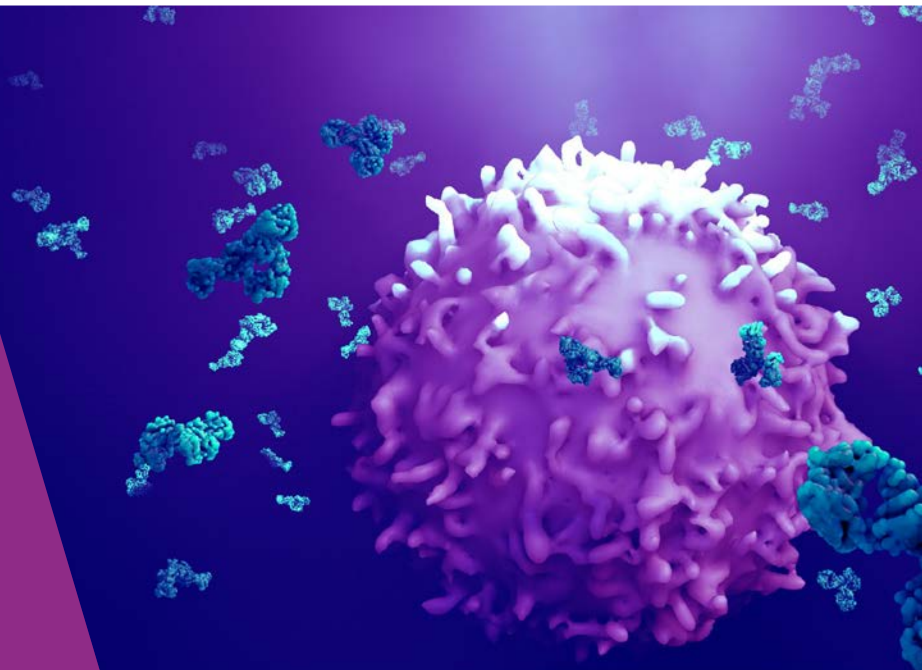
IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 2.8
2nd 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.

imSAVAR

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.

This deliverable report summarizes the progress that has been made since the last report (D2.5) regarding the refinement and development of different *in vitro* and organ-on-chip models to be used for CAR T cells and BiTEs as exemplary mode of actions (MoAs) and cytokine release syndrome (CRS) as their most frequent adverse outcome.

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1. Methods

irAOP Development

A joint WP2/WP3 AOP working group (led by K. Sewald, Fraunhofer ITEM) was established to harmonize and refine all irAOPs in WPs 2 and 3. This AOP WG recently reached out to members of the CIAO project in order to join forces. Within the CIAO project several AOPs for the different adverse outcomes mediated by SARS CoV-2 were developed, including also an AOP for CRS mediated by SARS-CoV-2. In addition, we will further refine the CRS irAOP for CAR-T cells and other modalities and implement updates in the disease maps developed by partner UniLu in a dedicated study-a-thon in June 2023.

Experimental Campaign with bispecific T cell engagers

A detailed study plan was established for testing bispecific T cell engagers in different cytokine release assay (CRA) platforms with the aim to identify differences between donors who are high or low responders following treatment.

Experimental Campaign with CAR-Ts

CAR-T cells generated from n=3 healthy donors (see also D2.3 and D2.5) were tested in different 2D/3D *in vitro* models across the consortium. Models were further refined, e.g. by incorporation primary tumor samples from cancer patients.

Correlation to "real world" data sets

Identification of new biomarkers for CAR-T mediated CRS is still ongoing. To this end, we collect patient samples at both partner sites (UKW; UKL) as well as clinical data. Results of flow cytometric analysis (and multiplex cytokine analysis) will be used as guide for sample selection for subsequent single cell RNA-seq analysis.

2. Results

irAOP Development

First results of the CAR-T patient research study, which aims at identifying new predictive biomarkers for CAR-T mediated CRS in the context of the established irAOP, was and will be presented at several international meetings, including the 5th European CAR T-cell Meeting (February 2023), the 21st International AEK Cancer Congress (February 2023) and the annual meeting of the ISCT (International Society for Cell & Gene Therapy; May/June 2023). In addition, an abstract was also accepted for poster presentation at the upcoming 4th International conference on lymphocyte engineering (ICLE) in Munich (September 2023).

Results of the first imSAVAR study-a-thon shall be made available in form of a publication. Updated versions of disease maps will be made available on the MINERVA platform of UniLu.

Experimental Campaign with bispecific T cell engagers

For the study plan established to identify differences between donors who are high or low responders following treatment with T cell engager molecules, there were four CRA platforms selected for evaluation: MIMIC® (Sanofi), BOEC (LabCorp), whole blood (Novartis) and PBMC/endothelial cell co-culture (BI). For

evaluation, three T cell engagers were selected that all bind to CD3 on the surface of T cells and receptors that are known targets for hematological malignancies (CD19, CD123, CD20). A non-targeting molecule was included as a negative control. Initial pilot studies were completed in each of the CRA platforms with the four molecules to identify doses of each molecule where donor variability is maximized. Concentrations tested ranged from 0.01 ng/mL to 100 µg/mL, and panels of cytokines were tested using established methods in each test facility. The number of donors evaluated in the pilot studies at each site ranged from four to 11. Analysis of the pilot study data is ongoing to select appropriate doses for evaluation in the next studies that are planned to evaluate a larger number of donors (~N=30) and collect additional endpoints (i.e. – identification of cell populations by flow cytometry, whole transcriptome profiling by single cell RNA-seq) for correlation with the cytokine levels. Work is ongoing to finalize the flow cytometry panels and confirm methods for conducting single cell RNA-seq.

Experimental Campaign with CAR-Ts

1) Tumor-on-Chip

A tumor-on-chip model was developed to recapitulate the efficacy aspect of CAR-T cell therapy against solid tumor as well as safety, namely by mimicking the resulting cytokine release syndrome (CRS) on the chip systems over one week period of perfused culture. Specifically, the model is able to incorporate ROR1-expressing tumor spheroids/organoids and a perfusable endothelium that allows CAR-T cell perfusion and extravasation. To capture patient-specific effects from the patient-derived cancer organoids, the model was refined by integrating human induced pluripotent stem cell-derived endothelial cells that are sourced from the same donor as CAR T cells, thereby excluding alloreaction between T cells and endothelial cells in the system.

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. Over a one week period, (CAR) T cell migration, infiltration, and cytokine release (IL-2, IL-6, IL-10, TNFα, IFN-γ, granzyme B) were monitored. The model has now been utilized to test CRS intervention using dasatinib at different time points and durations during the CAR-T cell treatment period. As dasatinib enabled a transient deactivation of CAR-T cells during their interaction with the tumor cells, a suitable time point and duration of its application enabled the management of cytokine release to ensure safety while keeping the efficacy of CAR-T cells minimally affected. Further, we found that the relative ROR1 expression on the patient-derived organoids positively correlates with the persistence of CAR-T cells within the tumor bulk and cytokine secretion. The integration of patient-derived organoids may therefore recapitulate target-antigen heterogeneity and patient-specific response. Future work will focus on the integration of innate immune cells such as macrophages and monocytes into the model to capture and study their contribution to the amplification and perpetuation of the inflammatory cycle that would lead to CRS. Current results were and will be presented at international conferences, including the 5th European CAR T-cell Meeting (February 2023) and Microphysiological System (MPS) World Summit (June 2023).

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 obtained from the University of Tübingen. The IPSC derived endothelial cells missed crucial growth factors in this medium, causing incompatibility that could be solved

by addition of these growth factors. At the moment experiments are continuing with the optimized medium, with readouts of markers for barrier permeability and endothelial dysfunction. In addition, a multiplexed chip with 16 channels and automated medium refreshment was developed to systematically study different cytokine mixtures. First prototypes of this chip were fabricated and used for automated cell seeding. Further experiments will focus on further optimization of the cell culture conditions, systematic testing of cytokine concentrations, and performing different perfusion times.

3) MIMIC® CRA platform

Test runs with n=3 CAR-T donors on the MIMIC® CRA platform were completed incl. multiplex cytokine and flow cytometric analysis as already described (see also D2.5). A manuscript to be published as part of the joint imSAVAR issue is under review.

4) Gut-on-Chip

Originating from the already characterized luminal concentration of short-chain fatty acids (SCFA), serum level concentrations were tested in the before-established pre-treatment condition. CD19 CAR T cells were added as an additional negative control condition as no CD19-expressing target cells are present in the Gut-on-Chip model. Additionally, antagonist studies to assess the mechanisms of SCFA-mediated pathways have started. A manuscript summing up the current results is currently under preparation to be submitted within the next six months. Further work will focus on the possibilities and characterization of potential stem cell-based models.

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

Up to now, peripheral blood and serum samples from over 130 patients receiving FDA/EMA approved CAR-T cell products were collected at both partner sites (UKW/UKL; for details on study design see D2.5). Flow cytometric analysis to detect CAR-T cells as well as other relevant immune cell subsets (e.g. monocytes, NK cells) were conducted and will be used along with the clinical data to decide which serum samples will be selected for multiplex cytokine analysis (Luminex platform, 44 different cytokines/chemokines) and genomic analysis using single cell RNA.

Figure 1 illustrates the detection of CAR-T cells in peripheral blood in a MM patient treated with BCMA-specific CAR-T cells (idecabtagene vicleucel, Abecma®).

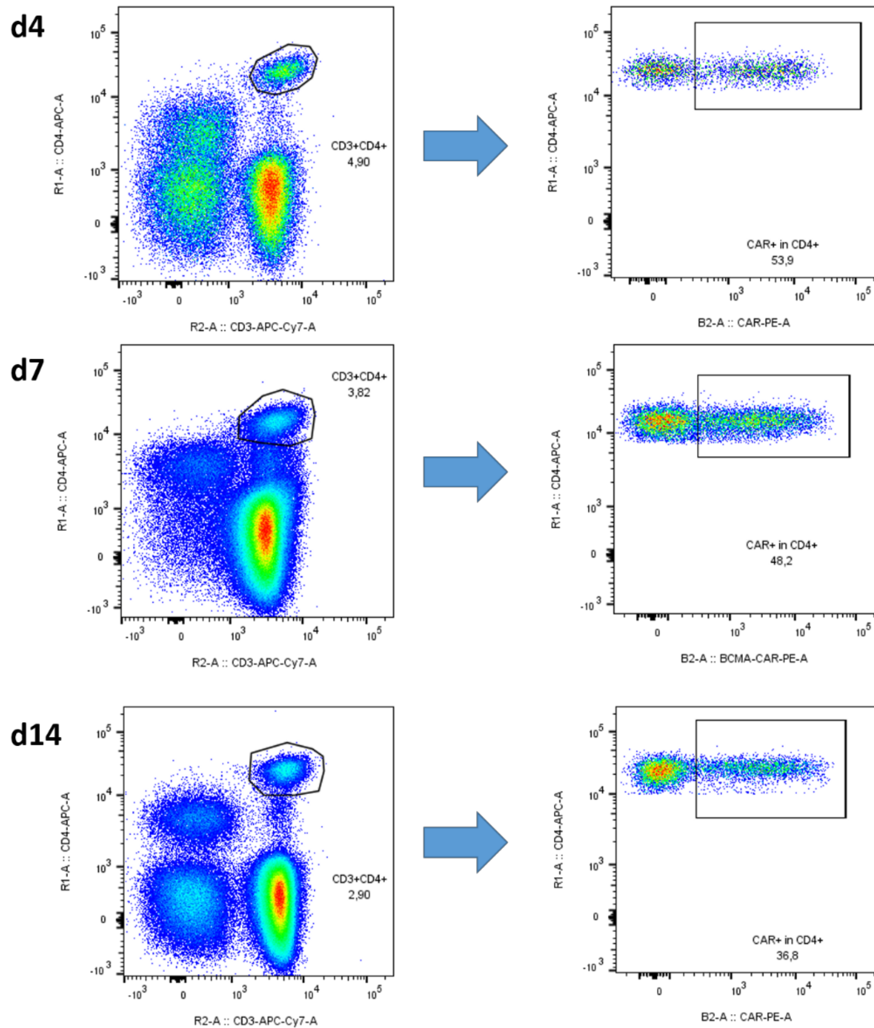

*Figure 1: **Frequency of BCMA-specific CAR-T cells within the CD3⁺CD4⁺ T cell subset in peripheral blood of a MM patient.** Peripheral blood was obtained from the patient after written informed consent. Flow cytometric analysis was conducted to analyse the frequency of CD3⁺CD4⁺ T cells and the frequency of BCMA-specific CAR-T cells using fluorophor-conjugated anti-CD3 and anti-CD4 antibodies (both from Biolegend) and PE-conjugated BCMA protein (Acro Biosystems). BCMA=B-cell maturation antigen; MM=multiple myeloma*

6) BOEC Autologous Assay

$CD4^+$ and $CD8^+$ CAR-T cells were generated targeting CD19 from two donors, which were cryopreserved and shipped to Labcorp along with untransduced (UTD) and Peripheral Blood Mononuclear Cells (PBMCs) from the same donors. PBMCs were then used to generate Blood Outgrowth Endothelial Cells (BOECs), serving as an autologous endothelial cell line for use in assays. Initial development runs of a CAR-T cytokine release assay focused on cellular response to endothelial, PBMC and CAR-Ts to an anti-CD3 stimulant in the absence of target cells, to establish assay performance in comparison to using in-house, fresh donors. A heterologous system using Human Umbilical Vein Endothelial Cells (HUVECs) in place of BOECs was also run to assess any difference in cytokine response in the two approaches. This assay demonstrated higher raw cytokine responses in the heterologous system compared to the autologous system (and thus a higher assay sensitivity in the BOEC assay), and recapitulated the expected levels of cytokine response to in-house assays. Subsequent runs added in $CD19^+$ Raji target cells at an effector to

target ratio of 2.5:1 and 1:1, demonstrating higher cytokine response in conditions of higher target concentration in line with higher clinical cytokine response to increased tumour burden. CAR-Ts used in these assays focused on $CD8^+$ cells, but future work on this project will include a 1:1 of $CD4^+$:$CD8^+$ CAR-Ts, running 4:1, 2:1 and 1:1 with a Raji-GFP line to visualise target killing. The assay will then be expanded to include more donors and transferred into a 3D setting to further recapitulate the *in vivo* setting, utilising the Mimetas 2-lane vessel-on-a-chip platform to generate autologous vessels from BOECs. Anti-IL-6 treatment tocilizumab may be included to assess the ability to reverse cytokine release in the model, to reflect the clinical treatment of cytokine release syndrome. End-points will include cytokine analysis and a vascular leakage assay to assess any impact to barrier integrity, a known clinical effect seen with higher levels of cytokine release. Later advances to the project will focus on a solid tumour model using ROR1-targeting CAR-T cells on the Mimetas OrganoPlate Graft platform.

3. Discussion

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

4. Conclusion

imSAVAR established an irAOP enabling the development and validation of improved nonclinical models that aim to enhance the characterization of CAR-T associated CRS. This effort is ultimately anticipated to enhance the safety assessment of therapeutic CAR T products, thus potentially accelerating patient access to CAR-T products with an enhanced therapeutic index. This CAR-T irAOP strategy forms part of imSAVAR’s broader efforts to enhanced nonclinical safety assessment algorithms for additional immunomodulatory therapeutic modalities (e.g. bispecific T cell engagers, immune checkpoint inhibitors), and a range of immune-related toxicities (e.g. ICANS, infections).

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