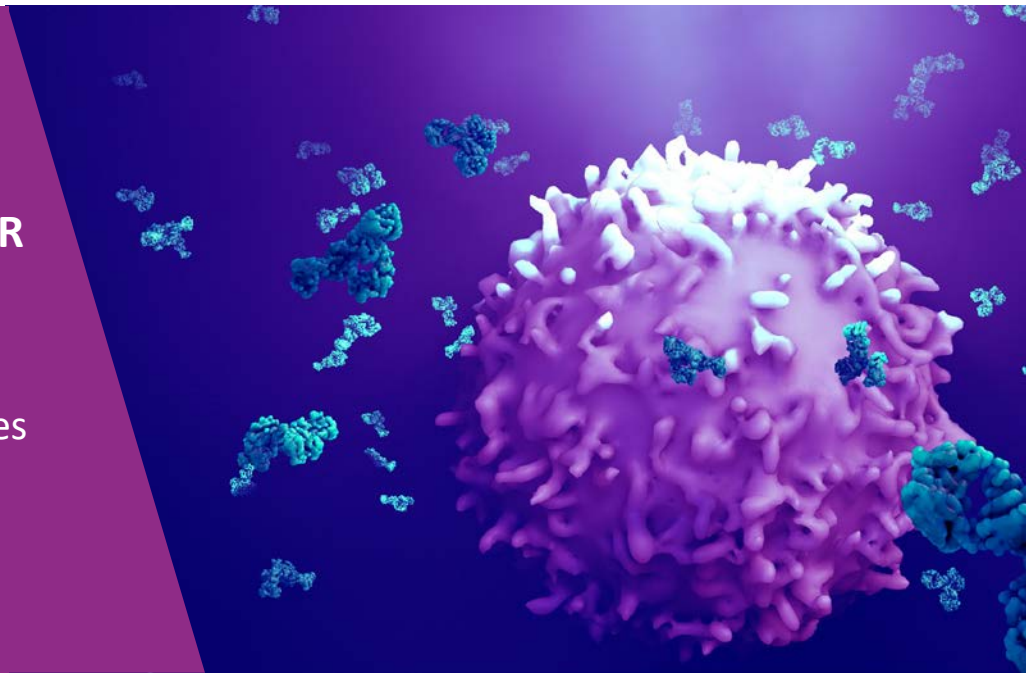
imSAVAR

IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 2.5
1st iteration in vitro & MPS models

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.

imi innovative medicines initiative

efpia

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.

Abstract

A key objective in imSAVAR is to create a platform with novel tools, models and resources for early non-clinical prediction of possible immune-related adverse events (irAEs) of immunomodulatory therapies. WP2 focuses on the refinement/adjustment/development of models for three different immuno-oncology modes of action (MoA) with a high medical need for improved non-clinical safety assessment: chimeric antigen receptor (CAR) T cells, bispecific T cell engagers (BiTEs) and immune checkpoint inhibitors (ICIs). The following immune related adverse outcomes are in the focus of the work program: cytokine release syndrome (CRS), neurotoxicity (CAR T cells, BiTEs) and hepatotoxicity (ICIs).

This deliverable summarizes the progress that has been made in the refinement and development of different *in vitro* and organ-on-chip models to be used for CAR T cells as an exemplary MoA and CRS as its most frequent adverse outcome.

Document Information

Deliverable Report	**D2.5: 1st iteration in vitro & MPS models**
Date	**31.05.2022**
Report prepared by	**Universitaetsklinikum Wuerzburg – Klinikum der Bayerischen Julius-Maximilians-Universitaet** Universitaetsklinikum Jena, Universiteit Twente, Eberhard Karls Universitaet Tuebingen, Labcorp Early Development Laboratories Ltd., Boehringer Ingelheim International GmbH, Novartis Pharma AG, Roche, Sanofi-Aventis Recherche & Developpement
Project	**imSAVAR - Immune Safety Avatar: nonclinical mimicking of the immune system effects of immunomodulatory therapies** Grant Agreement No.: 853988 (IMI2-2018-15-04)
Project Coordinator	**Fraunhofer-Gesellschaft zur Foerderung der angewandten Forschung e.V.** Prof. Dr. Dr. Ulrike Köhl **Novartis Pharma AG** Dr. Jonathan Moggs
Type	**Deliverable Report | Public**

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.

Table of Contents

1. Methods

irAOP Development

A key deliverable is the development of a conceptual map of CRS pathogenesis as an immune-related adverse outcome pathway (irAOP) comprising key molecular and cellular events identified through a systematic literature review on pathophysiology and clinical occurrence of CRS after CAR T cell therapy. To each event, existing and emerging non-clinical assays were allocated. imSAVAR is engaging all stakeholders in CAR-T immunotherapy, including clinicians, scientists, model developers, bioinformaticians, data analysts as well as patients through surveys and workshops for patients and health care professionals (for details see Deliverable Report D2.3).

Experimental Campaign with CAR-Ts

We established a roadmap for studies establishing a new set of non-clinical assays and endpoints that will be validated incorporating the feedback from multi-stakeholder workshops to help mitigate clinical safety concerns (see also Deliverable Report D2.3). To this end, an experimental campaign was conducted with n=3 CAR T cell products in different test systems from within the consortium. Test systems available in imSAVAR include *in vitro* co-culture assays, genomic approaches, and organ-on-chip models.

Correlation to "real world" data sets

All data obtained in the test systems will be correlated with clinical data to identify optimal non-clinical assays for enhancing assessment and prediction of CRS. To this end, we are collecting blood and serum of patients undergoing CAR T cell therapy at the UKW partner site and in future also at the Leipzig and Hannover partner sites. FACS analysis as well as multiplex cytokine analysis will be accompanied by in-depth RNA-seq analysis and correlated with adverse events (mainly CRS and neurotoxicity) to identify new biomarkers that will be then incorporated in the test systems.

2. Results

irAOP Development

The CAR-T mediated CRS irAOP was presented as a Poster on several international meetings including the in-person meeting of the ICLE 2022 (International Conference on Lymphocyte Engineering) in Munich. Here, we were able to gain the Jackson Laboratory, an independent, non-profit biomedical research institution that has a long-standing expertise in cancer research and offers more than 11,000 genetically defined mouse strains to the research community, as an external partner for the imSAVAR stakeholder community. As the Jackson Laboratory also offers mouse strains to study CRS, this will be of particular interest for imSAVAR in the upcoming project years.

Furthermore, based on the linear CRS irAOP for CAR T cells a so-called disease map was developed in a joint project with WP4 using the MINERVA Platform provided by partner UNILU. This platform enables the visual exploration, analysis and management of molecular networks (see also https://minerva.pages.uni.lu/doc/) and will also enable gathering input from external partners.

Experimental Campaign with CAR-Ts

1) Tumor-on-Chip

A tumor-on-chip model was developed to incorporate ROR1-expressing tumor spheroids and a perfusable endothelium that allows CAR T cell perfusion and extravasation. Methods were developed to generate 100-150 µm tumor spheroids off-chip. Initial designs were iteratively optimized to yield reproducible loading of the generated spheroids without impacting the viability. A dextran-based hydrogel that retains a compact spheroid structure over a week and simultaneously enables T cell trafficking was selected. Endothelial barrier adjacent to the tumor was characterized by CD31 and VE-Cadherin expression prior to CAR T cell perfusion.

Following the tumor-on-chip establishment, an equal ratio of $CD4^+$ and $CD8^+$ ROR1-specific CAR T cells (or control untransduced T cells from the same donor) was labeled and perfused through the chips. During the first 15 h of infusion, CAR T cells infiltrated the tumor spheroid chambers faster than the control T cells. Quantitative imaging analysis showed that CAR T cells hampered tumor growth and infiltrated the tumor at a higher density over one week period. On the contrary, the tumor continued to grow when treated with the control T cells despite their infiltration into the tumor at earlier time points. Treatment with dasatinib at different time points enabled the modulation of CAR T cells activity against tumor, as shown by the image-based quantification of infiltration density. Additionally, cytokine levels (IL-2, IL-6, IL-10, TNFα, IFN-γ) typically observed during CAR T cell-associated cytokine release syndrome were recapitulated, which were significantly higher than in the control T cell condition at almost all measured time points. Future work will focus on the integration of patient-derived cancer organoids into the model to evaluate patient-specific response, studying the effect of dasatinib on the kinetics of CAR T cell-mediated cytokine release, as well as optimizing additional endpoints to evaluate CAR-T/T cells activation status upon one-week interaction with the tumor.

2) Vessel-on-Chip

A previously established 3D vessel-on-chip model was used to model endothelial dysfunction, after exposure to CAR T cell derived culture media. IPSC derived endothelial cells were seeded on cylindrical collagen lumens and stimulated with different concentrations of cytokines. CAR T cell derived culture media were obtained from the university of Tübingen, with varying levels of cytokines like TNF-α, IFN-γ, IL-2, and IL-6. After stimulation, immunofluorescent stainings were performed for VE-cadherin and F-actin. Cell viability decreased if the cytokine concentration was higher, but optimization of media compatibility is needed for further analysis. In addition, a chip with 16 multiplexed channels and automated medium refreshment is being developed to systematically study different cytokine mixtures. These chips are based on Quake valve technology combined with rapid prototyping methods (in particular micromilling) for the endothelial cell channels. First prototypes of this chip were fabricated and the automated cell seeding protocol is currently being optimized to obtain a uniform seeding density throughout the channels.

3) MIMIC® CRA platform

Work was conducted to evaluate the immunostimulatory potential induced by CD19 and ROR1 CAR T cells in the MIMIC® CRA platform. The MIMIC cytokine release assay (CRA) is designed to elucidate cytokine/chemokine responses of drugs intended for i.v. administration. This assay is an automated 3D co-culture assay comprised of fresh RBC-depleted whole blood, autologous plasma, and EA.hy926 human endothelial cells pre-cultured to confluency on a collagen bed, and was qualified for evaluating cytokine secretion. CAR T cells generated from two healthy donors were expanded and applied onto the MIMIC-CRA, and a multiplex array measured cytokine secretion in the culture supernatant after 20-22hr. Twenty-one normal whole blood donors were assessed and the cytokines measured were IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, MIP-1β, MCP-1 and TNFα. A dose-dependent response was observed under these

conditions for all of the cytokines, and there was a dose-dependent reduction in total $CD19_+$ B cells observed in conditions with CD19 CAR T cells indicating expected pharmacodynamic activity. We further removed endothelial cells or red blood-depleted whole blood from the assay to explore dependencies and confirmed that cytokine induction is primarily dependent on blood cells and minimally on endothelial cells. Future work will be done evaluating a third CAR T cell donor in the same manner as the above two donors to evaluate variability between donors and potentially explore additional biomarkers.

Furthermore, discussion is ongoing to design potential studies using *in vitro* assays such as the MIMIC® CRA platform as tools to identify biomarkers that correlate with individuals that demonstrate a high level of cytokine secretion following treatment with T-cell engaging (TCE) bispecific antibodies and may represent individuals at higher risk of developing a cytokine storm response. It would be highly impactful to identify factors that correlate with risk of acute, high levels of cytokine release after TCE antibody treatment that could be clinically assessed prior to treatment initiation as part of the risk mitigation strategy for cytokine release syndrome.

4) Gut-on-Chip

$CD4_+$ and $CD8_+$ CAR T cells were analysed either individually or in its combination in the Gut-on-Chip model containing ROR1 expressing target cells. CAR T cells mediated in contrast to non-modified naive donor T cells from healthy donors a high degree of tissue damage by the deregulating VE-Cadherin and E-Cadherin expression on the endothelial or epithelial tissue layers. A pre-treatment of the CAR T cells with small chain fatty acids (SCFA) limited CAR T cell-mediated tissue damage in a dose dependent manner. Acetate, propionate and butyrate have increasing protecting capacity in limiting tissue damage by CAR T cells. The gut-on-chip model was optimized in its cellular composition, use of perfusion protocols for engineered and native T cells as well as for SCFA treatment. Detection limits for biomarkers of tissue damage and cytokine release were improved and handling protocols optimized. SOPs for T cell phenotyping and image based quantification of T cell infiltration were generated. Future work will focus on *in situ* characterization of SCFA mediated modulation of T cell plasticity and the integration of human stool sample filtrates to the model. Further, mechanistic analysis of SCFA receptor mediated signalling and associated modulation of the CAR T cell immune response will be performed.

5) Characterization of Immune Cell Subsets in patients undergoing CART treatment (*Biomarker Development for CAR-Ts)*

Blood and serum samples were collected from 25 patients at the UKW partner site. To characterize the immune cell subsets prior to CAR-T therapy, samples were collected at two time points before CAR-T treatment, i.e. when patients undergo apharesis to collect leukocytes for the production of the CAR-T product and prior to the induction of the lymphodepleting treatment that enables the engraftment of the CAR-T product. Additionally, to characterize immune cells and infused CAR T cells, samples were also collected at two time points after infusion of CAR T cells, that is early after CAR-T treatment (day 3 to 7) and on day 14 of CAR-T treatment. In-depth RNA-seq and multiplex cytokine analysis will then be preferentially conducted with samples from patients with CRS symptoms to identify novel non-clinical biomarkers. The original scientific study protocol and further amendments were approved by the local Ethical Committee of the Medical Faculty, University of Würzburg, Germany. All patients provided their written informed consent, in accordance with the Declaration of Helsinki. All data were decoded and maintained in secure databases.

Figure 1 shows the frequency of $CD3_+CD4_+$ T cells as well as the frequency of CD19 CAR T cells in the peripheral blood of a patient treated with the Axicabtagene ciloleucel CD19 CAR-T product (Yescarta®) on day 7 and day 14 after infusion of the CD19 CAR-T product.

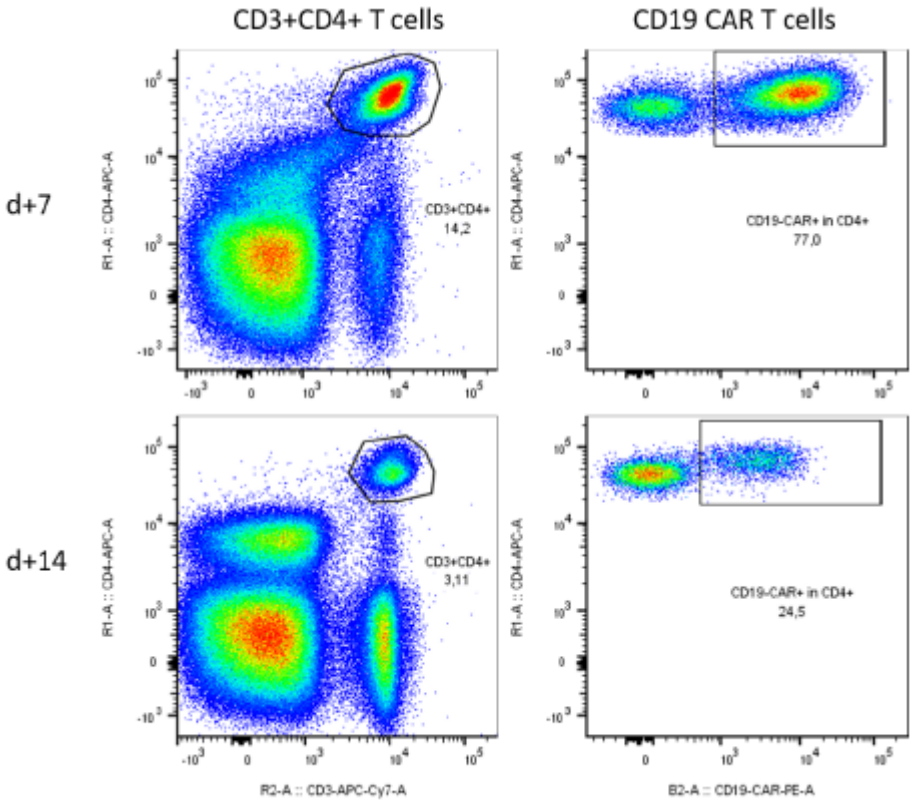


FIgure 1: ***Frequency of CD19 CAR T cells within the CD3+CD4+ T cell subset in peripheral blood of a DLBCL patient.*** *Peripheral blood was obtained from the patient after written informed consent. Flow cytometric analysis was conducted to analyse the frequency of CD3+CD4+ T cells and the frequency of CD19 CAR T cells using fluorophor-conjugated anti-CD3 and anti-CD4 antibodies (both from Biolegend) and the biotinylated CD19 CAR Detection Reagent with a PE-conjugated anti-Biotin antibody (both from Miltenyi Biotec).*

3. Discussion

No relevant deviations from the Description of Action and contingency plans identified.

4. Conclusion

imSAVAR established an irAOP for CAR-T mediated CRS that will enable the development and validation of novel non-clinical assays that aim to enhance the characterization of CAR T cell associated CRS during non-clinical development. In particular, we studied CAR-T/T cell interaction with appropriate target cells in a variety of models, ranging from 2D co-culture assays to advanced 3D and organ-on-chip models from within our consortium for the first time and were able to recapitulate cytokine patterns also seen in patients treated with FDA/EMA approved CAR T cell products. Furthermore, we are collecting samples from patients treated with such CAR T cell products for an in-depth genomic analysis with the aim to identify new biomarkers for nonclinical test systems. This effort is ultimately anticipated to enhance the safety assessment of therapeutic CAR T cell products, thus potentially accelerating patient access to CAR T cell products.

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.