell mediated CRS

The same state of the art assays and MPSs described previously were analysed in the context of safety and/or efficacy testing for CAR T-cell therapy irAOP and appliable to the different KEs elucidated in Figure 7. As is evident from Table 2 and Table 3, there are some recurring limitations to the assays and systems pertaining to certain important cells missing and confounding results due to HLA mismatch when using cells or cell lines from different donors.

Test system	Purpose	Possible read-outs	Controls (examples)	Limitations
Co-culture of tumor and immune (CAR T) cells	Efficacy testing. Useful to study CAR-T MoA and KE1/2	• ELISA • Multiplex cytokine analysis • Flow cytometry	• Cell culture medium • Mitogens (e.g. PHA) • Tumor cellslacking antigen expression	Bystander (immune) cells missing
PBMC assay(incl. high cell density culture „RESTORE“)	Efficacy and safety testing of mAb. Could possibly be adapted for CAR-T to assess KE1/2	• ELISA • Multiplex cytokine analysis • Flow cytometry	• Cell culture medium • Mitogens • Isotype controls • anti-CD28 (e.g. TGN1412)	Described for cytokine release mediated by mAb. Might be difficult to adapt for preclinical testing of CAR T cells
Co-culture assay ofe.g. BOEC or HUVEC and PBMC [and tumor cells]	Safety testing possible. Could be adapted for CAR-T to assess MoA, KE1/2/3	• ELISA • Multiplex cytokine analysis • Flow cytometry	• Cell culture medium • Mitogens • Isotype controls • anti-CD28 (e.g. TGN1412)	Confounding results due to HLA-mismatch might occur if cells are obtained from more than one donor
Whole-blood assay	Safety testing for mAb possible. Might be useful in an adapted versionfor CAR T cells (MoA, KE2/3)	• Same as above	• Same as above	Described for cytokine release mediated by mAb. HLA-mismatch if using 3rd party donor.

Table 2. Integration of assays into CAR T-cell irAOP

Test system	Purpose	Possible read-outs	Controls (examples)	Limitations
Cancer on chip	Efficacy testing. Useful to study CAR-T MoA and KE1/2	• Imaging (monitor cell migration) • Multiplex Cytokine Analysis	• Cell culture medium, conditioned • Tumor cells lacking antigen expression	• Bystander (immune) cellsmissing
Vessel on chip	Safety testing possible. Could be adapted for CAR-T to assessMoA in a patient specific fashion (KE2/3)	• Live imaging (monitor membrane integrity) • Multiplex Cytokine Analysis	• Cell culture medium, conditioned from cancer-chips • Membrane disruptors • Endothelial cells stressors(e.g. TNF -α)	• Cancer cellsmissing • HLA-mismatch if using3rd party donors
Vascularized cancer chip	Safety testing possible. Could be adapted for CAR-T to assessMoA in a patient specific fashion (KE1/2/3)	• Live imaging (monitor membrane integrity) • Imaging (monitor cell migration) • Multiplex Cytokine Analysis	• Same as above.	• To evaluatethe order of the observed events. • HLA-mismatch if using3rd party donors
Vascularized cancer chip connected to vessel on chip	Safety testing possible. Could be adapted for CAR-T to assessMoA in a patient specific fashion (KE1/2/3/4)	• Same as above.	• Same as above.	• HLA-mismatch if using3rd party donors

Table 3. Integration of MPSs into CAR T-cell irAOP

3.3 Selecting the right assay or drug development

The use of assays in the context of drug development provides a broader picture of the types of considerations that are in play. Performance of a CRA is considered a regulatory expectation to evaluate the potential risk for cytokine release for any given molecule. Yet, there is no consensus on when and how to perform CRAs but it is clear that they should be driven by mechanistic considerations such as:

- expected pharmacology activity;
- molecule structure;
- agonism vs antagonism;
- presence of Fc effector function;
- likelihood of cross-linking;
- potential for cell activation; and
- target expression (cell-based vs soluble).

Each biopharmaceutical company adopts its own decision process on whether to perform CRAs and what formats are used. The rationale behind both stages is intricate and involves numerous parameters, to ensure CRAs are performed for the right reasons and under the right conditions. Other assay specific conditions beyond the format include:

- test concentrations;
- controls;
- cytokine detection;
- which cytokines to measure;
- and donor number.

Data interpretations and potential follow-up activity related to positive results for an *in vitro* CRA are based on the likelihood of cytokine release related to the MoA or not; leading to further investigations and development of risk mitigation strategies.

3.4 Panel discussion on assessing and choosing the right model systems

Discussion was based on how to choose and grade the right assay where the grading depends on how predictive the model is of the clinical situation. In addition focus was placed on biomarkers and how they can facilitate the selection and grading of assays.

Timepoints used for the assays

This can be a struggle but the assays have been set up with positive controls and the best timepoints for when to look for these cytokines. For example IL-2 has to be looked at earlier than others. Typically assays run for 48 hours to at the most 72 hours, although there is limited difference between these timepoints. In order to view changes in cytokine release, early timepoints (i.e. at 6-8 hours) or at 48 hours.

Inclusion of tissue resident immune cells in CRAs

In most cases for CRA these cells are not included for classical CRAs. Upon review of the entire mechanism of action and the target, if of concern, the CRA would then be adjusted. The high density PBMC assay may also address this issue. There will always be trade off between more fit-for-purpose assays versus the holistic models and both have their advantages and disadvantages.

Use of isogenic models

Isogenic human disease models are a family of cells selected/engineered to accurately model the genetics of a specific patient population, *in vitro*. They are provided with a genetically matched 'normal cell' to provide an isogenic system to research disease biology and novel therapeutic agents. These models have not been considered in general within industry and for imSAVAR. It may be important to include some diversity which is reflected in the relatively high number of donors included in the assays used. There are fit-for-purpose assays and holistic models—both have their merits and there need to be cost benefit trade-offs.

Predictive capacity of are preclinical models at mimicking what will be observed in patients following novel treatments

Translatability of these assay is a struggle for industry along with which assays to use. Currently there are some industry initiatives and consortia attempting to advance in this area but presently there are no studies that correlate data from *in vitro* assays and clinical settings. As assays have to be fit-for-purpose, correlated with preclinical data, validated and reproducible; their development is not an easy feat. The same holds true for biomarkers but this is where the irAOP networking can come into play by spreading across the key events. Hence identification of biomarkers for key event can help set up criteria to develop assays.

Can biomarker development be facilitated by expanding the number of molecules preclinical models are able to measure.

Both in the clinical and preclinical setting**,** only a very small number of molecules are tested. From the clinical side, it is likely technically feasible to expand the number of molecules able to be measure but it is expensive and burdensome on patients. In addition with more data collected there can be data interpretation issues. The lack of correlation for example with cytokines measures does not always correlate with CRS in patient which raises the question if this the correct biomarker.

Developers struggles with which models to create but industry also struggle with model selection. Therefore the prospect of the imSAVAR grading system could in addition to providing guidance to developers feed into the regulatory context.

4. Multistakeholder perspectives on irAOPs

As one of the aims of imSAVAR is to bring a degree of harmonisation and alignment to the process of immune safety assessment it is important that we engage with diverse stakeholders. It is also of critical that we gather stakeholder input to inform the effort to refine and develop the irAOPs, and models. This is particularly relevant in terms of regulatory advice. Building a community around the irAOP concept also helps build momentum for their subsequent uptake.

4.1 Patient stakeholder perspective

Patient perspective in the research setting is often underrated based on various assumptions and not sought in a timely manner. However, patient organisations, such as Melanoma Patient Network Europe (MPNE) and others demonstrate that the patient voice is very well informed and organised. MPNE works according to network principles where science is given utmost importance and having participated in multiple medical research consortia, it can hold a research agenda accountable to patient centeredness. Patients are encouraged and empowered to take an interest in research which involves their well-being rather than blindly trusting their medical expert. The ability of patient stakeholders to shape anything depends on the level of awareness of the setting and processes. What can patient stakeholder contribute to research? They can:

- ensure that projects get their initial research questions right, monitor the translation and ensure the transition in between is successful;
- draw attention to usual incentivisation of publications does not lead to improving patient outcomes; and
- draw attention to siloed data and results within consortia due to lack of leadership.

Why should patient involvement occur in research? This is a two-fold concept with a (1) “pull” or passive side and (2) “push” or proactive side. The first part is purely about accountability as most research is funded by citizens and society today expects transparency. The second part is more focused on getting the best possible outcomes from research for the patient. Patient stakeholders can anticipate the needs of regulators and HTA. Therefore patient organisations are more interested in the overall project and its outcome rather than communications/dissemination work. Making research results more accessible is something of great interest to patient communities and more often than not, patients provide unusual perspectives of great value.

4.2 Academic perspective

As previously demonstrated, AOPs are modular networks that link molecular, cellular, and organ/organism level effects across complex biological space to an adverse outcome and may be implemented for *in vitro* systems. They have a common structure consisting of a molecular initiating event, a series of KEs connected by key event relationships (KERs) and an adverse outcome. The AOP concept is a new toxicity testing paradigm requiring mechanistic insights. The international AOP programme is spearheaded by the OECD. From the academic perspective, there is currently an urgent need to better understand the risk of therapeutics for immune-oncology and immune-inflammatory diseases, including infection, CRS, malignancies, and autoimmunity. imSAVAR wants to improve the productivity of nonclinical safety testing methods by the application of appropriate test models. Immunotoxicity is described for many different types of drugs, with immunotoxicity of small trucks being relatively well understood, in particular regarding their mode of action. As shown in Figure 8, new therapies yield new immunotoxicities.

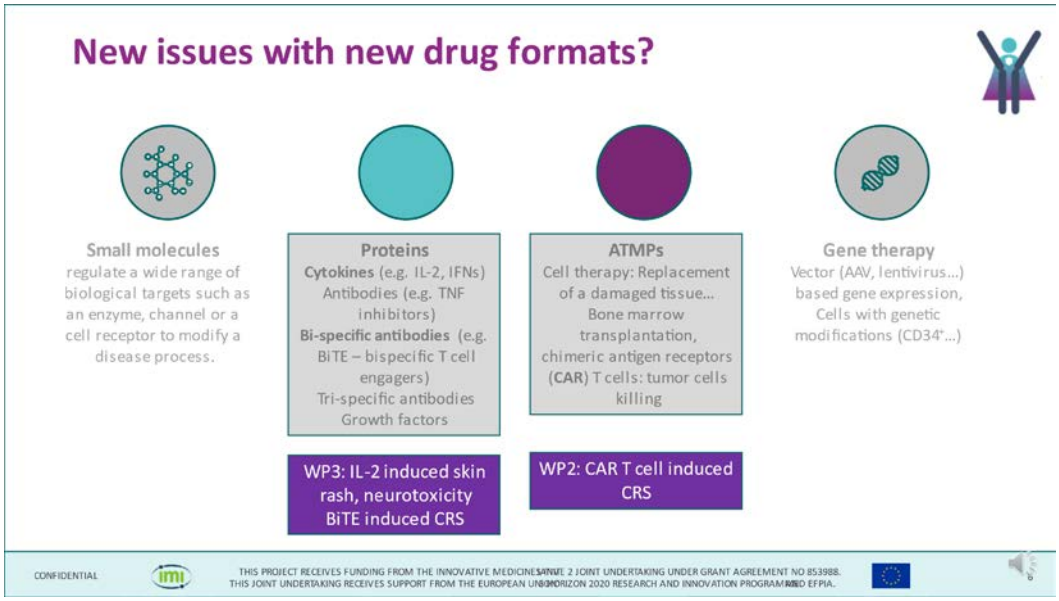

Figure 8. Drug immunotoxicities

Another aspect of imSAVAR is to support the evaluation of preclinical models by the identification of biomarkers. Biomarkers will be used as indicators for studying the underlying immunological process leading to toxicities.

The current status of imSAVAR after development of the previously presented irAOPs merits the following questions:

- is prioritization needed?;
- is further development and linkage evaluation of the hypothesized AOP possible?;
- what experiments are needed to fill gaps and/or add to the weight of evidence?;
- and will AOP information be deposited in an appropriate knowledge base.

4.3 Industry perspective

Everyone agrees that there is limited understanding of human immunology although with the help of technology progress is being made. It is interesting to see, based on Sars-CoV-2 AOP work which seems relatively straightforward, how a linear process can be turned into a network. Thus, for immune-mediated and inflammatory AOPs, the complexity would be even greater.

From biopharma perspective the relevance of irAOPs is linked to the broad portfolio of cancer immunotherapy drugs where immunotoxicities will likely become more common and require better prediction and mitigation strategies. In particular, the AEs linked to CPIs have become better described as they have been in clinical practice for a period of time. The use of CPIs is rising exponentially—with approximately 50% of cancer patients in 2019 in the USA deemed eligible—and nearly 50% of patients treated will experience some form of irAE. Designing irAOPs may help the refinement and validation of *in vitro* models applied to the safety of immunomodulatory drugs. The key advantage is it can create a research framework for hypothesis testing in a collaborative manner such as a consortium like imSAVAR. A potential limitation of irAOPs could be trying to fit a square into a round hole—in other words the irAE are too complex for AOPs.

A basic internal company survey yielded insight into the general lack of awareness irAOP concept of and the limited awareness that existed, lay with toxicologists. Despite the lack of awareness of the concept,

most of the surveyed group perceived the relevance and helpfulness of the irAOPs highly. The top advantages and disadvantages beside the ones previously listed are shown in Table 4.

Advantages	Disadvantages
Identify gaps in terms of assays/technologies	Favour a biased scientific approach towards what we think is happening →look for cytokines that are the usual suspect when we may have more exotic contributors that are not considered. How to test key events with an open-minded fashion?
Facilitate interactions with regulatory agencies and may also facilitate interactions with clinicians and patients Identify gaps in terms of assays/technologies	

Table 4. irAOP advantages and disadvantages

4.4 Regulatory perspective

The AOP concept is not new and has been described previously as 'toxicity pathways', 'mode of action' and 'mechanism of action'. The critical defining factor of an AOP is it links a MIE to an adverse outcome, which is of importance within a regulatory context. It must contain an appropriate level of information regarding the causal key events that links the MIE and an adverse effect. Due to these unique properties the AOPs could be of tangible benefit to the development of new strategies for the safety assessment of new drugs. In addition, AOPs will not likely be linear pathways but cascades and this is an important message to reinforce—AOPs should not be simplistic otherwise important details will be lost.

The high specificity of the interactions of immunomodulatory biologics with their relevant immune targets (on-target effects) should nullify off-target effects. Despite generally superior safety profiles compared with small-molecule immunomodulatory drugs, clinical concerns relating to adverse reactions for immunomodulatory biologics have emerged. This deters development and early clinical investigation of many immunomodulatory drugs. Understanding limitations of nonclinical species for human safety assessment and supplementation of *in vivo* safety assessment with appropriate *in vitro* human assays is frequently needed. ICH S8 (Immunotoxicity Studies for Human Pharmaceuticals), which came into operation in 2006, provides testing recommendations. The TGN 1412 disaster also occurred in 2006 and the trial sponsor had conducted a nonclinical package compliant with ICH S8 guidelines. The effects seen in humans is rarely predicted in animals. As immunotherapies are becoming frontline treatments, it is crucial to separate adverse events from intended pharmacology.

Investigators and regulators suggest having a tiered approach to assess the effect of immune system, function and risk infection in cancers and we need to have a really good understanding of the features of these immunomodulatory drugs. With more data generated, interpretation becomes a challenge and thus an AOP approach put it in context and get a much thorough understanding of the comparative immunology, not just between animals and humans, but also different people.

Standardising irAOPs will be needed if wider uptake is desired. In turn this leads to:

- reduction in use of animal models which are not predictive;
- help industry with drug attrition reductions; and
- and lower adverse events.

However, the mentality of ticking boxes should be abandoned and reliance on regulatory guidelines should be sought as last refuge. Convincing many that the irAOPs is the best way forward will be challenging but a better way of drug development which is more patient oriented. Regulatory agencies are becoming more patient centric by moving towards patient relevant clinical endpoints.

It is also key to communicate and engage regulators irrespective of the type of stakeholder to resolve challenges. irAOPs are the future as they help consolidate the data better and present a bigger connected picture.

4.5 Physician perspective, uptake of AOPs and link to 3Rs

The Physicians Committee for Responsible Medicine (PCRM) is a member supported advocacy group that undertakes collaborative activities to progress modernisation of toxicology in medical research. They want to see patients reflected in nonclinical models and improvements in drug development which can be achieved by using more human relevant systems rather than animal models. AOPs are viewed as key to this shift which is why PCRM is invested in AOP development, training, and outreach to support the OECD AOP program and concept and to make sure it succeeds. Given imSAVAR's aims, it is evident that PCRM supports the project. In addition, there is stakeholder support for non-animal methods, advancing AOPs and building regulatory support which can help imSAVAR efforts.

The AOP stages are now clear to all in the workshop but an element that is as important as KEs are KERs. Information about the transition from one KE to another would be captured at this level-–including evidence for causality and quantitative dose-response or threshold. This plays an important part of helping to support testing strategies based on the AOPs.

AOPs were taken up by the OECD to solve an issue in the chemical toxicology to link mechanistic data to *in vivo* knowledge and benefit from the immense biological knowledge we already have that might not be easily accessible. The AOP wiki is a publicly accessible tool used by most AOP developers and is essential to advancing the programme.

AOPs are being employed in regulatory contexts to support development or use of New Approach methodologies for OECD test guidelines and case-by-case approaches at US EPA. There are ongoing developments to increase the application of more AOPs. However a balance is needed in the level of effort put into developing an AOP compared with the needs of the AOP as a tool for a use case (see Figure 9.).

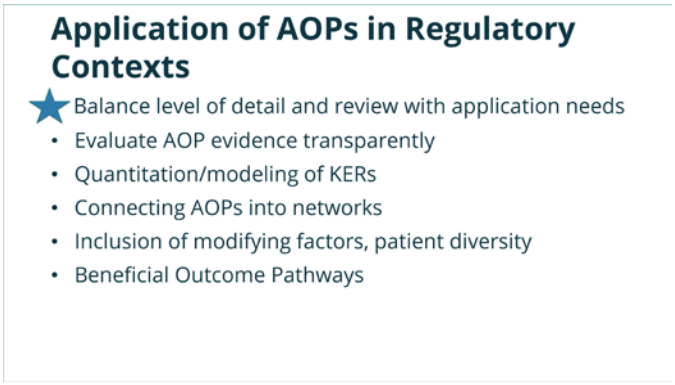

Figure 9. AOPs in regulatory context

The concept of a beneficial outcome pathway is being investigated in the EC JRC initiated CIAO project focusing on AOPs for COVID-19. The AOP framework is used to map the impact of positive stressors (e.g. treatments/therapies, masks) to assess and compare the outcomes. Another topic being investigated is gaining a better understanding of why the disease affects people differently by capturing modifying factors (e.g. diet, existing comorbidities, genetic makeup) that may make certain KEs more or less likely to occur and how to incorporate this in the AOPs.

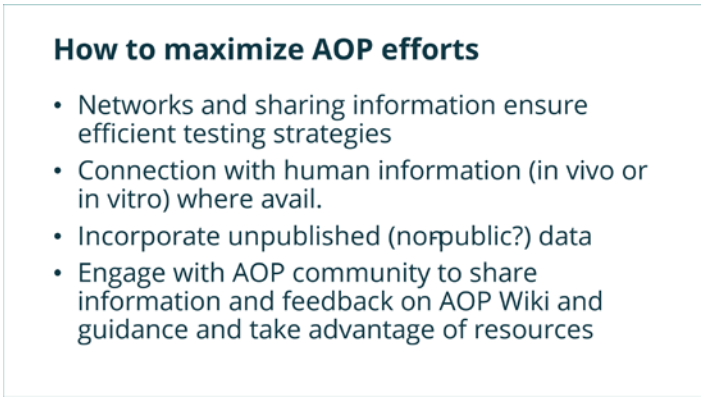

Figure 10. Maximise AOP efforts

As mentioned in earlier discussion, AOPs are the future—it is vital to maximise the impact of the ongoing AOP work (see Figure 10.).

4.6 Panel discussion

Opportunities to maximise AOP efforts

Do mitigation strategies fit within the concept of beneficial outcome pathways?

Mitigation of unintended adverse events is a good application of this concept.

A lot of data is required to build irAOPs

This is limiting but it is key to understand that the irAOPs should be used in a flexible and transparent way based on the level of data available and can still be useful.

OECD guidelines do not provide a clear guidance on how to incorporate biomarkers within AOPs

Not a lot of biomarker development information is included in the OECD guidance due to the origin of the AOP concept being based on chemicals. imSAVAR work on biomarker identification is an important step in maximising the value of the AOPs.

Patient context

Could irAOPs be used to teach/inform oncologists about safety issues?

This is again linked to better dissemination strategies and earlier on.

Patients know that there will not be a 100% safe drug but how to find a balance for an overarching scheme that is valid for all patients but includes individual variability (i.e. personal modifiers)?

Patients are desperate to know more about therapies and must weed through a plethora of misinformation. Patient no longer perceive doctors as gods. This is also why regulators are trying to move towards patient relevant clinical endpoints. Patient forums are good to pick up on side effects for patients to become more aware. The key issue is that the clinical trial setting is different from real world setting and it is enormously difficult to pick-up side-effects and adverse events in small populations of clinical trials. Key example of this is with the COVID-19 vaccines and links specific clots—it is not clear if the clots are linked to the vaccine or not. This is why pharmacovigilance is an extremely important concept as trials cannot be done in millions and drugs must get to the market as quickly and safely as possible to prevent deaths. It all comes down to balance between benefit and risk.

Regulatory context

Early communication of irAOPs to regulators

It is not necessary that all regulatory bodies and relevant staff are aware of irAOPs and this highlights that early communication with regulatory bodies is needed and encouraged. Companies look at the intended target effects whilst developing products and often neglect off target side effects, where the target is expressed in a different organ or in a different issue. The irAOP could make it easier to visualise or capture this and thus be useful for regulators.

How important is it that there is broad community buy in for irAOPs for accepting the concept?

Pharmaceutical development is a global process. The ICH try to create guidelines which everyone can buy into but this is a complicated process and depends on countries and regions buying-in. There are many topics in which regulators do not always agree or follow the same principles. The OECD AOP guidelines are nice but mainly suited to chemicals and are not well received guidance for other treatment development. It is also challenging to get biopharma to buy-in to new concepts as they wait for the regulators to mandate them. However, regulators cannot mandate addition of new concepts unless they are backed by adequate data. The risk assessment behind chemicals versus pharmaceuticals is very different since you do not want human exposure to chemicals in the first place.

irAOPs were also originally developed to avoid animal testing

This is correct in general; the pathways are there to help contextualise information from high throughput assays and *in vitro* assays to improve their regulatory acceptance. In fact some of the early irAOPs were actually not only based on chemicals only but did include some pharmaceuticals.

However, for the regulatory perspective, “saving animals”, will not be very helpful for irAOP uptake or acceptance. It is better to highlight that irAOPs will actually improve science. There are regions in the world where saving animals is not a priority. Even the 3R are being promoted by regulators in these regions as improving science and providing better risk assessment processes and, in this way, there is slowly buy-in.

5. How to bring regulatory perspective and irAOPs together - strategic plan

5.1 Scene setting

It is important to provide structure to the valuable interaction within the Workshop—specifically relating to the feedback and discussion on the irAOP work, potential for the assay grading concept and notably the multistakeholder perspectives. A group dialogue to build this framework for concrete next steps within the context of imSAVAR strategy.

The vision and objectives of imSAVAR were explained earlier. In particular, imSAVAR objective 2, as described in Figure 8, is paradigmatic of a dual pronged objective. There are two types of goals or objectives within projects, some are very concrete and feasible and there is a tension between objectives being smart versus being more aspirational which could be referred to as a high hard goal. The first part of objective two, "*refining and building new models*", is more actionable whereas the second part, "*stopping the development of unsafe immunomodulatory therapies early",* is more emblematic of these a high hard goal which is unlikely to be achieved during the project.

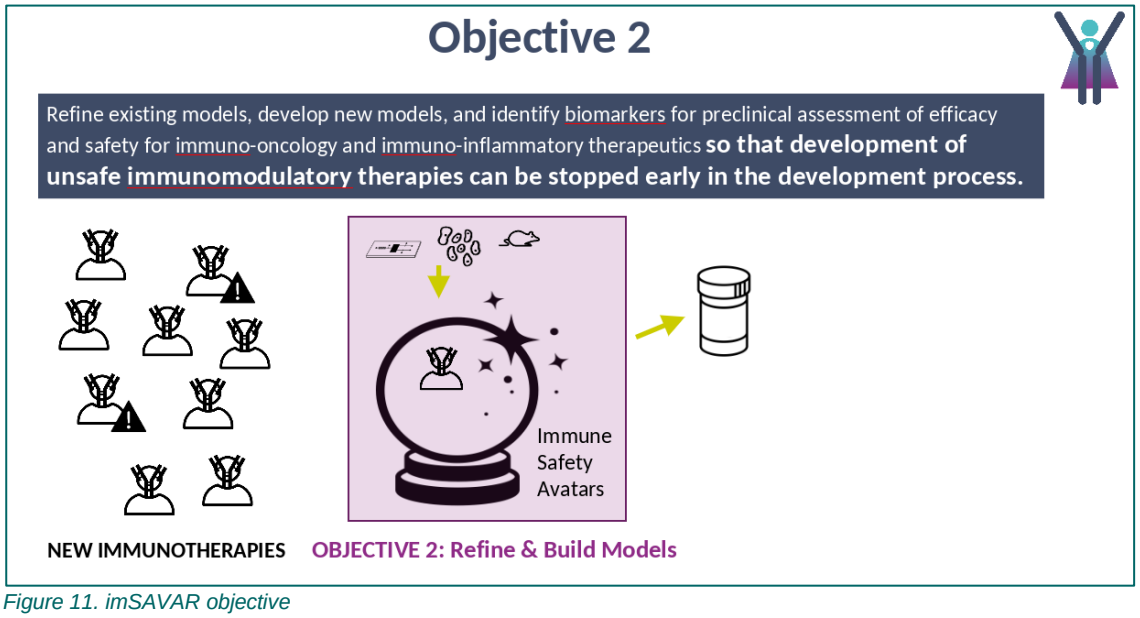

Figure 11. imSAVAR objective

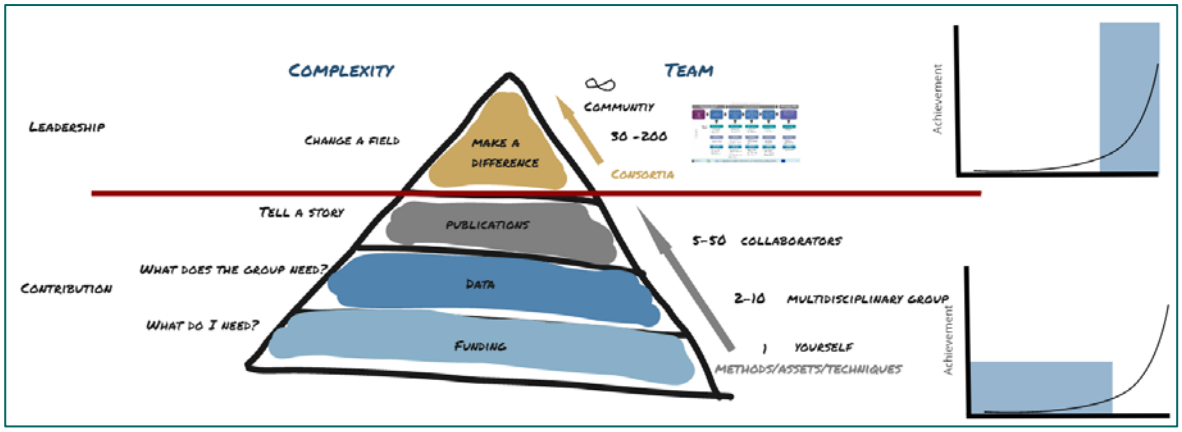

Figure 12. Pyramid graph

Figure 12 demonstrates what is attempted via biomedical research or innovation which include the typical core elements (e.g. funding, data, publications). As you move upwards along the pyramid the complexity increases along with the number of people to engage and methods and assets required. The crux of a consortium is how to create an impact or make a difference and this requires building a community, which in the case of imSAVAR means raising awareness around the AOPs. Based on the discussions in this Workshop, the galvanising element to convene and unify this global community is the irAOPs. This was demonstrated through the different external stakeholders that participated in the Workshop and in particular those working on the irAOPs in a different area where various synergies were apparent. After three stakeholder Workshops, the build-up of a stakeholder community can be elaborated for sustainability as it is also one of the four imSAVAR objectives.

Two fundamental concepts that surfaced out of the Workshop discussions are (1) aligning around the irAOPs and concept of grading assays and (2) the ethos of holistic assessments which highlights the tension between flexibility versus synergy. In Figure 13 summarises various topics discussed under each of these concepts and highlights the need to find balance between demonstrating the complexity of the immune system versus creating alignment around models to achieve synergies.

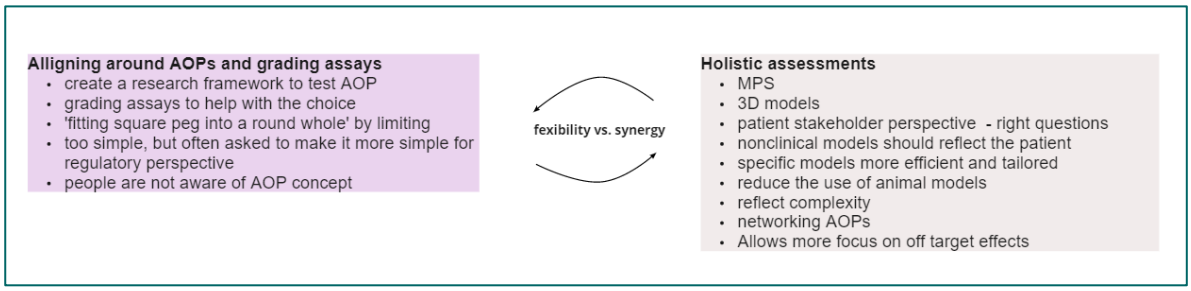

Figure 13. imSAVAR concepts

5.2 Group Dialogue

In order to drive the concepts forward, a simple mapping of actions (in black) was presented as a base for the group dialogue to expand upon (in green), to form a base for a strategic plan. As these concepts form the cornerstone of imSAVAR strategy careful evaluation is needed (see Figure 14.).

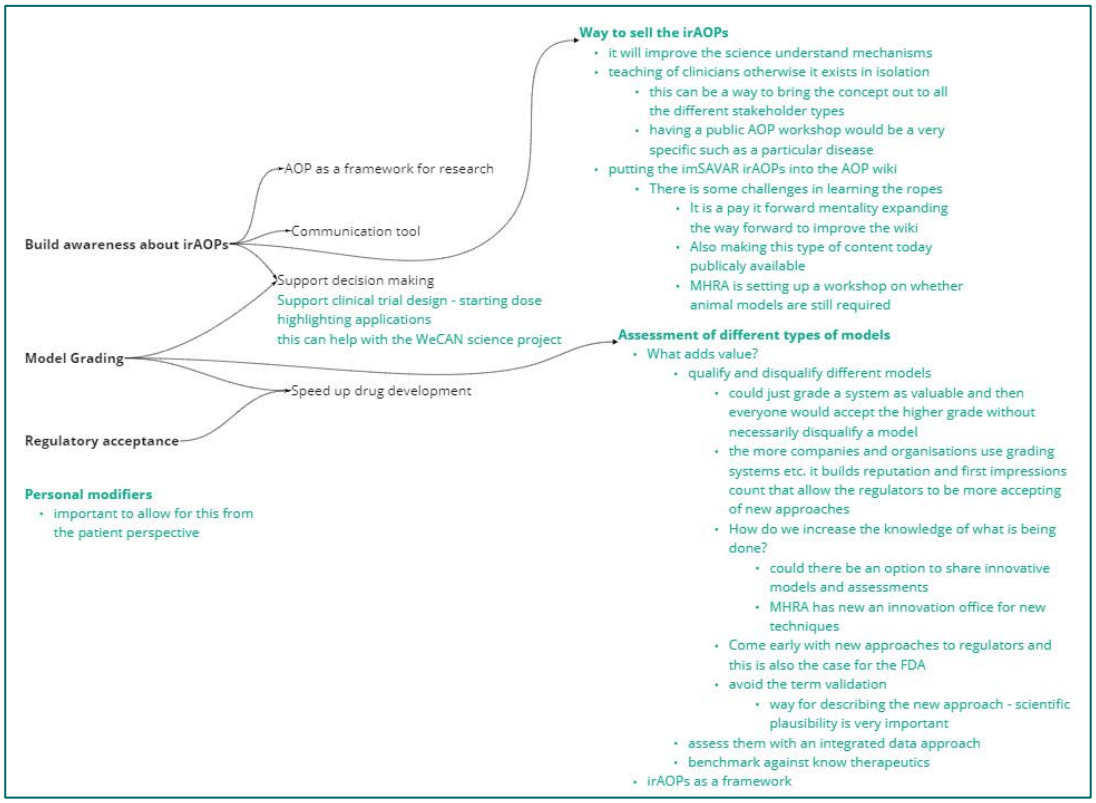

Figure 14. Mindmap following group dialogue

Model Grading:

Model grading refers to assessment of different types of models and of different levels of complexity. From the imSAVAR perspective, we want to focus on what adds value and take an unbiased approach on qualifying good models and/or biomarkers and disqualifying bad models and/or biomarkers. Perhaps a more amenable way of viewing this would involve focusing on qualifying good models instead since a large-scale consortium backed qualified model bring immediate value to developers.

Build awareness of the irAOPs:

Awareness building of the irAOP concept is not concentrated at the level of specific stakeholders but each stakeholder. This leads to the obvious need of tools and conduits to raise awareness and better educate stakeholders about this concept. It would be important to carefully tailor content and events to the stakeholder and have a clear disease or treatment focus, as being too broad will make the messaging diffuse. In addition organising public level multistakeholder Workshop are key. Part of raising awareness also involves building collaborations. In the context of the AOP wiki, they also offer coaching for input and so forth and this can act as a complementary activity to expected publications. In addition, the OECD subgroup EAGMST, offer training in various forms (resources and materials) which could be taken advantage of.

Regulatory acceptance:

Validation is a term that has specific meaning in the regulatory context and in general should be avoided. It is unlikely that the irAOP approach or grading system for models could be validated but that it should be fit for purpose. Scientific plausibility is probably very underrated but is really a key aspect to consider. It would be challenging from the regulatory perspective to push a specific agenda. If there is growing

uptake of these novel processes by organisations and companies that is when regulatory acceptance comes into play. Reticence to avoid innovation should be avoided by companies; from the regulatory perspective the motivation should not lie only at the level of regulatory approvals. Another element is interaction with regulators which can help augment regulatory acceptance of new processes as it is beholden on regulators to provide course-correcting guidance. In addition, regulators are no longer taking a back seat on innovation, and it is important to openly communicate at an early stage. In general different regulatory bodies are also aligned on this notion of being more open towards innovation.

Stakeholder focused concepts:

Organising public level multistakeholder workshops would help align the research community and the relevant stakeholders. As an example creating a bridge between basic scientists and toxicologists.

There are also upcoming regulatory workshops by MHRA which imSAVAR would likely be invited to participate in which helps with raising awareness on irAOPs.

Uptake of irAOPs within the biopharma sector could involve highlighting that there are advantages linked to decision making as well and potentially clinical trial design; basically speeding up the process to get to clinical settings.

Patient stakeholders can help with approaches to regulatory agencies. In addition, there is educational value for patient advocates involved within the trial design projects so scientific training programmes through WECAN Science could be an opportunity. From a patient perspective, greater interest lies in what functions for individual patients. If irAOPs can consider personal modifiers (e.g. genetic background, microbiomes), that may drive certain safety profile in particular direction.

There is ample scope for an imSAVAR stakeholder community, as outlined in the fourth objective to drive forward work on the irAOPs, model grading assessments and incorporating holistic perspectives into the models.

6. Conclusion

imSAVAR is tackling the challenging space of safety assessment of novel immunomodulatory therapies through a collaborative process. There is an existing repertoire of novel approaches to which imSAVAR added bandwidth and significant resources via its partners. The multi-year project tenure allowed the consortium to set strategy and expand outreach to a wide range of stakeholders to build momentum and use resources to maximise impact. This Workshop reinforces interest in imSAVAR outputs to enhance R & D of immunomodulatory drugs, divest earlier from inferior medicines so ultimately patients benefit form high therapeutic index treatments sooner.

The irAOPs work being solidified by imSAVAR focuses on a few therapeutic modalities which may be limiting but also open to new and additional insights to ensure they are fit for purpose; providing ample opportunity for a stakeholder community to build upon.

Importantly, the commitment of external stakeholders to continue engagement through the project duration helps maintain relevance of the imSAVAR research agenda. In parallel, imSAVAR is dedicated to utilizing its assets to better inform and educate the broader community of its work.

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