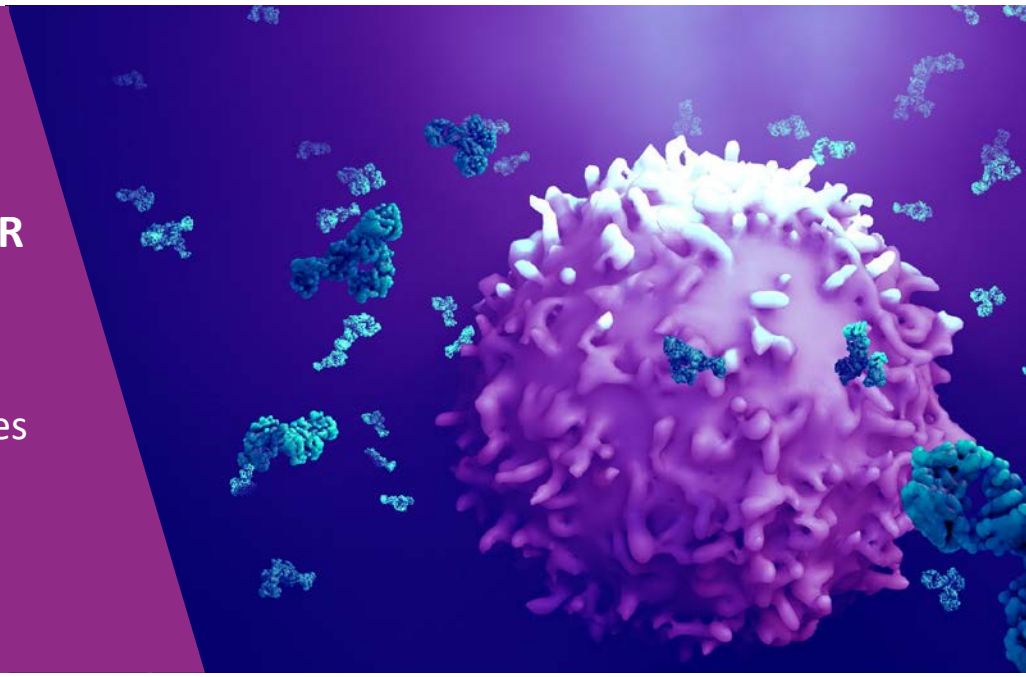
imSAVAR

IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 3.1
Preliminary irAOP – IL-2-TREG

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 and will refine, standardize, and develop models that incorporate the complexity of immune-mediated diseases, such as autoimmune and inflammatory diseases. With this, desired and undesired adverse clinical immune responses will be predicted more accurately. To approach this, we follow (similar and in close cooperation to WP2) the concept of structuring the effort in the framework of immune-related adverse outcome pathways (irAOPs) based on mode of action (MoA) case studies to guide the development of novel test systems. First focus was the development of an irAOP for interleukin-2 (IL 2). Besides the irAOP for IL-2-induced skin rash and vascular leak syndrome addressed in this report, irAOPs for the MoAs of cytokine release syndrome (CRS) associated with CAR T cells (T cells genetically engineered to express a chimeric antigen receptor [CAR]) or CD3-targeted bispecific T cell engager antibodies (BiTEs) will be developed, both in close cooperation to WP2.

Although high doses of recombinant human IL-2 (rhIL-2), marketed as Proleukin® (aldesleukin), were shown to be an effective anti-cancer therapy in some patients, severe side-effects not seen in rodent and non-rodent preclinical studies (e.g. capillary leak syndrome) led to a drawback of the treatment. Longer-term repeated dosing of rhIL-2 additionally resulted in skin rash, even though the relationship between rhIL 2 dose level and dosing schedule, duration to the onset of skin rash, and additional toxicities is unclear. However, clinical experience confirms that low-dose rhIL-2 can effectively expand functional regulartory T cells (Tregs) and therefore, low-dose rhIL-2 treatment is currently developed as medication against autoimmune diseases. Optimal dose selection is likely to be further confounded due to specific disease states and inter-patient variability. Thus, additional non-clinical mechanistic insights into the sequence of events leading to clinical pathologies such as 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.

Document Information

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1. Methods

Based on the OECD Guidance Document for irAOPs, we developed a preliminary irAOP for the MoAs of rhIL-2. This irAOP outline is based on extensive literature research and will guide the evidence-based development of methods and assays. It will further support the utility of in vitro methods and the identification of biomarkers.

In a first step, we searched for information what is already known about the biology of the system, set bounds of the biological system and identified potential key events. We outlined our fundamental understanding of the normal vs. disturbed regulation and identified different elements that will impact overall outcome. The identification of key events was 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). By this, we further identified data gaps that will guide our experimental work.

2. Results

At first, we developed a list of immuno-inflammatory MoAs that require better non-clinical safety assessment. With this at hand, the first two case studies (rhIL-2 and CRS) were chosen and specific irAOPs for immune-related adverse pathways were researched in literature and graphically outlined. Further, for the case study, patterns of bio-related parameters that are specific for the defined MoA and key events (KEs) and that need to be assessed were identified. At last, case study specific test batteries that allow the experimental detection of different KE relevant in immune-related adverse pathways were determined.

Outline and evaluation of scientific evidence of an immune related irAOP for rhIL-2:

This outline of the irAOP depicts what is known about the function of that biological system with regards to rhIL-2 treatment leading to skin rash and vascular leakage as pathological outcomes. The fundamental understanding of the normal regulation is the basis for understanding how perturbation of different elements will impact overall function.

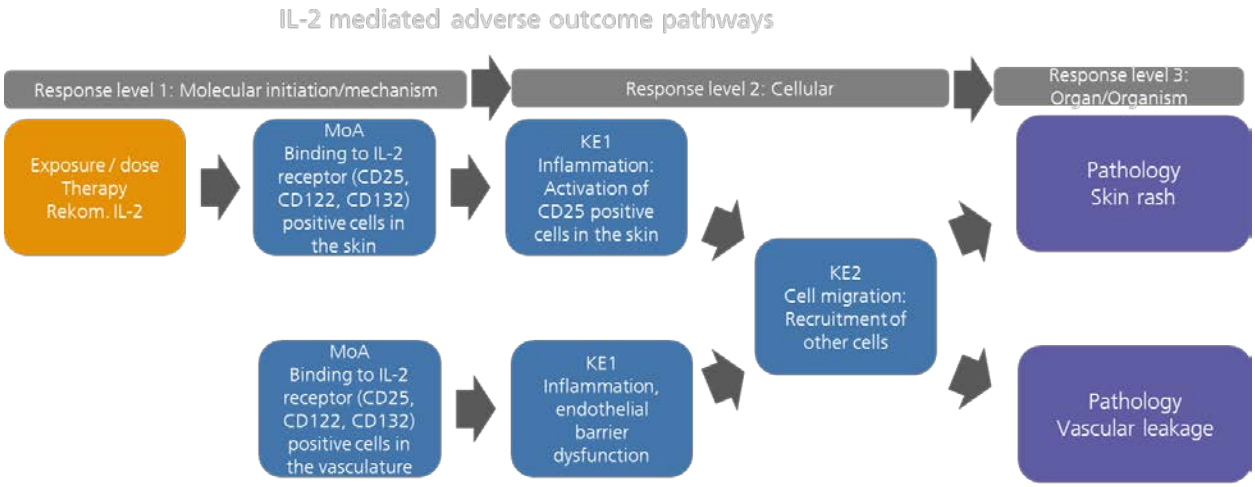

Figure 1: irAOP development for rhIL-2-treatment leading to skin rash and vascular leakage as two adverse outcomes.

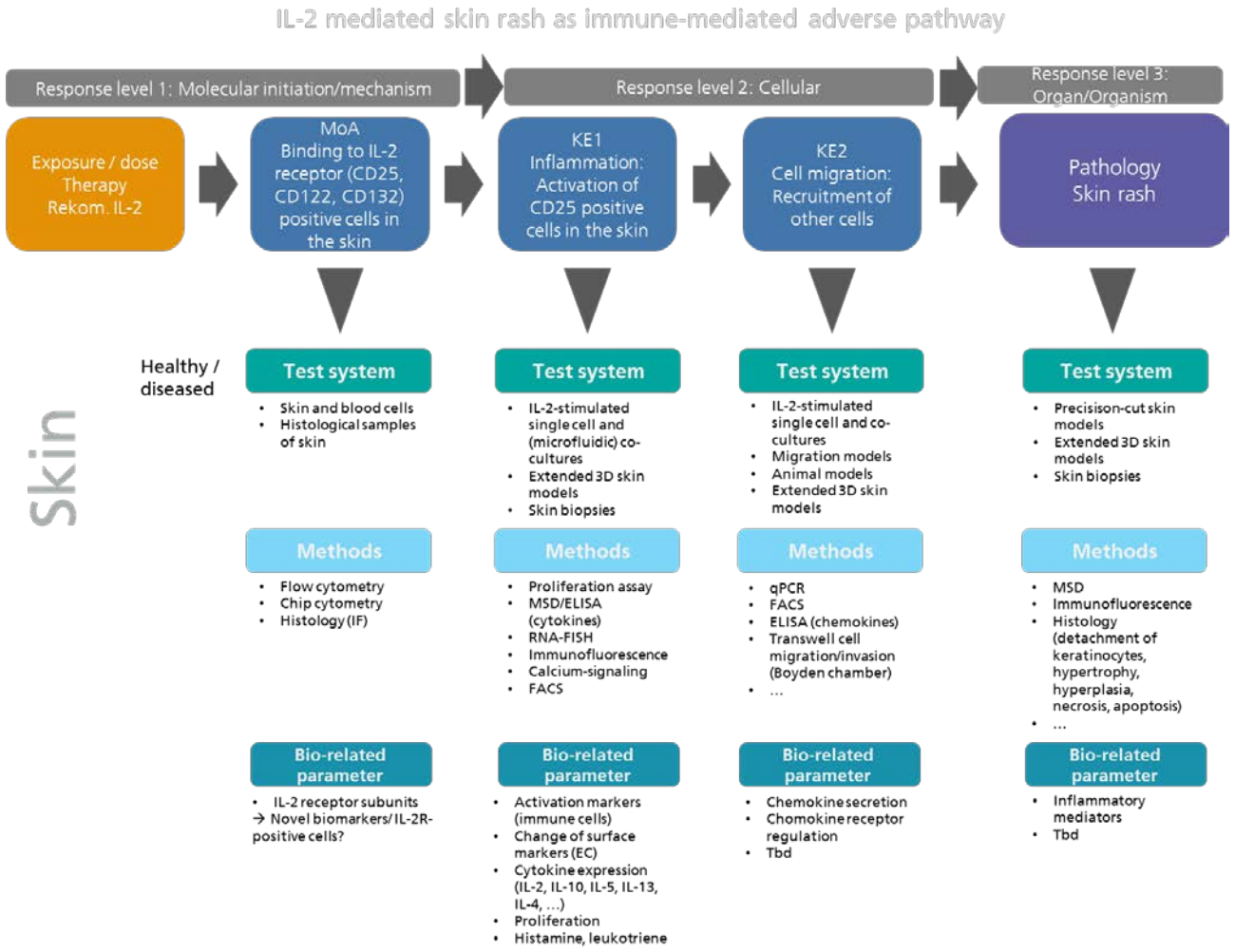

Figure 2: irAOP development for case study no. 1, rhIL-2-treatment leading to skin rash.

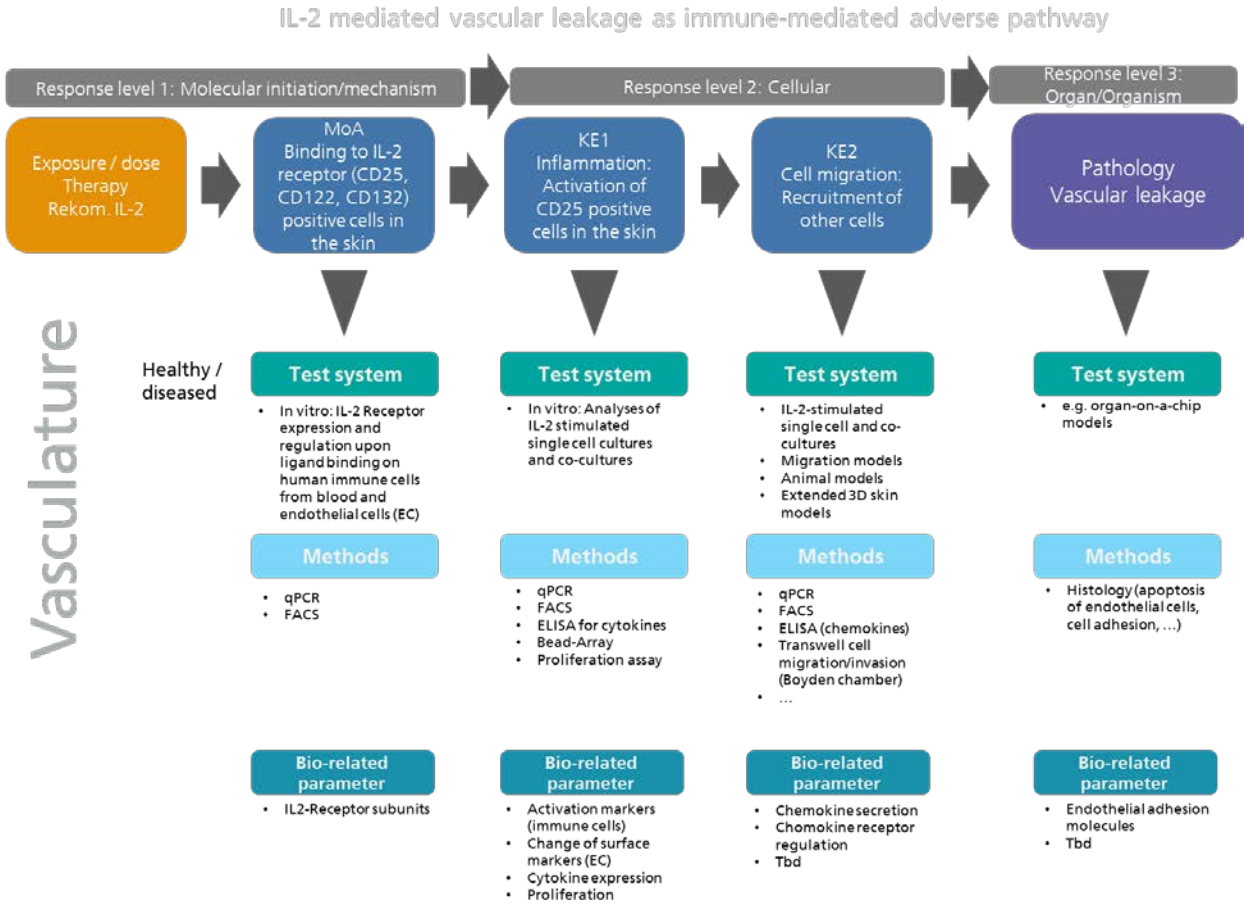

Figure 3: irAOP development for case study no. 2, rhIL-2-treatment leading to vascular leakage.

For this and further case studies, a team, interested especially in that irAOP, has formed to work together to draft the outlines. rhIL-2 mediated pathologies will be addressed from different perspectives, which in the end will contribute to a detailed picture and a thoroughly analyzed/constructed irAOP. According to our extensive literature research, the irAOPs of the two case studies of skin rash and vascular leakage converge at KE2 (Figure 1). Based on this literature research, experiments will cover analyses of single cell and co-culture systems representing different relevant compartments from lymph node over blood cells and endothelia to the end organ/tissue (Figure 2 and 3). While one focus here will be set to the skin (Figure 2) and vasculature (Figure 3), another approach investigating rhIL 2-mediated hepatotoxicity is being established. With this, clinically relevant adverse outcomes of rhIL-2 treatment will be illuminated to on the one hand confirm state of the art data. On the other hand and predominantly, gaps in the understanding of rhIL-2-mediated processes and on the path towards valid test systems for reliable adverse event prediction during rhIL 2-therapy shall be identified and filled. In this course, the experimental work based on the KE and test systems available will be coordinated and arranged to cover test systems and the biomarker not only with the expertise within WP3 but from the whole imSAVAR consortium.

3. Discussion

So far, the literature research gave different insides in KE that we will be focusing on. To elucidate and mimic these KE, experimental work as outlined above will be started next. This experimental phase will

be conducted in close cooperation within the WP3 members. Before, principle terms will be arranged and concerted where possible, such as the use of the same molecule (e.g. IL-2 batch from the same distributor), the exchange of principle protocols, and meetings and discussions on a regular basis. Additionally, correlation of irAOPs to application route (intravenous vs. intradermal) as well as dosing is of importance and thus will be investigated in more detail. Within this work package, a close coordination will be essential in order to compare and evaluate the results and to bring the data into a plausible context to understand the irAOPs of the chosen case studies.

In parallel, we will conduct a survey among the EFPIA partners involved in WP3 and WP2 regarding information for the currently available test systems (in vitro and in vivo) which will be helpful for further refinement.

4. Conclusion

The work that has been conducted so far sets the base for the experimental phase. The literature research showed that there are a lot of “black boxes” that need to be elucidated and that are responsible for unexpected irAOPs when it comes to clinical testing. The experimental phase will show to what extend the presumed outlines reflect the irAOPs and if the chosen test systems are sufficient to display the KE or if they need adjustments.

Abbreviations

BiTEs	bispecific T cell engagers
CAR	chimeric antigen receptor
CRS	cytokine release syndrome
irAOP	immune-related adverse outcome pathways
KE	key event
MoA	mode of action
rhIL-2	recombinant human interleukin-2
Tregs	regulatory T cells

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