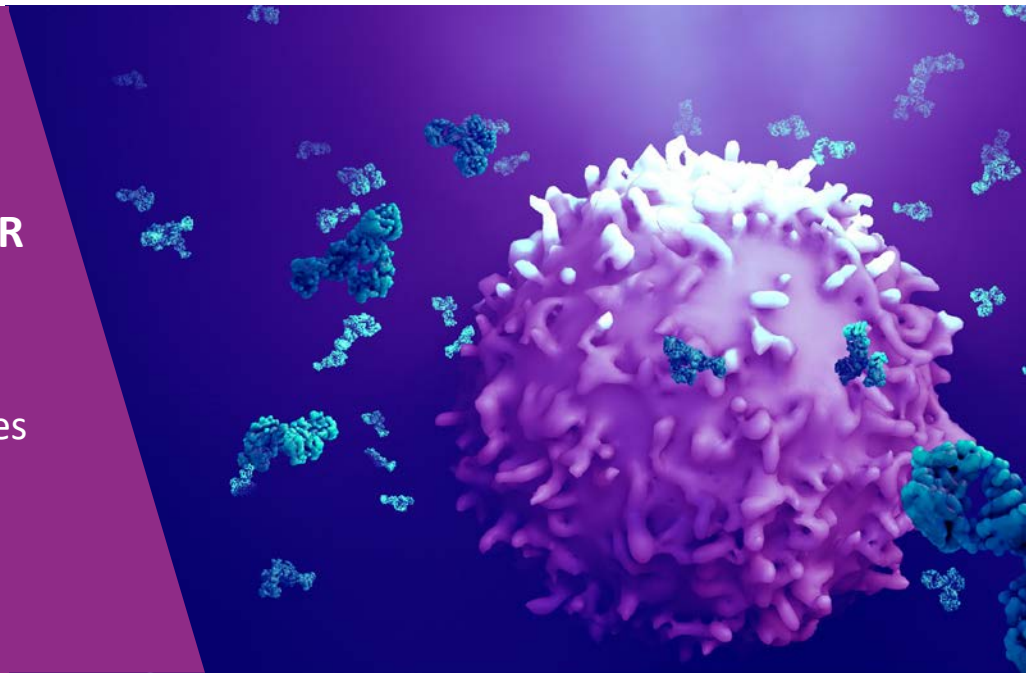
imSAVAR

IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 2.7

Strategies and experimental in vivo and in vitro immune-competent target organ models to study the MoA of immune checkpoint inhibitors (CPI)

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 this report. Furthermore, a CPI-mediated (Avelumab-mediated) AOP for the adverse event of hepatotoxicity is currently being generated. 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	**D2.7: 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

Based on the OECD Guidance Document (available at: https://www.oecd-ilibrary.org/content/paper/5jlv1m9d1g32-en) for developing and assessing AOPs, and the Deliverable report "D2.2 Preliminary irAOP - CPI MoA", V1.1, an updated version of the irAOP for the CPI-mediated CRS has been developed (Figure 1), further highlighting biomarkers relevant to each key event. An irAOP for the CPI avelumab is being generated and used for the identification of potential assay systems. However, as it is still in preliminary stage, it is not presented in this report.

In vitro models to analyse the MoA and toxicities of CPIs comprise organ-specific immunologic cell cultures. Specifically for the CPI-mediated hepatotoxicity adverse outcome, the models generated and used comprise mono-cultures, co-cultures and triple-cultures of primary immune cells and hepatic cell line or primary hepatocytes. Avelumab (anti-PD-L1) and atezolizumab (anti-PD-L1) are used as test biologicals. Primary human immune cells were screened for expression of PD-1 and PD-L1 and the effect of CPIs on various immune cells regarding surface marker expression, cytokine release and PD-1/PD-L1 mRNA expression was investigated. CD8 T cells were selected as the most interesting immune cell type regarding immune modulatory effects mediated by anti-PD-L1 biologicals. Further, the effect of CPIs in a triple-culture model consisting of CD8 T cells, macrophages and hepatocytes is currently being explored. These in vitro approaches will be complemented with respective microphysiological Organ-on-Chip (OoC) systems and in vivo models, and specifically for the hepatotoxicity adverse outcome, a cross cutting cooperation with project partner Dynamic42 will enable complementary analyses with a Liver-on-Chip model.

To further increase the in vitro assay development, a model system has been set up for the investigation of CPI-mediated CRS, which is an adaptation from Reed et al 1; this comprises of autologous PBMCs, serum and blood outgrowth endothelial cells (BOECs) isolated from donors' blood. Healthy donors' blood has been used to set up and optimise the conditions of this model and currently the same protocol is tested with Head and Neck Squamous Cell Carcinoma patients' blood. This co-culture system aims to mimic the CRS pathology, following stimulations with Avelumab and Nivolumab (anti-PD-1). Additionally, attempts to translate the 2D model into a 3D nanofiber system, using a nanofiber technology by Cellevate AB (Lund, Sweden) are initiated, with the aim to better replicate the CRS pathology and include more blood-related parameters. The experimental in vitro models used to analyse CPI-mediated toxicities are summarized in Table 1.

Toxicity associated with immunomodulatory therapies is often detected late in drug development due, in part, to the absence of the intended targets (molecular and functional) in preclinical animal models. Cord blood derived, humanized immune system mice are being explored as a potential translational (more human-relevant) risk assessment tool. Synergistic interactions have been observed for small molecule/CPI combination therapies where serious adverse events manifested either as increased incidence of injury, or targeted alternative organs compared to monotherapy. Patients treated with the CPI Ipilimumab in combination with the BRAF kinase inhibitor vemurafenib experienced grade 3 elevations in Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) prompting early termination of the trial. When this combination was administered to wild type mice at clinically relevant exposures the histomorphologic and clinical chemistry results were not remarkable. Pilot studies in the humanized model revealed evidence of immune infiltration of the liver and spleen. These findings were not observed with monotherapy and were not accompanied by of a graft vs. host response in either the lung or GI tract (Figure 2). More detailed mechanistic and biomarker studies are planned.

2. Results

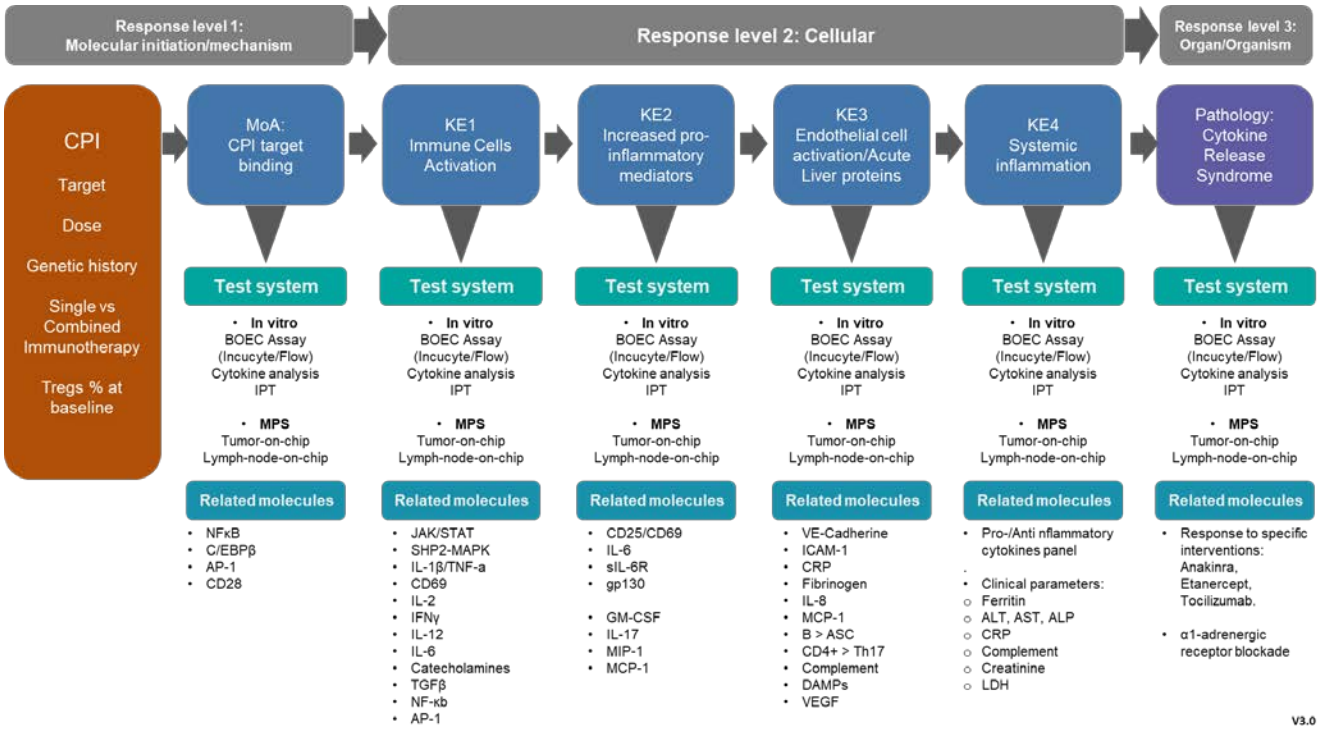

Figure 1: Updated (V3.0) irAOP for MoA CPI and adverse outcome CRS. In this irAOP version additional biomarkers relevant to each key event have been identified.

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

Partner	Pathology	Methods/Model
Fraunhofer ITMP	Hepatotoxicity	➢ Mono-Cultures of immune cells and hepatic cell line (HepaRG) to analyse the direct effect of CPIs on immune cells or HepaRG cells regarding surface marker expression/cytokine release and to determine the mRNA expression of PD-1 and PD-L1. ➢ Triple-Co-Cultures of hepatic cell line (HepaRG) and primary human CD8 T cells and macrophages to determine hepatocytotoxicity, surface marker expression on hepatocytes and function of hepatocytes.
Lund University	CRS	➢ Cocultures of autologous PBMCs, serum and blood outgrowth endothelial cells (BOECs), stimulated with different CPIs biologicals. Collection of supernatants after different timepoints and multiplex analysis for a panel of cytokines.

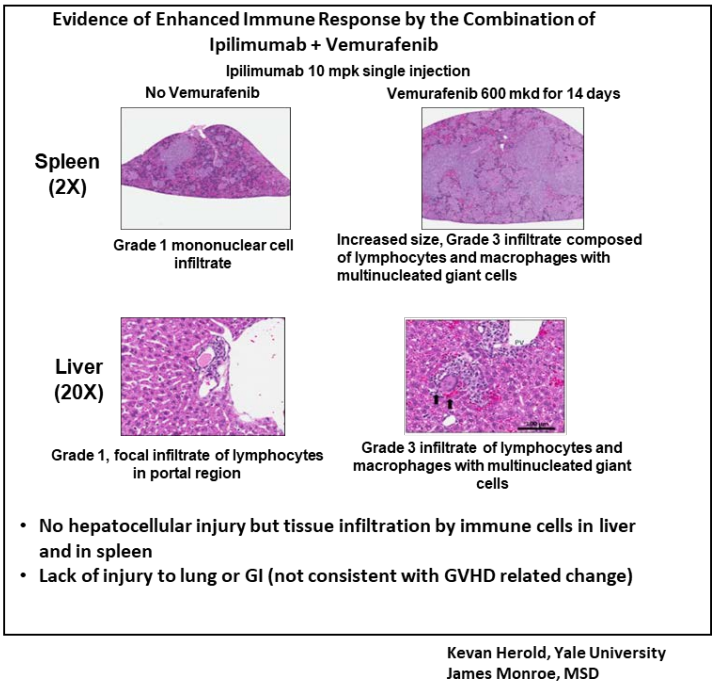

Figure 2: Modeling in cord blood engrafted humanized immune system mice.

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).

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