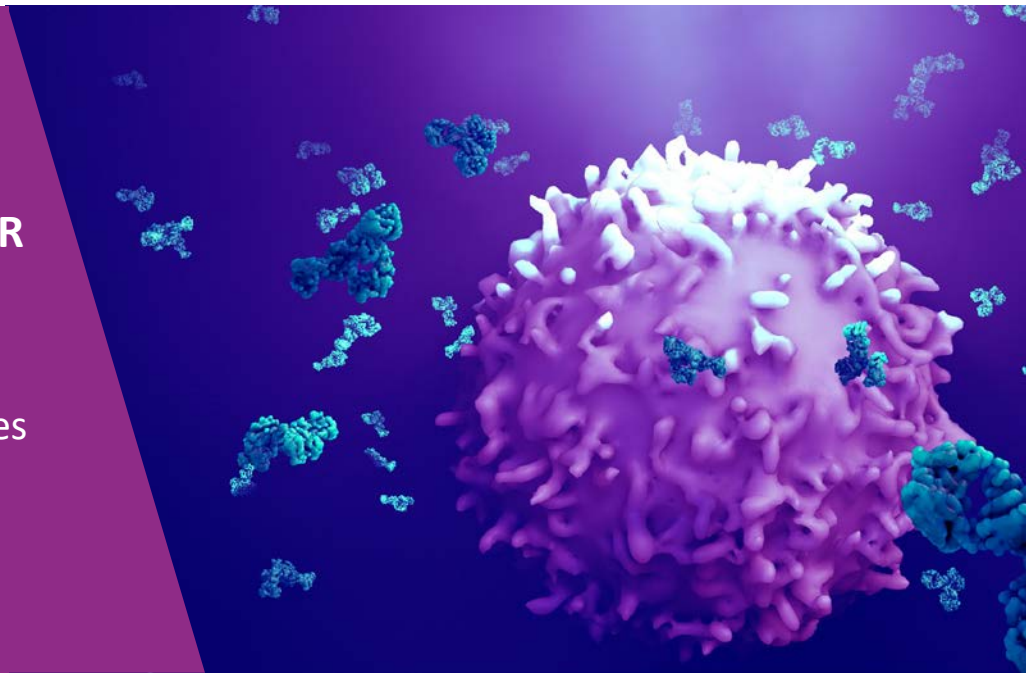
imSAVAR

IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 2.6
1st iteration refined CRS *in vivo* models

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

A key objective in imSAVAR is to create a platform with novel tools, models and resources for early nonclinical prediction of immune-related adverse events (irAEs) of immunomodulatory therapies. Here, we adapted the adverse outcome pathway (AOP) concept used in the toxicology field to describe adverse effects mediated by (cellular) immunotherapies. During the first imSAVAR study-a-thon, the immune related AOPs describing cytokine release syndrome (CRS) as an adverse outcome of chimeric antigen receptor (CAR) T cell, bispecific T cell engager (TCE) and immune checkpoint inhibitor (CPI) therapy were harmonized. In the past few years, several new animal models to study CRS mediated by CAR T cells were described. Although there are still some drawbacks regarding translatability, such models can be a useful tool to study new mitigation strategies to reduce or even prevent side effects such as CRS.

This deliverable report summarizes the lessons learned from use of in vivo models by academia and industry and plans for use of in vivo models to assess CRS and cytopenias induced by CAR T cell treatment within imSAVAR.

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1. Methods

irAOP Development

During the first imSAVAR study-a-thon in June 2023 in Leipzig the three separately developed irAOPs for CRS meditated by CAR T cells, TCEs and CPIs were harmonized, suitable test systems and biomarkers to describe specific key events leading to CRS were assigned and disease maps developed (see Figure 1 below; Mazein et al., *manuscript in preparation*). For CAR T cells, a review article on the use of the irAOP concept to guide model development has been recently submitted for the joint imSAVAR issue in the Journal of Immunotoxicology (Alb et al., *manuscript under revision*). Here, we also outlined which animal models might be suitable to assess not only efficacy but also safety of CAR-T cell therapy (see Table 1 below).

In vivo models to study CRS mediated by CAR T cells

In vivo models have limitations in assessing the safety of CAR T cell therapies due to potential graft-versus-host and host-versus-graft reactions when human cells are administered, making interpretation and distinguishing CRS-related effects challenging[1]. Novel humanized mouse models, such as NSG-SGM3 mice reconstituted with human hematopoietic stem cells (HPSC) and utilizing xenotolerant T cells generated during humanization, enable investigation of CAR T cell toxicities including CRS and neurotoxicity, by minimizing the risk of graft-versus-host disease. However, these models have limitations as human immune cells are not fully differentiated and functional, making it unclear how well this predicts CRS risk in humans[2]. The PBMC-engrafted NSG MHC I/II double knockout model developed by Jackson Lab minimizes the risk of graft-versus-host disease and thus increases confidence in attributing toxicities specifically to CAR T cell therapy[3]. However, while this model offers the opportunity to pre-screen cytokine induction profiles and allows for experiments on modified CAR structure changes, its clinical relevance in terms of safety risk assessment still requires further determination. As outlined in Table 1 (see Results section) the animal models that are available to study CRS mediated by CAR T cells need to be carefully chosen depending on the desired readout.

Correlation to "real world" data sets from CAR T cell patients

For predictive biomarker development in nonclinical models, it is essential to benchmark results obtained in these models to "real-world" data sets. To this end, we obtained ethics approval at two partner sites (UKW and UKL/IZI) to enrol patients that receive approved CAR-T products (Kymriah, Tecartus, Yescarta, Breyanzi, Abcema, and Carvykti) for a correlative longitudinal study. Here, we collect blood and serum samples at four different time points prior (apharesis and prior to lymphodepletion) and post CAR-T cell infusion (day 3-7 and day 14). Immune cell phenotyping via multicolour flow cytometry (for all patients) and multiplex cytokine analyses (for a subset of patients at the UKW partner site) have been conducted and were correlated with adverse events, best response rate, progression free and overall survival among others. In addition, peripheral blood mononuclear cells (PBMC) from certain patients were selected for single cell RNA sequencing. These results will form the base for the anticipated *in vivo* experiments at the UKW partner site (see Results).

2. Results

Animal models ranked for their suitability to recapitulate CRS mediated by CAR T cells

Figure 1 illustrates the harmonized irAOP for CRS mediated by CAR-T cell therapy. Here, we also listed test systems (incl. animal models) and related molecules (biomarkers) that can be assigned to the different key events within the irAOP.

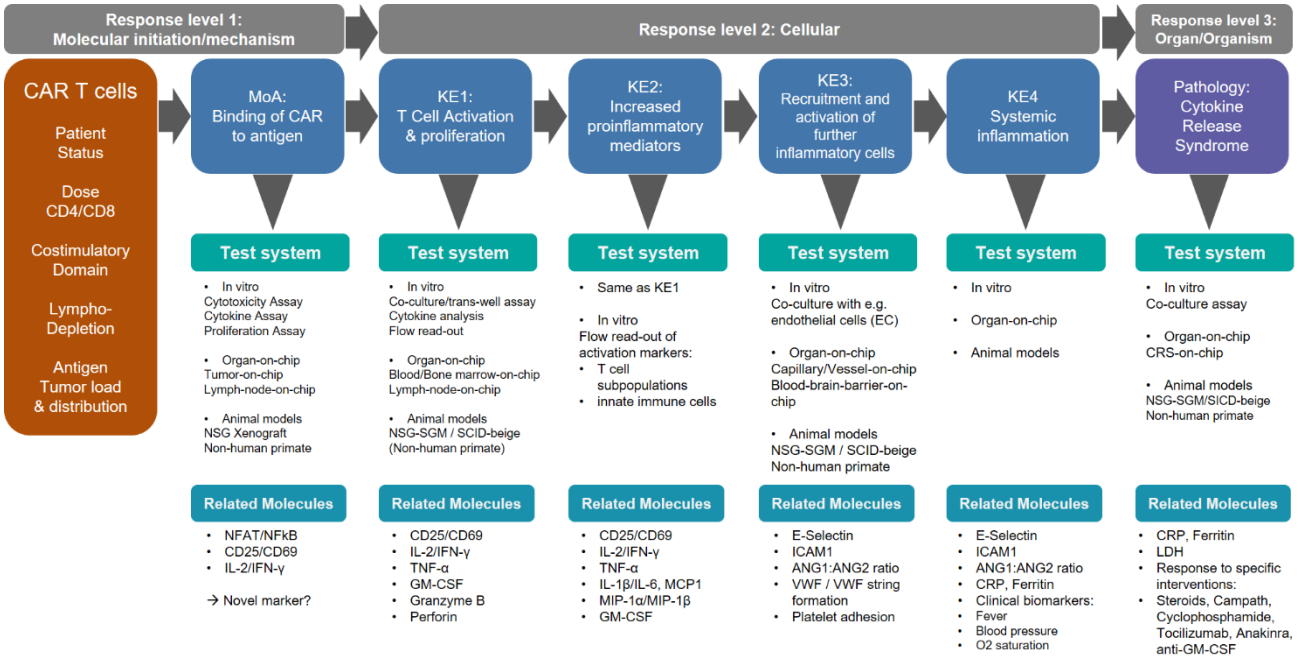

Figure 1: CRS mediated by CAR T cells as graphical irAOP representation including defined biological key events, test systems and biomarkers on the different response levels (molecular, cellular, tissue, organ, and organism) included in the pathophysiology of CRS. ANG, Angiopoietin; CAR, chimeric antigen receptor; CRP, C-reactive protein; EC, endothelial cell; GM-CSF, granulocyte-macrophage colony stimulating factor; ICAM1, intercellular adhesion molecule 1; IFN, interferon; IL, interleukin; KE, key event, LDH, lactate dehydrogenase; MCP1, monocyte chemoattractant protein 1; MIP-1, Macrophage Inflammatory Protein-1; TNF, tumor necrosis factor; VWF, von Willebrand factor.

Most of the currently used animal models utilize immunocompromised mice to allow for the engraftment of human tumour and effector immune cells, e.g. CAR T cells[4]. Such models, however, do not reflect the complexity of the human immune system and can therefore only be predictive of adverse immune effects if the mice are humanized, for example by engraftment with peripheral blood mononuclear cells[2,3,5].

Table 1: Currently available animal (mouse) models for CAR T cell therapy

Model	Description	Limitations	Purpose
Syngeneic mouse models	Immunocompetent model. Tumor cells and CAR T cells both of murine origin.	Translatability to the clinic might be limited if CAR vector is not identical to the CAR vector in the clinic	Efficacy and safety testing of CAR T cells (off-tumor toxicity). Assessment of MoA and KE1/2 possible (Fig. 1).

NSG mouse model	Immunocompromised model lacking T, B and NK cells. Tumor cells are engrafted followed by CAR T cell administration (both of human origin).	Without engraftment of PBMC or other relevant immune cell subsets assessment of CRS not possible; Risk of GvHD reaction	Efficacy testing of CAR T cells (MoA, KE1). Safety testing possible if mice are additionally engrafted with human immune cells (MoA, KE1/2; Fig. 1).
NSG MHC I/MHC II double knockout mouse model	NSG mice that lack murine MHC class I and II molecules	Without engraftment of PBMC or other relevant immune cell subsets assessment of CRS not possible; Risk of GvHD reaction	Efficacy testing of CAR T cells (MoA, KE1). Safety testing possible if mice are additionally engrafted with human immune cells (MoA, KE1-4, AO).
SCID-beige mouse model	Engraftment with 3 x 10^6 Raji tumor cells (*i.p.*; lymphoma cell line, human origin). After three weeks *i.p.* injection of 30 x 10^6 CAR T cells (human origin).	Artificial tumor site. Tumor engraftment in bone marrow ("physiological" tumor niche) might give different results.	Efficacy and safety testing of CAR T cells (CRS). Assessment of MoA, KE1-4, AO possible (Fig. 1).
HuSGM3 mouse model (PDX model)	Sublethally irradiated newborn triple transgenic NSG mice engrafted with HSPCs and ALL cells of human origin. Four or seven weeks (low or high tumor burden) later administration of CAR T cells of human origin.	Availability of matched HSPCs and tumor cells might be limited.	Efficacy and safety testing of CAR T cells (CRS). Assessment of MoA, KE1-4, AO possible (Fig. 1).

ALL, acute lymphoblastic leukemia; AO, adverse outcome; CAR, chimeric antigen receptor; GVHD, graft versus host disease, HSPC, hematopoietic stem and progenitor cell; i.p., intraperitoneal; KE, key event; MHC, major histocompatibility complex; MoA, mode of action, NOD-SCID, Nonobese diabetic/severe combined immunodeficiency mouse model, NSG, NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ mouse model [NOD-SCID mice with lack of IL-2 receptor expression]; PDX, patient-derived xenograft

Anticipated Experimental Campaign with CAR-Ts in vivo

As outlined above, CAR-T therapy can lead to serious side effects such as CRS, but also neurotoxicity and secondary infections due to prolonged cytopenias. All of these side effects are associated with potentially fatal consequences. In the interests of patient welfare, it is therefore essential to develop models that not only ensure adequate nonclinical safety assessment, but also to understand the basic pathomechanisms of these side effects and thus to develop new therapeutic interventions to prevent these potentially fatal side effects. The side effects mentioned above are highly complex interactions between a large number of different cell populations, which as a result of systemic inflammation and as part of the anti-tumour response interact with each other and thus trigger the adverse effects mentioned above.

Therefore, we would like to address the question of the extent to which the side effects mentioned are attributed to systemic effects or the recognition of existing target structures in toxicity-relevant tissues. We would then like to use these findings to identify new substances that could possibly lead to a mitigation in the clinical setting. A mechanistic investigation and clear information as to whether these substances are actually suitable for alleviating CRS symptoms is only possible in animal models, as the complex environment of systemic CRS cannot be studied *ex vivo*.

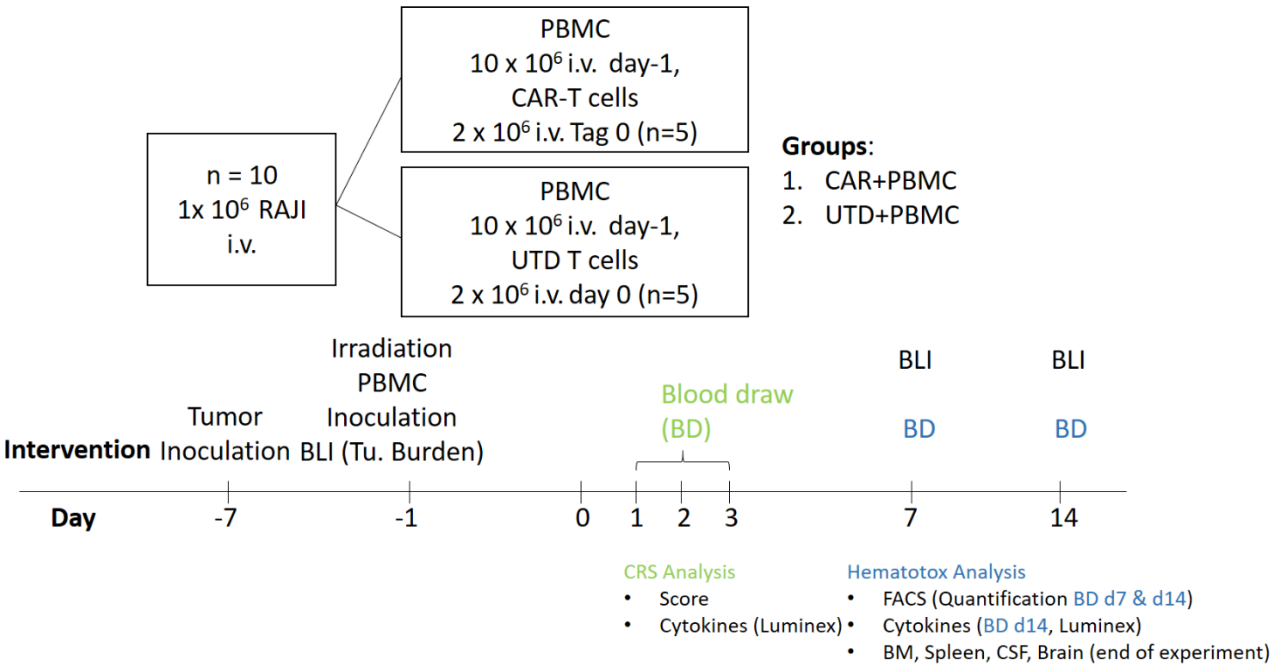

Figure 2: Overview of the anticipated *in vivo* experiment using NSG mice with MHC I/II knockout to assess adverse events of CAR-T cell therapy. BD, blood draw; BLI, bioluminescence imaging; BM, bone marrow; i.v., intravenous; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cells; Raji, Burkitt Lymphoma Cell Line; UTD, untransduced

3. Discussion

Recently, a recommendation to reduce the use of laboratory animals prior to first-in-human studies has been published (*The Food and Drug Administration commits to exploring alternative methods to replace laboratory animals in developing new drugs and products.* doi: https://doi.org/10.1038/d41586-022-03569-9; doi: 10.1126/science.adg6264). In light of this recommendation, the 3R principle (reduce, refine, replace) and our efforts since the start of the imSAVAR project to develop, improve and refine *in vitro* models capturing cytokine release in relevant human *in vitro* settings (see Deliverable Reports D2.5 and D2.8), we decided to reduce the number of *de novo* animal experiments.

4. Conclusion

Although there is a common wish within the scientific community to reduce the use of *in vivo* models, some aspects of therapeutic interventions such as biodistribution of drugs are still not easy to recapitulate using *in vitro* test systems. Even in advanced models like organ-on-chips there are still limitations (relevant organs not included, e.g. liver, kidney, heart or relevant sites for re-trafficking of immune cells like lymph nodes missing). Therefore, we propose to use an *in vivo* model that should allow to capture side effects and enable testing of new mitigation strategies that cannot be covered by *in vitro* test systems at this point in time.

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