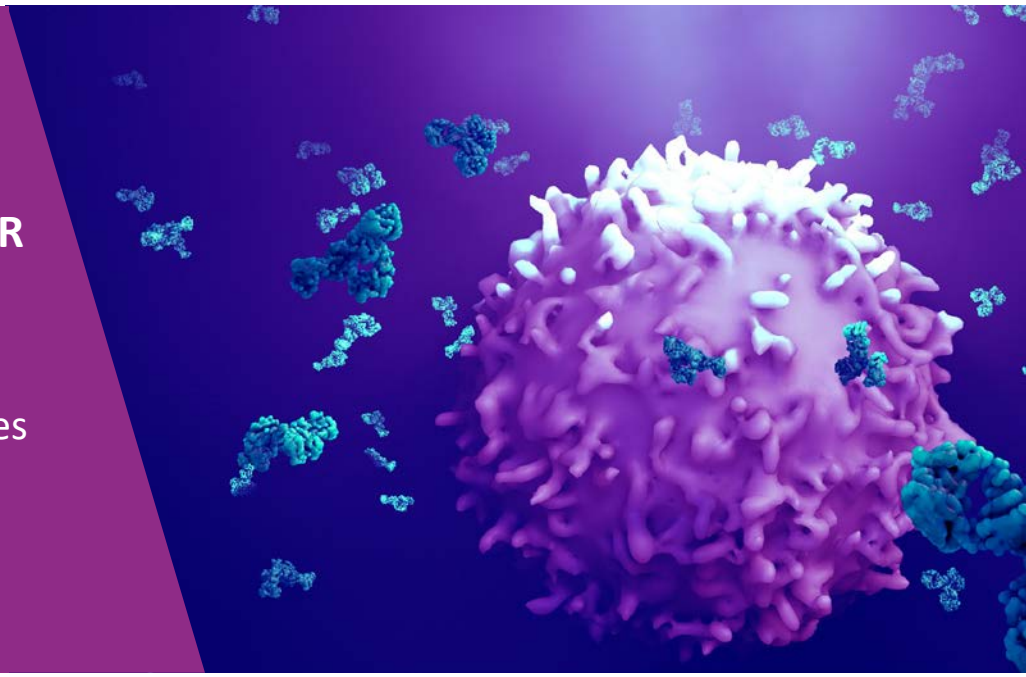
imSAVAR

IMMUNE SAFETY AVATAR

Nonclinical mimicking of the immune system effects of immunomodulatory therapies



Deliverable 5.3

Network of disease domain providers for providing samples

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.

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Abstract

The goal of this deliverable was to create a network that will facilitate the sourcing of human-derived liquid and tissue samples for the use in WP 2, 3 and 4. We successfully built a network of biobanks, disease domain providers from the Hannover Medical School (MHH) and partners from the imSAVAR consortium which started to provide samples and are available for future inquiries.

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1. Methods

In case of a biosample request from WP 2, 3 or 4, suitable collections of biosamples will be searched in the existing biobank network. The collections of Biobanks of the German Biobank Alliance (GBA) and the European Biobank Network (BBMRI-ERIC) can be searched using the BBMRI-ERIC Directory which provides a central listing of the participating biobanks and their collections (see section results 2). As for the GBA Biobank the collections of the Hannover Unified Biobank (HUB, Hannover Medical School) are published in the BBMRI-ERIC Directory. In addition several imSAVAR partners have already existing cooperation for the sourcing of biosamples which can also be used in the imSAVAR project. We will check availability and suitability of existing biosample collections. The requirements for the suitability of samples depend strongly on what they are used for and can differ from case to case. The basic parameters for the suitability are: an existing ethics vote, consent allows use of samples for imSAVAR, collection and freezing date / time as well as sample type and number of freeze / thaw cycles is documented. The samples have a unique ID and a clear assignment to the donor is given. In case of patient derived samples the year of birth, gender and the exact diagnosis has to be available. However, depending on the requirements of the requesting imSAVAR partner additional parameters defining the suitability may be necessary. In case the appropriate biosample collections are not available, we will establish suitability of an existing / ongoing collection. For example, the ethics vote can be extended to allow collection of an additional sample type / additional data or the documentation can be improved so that newly obtained samples meet the requirements. If the adjustment of an ongoing collection is not possible we will start new collections for imSAVAR in cooperation with partners from Hannover Medical School or other GBA locations to deliver the requested biosamples.

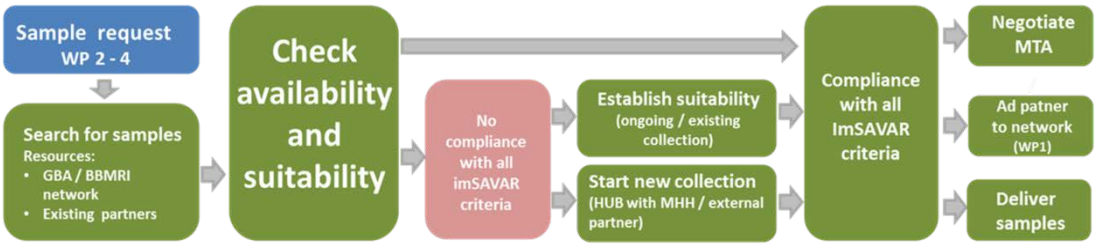

Figure 1: Workflow sourcing of biosamples

2. Results

In accordance with the previous requirements a network of disease domain providers for providing samples was established. The current providers for biosamples are:

1) The Biobanks of the German Biobank Alliance (GBA)

The German Biobank Alliance is a network of currently 20 excellent German Biobanks funded by the Federal Ministry of Education and Research (Table 1). The alliance partners establish uniform quality standards, common IT structures and make their biosamples available for biomedical research throughout Europe (https://www.bbmri.de/about-gbn/german-biobank-alliance/?L=1). Prof. Dr. Thomas Illig (WP5 lead) is the deputy speaker of the GBA. He presented the imSAVAR project to the GBA steering committee. All biobanks confirmed their full support.XYZ

Table 1: List of all GBA Biobanks

Location	Name of Biobank	Focus of the Biobank	Head of Biobank
Aachen	Centralised Biomaterial Bank of the RWTH Aachen University	Clinical Biobank, Tissue Biobank, Liquid Biobank	Prof. Dr. Edgar Dahl
Berlin	Central Biobank of the Charitè	Clinical biobank (liquid, tissue), study biobank, cell cultures (cells, stem cells), animal biobank	Prof. Dr. Michael Hummel
Bonn	BioBank Bonn	Clinical Biobank, Tissue Biobank, Liquid Biobank	
Dresden	BioBank Dresden	Clinical Biobank, Tissue Biobank, Liquid Biobank	Dr. Heidi Altmann
Essen	Westdeutsche Biobank Essen	Clinical Biobank, Tissue Biobank, Liquid Biobank	Dr. Katharina Jockers
Frankfurt am Main	Interdisciplinary biomaterials and database Frankfurt (iBDF)	Clinical Biobank, Tissue Biobank, Liquid Biobank	Prof. Dr. Christian Brandts
Freiburg	FREEZE-Biobank Freiburg	Clinical Biobank, Tissue Biobank, Liquid Biobank	PD Dr. Alexandra Nieters
Göttingen	Central Biobank UMG	Hospital-integrated clinical biobank (liquid samples, solid biosamples,cells)	PD Dr. Sara Y. Nußbeck
Greifswald	Integrated Research Biobank of the Universitätsmedizin Greifswald	Epidemiological Studies, Population-based Studies, Liquid Biobank	Dr. rer. nat. Theresa Winter
Hannover	Hannover Unified Biobank	Clinical Biobank, Tissue Biobank, Liquid Biobank	Prof. Dr. Thomas Illig
Heidelberg	BioMaterialBank Heidelberg	Clinical Biobank, Tissue Biobank, Liquid Biobank	Prof. Dr. Peter Schirmacher
Jena	Integrated Biobank Jena	Clinical Biobank(Liquid), Study biobank	PD Dr. Dr. Michael Kiehntopf
Leipzig	Leipzig Medical Biobank	Population-Based Biobank, Clinical Biobank, Tissue Biobank, Liquid Biobank	Dr. Ronny Baber
Lübeck	Interdisciplinary Centre for Biobanking - Lübeck	Clinical Biobank, Tissue Biobank, Liquid Biobank, Population-Based Biobank, Cell cultures (cells, stem cells)	Dr. Martina Oberländer
Mainz	BioBank Mainz	Tissue Biobank, Liquid Biobank, Pathology, Health Care, Clinical and Population-Based Studies, Epidemiology	Prof. Dr. Wilfried Roth
Marburg	Comprehensive BioBank Marburg	Clinical Biobank, Project and Study Biobanks, Tissue Bank, Liquid Bank, Cells	Prof. Dr. Dr. Petra Ina Pfefferle
München	Joint Biobank Munich	Clinical Biobank, Tissue Biobank, Liquid Biobank, Populationsbasierte Biobank	Dr. Christian Gieger
Regensburg	Central Biobank Regensburg	Disease-specific biobank, research biobank, Tissue Biobank	Prof. Dr. Christoph Brochhausen
Tübingen	Stuttgart	Clinical Biobank, Tissue Biobank	Prof. Dr. Thomas Iftner
Würzburg	Interdisciplinary Biomaterials and Database Würzburg	Clinical Biobank (Tissue und Liquid), Study Biobank, Population-based Biobank	Univ. Prof. Dr. med. Roland Jahns

- **Current status: Network available, awaiting requests from WP2, 3 and 4.**

2) The Biobanks of the European Biobank network (Biobanking and Biomolecular Resources Research Infrastructure – European Research Infrastructure Consortium (BBMRI ERIC))

Via the German Biobank Alliance / the German Biobank Node the HUB is partner of the European research infrastructure for biobanking BBMRI-ERIC (https://www.bbmri-eric.eu/). Currently Biobanks from 16 European countries are member of the BBMRI-ERIC (Figure 2). The national biobank nodes coordinate the activities of the local biobanks in the respective countries. The BBMRI Directory is a tool that collects and makes available information about biobanks throughout Europe that are willing to share their data and /or samples and provides a central listing of biobanks and their collections https://directory.bbmri-eric.eu/menu/main/app-molgenis-app-biobank-explorer. In case of a specific request from WP 2-4 this tool can be used for a comprehensive pan-European search across all locations.

BBMRI-ERIC national nodes

BBMRI-ERIC, all participating biobanks

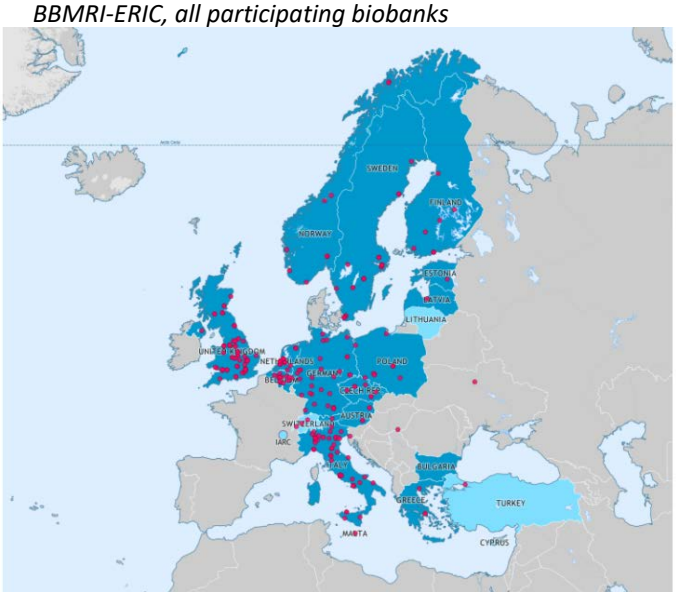

Figure 2: National Nodes and Biobanks of the BBMRI-ERIC network

3) New partners from the Hannover Medical School (not funded imSAVAR partner)

3.1) Primary Human Hepatocyte Core Facility, Prof. Vondran, Department of General, Visceral and Transplant Surgery, MHH

Located at MHH, the Primary Human Hepatocyte (PHH) Core Facility provides DZIF (German Center for Infection Research) research groups with liver cells freshly prepared from patient resectates. The isolation of PHH is a standardized, technically complex procedure and PHH are only of limited availability. Amongst other purposes, PHH are mainly required for studying infections of the liver as well as for the pre-clinical development of drugs. The Core Facility supplies an ever growing number of research groups of DZIF, mainly of the TTU Hepatitis, with high-quality hepatocytes, with cell populations from the non-parenchymal fraction of the liver (NPC) as well as with liver stem cell populations (LSC) derived from primary patient hepatocyte preparations. The samples are derived from patients with clinically well-defined characteristics and diseases; isolated PHH and liver stem cells are characterised further in vitro by the Core Facility. Prof. Florian Vondran has established a "Standard Operating Procedure" (SOP) for the logistical and methodical standardisation of handling of the patient specimens from the operation theatre of the Department of General, Visceral and Transplant Surgery at MHH into the PHH laboratory. A robust protocol for PHH isolation was refined and established, staff is employed and trained. A fast and efficient distribution of cell samples to collaboration partners is established, rendering the PHH Core facility a unit with unique expertise for regular provision of fresh patient liver cell samples in one of Germany's leading centers for the treatment of liver diseases.

- **Current status: Request from WP3 for primary hepatocytes. Prof. Vondran is very open to cooperation. Currently modalities for the cooperation are clarified.**

3.2) Prof. Könecke, Department of Hematology, Hemostaseology, Oncology and Stem Cell Transplantation, MHH

The Department of Hematology, Hemostaseology, Oncology and Stem Cell Transplantation of the MHH offers a CAR T cell consultation for adult patients with relapsed or refractory diffuse large B cell lymphoma (DLBCL) after two or more lines of systemic therapy. Plasma, urine and viable PBMCs are collected from

these patients during the CAR T cell therapy at several time points in cooperation with HUB. Currently over 1,400 sample aliquots (plasma, urine, viable PBMC) from 14 DLBCL Patients have been collected. Prof Könecke, the gatekeeper of the collection, is very open to cooperation.

Table 2: Number of currently available samples from Prof Könecke

Sample type	Number of samples
Plasma	863
viable PBMC	489
Urin	78

- **Current status: Awaiting requests from WP2, 3 and 4.**

3.3) Prof. Werfel, Department for Immunodermatology and experimental Allergology

To investigate mechanisms and responsible cell types leading to skin rash in response to IL-2 therapy, skin-resident cells responsive for IL-2 need to be characterized. Skin biopsies of different donors and body regions are used at Fraunhofer ITEM to phenotype healthy skin regarding IL-2 receptor (IL-2R) positive skin-resident cells. Healthy tissue of biopsies taken from patients bearing skin tumors are given to the group of Prof. Werfel and are kindly distributed to Fraunhofer ITEM (approx. every or every other week). Skin samples are then processed into single cell suspensions to quantify IL-2R+ cells using flow cytometry. Additionally, these IL-2R+ cells will be characterized further with regards to their (surface) marker expression to distinguish different cell types. In a second step, to define localization of IL-2R+ cells in full-thickness skin, skin sections are prepared and stained using immunofluorescence and are subsequently analyzed by confocal laser scanning microscopy.

- **Current status: Regular supply of skin samples for WP3 (Fraunhofer ITEM)**

4) Resources provided from partners from the imSAVAR Consortium

4.1) Prof. Jonigk, Institute of Pathology; MHH, WP5

The Institute of pathology offers a unique infrastructure for generation and accessibility of various human tissue samples for a multitude of research projects. The work group of Prof. Jonigk established a 24/7 comprehensive work-up of human end-stage disease lungs following organ transplantation and comprehensive work-up of tumor-resection specimen as control-material during the work-days.

Since the start of imSAVAR, 72 explanted lungs (28 fibrosis, 23 emphysema, 8 pulmonary hypertension, 6 redo transplantations, 5 cystic fibrosis, and 2 other cases) and 103 tumor resection specimen were worked up immediately after pick-up for research projects. The imSAVAR project focuses on the generation of precision cut lung slices (PCLS) for in-vitro experiments of human lung tissue in cell culture. This method offers experiments on native lung tissue closest to the state in living patients. This method requires a minimum size for the resection specimens to be filled with liquid agarose, and some end-stage diseases – e.g. emphysema – are not suitable for this method. Further, this method requires a very small time window (1-2hours) between specimen retrieval and filling with agarose. Therefore, lung explants and tumor resection specimen retrieved during the night-time could not be included. Workup of 3 suitable explanted lungs (all lung fibrosis cases) and 16 tumor resection specimen was performed since the start of the imSAVAR project (Figure 3).

Besides PCLS, a multitude of other compartment-specific biosamples are available (Figure 4). On routine admission, compartment specific (artery, bronchus, lymph nodes, lung parenchyma) fresh-frozen tissue

of every lung explant and a majority of tumor resection specimen (tumor and unaffected tissue) are stored and readily available. Further, formalin-fixed paraffin embedded tissue of every specimen is preserved. Further, individual fixation and preservation methods can be applied individually, ensuring high flexibility and high-quality biosamples for the research-community especially for imSAVAR.

In addition, through histomorphological workup of every specimen administered by a practiced pathologist, quality and biosafety (e.g. exclusion of infectious diseases) of each specimen is guaranteed. Further, detailed compartment-specific histomorphological characteristics are generated and distributed through the routine histological workup and can be linked to various clinical information (e.g. underlying disease, ischemia time).

Taken together, highly characterized, compartment specific, individually preserved and safe biosamples of various end-stage lung diseases, as well as suitable control material in a high quantity can be provided by this unique infrastructure.

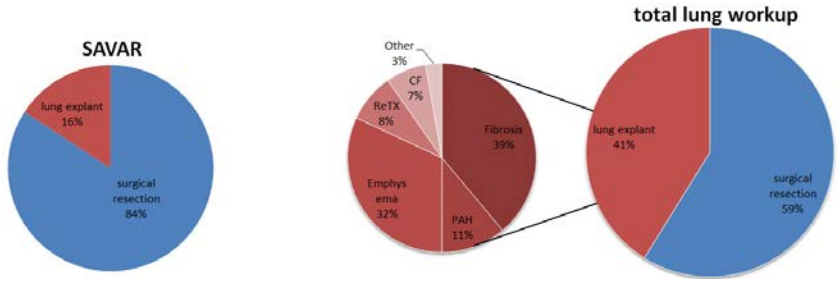

Figure 3: Total lung specimen and provided PCLS-specimen for the imSAVAR project in comparison.
Pie charts showing relative proportions of specimen provided for the imSAVAR project and total lung workup of the Institute of Pathology. CF = cystic fibrosis, PAH = pulmonary hypertension, ReTX = redo transplantation

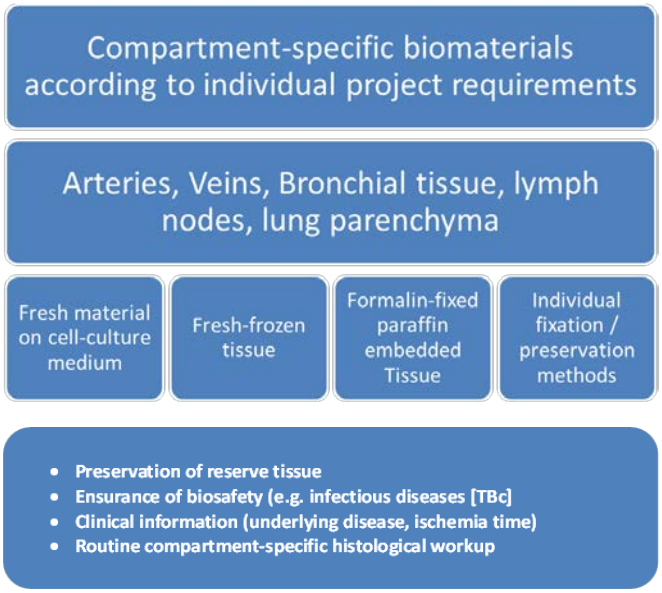

Figure 4: Capabilities of biomaterial generation for researchers from Prof. Jonigk

- **Current status: Regular supply of biosamples for WP3 (Fraunhofer ITEM)**

.2) Prof Hudecek, Dr. Miriam Alb; UKW, WP2

Peripheral Blood Mononuclear Cells (PBMCs) were isolated using density gradient centrifugation from a leukapheresis of a healthy donor which was obtained from the German Red Cross (DRK) Center in Frankfurt. Part of the PBMCs were directly cryopreserved. CD4 and CD8 T cells were isolated from PBMCs using MACS technology (Miltenyi Biotec). Afterwards, T cells were stimulated with Dynabeads® Human-T-Activator CD3/CD28 (Thermo Fisher Scientific). CAR T cells were then generated via lentiviral transduction (lentiviral particles encoding the CAR construct of choice) using our internal SOP: Non-transduced T cells were included as a negative control. CD3/CD28 Beads were magnetically removed after seven days in culture. Transduction efficacy was analyzed using flow cytometry as CAR T cells carry a transduction marker (truncated EGFR) which can be visualized using a fluorophore-conjugated anti-EGFR antibody (e.g. clone AY13 from Biolegend). CAR-positive T cells were then purified using MACS technology (Miltenyi Biotec) according to our internal SOP. CAR-positive T cells as well as control T cells were then further expanded (antigen-specific in case of CD19-specific CAR T cells; CAR T cells targeting other antigens and control T cells are usually expanded using third party PBMCs as feeder cells) and cryopreserved according to our internal SOPs.

Furthermore, unmodified as well as CAR-modified T cells, which were not expanded yet, were cryopreserved. These cells can be thawed and expanded if the below listed T cells are no longer available (see Table 3).

Additionally, Partner UKW can provide Raji lymphoma cells (obtained from DSMZ-German Collection of Microorganisms and Cell Cultures GmbH), either native or modified to co-express green fluorescent protein (GFP) and firefly luciferase (ffluc).

Partners JUH (Alexander Mosig), Covance (Chris Cooper) and Sanofi (Thuvan Dinhle) already received cells from Partner UKW to be tested in their in vitro models. Partners BI (Birgit Fogal) and PEI (Patricia Gogesch) will receive cells in the upcoming weeks. Further partners within the imSAVAR consortium can receive cells upon request.

Partner UKW plans to provide the imSAVAR consortium with CAR T cells from up to n=3 healthy donors to establish the preclinical test systems to study CAR-T mediated cytokine release syndrome as well as CAR-T mediated neurotoxicity.

When the first iteration cycle for the in vitro test systems is completed, we also plan to provide the imSAVAR consortium with CAR T cells generated from patient samples (e.g. Multiple Myeloma patients).

Table 3: Cells available from Partner UKW (WP2)

Cell type	No. of vials	Cell number per vial
PBMC	17	50 x 10e6
PBMC	15	100 x 10e6
CD4 control (expanded)	26	10 x 10e6
CD4 CD19-CAR (expanded)	20	10 x 10e6
CD4 ROR1-CAR (expanded)	26	10 x 10e6
CD8 control (expanded)	26	10 x 10e6
CD8 CD19-CAR (expanded)	26	10 x 10e6
CD8 ROR1-CAR (expanded)	18	10 x 10e6
Raji lymphoma native (DSMZ)	8	2.8 x 10e6
Raji lymphoma GFP-ffluc	5	2.8 x 10e6

- **Current status: Regular supply of biosamples for WP2**

3. Discussion / Conclusion

We have successfully established a network that will facilitate the sourcing of human-derived liquid, cell and tissue samples for the use in WP2, 3 and 4. In the past project period regular deliveries of biosamples have been established and we built a network of biobanks and disease domain providers which is available for further inquiries. The established cooperation for sourcing of biosamples listed in the results 3) and 4) are due to specific requests / requirements from the consortium. The GBA and BBMRI-ERIC biobank network are available for future enquiries. The current sources are sufficient to fulfil the present requirements of the project. The network of disease domain providers for providing samples will not be finalised at a certain time but will continue to develop during the whole project along with the achievements resulting in new requirements from WP2, 3 and 4 which are not yet clearly foreseeable. However, with our existing network we are confident to be able to handle future sample requests.

Acknowledgement

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