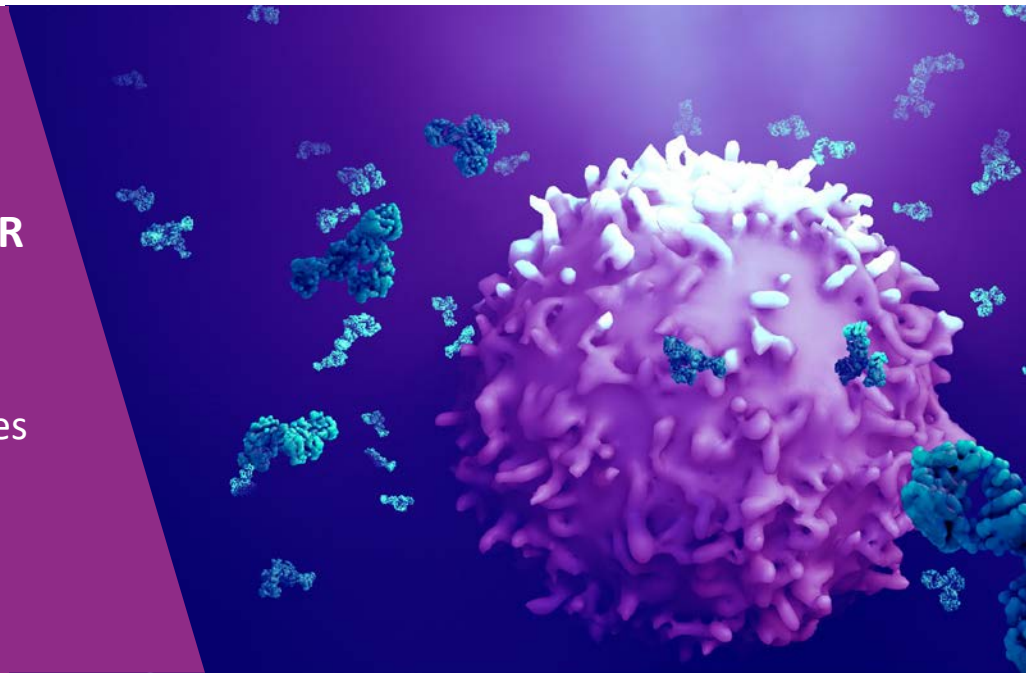
imSAVAR

IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 2.10

2nd iteration of strategies and experimental in vivo and in vitro immune-competent target organ models to study the MoA of immune checkpoint inhibitors

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 2 (WP2) has been focusing on the refinement and development of in vitro and in vivo immuno-oncology models for three different modes of action (MoA), one of them being the immune checkpoint inhibitors (CPIs). Within the imSAVAR project we have developed immune-related adverse outcome pathways (irAOPs) for different MoAs, to guide us in identification of gaps in knowledge, and in the development and evaluation of test systems, assays and biomarkers related to each key event, that lead to an adverse pathology. In Deliverable 2.1, a preliminary version of the CPI-mediated cytokine release syndrome (CRS) was developed, which was further explored and presented in Deliverable 2.7. Furthermore, a preliminary CPI-mediated (PD-1/PD-L1-mediated) AOP for the adverse event of hepatotoxicity has been generated and presented in this report. Based on these AOPs, we have identified, refined and developed in vitro and in vivo potential assay systems that will aid in the analysis of CPI-mediated toxicities.

Document Information

Deliverable Report	**D2.10: 2nd iteration of strategies and experimental in vivo and in vitro immune-competent target organ models to study the MoA of immune checkpoint inhibitors (CPI)**
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1. Methods

In vitro models are used to analyze the mechanism of action (MoA) and toxicities of CPIs. These models consist of organ-specific immunologic cell cultures. For studying CPI-mediated hepatotoxicity, different types of models are generated and used, including mono-cultures, co-cultures, and triple-cultures of primary immune cells, hepatic cell lines, or primary hepatocytes. Avelumab and atezolizumab are used as test biologicals. As control unspecific IgG antibody was applied. Primary human immune cells are screened for PD-1 and PD-L1 expression, and the effects of CPIs on various immune cells are investigated, including surface marker expression, cytokine release, and PD-1/PD-L1 mRNA and protein expression. CD8 T cells are selected as the most interesting immune cell type for studying the immune modulatory effects of anti-PD-L1 biologicals. Additionally, the effects of CPIs are being explored in a triple-culture model consisting of CD8 T cells, macrophages, and hepatocytes.

The relevance of comedication was investigated by pre-treating hepatocytes with drugs known to cause hepatotoxicity, such as acetaminophen. To simulate a diseased condition, hepatocytes were pre-treated with an inflammatory cytokine and CD8 T cells were pre-activated. Interestingly, cytokine treatment of hepatocytes led to an increase in the expression of PD-L1. These in vitro approaches will be complemented with microphysiological Organ-on-Chip (OoC) systems and in vivo models. For hepatotoxicity analysis, there will be cooperation with Dynamic42 for complementary analyses using a Liver-on-Chip model.

A model system that has been set up for the investigation of CPI-mediated CRS, an adaptation from Reed et al [1], which comprises autologous PBMCs, serum and blood outgrowth endothelial cells (BOECs) isolated from donors' blood has been optimised with healthy and diseased donors' blood. It is now implemented for the use of blood samples isolated from patients with tonsillar cancer, before and after they are treated in the clinic. The coculture system aims to mimic CRS pathology, following stimulation with Nivolumab ex vivo and this approach is a "proof-of-model", as in due time it will be possible to see if those tonsillar cancer patients who received immunotherapy, will develop CRS. This study was initialised in 2024, so the supernatants from the cocultures are currently being collected.

The experimental in vitro models used to analyse CPI-mediated toxicities are summarized in table 1.

2. Results

In the initial stage, a preliminary immune-related adverse outcome pathway (irAOP) was developed for CPI-mediated hepatotoxicity (Figure 1). This irAOP will serve as a tool to identify the cell types and molecules that may be relevant in CPI-mediated hepatotoxicity and to provide suitable test systems. Based on the irAOP, it is suggested that CD8 T cells and macrophages may play a role in CPI-mediated hepatotoxicity. Therefore, our first step was to establish in vitro models using macrophages and CD8 T cells obtained from healthy donors. However, we have now initiated a collaboration with the University of Leipzig, who will provide us with primary macrophages and CD8 T cells isolated from the liver. By using these cell types, we aim to mimic the in vivo situation more accurately.

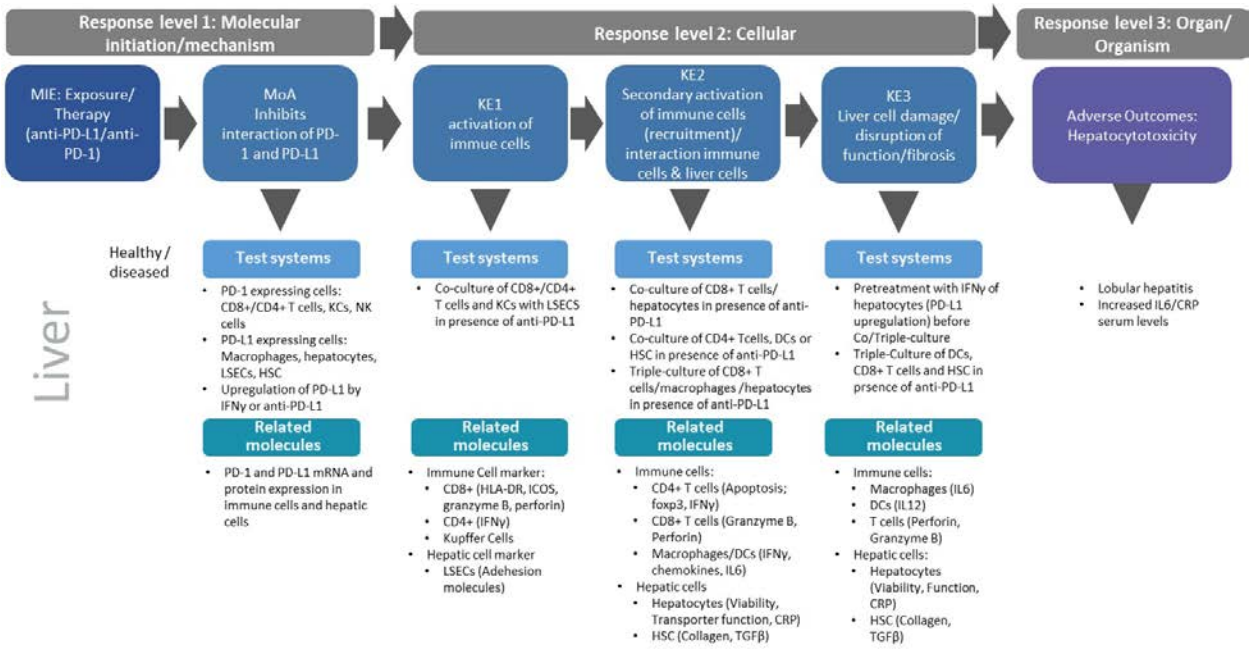

Figure 1: Preliminary version of the irAOP for MoA CPI and adverse outcome hepatotoxicity.

Table 1: Experimental in vitro methods and models conducted by the different partners.

Partner	Pathology	Methods/Model
Fraunhofer ITMP	Hepatotoxicity	➢ Co-Cultures of CD8 T cells and hepatic cell line (HepaRG) to determine hepatotoxicity, surface marker expression and function of hepatocytes. ➢ Triple-Co-Cultures of hepatic cell line (HepaRG), primary human CD8 T cells and macrophages to determine hepatocytotoxicity, surface marker expression on hepatocytes and function of hepatocytes. The optimal time point for culturing CD8 T cells was determined to prevent T cell exhaustion.
Lund University	CRS	➢ Cocultures of autologous PBMCs, serum and blood outgrowth endothelial cells (BOECs), stimulated with nivolumab and control biologics, known to induce CRS. Collection of supernatants after different timepoints and multiplex analysis for a panel of cytokines.

3. Discussion

The findings presented in this deliverable build upon previous work in the imSAVAR project, particularly from Deliverables 2.2 and 2.7. The development of irAOPs for CPI-mediated CRS and hepatotoxicity represents a significant advancement in understanding the immune-modulating effects of CPIs. By refining these models, we have identified key immune cell interactions that contribute to CPI-induced adverse effects, particularly the role of CD8 T cells and macrophages in hepatotoxicity.

The focus has expanded on incorporating microphysiological OoC technology, and specifically liver-on-a-chip, which will be necessary to improve the predictive capacity of the models. Future work should focus on enhancing these models by integrating additional patient-derived data, refining co-culture conditions, and expanding the range of tested CPIs. Moreover, continued collaboration with clinical partners will be crucial for validating these findings in a translational context.

4. Conclusion

Moving forward, further validation using patient-derived samples will be essential. Additionally, integrating these models into regulatory and industry workflows will enhance their applicability in nonclinical safety testing. The continued refinement of in vitro and ex vivo models, in conjunction with emerging technologies like OOC systems, will support the broader goal of improving the safety and efficacy of CPIs.

Abbreviations

MoA – mode of action
CPI – immune checkpoint inhibitor
irAOP – immune-related adverse outcome pathway
CRS – cytokine release syndrom
PD-1 – programmed cell death protein 1
PD-L1 – programmed death-ligand 1
OoC – organ-on-a-chip
CD8 – cluster of differentiation 8
PBMCs – peripheral blood mononuclear cells
BOECs – blood outgrowth endothelial cells

References

1 Reed, D. M. et al. An autologous endothelial cell:peripheral blood mononuclear cell assay that detects cytokine storm responses to biologics. FASEB J **29**, 2595-2602, doi:10.1096/fj.14-268144 (2015).

Acknowledgement

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