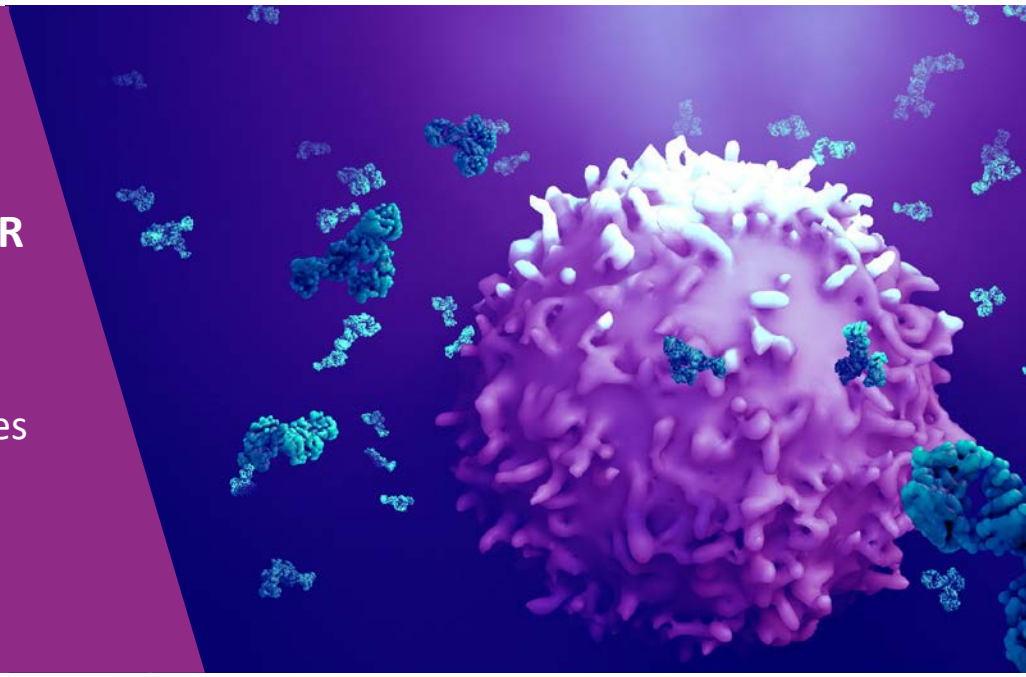
imSAVAR

IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 3.2

TReg-IL2 immune safety assessment research roadmap

DELIVERABLE REPORT

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 853988.
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Abstract

Work package 3 focuses on the safety assessment of immuno-inflammatory therapeutics. Regulatory T cells (Treg) are important for the maintenance of immune homeostasis and stimulation of Treg is considered as a therapeutic treatment option for a number of diseases (i.e. autoimmunity, transplant rejection). IL-2 and IL-2 signaling are implicated in the maintenance of Treg levels and function (limit the adaptive immune response through expression of co-inhibitory molecule CTLA-4 or anti-inflammatory cytokines (IL-10), acting on monocytic cells, triggering effectory T cells (Teff) apoptosis, and dampening Teff proliferation and activity). The development of low dose IL-2 for the treatment of autoimmune diseases is of interest. Clinical experience confirmed that low dose rhIL-2 can effectively expand functional Treg, however unmodified IL-2 (e.g. Proleukin) has a very narrow therapeutic index with activation of Teff cells and is poorly tolerated. There is a need for non-clinical mechanistic insights into the sequence of wide range of adverse events (i.e. skin rash, vascular leakage or hepatotoxicity) as the relationship between IL-2 dose levels and the onset of adverse reactions is unclear. Optimal dose selection is likely to be further confounded by specific disease states and inter-patient variability. Thus, additional non-clinical mechanistic insight into the sequence of events leading to skin rash, and the potential for discovery of biomarkers that could be used for clinical safety monitoring in healthy volunteers and patients, would add significant value to the ongoing development of therapeutic IL-2 modulators. The objective of this WP is the establishment of a series of appropriate test systems with readouts and biomarkers that ultimately allow predictions on the adverse influence caused by IL-2 therapy and later by other immunomodulatory agents.

Establishment of immune-related adverse outcome pathways (irAOPs) for e.g. IL-2 mediated skin rash, vascular leakage or hepatotoxicity was performed. Development of *in vitro* and *in vivo* models/test systems based on these irAOPs is ongoing. These models/test systems, allowing the reflection of adverse effects, will enhance readouts (e.g. molecular/cellular immune endpoints for Treg vs Teff subsets) in non-clinical animal models and support clinical studies of rhIL-2. Furthermore, the model systems developed will provide mechanistic insight in adverse events and allow biomarker discovery and ultimately include specific disease states using patient-derived samples within human *in vitro* model systems. Such test systems might be used *in vivo* to recapitulate adverse events and generate optimal samples for biomarkers but also complex *in vitro* models combining immune cells with cells/tissues of interest to profile/predict safety concerns potentially associated with new rhIL2s or any other immunomodulatory drugs targeting Treg.

A document will be compiled following the outcome of a planned imSAVAR workshop on TR-IL2 MoA (likely timing = Q1 2021). It will contain a description of the highest priority irAOPs for TR-IL2 MoA, and input from pharma industry stakeholders and regulators. This document will also highlight the most important areas where further research and development is needed with specific representation of what is planned for imSAVAR's efforts to enhance the safety assessment of therapeutic Treg modulators.

Document Information

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Table of Contents

1. Methods

irAOP for the MoAs of rhIL-2 were developed based on the OECD Guidance Document and extensive literature research and will guide the evidence-based development of methods and assays and support identification of biomarkers. The identification of key events in the irAOPs are based on two important characteristics: 1) they have to be measurable or observable, and 2) they should be necessary to functions whose disruption can be causally linked to the adverse outcome (either direct evidence or biological plausibility).

The highest priority irAOPs and the input from different pharmaceutical industry stakeholders have been reviewed and the most important areas to focus research and development in imSAVAR were discussed.

2. Results

Multiple irAOPs were drawn and refined for skin rash, hepatoxicity, vascular leakage in order to identify key events (KEs) and potential biomarkers (see D3.1 and figures 1-3). Among all these ongoing irAOPs, skin rash is the most important and urgent to understand as this is observed in preclinical and clinical studies with unmodified IL-2 as well as with rhIL-2. The understanding of any relationship between eosinophilia and such skin rash event will also be important. Hepatotoxity and vascular leakage will be important, as patient specificity is an unknown factor that needs to be further explored.

Literature research in regards to irAOP and relevant biomarkers (facilitated by WP4- Kristin Reiche) were performed and are currently basis to publish reviews describing immune related adverse outcome pathways (AOP) for IL-2 induced adverse toxicity (skin rash, vascular leakage and hepatotoxicity). Developed irAOP, reviews and information from pre-clinical and clinical Proleukin studies performed by Novartis were used as to draw up experimental plans, which were discussed and harmonized in biweekly meetings including Fh institutes (IME, IZI, PEI, IGB), PEI, universities (Lund, Jena) and industry partners (Covance, BI, Novartis).

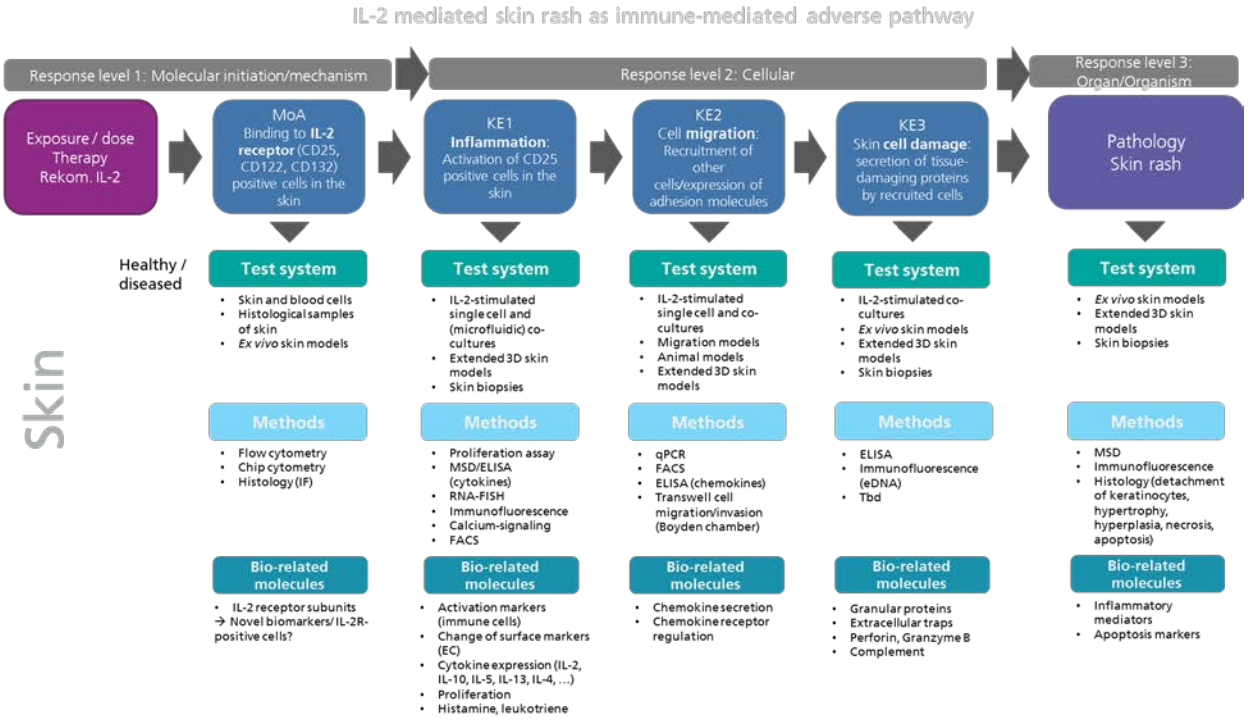

Figure 1: irAOP development for case study no. 1, rhIL-2-treatment leading to skin rash.

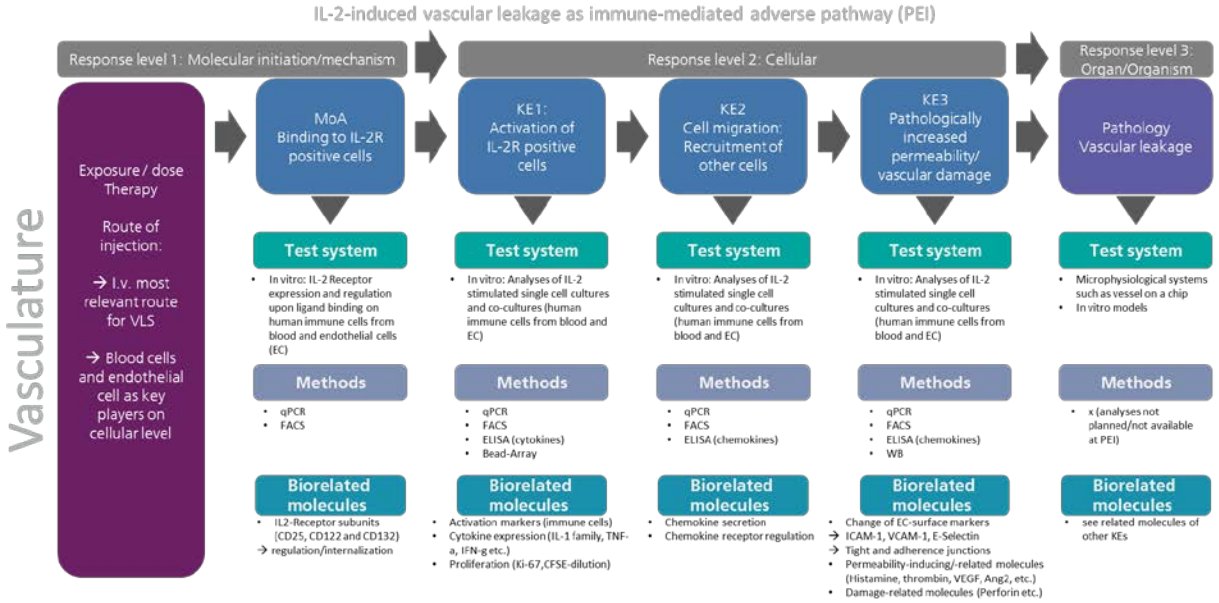

Figure 2: irAOP development for case study no. 2, rhIL-2-treatment leading to vascular leakage.

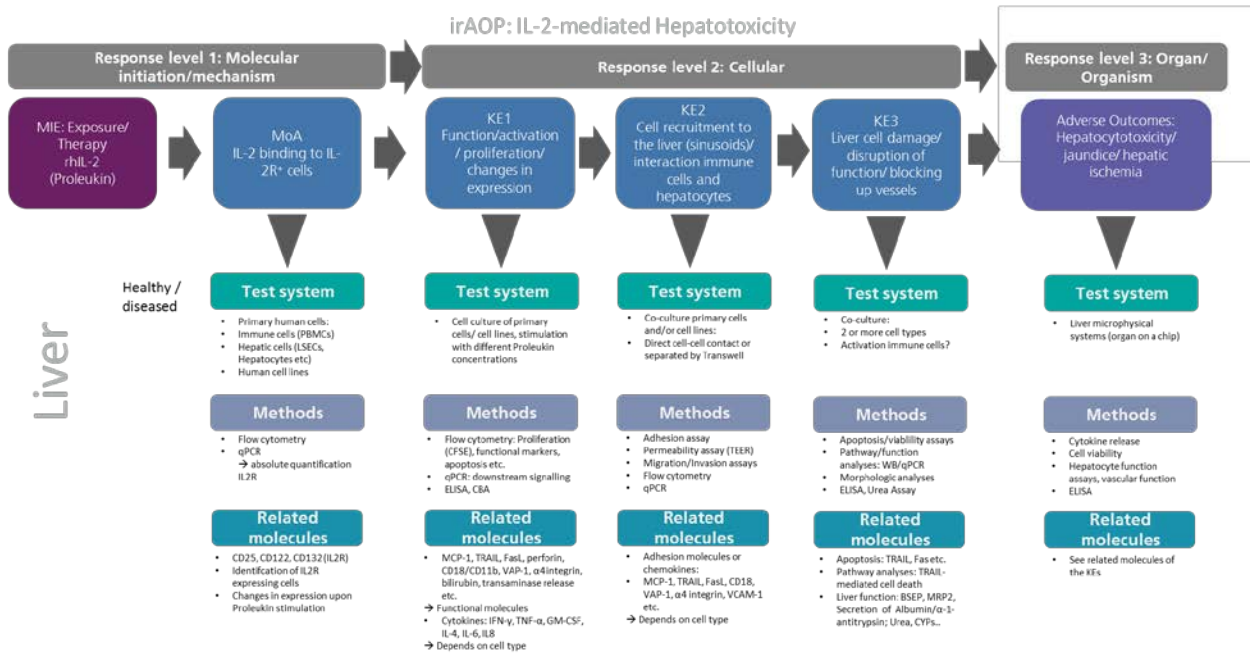

Figure 3: irAOP development for case study no. 1, rhIL-2-treatment leading to hepatotoxicity

Mechanistic insights using *in vitro* systems will help to determine onset and drivers of each KEs, but also safe and adverse doses of rhIL-2 in healthy and later in diseased models. These mechanistic insights are important for pharmaceutical companies to better define the therapeutic dose and predict any adverse events (AEs), but also for health authority, as AEs are better understood and biomarker strategy is in place to prevent such events.

Representative tool compounds for therapeutic IL-2 mimetics exhibiting different degrees of Treg vs T effector cell selectivity (including the marketed drug Proleukin) have been identified by industry partners to support *in vitro* and *ex vivo* skin rash model development at the Fraunhofer-Institute for Toxicology and Experimental Medicine (ITEM). Molecular and phenotypic responses observed within the resultant *in vitro* skin rash models will be benchmarked versus therapeutic rhIL-2 non-clinical and clinical experience regarding skin rash phenotypes and mechanistic hypotheses.

To develop skin rash models, a cooperation with Prof. Werfel from the medical school Hannover was established to get access to *ex vivo* skin biopsies. As can be seen in Figure 4, dissociation of skin biopsies from three different donors yielded tremendous differences in the CD45+ immune cell population. Regarding IL-2R expression, the subunit of the high affinity receptor CD25 was identified to a low degree in all skin biopsies investigated. However, as with CD45, this expression was highly variable between the donors. Donor- but also body region-specific frequencies of cell types bearing IL-2R subunits (including CD122 and CD132) and identification of receptor-bearing cells will be a subject of future investigations. Additionally to quantitative analysis using flow cytometry, skin biopsies were asservated for immune fluorescence studies as cryo-embedded preparations to study location of IL-2R positive cells within the skin. Furthermore, *ex vivo* skin tissue cultures will be set up to analyse IL-2-induced modulation of skin resident (immune) cells. Mediators and biomarkers leading to skin rash will be investigated.

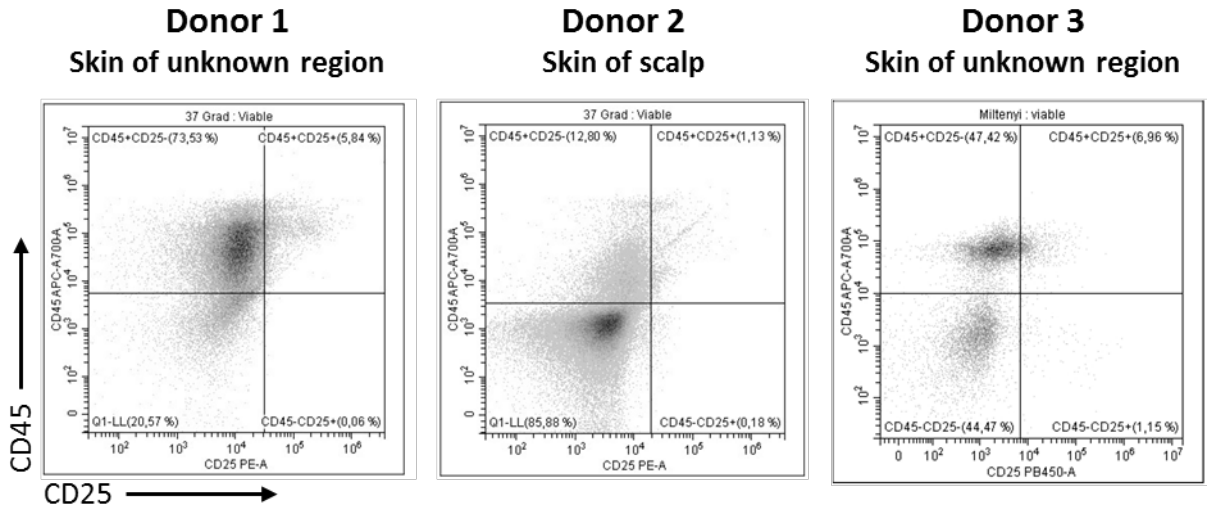

Figure 4: Patient- and body region-specific expression of IL-2Rα (CD25).

It is aimed to analyse interactions of immune cells and structural skin cells, as well as the influence of soluble mediators (e.g. histamine, LT, proinflammatory cytokines, perforins, complement proteins) on skin cell damage and inflammation in 3D skin model (Fraunhofer IGB) and relevant *in vitro* co-culture models.

3. Discussion

In parallel to the progress being made in establishing *in vitro* model systems for recapitulating elements of the therapeutic IL2-associated clinical skin rash phenotype, the WP3 team will continue to collate *in vitro* and *in vivo* data (non-clinical and clinical) for therapeutic rhIL2 safety assessment case studies for a broader range of irAEs (e.g. Establishing a vascular leakage syndrome rat model for investigating mechanisms associated with aldesleukin-induced vascular leakage syndrome in patients; presented within WP3 by Novartis; October 28th 2020).

4. Conclusion

The irAOPs reflect the needs to better understand AEs, select appropriate therapeutic doses and put in place a biomarker strategy for the development of rhIL-2 and later other immunomodulatory Treg therapies. Tool IL-2 compounds and supporting non-clinical and clinical safety data have been identified/ shared within the consortium team to support *in vitro* skin rash model development.

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.