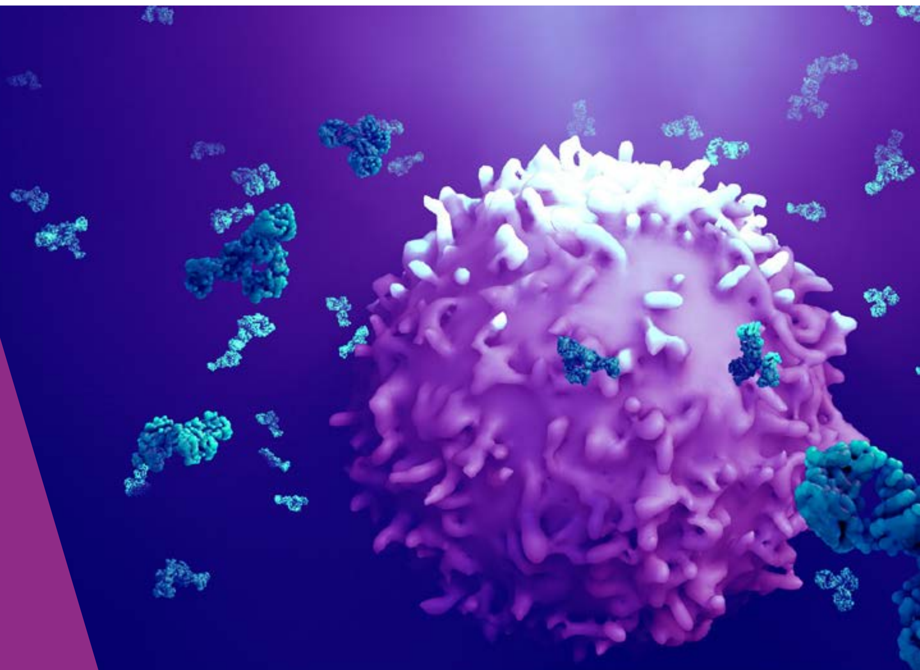
IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 4.4

3rd iteration of advanced immune cell profiling

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

Work package 4 (WP4) focuses on the development of biomarkers for predicting the risk of observing harmful adverse outcomes in first-in-human (FIH) studies of immunomodulatory therapeutics. Current preclinical models to assess safety of immunotherapies are often species-dependent and incomplete, since they reflect only limited areas of the human immune system, which often leads to wrong predictions of human immune-related adverse events (irAEs). Hence, WP4 aims at establishing biological characteristics (biomarkers) that are measurable and evaluable and can be integrated into safety models in order to (i) assess if the model mimics the underlying human biological processes leading to an immune-related adverse outcome as closely as possible, to (ii) assess if the biomarker is reliably predicting the risk of harmful adverse outcomes in FIH studies, and to (iii) support safe starting dose selection for FIH studies.

In imSAVAR, we will focus on four initially defined modes of action (MoAs) of immunomodulatory therapeutics, which require the development and validation of biomarkers: (i) CAR (chimeric antigen receptor) T-cells, (ii) BiTEs (bispecific T-cell engagers), (iii) CPI (checkpoint inhibitors), and (iv) IL-2. By aligning biomarker development with immune-related adverse outcome pathways (irAOPs), we promote a shared understanding of the processes initiated by a molecular event that may ultimately result in adverse outcomes.

In Deliverable D4.4, we present a third version of datasets that have been processed through bioinformatics. These datasets are valuable for identifying biomarkers and gaining mechanistic insights from the models being refined and developed in imSAVAR. To this end, we advanced studies previously described in D4.3 (2nd iteration of advanced immune cell profiling) and initiated a new study.

Document Information

Deliverable Report	**D4.4: 3rd iteration of advanced immune cell profiling**
Date	**05.03.2025**
Report prepared by	**Fraunhofer IZI** University of Leipzig, University of Oslo, Medical University of Innsbruck, Paul-Ehrlich-Institute, University Hospital of Würzburg, LabCorp, Boehringer Ingelheim International GmbH, Novartis, 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 Dr. Kristin Reiche **Novartis Pharma AG** Dr. Jonathan Moggs Hannah Morgan, PhD
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

Abstract....2
1. Background....5
2. Data....5
3. Results....8
3.1 Study 1 – Single cell RNA and bulk sequencing of in vitro stimulated PBMCs (CD3/CD28) in healthy humans and non-human primates (NHPs)....8
3.2 Study 2 – Roche Immune Cell Atlas....8
3.3 Study 3 – Patient Research Study....9
3.4 Study 4 – CAR T cell atlas....9
3.5 Study 5 – In vitro assay to study vascular leakage....10
3.6 Study 6 – Re-analysis of Deng et al.....10
3.7 Study 7 – TCE CRA Assessment....11
3.8 Study 8 – Dose-response assessment of cytokine release by T-call engagers....11
3.9 Study 9 – Deep learning-aided inter-species-comparison of immune response in drug development involving cynomolgus monkey....11
4. Summary....12
5. Abbreviations....12
6. References....14
Acknowledgement....15

1. Background

The project imSAVAR (Immune Safety Avatar: nonclinical mimicking of the immune system effects of immunomodulatory therapies) aims at creating a platform that provides novel tools, models and resources for early nonclinical prediction of adverse events of immunomodulatory therapeutics.

Within imSAVAR this is implemented by defining immune-related adverse outcome pathways (irAOPs) to guide the development of novel test systems. irAOPs describe the interconnection of a molecular initiating event (MIE) with a series of key events eventually leading to an adverse outcome. The interconnection between two subsequent key events (KEs) is defined by key event relationships (KERs).

Biomarkers are measurable and assessable biological characteristics that evaluate the molecular initiating event MIE, key events KEs, or adverse outcomes of an immune-related adverse outcome pathway irAOP. The initial step in biomarker development involved systematic reviews of existing datasets on human and non-human immune cell subsets to identify knowledge gaps and inform future research directions (see Deliverable D4.1). In the second step, specific research questions were formulated, and suitable datasets were identified (D4.2). Subsequently, we present the second round of available datasets and provide details on their processing status (see Deliverable D4.3). Here, we provide a third iteration of advanced profiling of immune cells, that includes an advanced status of previously described studies (see Deliverable D4.3) and a newly initiated study.

2. Data

The following datasets are included in the second round of biomarker development. They have been identified by systematic reviews (as described in D4.2) or have been newly generated in close cooperation with WPs 2 and 3.

Table 1: Ongoing biomarker studies in WP4.

No.	Study	Aim of study	Study design	Status of data (raw / (pre)processed)	MoA
1	Single cell RNA and bulk sequencing of in vitro stimulated PBMCs (CD3/CD28) in healthy humans and non-human primates (NHPs) -ongoing-	Determining baseline characteristics of CD3/CD28 engagement of T cells, cross-species comparison	Isolation of PBMCs of healthy donors (human, NHP), incubation with CD3/CD28 and subsequent single cell sequencing of RNA	Single cell RNA sequencing: preprocessed Bulk RNA sequencing: preprocessed	Baseline Immune Cell Profiles
2	ROCHE Immune Cell Atlas -completed-	Baseline characteristics of immune cells (bulk RNA sequencing)	Characterize immune cells of healthy H. sapiens, M. fascicularis, and M. musculus	Processed according to FAIR principles and provided to the consortium	Baseline Immune Cell Profiles

3	Patient Research Study -ongoing-	Identify biomarkers for CRS development (and other adverse events) in CAR T cell patients	Collect blood of patients receiving FDA/EMA-approved CAR T cell products, characterize cytokine/chemokine profile, immune cell composition, transcriptome on single cell level	Flow cytometry data: raw/processed Cytokine data: processed, manuscript in preparation scRNA seq data: preprocessed	CAR T cells
4	Single cell CAR T cell atlas -ongoing-	Develop a specialized single-cell atlas for CAR T-cells with a large number of samples to distinguish the spectrum of transcriptional states that may explain the irAOPs.	Re-analysis of publicly available single-cell data including infusion products as well as pre- and post-infusion samples	Gene expression and clinical metadata were processed and harmonized across studies	CAR T cells
5	In vitro assay to study vascular leakage – -Assay establishment: completed -Characteriza-tion-study of HUVEC-monocultures: completed (Gogesch and Ortega Iannazzo et al. submitted) -Analyses of HUVEC:immune cell co-cultures upon stimulation: ongoing	Identification of potential novel biomarkers to better predict/manage vascular leakage e.g. after IL-2 therapy.	Characterization-study of HUVEC-monocultures: HUVECs were treated with 16 leakage-associated stimuli, for selected stimuli in presence peripheral blood mononuclear cells (PBMCs); Regulation of 5 surface markers, cellular viability, and 11 cytokines as well as permeability of HUVECs assessed	Data of surface markers, cell viability, cytokine levels, and leakage for multiple stimuli and unstimulated controls for all time points processed, analysed and manuscript with results submitted for publication	Drug- and immune-related induction of vascular leakage (including IL-2)

6	Re-analysis of Deng et al., 2020 -completed-	Adaptation and optimization of the oposSOM data portraying analysis workflow for single-cell data	Modularization of gene expression patterns in infusion product T cells and characterization of cellular subpopulations using single-cell oposSOM	Original data publication by Deng et al. 2020 (PMID: 33020644). Processed meta-cell data and analysis results were integrated into the oposSOM-browser and published in PMID: 36248848	CAR T cells
7	TCE CRA Assessment -ongoing-	Identification of potential differences in healthy donor baseline phenotypes that correlate with high or low responses in four different in vitro cytokine release assays	Baseline phenotype characterization will include hematology, clinical chemistry, flow cytometry assessment of immune cell subsets and scRNA-Seq of high and low responding donors	Baseline phenotyping data generated (flow cytometry, hematology, clinical chemistry) Cytokine release assay experiments: data are generated; low/ high responders are identified. scRNAseq on high and low responders initiated	BiTEs
8	Dose-response-assessment of cytokine release by a T-cell engager in multiple novel CRS models -ongoing-	support the transfer of new immunomodulatory drugs from pre-clinical development to first-in-human studies using novel biomarkers and endpoints to determine meaningful readouts	Systematic utilization of assays (whole blood assay, lymphoid model, Vascular models) that reflect the individual KEs in the irAOP of CRS induced by BiTEs, assessing cytokine release after treatment with a T-cell engager to calculate dose-response-curves	Data to be generated	BiTEs
9	Deep learning-aided inter-species-	identifying shared characteristics and divergences within	T cell activation in PBMCs from humans and macaca	Single cell RNA sequencing: processed	Baseline Immune

	comparison of immune response in drug development involving cynomolgus monkey -ongoing-	T Cell-triggered immune response between cynomolgus monkeys and humans using deep learning and 'traditional' bioinformatics	fascicularis by anti CD3/anti CD28 stimulation, single cell RNA sequencing of baseline and stimulated PBMCs, set-up of computational workflow for similarity analyses		Cell Profiles

3. Results

In the following we provide an overview of the current results for each of the individual studies listed in Table 1.

3.1 Study 1 – Single cell RNA and bulk sequencing of in vitro stimulated PBMCs (CD3/CD28) in healthy humans and non-human primates (NHPs)

In the NHP working group, this study aimed to identify specific gene signatures for each cell type, particularly immune cell types, in order to develop a validated and reliable cell-decomposition method for non-human primate models, addressing a currently unmet need. Deconvolution methods for NHP models will facilitate the assessment of cell type subset changes under different conditions. Single-cell data for non-human primates were preprocessed and clustered using the Seurat R package. Based on highly expressed marker genes for each identified cell cluster, the clusters were annotated with specific cell types. The identity of cells was further confirmed using cell signatures from the Roche Immune Cell Atlas and the publicly available Non-Human Primate Cell Atlas (NHPCA) (https://db.cngb.org/nhpca/). The identified phenotypes contribute to the refinement of the reference cell-type signature, which is essential for developing effective deconvolution methods. Further validation and testing of the proposed methods are currently ongoing.

3.2 Study 2 – Roche Immune Cell Atlas

The NHP working group used the RNAseq data for non-human primates from the Roche Immune Cell Atlas to create preliminary gene signatures for available immune cell types and evaluated its enrichment in the bulk RNAseq datasets (of the Roche Immune Cell Atlas and a the publicly available NHPCA atlas) in order to validate their specificity and accuracy. We also used the Roche Immune Cell Atlas to compare the cell type signatures of the human (14 different immune cell types) and NHP (9 different immune cell types) data for the intersecting cell types to highlight the differences in the number and type of the marker genes.

The dataset has been processed according to FAIR principles in collaboration with FAIRPlus. It is now available from https://datacatalog.elixir-luxembourg.org/develop/e/study/f85998b0-7479-11ed-a0d3-acde48001122.

3.3 Study 3 – Patient Research Study

With this study we aim to identify biomarkers that discriminate patients that will develop high grade CRS (grade 3-4) after CAR T cell infusion from those that will present with lower grades of CRS (grade 1-2). These biomarkers will eventually be transferred to the in vitro models developed in imSAVAR and will allow for a more reliable prediction of CRS. To this end, we collect peripheral blood samples of patients receiving FDA/EMA-approved CAR T cell therapies at the University Hospitals Würzburg and Leipzig, twice before and twice after CAR T cell infusion. Additionally, extensive clinical data sets of these patients are collected. Immune cell composition of the fresh sample as well as CAR positive T cells are analyzed using flow cytometry. The patients' sera and their peripheral blood mononuclear cells (PBMCs) are retrieved to determine cytokine levels and conduct single cell transcriptome sequencing using next generation sequencing (NGS), respectively.

The flow cytometry as well as the relevant clinical data from the two centers have been processed and correlated. The data show a high inter-patient variability in immune cell composition and CAR T cell expansion after infusion. Patients that present with CRS grade 1 or 2 are treated with dexamethasone and/or tocilizumab (anti-IL-6 receptor antibody) and thus rarely develop higher grades of CRS.

Besides CRS, many patients present with various grades of cytopenias that may require intervention such as G-CSF support and blood transfusions. We subjected the serum samples of 78 patients of this cohort to an analysis of >40 cytokines in a multiplex cytokine assay. This profiling revealed that severe neutropenia, thrombocytopenia and anemia were highly associated with markers of endothelial dysfunction early after treatment with CAR T cells Further analyses revealed that patients with elevated markers of endothelial dysfunction and hyperinflammation prior to lymphodepletion were at high risk for prolonged severe neutropenias. A manuscript for publication is in preparation (Scheller et al.).

3.4 Study 4 – CAR T cell atlas

The purpose of this study is to clarify the functional integrity and activity of CAR T-cells to identify therapeutically relevant T-cells for safety assessments. By creating a specialized single-cell atlas for CAR T-cells, we aim to achieve the resolution necessary to differentiate the range of transcriptional states that may account for immune-related adverse events (irAOPs). In conjunction with other techniques, such as single-cell TCR sequencing to track T-cell responses to therapy, we aspire to establish a comprehensive and reliable CAR T-cell reference atlas.

We downloaded publicly available single-cell data from five studies (PMID: 33020644, PMID: 36097223, PMID: 36097221, PMID: 31924795, PMID: 35792801). After conducting quality control, we re-analyzed single-cell sequencing data from about 200 CAR T-cell samples, including infusion products and pre- and post-infusion samples, sourced from the PBMCs of 90 patients with Non-Hodgkin's lymphoma or acute lymphocytic leukemia. Following the filtering for CAR+ T-cells, approximately 400,000 cells were included in the downstream analysis. T cell subtypes were annotated using CD4 and CD8 single-cell atlases through transfer learning techniques. Clinicopathological metadata were harmonized, resulting in the first version of an integrated transcriptomic single-cell reference atlas. Additionally, we developed an initial version of a web application that enables users to analyze the CAR atlas by visualizing cell information and gene expression in reduced dimensions. Users can also explore gene co-expression and visualize expression patterns of multiple genes based on grouped categorical cell annotations (e.g., cell type, treatment response, time point post-treatment). For functional interpretation, users can analyze gene signatures to

understand coordinated variations among cells and capture transcriptional differences across various conditions of interest.

3.5 Study 5 – In vitro assay to study vascular leakage

Rationale of this study is to identify potential biomarkers or biomarker combinations that can be statistically correlated to increased endothelial permeability and hence, vascular leakage, a severe adverse outcome during e.g. immunotherapy. In this study, HUVECs are treated with 16 leakage-associated stimuli, including a cytokine cocktail derived from TGN1412-stimulated T cells (SNTGN1412) in a monoculture setting or in co-culture with PBMCs. We analyzed regulation of 5 endothelial-characteristic surface markers (ICAM-1, VCAM-1, E-selectin, JAM-3, and VE-cadherin), cellular viability, and 11 different cytokines. Also, we established an in vitro vascular leakage assay, with HUVECs as monoculture and in combination with PBMCs as co-culture, and analyzed changes in HUVEC-permeability upon treatment with the above-mentioned stimuli. The data was shared with WP4 and processed as follows: Datasets were analyzed for quality and outliers were identified and reviewed. Surface markers, cytokines, cell survival, and leakage were compared between stimulated cells and unstimulated controls at different time points. Measured levels were compared descriptively and by U-tests. Furthermore, association of surface markers, cytokines, and permeability were analyzed by Spearman correlation and respective correlation tests. With the current results, we could identify/confirm potent HUVEC-activating compounds (IL-1β, TNF-α, SNTGN1412, thrombin, and LPS). This activation is characterized by positive correlation of ICAM-1-, VCAM-1-, and E-selectin-expression and secretion of IL-6, IL-8, and MCP-1 in all pairing-combinations suggesting a conserved pattern HUVECs respond with upon activation. Moreover, strong HUVEC-activation results in decreased HUVEC viability and a slight downregulation of JAM-3. Certain stimuli were able to induce vascular leakage (thrombin, VEGF, and SNTGN1412). Interestingly, for certain (but not all) immunological stimuli investigated, including IL1-β or LPS, PBMCs were required for the induction of increased permeability indicating that immune cells play a central role in the induction VL, most probably with different mechanisms. The results were compiled in a manuscript and submitted for publication (Gogesch and Ortega Iannazzo et al.). Currently, studies analyzing immune-cell mediated HUVEC-permeability upon treatment with immunotherapeutic models are ongoing.

3.6 Study 6 – Re-analysis of Deng et al.

Rationale of this study was to extend the bulk data-focused analysis framework ‘oposSOM’ (PMID: **26063839)** with focus on challenges of single-cell analyses and on specifics of immune-related liquid biopsies, and to apply this novel methodology to single-cell transcriptome data of CAR T cell therapy infusion products to extract patterns of transcriptional activity and cellular subpopulations. Therefore, we adapted already implemented routines for processing the large-scale input of single-cell data sets, and extended functionalities especially by down- and upscaling procedures and visualization options.

The method was applied to data previously published by Deng et al. in 2020 (PMID: 33020644), comprising single-cell RNA sequencing data of 24 patients comprising more than 130,000 cells. Pre-processed data was obtained from Gene Expression Omnibus and processed using the novel single-cell oposSOM software. We demonstrated capabilities of our approach to disentangle transcriptional states using intuitive visualization, functional mining, molecular cell stratification, and variability analyses. Our analysis revealed that the T cell composition of the patient’s infusion product as well as the spectrum of their transcriptional states of cells derived from patients with low ICANS grade do not markedly differ from

those of cells from high ICANS patients, while the relative abundancies, particularly that of cycling cells, of LAG3-mediated exhaustion and of CAR positive cells, vary.

The results were published in Frontier Immunology Loeffler-Wirth et al. in 2020 (PMID: 36248848), and meta-cell data and analysis results uploaded to oposSOM-Browser, an interactive data and analysis browsing tool (PMID: 33076824).

3.7 Study 7 – TCE CRA Assessment

Rationale of this study is to conduct a cross-consortium study using four different in vitro cytokine release assay formats to identify potential differences in donor phenotypes that correlate with high or low responses in the in vitro assays.

Cell assay systems involved: WB, endothelial cell co-cultured with PBMCs or RBC depleted WB consisting of heterologous (HUVEC/EA.hy926) and autologous (Blood Outgrowth Endothelial Cell) platforms.

Three different TCEs have been tested at three concentrations in these models and absolute cytokine concentrations measured after stimulation in order to identify high and low responders Each model tested N=30 healthy donors. In order to facilitate comparison of the models a single concentration was shared between the experiments. In parallel, baseline phenotypes were determined by measuring standard hematology and clinical chemistry (including CRP and ferritin). In addition, multiple cellular subsets were enumerated by flow cytometry. High and low responders in the assay system were identified using hierarchical clustering methods and 7 clear high responders and 5 clear low responders were identified across the different models scRNAseq of the identified high and low responders has been initiated. In parallel, correlation analyses between the absolute cytokine responses in the model and the baseline phenotypes is ongoing.

3.8 Study 8 – Dose-response assessment of cytokine release by T-call engagers

This study aims to support the determination of parameters for the safe and efficient translation from preclinical development to first-in-human studies like therapeutic index (TI) / maximum recommended safe dose (MRSD) and minimal pharmacological active dose (mPAD) / minimum anticipated biological effect level (MABEL). To this end, we conduct a proof-of-concept study, where we use models that reflect the KEs of an irAOP to generate dose-response curves to determine if an immunotherapy is safe at a predicted therapeutic dose. We will employ the irAOP of CRS induced by BiTEs and identify assays available in the consortium that model each KE of the irAOP, especially anticipating the later KEs which require more sophisticated models like organ-on-chip. One defined BiTE-molecule will be used to ensure that deviations in the quality of the readouts are due to the assay formats, not the starting material. Using a BiTE molecule that has already been employed in human studies will allow us to draw conclusions of the transferability of the results we obtain in the nonclinical assays with the patient data on safety and efficacy. The study is currently in the planning phase and will be completed until the end of the imSAVAR project.

3.9 Study 9 – Deep learning-aided inter-species-comparison of immune response in drug development involving cynomolgus monkey

Assessing the efficacy and safety of novel therapeutic agents in the preclinical stage is integral to the early phase of drug development. Animal studies in NHPs like cynomolgus monkeys (Macaca fascicularis) are a

common in the preclinical phase for investigating the immune system. Still, translating the findings from animal models to humans remains a major challenge. In this study, we seek to identifying similarities and differences within T cell-triggered immune response between cynomolgus monkeys and humans. We established a computational workflow using deep learning and conventional bioinformatics to determine shared characteristics between human and monkey as well as unstimulated and stimulated PBMCs. The model will be extended with more features to better understand the underlying species-specific and shared biological mechanisms. Furthermore, we will explore the feasibility of our pipeline as a cross-species prediction model. This will support assessing the safety of immunomodulatory therapies in drug development strategies involving animal models.

4. Summary

In the third iteration of advanced immune cell profiling, we started, advanced and/or completed the (re-)analysis of nine datasets, that were either generated by imSAVAR partners or publicly available. These comprise either baseline immune cell data that allow insights in the mechanisms of the immune system, also comparing human and NHPs, or data for the MoAs BiTEs, IL-2 or CAR T cells.

5. Abbreviations

BiTE – bispecific T-cell engagers

BOEC – blood outgrowth endothelial cell

CAR – chimeric antigen receptor

CLL – chronic lymphocytic leukemia

CPIs – checkpoint inhibitors

CRA – cytokine release assay

CRS – cytokine release syndrome

HUVEC – Human Umbilical Vein Endothelial Cells

ICANS – immune effector cell associated neurotoxicity,

irAOPs – immune-related adverse outcome pathways

KE – key event

KER – key event relationships

LBCL – large B-cell lymphoma

MABEL - minimum anticipated biological effect level

MIE – molecular initiating event

MoA – mode of action

mPAD – minimal pharmacological active dose

MRSD – maximum recommended safe dose

NGS – next generation sequencing

NHL -- non-hodgkin lymphomas

NHP – non-human primates

NHPCA – non-human primate cell atlas

PBMCs – peripheral blood mononuclear cells

PCL – plasma cell leukemia

RBC – red blood cell

RNA-seq – RNA sequencing

scRNA-Seq -- single-cell RNA sequencing

TCE – Bispecific T cell engager

TI – therapeutic index

6. References

1. Moher, D., Liberati, A., Tetzlaff, J. and Altman, D.G. (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS medicine. First published on July 21, 2009, 10.1371/journal.pmed.1000097.

2. Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P. and Stewart, L.A. (2015) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Systematic reviews. First published on January 01, 2015, 10.1186/2046-4053-4-1.

3. Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P. and Stewart, L.A. (2015) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ (Clinical research ed.). First published on January 02, 2015, 10.1136/bmj.g7647.

4. Deng, Q., Han, G., Puebla-Osorio, N., Ma, M.C.J., Strati, P., Chasen, B., Dai, E., Dang, M., Jain, N. and Yang, H. et al. (2020) Characteristics of anti-CD19 CAR T cell infusion products associated with efficacy and toxicity in patients with large B cell lymphomas. Nature medicine. First published on October 05, 2020, 10.1038/s41591-020-1061-7.

5. Löffler-Wirth, H., Kalcher, M. and Binder, H. (2015) oposSOM: R-package for high-dimensional portraying of genome-wide expression landscapes on bioconductor. Bioinformatics (Oxford, England). First published on June 10, 2015, 10.1093/bioinformatics/btv342.

6. Sheih, A., Voillet, V., Hanafi, L.-A., DeBerg, H.A., Yajima, M., Hawkins, R., Gersuk, V., Riddell, S.R., Maloney, D.G. and Wohlfahrt, M.E. et al. (2020) Clonal kinetics and single-cell transcriptional profiling of CAR-T cells in patients undergoing CD19 CAR-T immunotherapy. Nature communications. First published on January 10, 2020, 10.1038/s41467-019-13880-1.

7. Li, X., Guo, X., Zhu, Y., Wei, G., Zhang, Y., Li, X., Xu, H., Cui, J., Wu, W. and He, J. et al. (2021) Single-Cell Transcriptomic Analysis Reveals BCMA CAR-T Cell Dynamics in a Patient with Refractory Primary Plasma Cell Leukemia. Molecular therapy: the journal of the American Society of Gene Therapy. First published on December 03, 2020, 10.1016/j.ymthe.2020.11.028.

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.