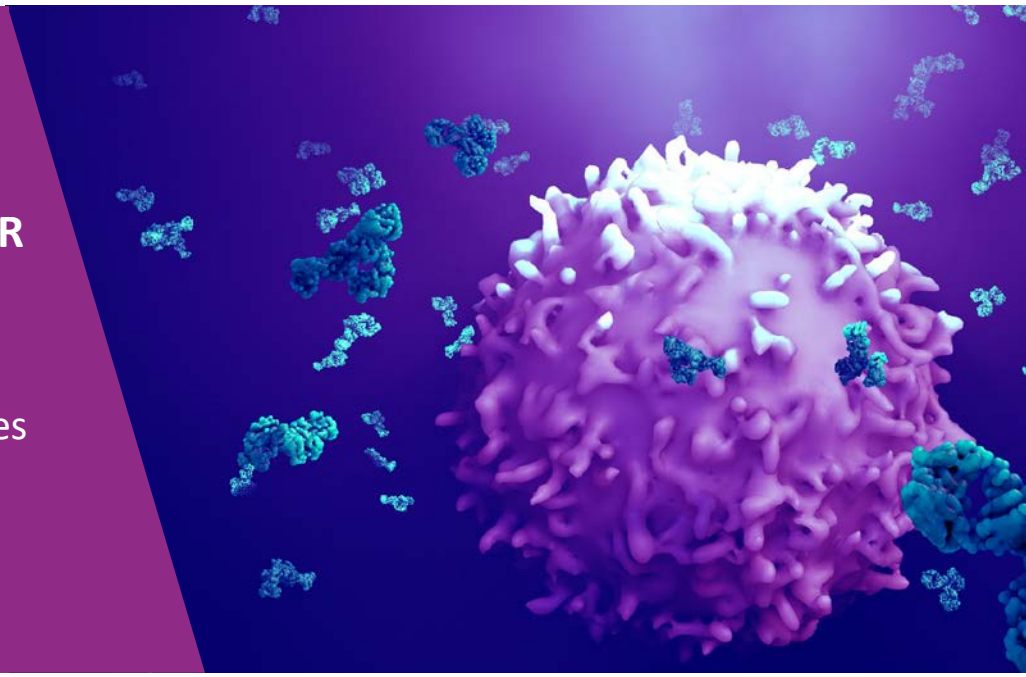
imSAVAR

IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 4.2
1st iteration of advanced immune cell profiling

DELIVERABLE REPORT

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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 four firstly defined mode of actions (MoAs) of immunomodulatory therapeutics will be addressed and require development and confirmation of biomarkers: (i) CAR (chimeric antigen receptor) T-cells, (ii) BiTEs (bispecific T-cell engagers), (iii) CPI (checkpoint inhibitors) and (iv) IL-2 as first chosen MoA for inflammatory disease therapeutics. We align biomarker development with immune-related AOPs (irAOPs) to foster a common understanding of the processes triggered through a molecular initiating event and eventually leading to adverse outcomes.

With Deliverable D4.2, we describe a first version of datasets that have undergone initial bioinformatic processings and are useful for identifying biomarkers as well as mechanistic insights from the models being refined and developed in imSAVAR.

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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 pre-clinical 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. Adverse Outcome Pathways 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 as measurable and evaluable biological characteristics evaluating the MIE, the KEs or the adverse event of an irAOP. The first step towards biomarker development were systematic reviews of pre-existing data sets on human and non-human immune cell subsets to identify knowledge gaps and to guide future research directions (see Deliverable Report D4.1). In a second step specific research questions were formulated and appropriate data sets identified. Here we report the first round of datasets available and provide details on their processing state.

2. Methods

To identify **publicly available datasets** we conducted systematic reviews. For this process we were guided by the concept of the PRISMA-P statement (as far as transferable to pre-clinical safety assessment) of the EQUATOR (Enhancing the QUAlity and Transparency Of health Research) network (1–3). We defined eligibility criteria to group the data sets by relevance. First-level studies describe biomarkers associated with adverse outcomes and reporting a clear causal effect between the biomarker and the adverse outcome in a statistical model. Whereas second-level studies report on adverse outcomes, not necessarily using a statistical model, but providing access to omic-wide data sets. We set additional criteria to assess the publications (data type, disease, treatment, side effects, species, quality of data). By employing a scoring scheme to evaluate and categorize the identified studies, we ranked the information content of the data sets therein according to our predefined criteria. For a first iteration of systematic reviews, we used a defined search term strategy and concentrated on studies with peer-reviewed publications listed in PubMed, because peer-reviewed publications allow detailed evaluation of the study and reported data. All publications were collected in a Citavi project (https://www.citavi.com/) and evaluated according to the above-defined eligibility criteria. For each study, we documented the rank in Citavi to ensure reproducibility of the systematic review as well as reviewing of the ranking process by independent experts.

We furthermore identified **existing datasets** of the EFPIA partners within imSAVAR and thirdly defined research studies with **newly generated datasets** by the partners of WP4.

3. Results

The **systematic review on publicly available data sets on the MoA of CAR T cell** yielded 50 articles employing our above described evaluating scheme and after removing articles of low rank. The full-text evaluation thereafter resulted in 15 publications. Five of these publications are first-level studies, describing biomarkers associated with adverse outcomes of CAR T cell therapy and reporting a clear causal effect between the biomarker and the adverse outcome in a statistical model. Ten publications are

second-level studies, that include assessment of CAR T cell constructs after treatment and reporting on adverse outcomes, not necessarily using a statistical model, but providing access to datasets (e.g. omic-wide data) to enable integration of the study in meta-analyses. Only for three of these datasets available metadata of the raw datasets also reported adverse outcome type and category (Table 1). The Deng et al. dataset (4) was integrated into the imSAVAR roadmap for biomarker development for MoA CAR-T utilizing the oposSOM R-package for high-dimensional portraying of genome-wide expression landscapes (5).

Table 1: Details of identified studies reporting omic-wide datasets either in preclinical models or in infusion products of patients retrieving CAR T-cell therapies. CRS: cytokine release syndrome, ICANS: immune effector cell associated neurotoxicity, NHL: Non-Hodgkin lymphomas, CLL: Chronic lymphocytic leukemia, PCL: Plasma cell leukemia, LBCL: Large B-cell lymphoma, scRNA-Seq: single-cell RNA sequencing.

Study	Target	Disease	Metadata	Staus of data	Model	Type
Sheih et al. (6)	CD19	NHL, CLL	CRS, ICANS	raw	Pre- and post-infusion CAR T-cell products	scRNA-Seq
Deng et al. (4)	CD19	LBCL	CRS, ICANS	raw	Infusion CAR T-cell products	scRNA-Seq
Li et al. (7)	BCMA	PCL	CRS, ICANS	raw	Infusion CAR T-cell products	scRNA-Seq

The **systematic reviews on publicly available data sets on the MoAs BiTE and CPI** yielded three and thirteen articles of high rank after employing our evaluating scheme, respectively. However, none of these reported adverse outcome type and category and are thus not eligible to be included in the respective roadmaps.

In addition, we identified **two existing datasets** at imSAVAR's EFPIA partners that are eligible for integration into the first round of research questions assessing human baseline immune cell profiles vs. non-clinical animal models (Table 2). Both datasets will be used to benchmark baseline immune cell profiles of non-clinical animal models with human biology.

Table 2: Existing datasets at imSAVAR's EFPIA partners and agreed to be shared with imSAVAR.
Abbreviations: NHP – non-human primates, PBMCs - peripheral blood mononuclear cells.

Study	Aim of study	Study design	Staus of data (raw / (pre)processed)	MoA
Single cell RNA and bulk sequencing of in vitro stimulated PBMCs (CD3/CD28) in healthy humans and non-human primates (NHPs)	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: raw Bulk RNA sequencing: preprocessed	Baseline Immune Cell Profiles
ROCHE Immune Cell Atlas	Baseline characteristics of immune cells (bulk RNA sequencing)	Characterize immune cells of healthy H. sapiens, M. fascicularis, and M. musculus	Processed	Baseline Immune Cell Profiles

Table 3 reports **ongoing studies** currently conducted by WP4 partners which will result in newly generated data identifying biomarkers for irAOPs of immunomodulating therapies.

Table 3: Ongoing biomarker studies on immunotherapies in WP4 with first round of datasets available.

Study	Aim of study	Status of data (raw / (pre)processed)	MoA
Blood Outgrowth Endothelial Cell (BOEC) to study cytokine release syndrome (CRS) study cytokine release syndrome (CRS)	Identification of potential novel biomarkers to better predict/manage CRS after CAR T cell therapy.	raw	CAR-T
In vitro assay to study vascular leakage	Identification of potential novel biomarkers to better predict/manage vascular leakage e.g. after IL-2 therapy.	raw	IL-2
ScRNA-Seq of (CAR-)T cells before and after manufacturing	Identification of cellular and molecular factors predicting risk for manufacturing failure	Processed	CAR-T

4. Summary

We identified existing data available in public databases or from EFPIA partners and integrated those into respective research questions for biomarker development (3 datasets). In addition, for the first research questions we started to generate new datasets (3 datasets). All reported datasets give mechanistic insights of the MoA of immunomodulating therapies and allow for a first step to identify biomarkers that can be used in the in vitro models already available or developed in imSAVAR. Next, we seek to implement these datasets into the ongoing studies and evaluate the data for the biomarker identification process.

5. Abbreviations

BiTE – bispecific T-cell engagers

BOEC – blood outgrowth endothelial cell

CAR – chimeric antigen receptor

CLL – chronic lymphocytic leukemia

CPIs – checkpoint inhibitors

CRS – cytokine release syndrome

ICANS – immune effector cell associated neurotoxicity,

irAOPs – immune-related adverse outcome pathways

KE – key event

KER – key event relationships

LBCL – large B-cell lymphoma

MIE – molecular initiating event

MoA – mode of action

NHL -- non-hodgkin lymphomas

NHP – non-human primates

PBMCs – peripheral blood mononuclear cells

PCL – plasma cell leukemia

RNA-seq – RNA sequencing

scRNA-Seq -- single-cell RNA sequencing

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