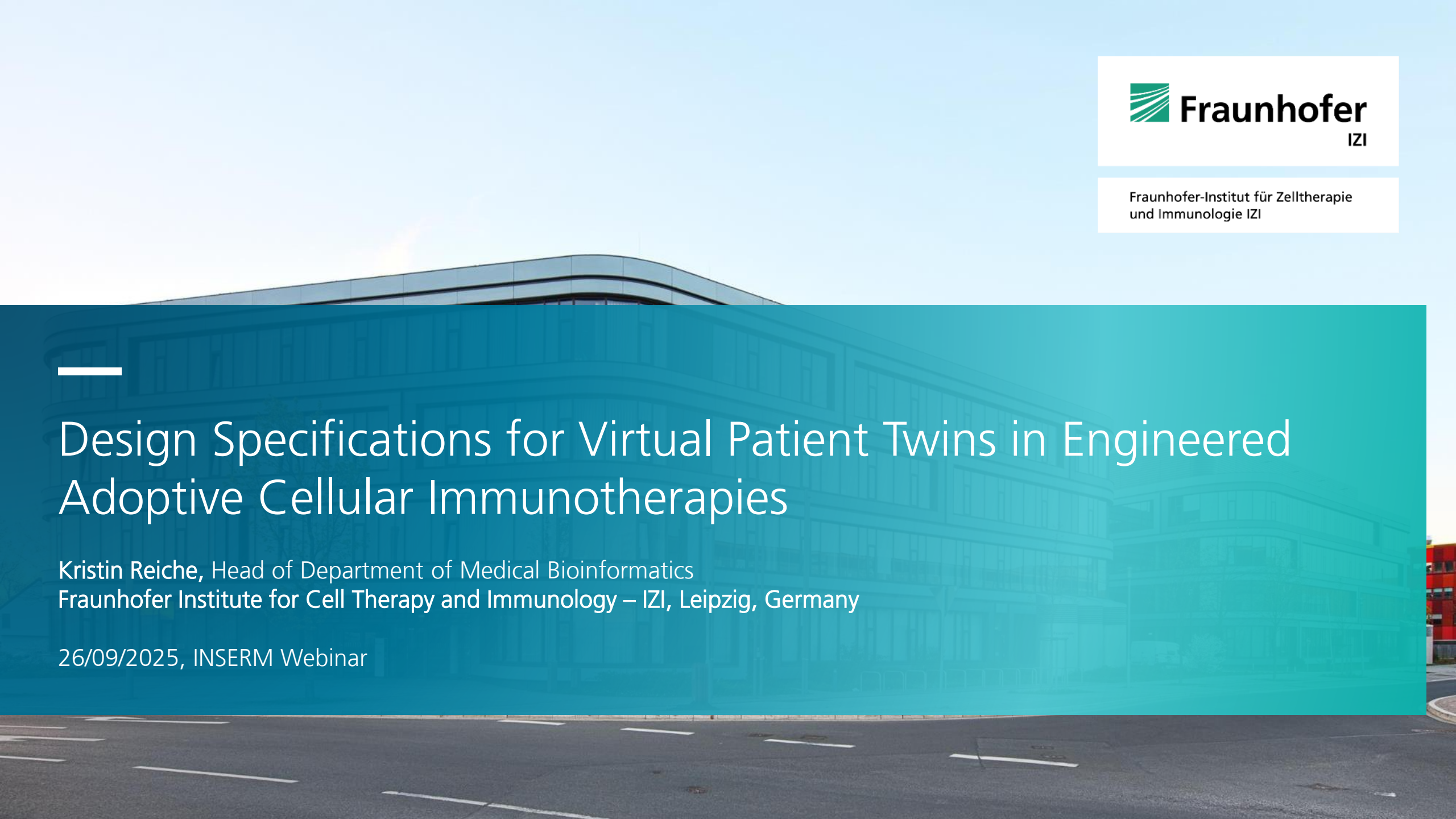

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Fraunhofer-Institut für Zelltherapie und Immunologie IZI

Design Specifications for Virtual Patient Twins in Engineered Adoptive Cellular Immunotherapies

Kristin Reiche, Head of Department of Medical Bioinformatics
Fraunhofer Institute for Cell Therapy and Immunology – IZI, Leipzig, Germany

26/09/2025, INSERM Webinar

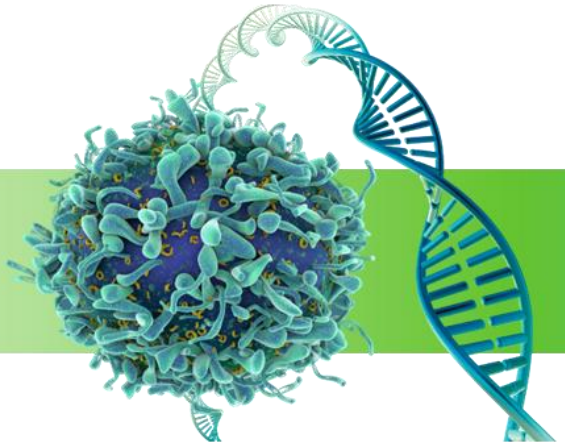
The Era of Precision Medicine

One-fits-all solution

- Chemical substances
- Regular intake
- Relief of symptoms

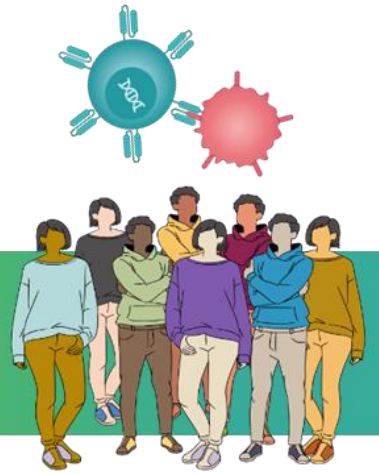
Living Drugs

Cell and Gene Therapies (CGTs)



Game changer

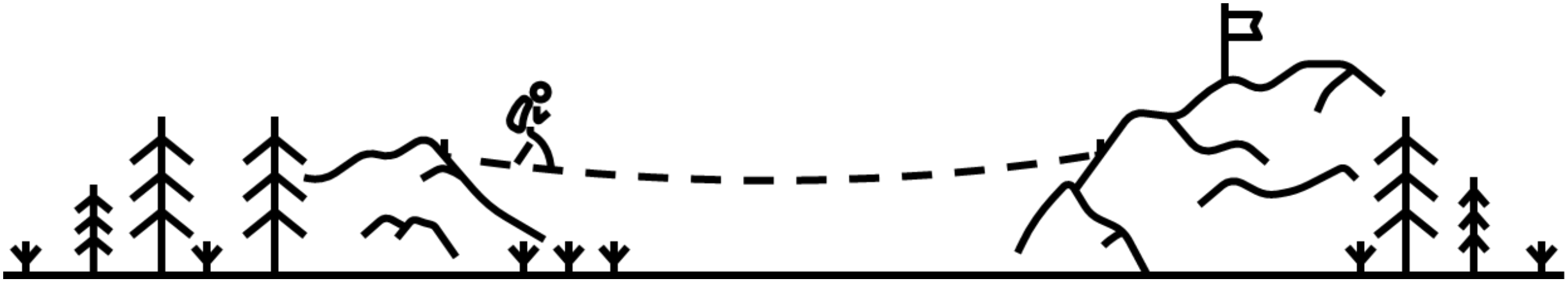
Tailored treatment



- One-off treatment
- Elimination of the cause
- Long-lasting effect

What treatments provide the longest, deepest, most durable response and with acceptable side effects to my specific type of disease?

Precision Medicine

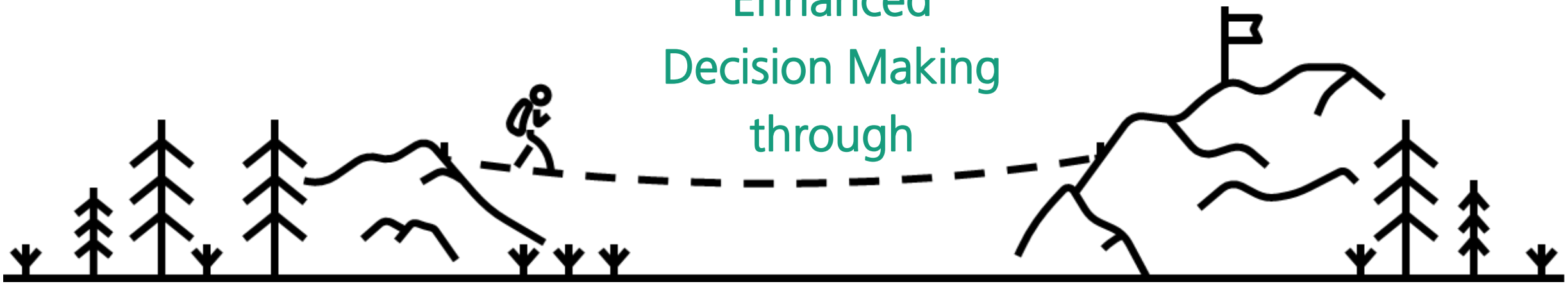


Visual inspired by app.napkin.ai

What treatments provide the longest, deepest, most durable response and with acceptable side effects to my specific type of disease?

Precision Medicine

Enhanced Decision Making through



Virtual Patient Twins?

Visual inspired by app.napkin.ai

Outline

Engineered Adoptive Cellular Immunotherapies

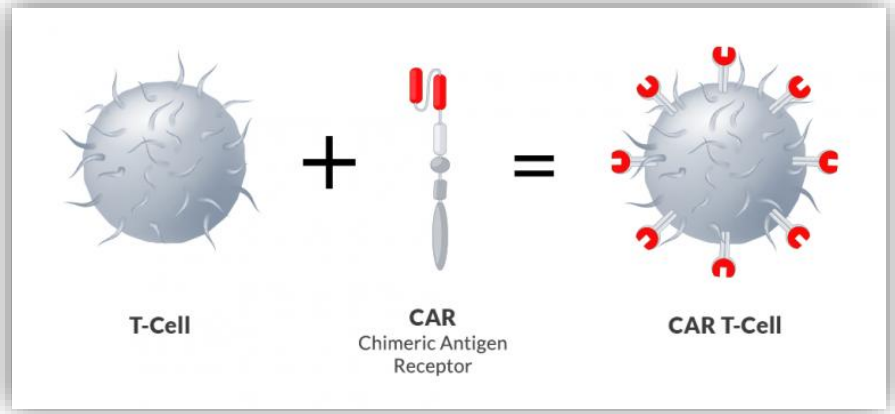
Design Specifications for Virtual Patient Twins in Engineered Adoptive Cellular Immunotherapies

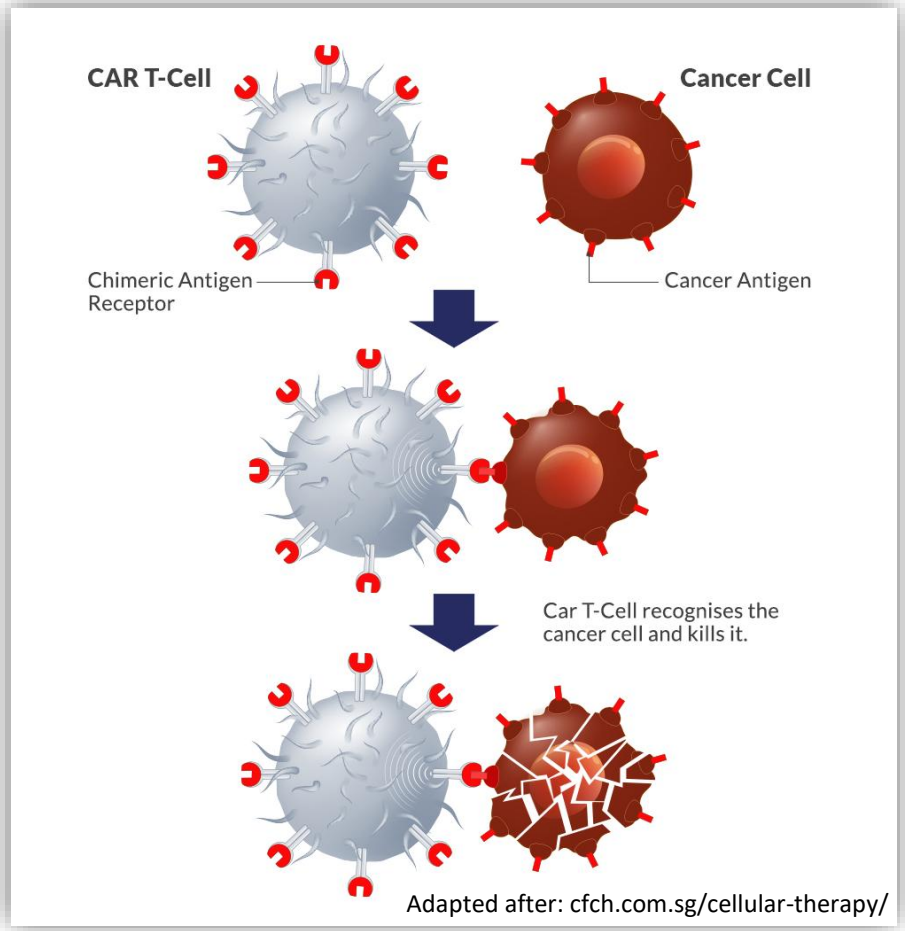
Towards a VT for Multiple Myeloma patients eligible for CAR T cell treatment

Engineered Adoptive Cellular Immunotherapies

CAR-T cells – Revolution of immunotherapy

© Emily Whitehead Foundation, 2022





Adapted after: cfch.com.sg/cellular-therapy/

CAR – chimeric antigen receptor | GVHD – graft versus host disease | CRS – cytokine release syndrome | CR – complete remission |ICANS Immune effector cell-associated neurotoxicity syndrome

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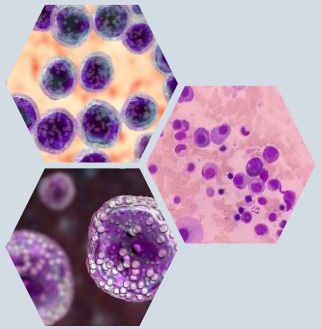
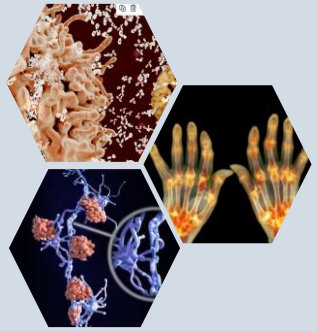
The Future
Indications



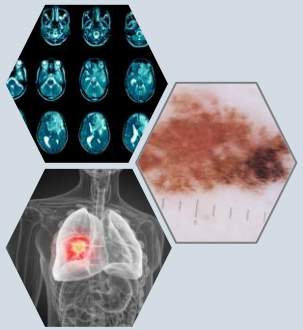
Hematological malignancies

- Move to earlier lines of treatment
- Increase time to relapse (MM)
- Other types of blood cancer, e.g., T cell lymphoma



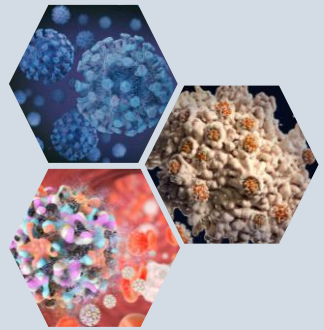
Autoimmune disease

- esp. when associated with autoreactive B cells; in clinical trials
- E.g., systemic lupus erythematosis, rheumathoid arthritis, multiple sclerosis, dermatomyositis



Solid tumors

- Overcome immunosuppressive tumor environment
- Increase accessibility of tumor tissue
- Tackle inter- and intra-tumor heterogeneity

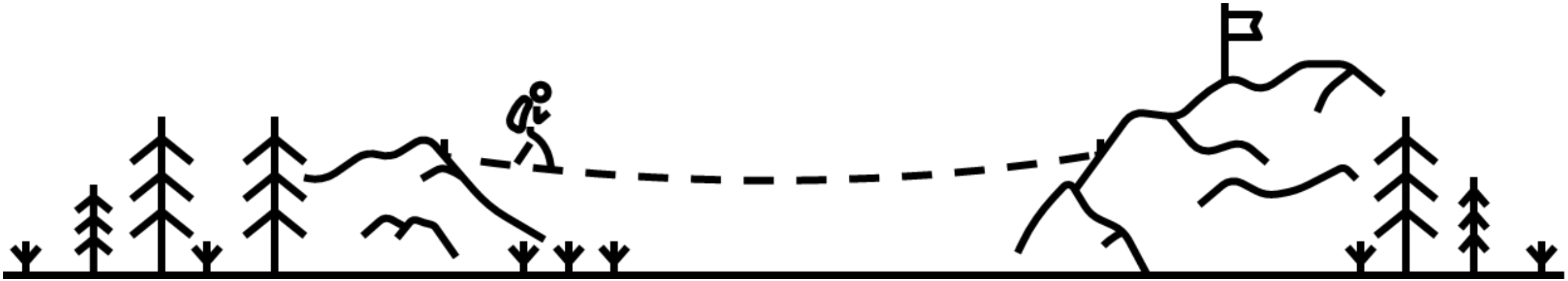


Infectious disease

- Primarily for viral infections; in clinical trials
- E.g., HIV,SIV, HBV, HCV, SARS-CoV-2, EBV

What treatments provide the longest, deepest, most durable response and with acceptable side effects to my specific type of disease?

Precision Medicine



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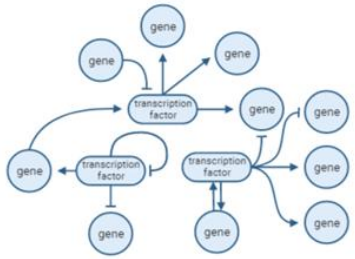
It is well accepted that studying (single) cells in different disease and health status of a patient advances precision medicine



Description of Status & Response to Cell Therapy
Genetics (e.g. genotoxicity)
Intra-Cellular / Molecular / Surfaceome
Inter-Cellular / Cell-cell communication
Tissue
Organ
Body

Figure created with Biorender.com

It is well accepted that studying (single) cells in different disease and health status of a patient advances precision medicine

For Cell and Gene Therapies (Living Drugs) this picture is incomplete!

Tissue

Organ

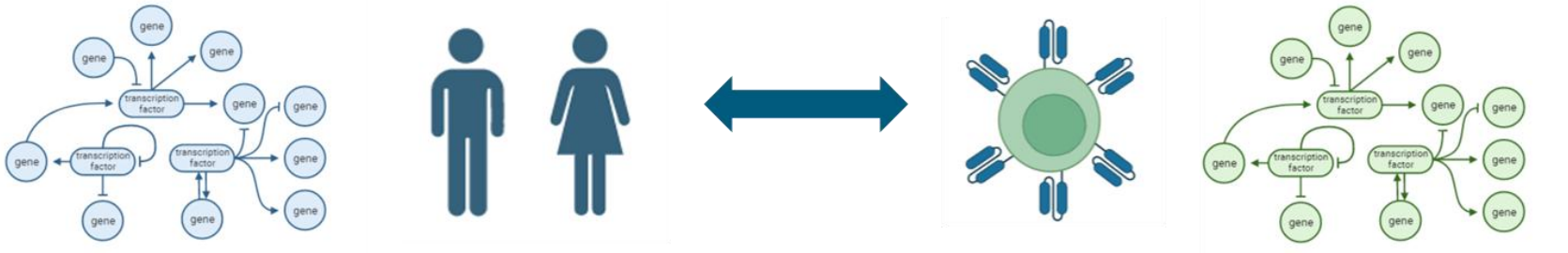
Body

Biomarkers

Matched Treatment

Figure created with Biorender.com

Studying the cells used as therapy is an essential requirement for advanced patient-specific models in Living Drugs



Description of Status & Response to Cell Therapy
Genetics (e.g. genotoxicity)
Intra-Cellular / Molecular / Surfaceome
Inter-Cellular / Cell-cell communication
Tissue
Organ
Body

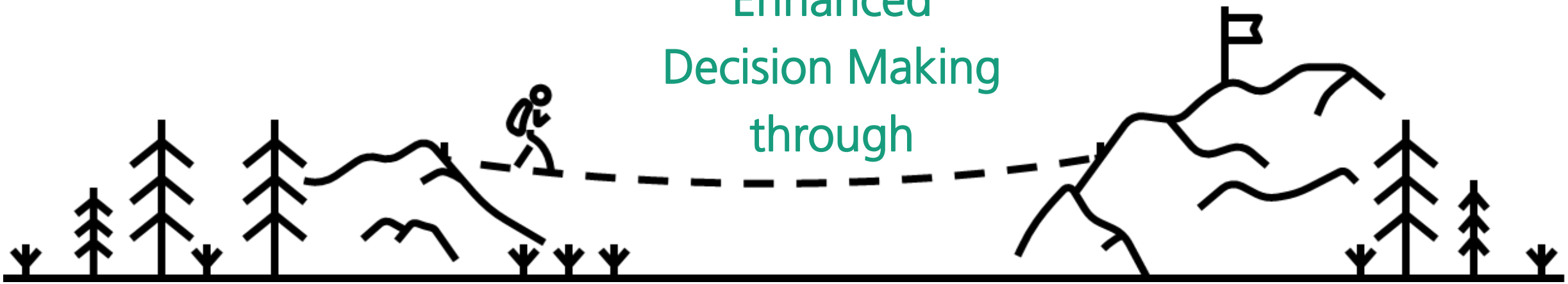
Description of Cell Product
Genetics
Molecular
Surfaceome
(Changes due to) Interaction with Host
Inter-Cellular / Cell-cell communication
Tissue
Organ

Figure created with Biorender.com

What treatments provide the longest, deepest, most durable response and with acceptable side effects to my specific type of disease?

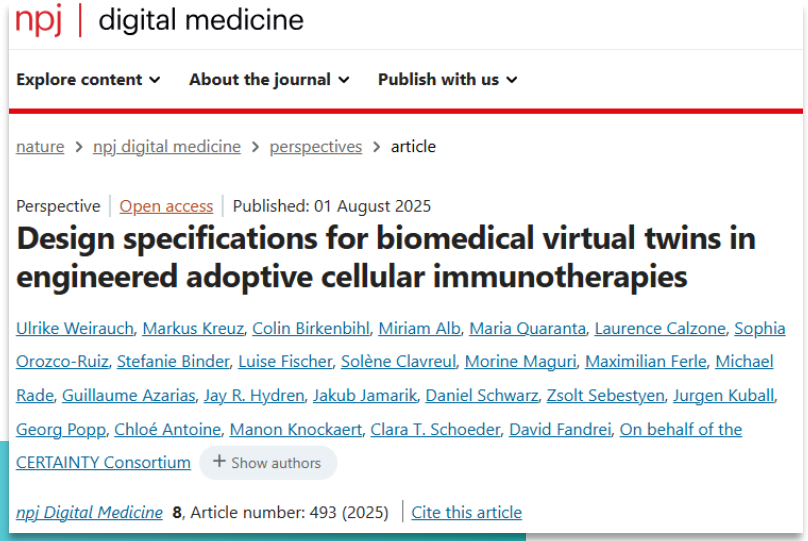
Precision Medicine

Enhanced Decision Making through



Virtual Patient Twins?

Visual inspired by app.napkin.ai



Design Specifications for Virtual Patient Twins in Engineered Adoptive Cellular Immunotherapies (Living Drugs)

Weirauch et al. Biomedical Virtual Twins for Living Drugs: Design Specifications for Virtual Twins in Engineered Adoptive Cellular Immunotherapies. NPJ Digital Medicine. 2025.

Digital Twin vs. Virtual Twin

Digital Twin

A digital representation of a real-world object qualifies as a Digital Twin (DT) if it includes:

(i) a **computational model of the object**,

(ii) a **dataset describing changes in the object**,

(iii) and **methods for continuously updating the computational model with data derived from its real-world counterpart**

→ A DT is expected to evolve in parallel with its real-world counterpart.

[1] Viceconti, M., Vos, M., de, Mellone, S. & Geris, L. Position paper From the digital twins in healthcare to the Virtual Human Twin: a moon-shot project for digital health research. *IEEE J. Biomed. Health Inform.* **28**, 491–501 (2023)

[2] Wright, L. & Davidson, S. How to tell the difference between a model and a digital twin. *Adv. Model. and Simul. Eng. Sci.* **7**, https://doi.org/10.1186/s40323-020-00147-4 (2020)..

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Virtual Twin

A Virtual Twin (VT) in healthcare as an application-specific in silico system covering:

(i) **at least two single(-organ) DTs** from the same patient **to simulate multi-organ** biomedical **interplay**.

→ **This makes VTs particularly well-suited for use in patients eligible for Living Drugs!**

[1] Viceconti, M., Vos, M., de, Mellone, S. & Geris, L. Position paper From the digital twins in healthcare to the Virtual Human Twin: a moon-shot project for digital health research. *IEEE J. Biomed. Health Inform.* **28**, 491–501 (2023)

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Real world

Different data types & categories

Weirauch et al. Biomedical Virtual Twins for Living Drugs: Design Specifications for Virtual Twins in Engineered Adoptive Cellular Immunotherapies. NPJ Digital Medicine. 2025.

Real world

Different data types & categories

Virtual representation

Different parameter & model types

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Real world

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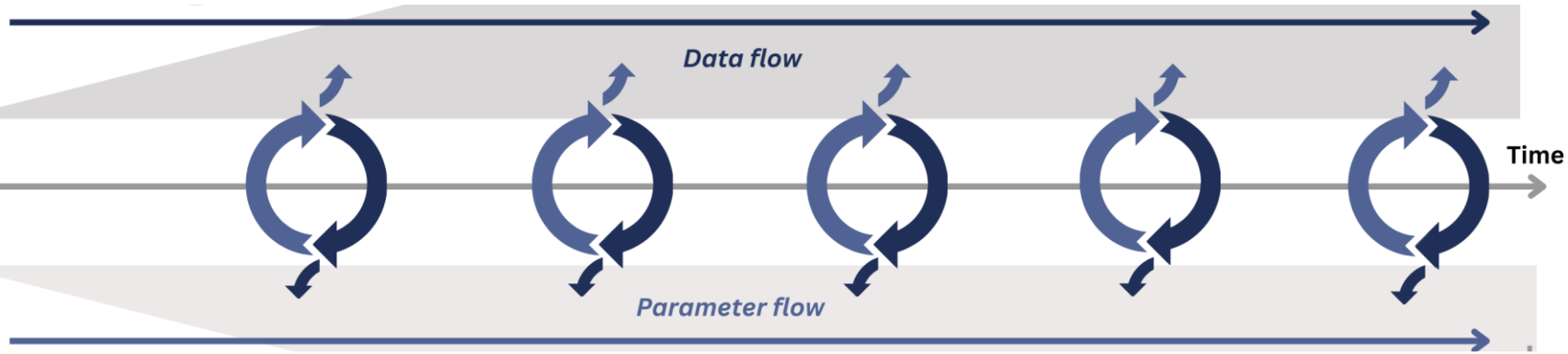

Bidirectional interactions
Types, categories, decision making, resolution, fidelity

Virtual representation

Different parameter & model types

CERTAINTY

Weirauch et al. Biomedical Virtual Twins for Living Drugs: Design Specifications for Virtual Twins in Engineered Adoptive Cellular Immunotherapies. NPJ Digital Medicine. 2025.

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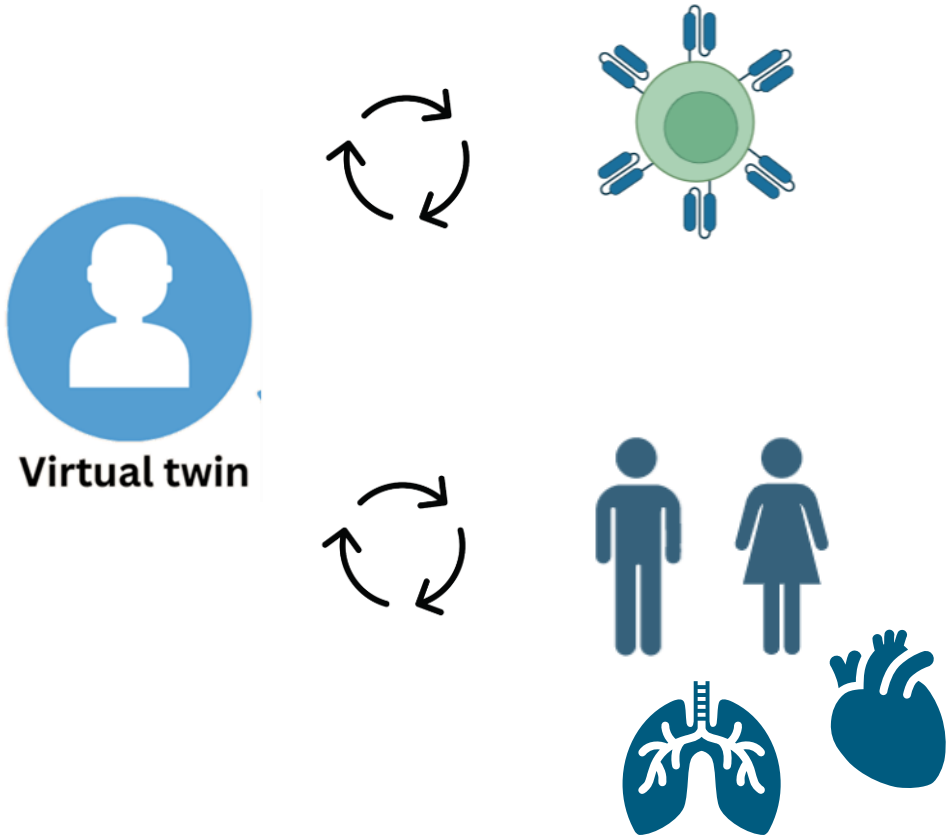
Virtual Twin in Engineered Adoptive Cellular Immunotherapies



Digital Twin(s) for modified immune cells

Digital Twins for organs of patient (incl. immune system AND normal tissue)

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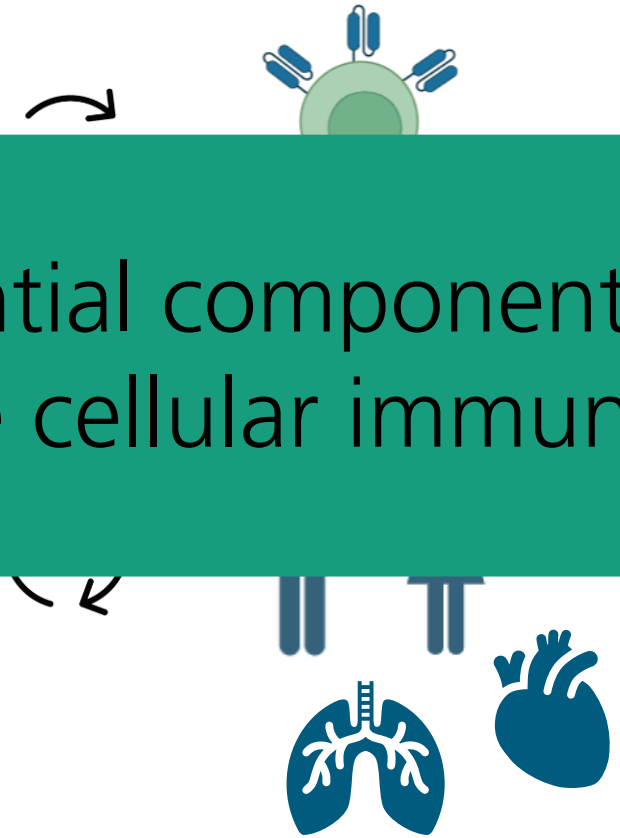
Virtual Twin in Engineered Adoptive Cellular Immunotherapies



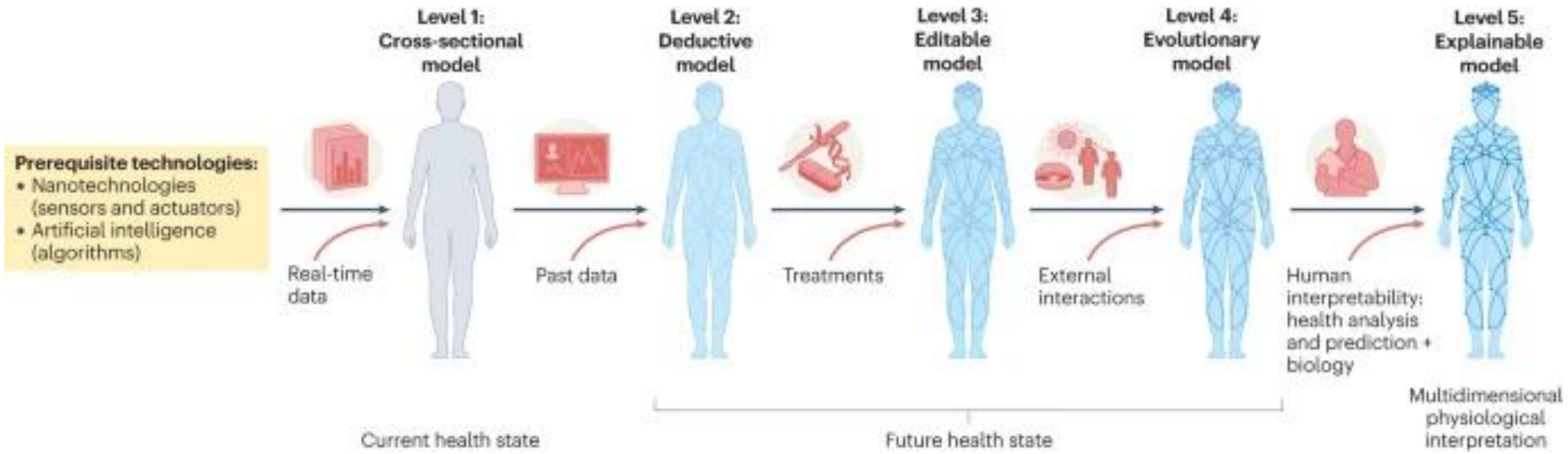
Digital Twin(s) for modified immune cells

(incl. immune system AND normal tissue)

Are all essential components for a VT in engineered adoptive cellular immunotherapies covered?

Weirauch et al. Biomedical Virtual Twins for Living Drugs: Design Specifications for Virtual Twins in Engineered Adoptive Cellular Immunotherapies. NPJ Digital Medicine. 2025.

Whole Body Virtual Twins for Humans



A roadmap for the development of human body digital twins. Chenyu Tang, Wentian Yi, Edoardo Occhipinti, Yanning Dai, Shuo Gao & Luigi G. Occhipinti. Nature Reviews Electrical Engineering volume 1, pages199–207 (2024)

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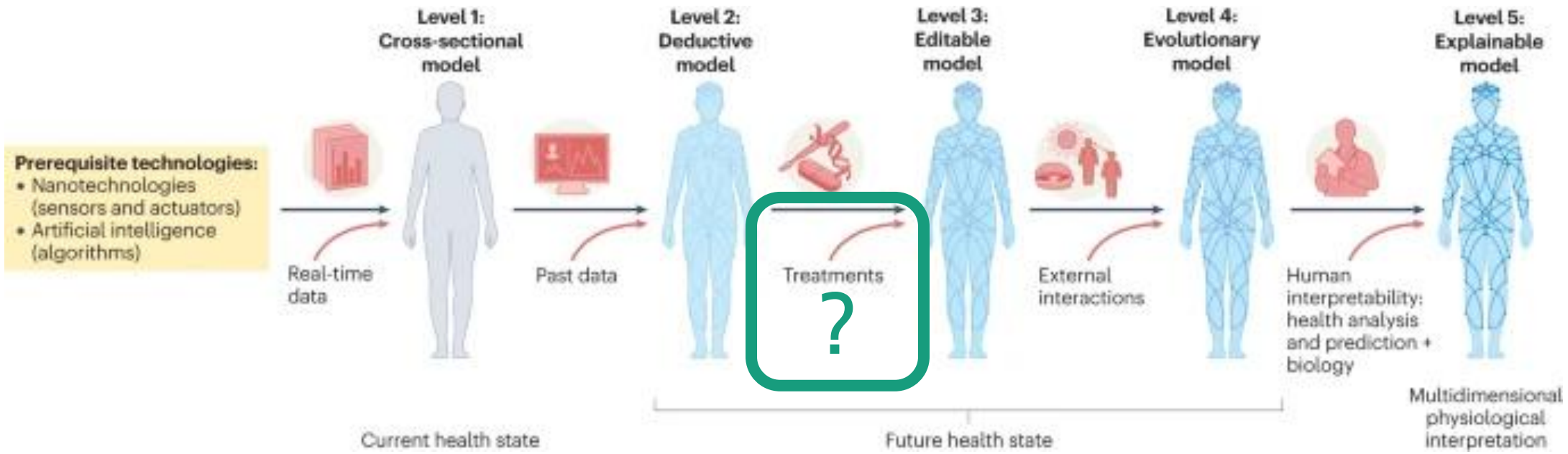

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Living Drugs compared to Conventional Drugs

Conventional Drugs

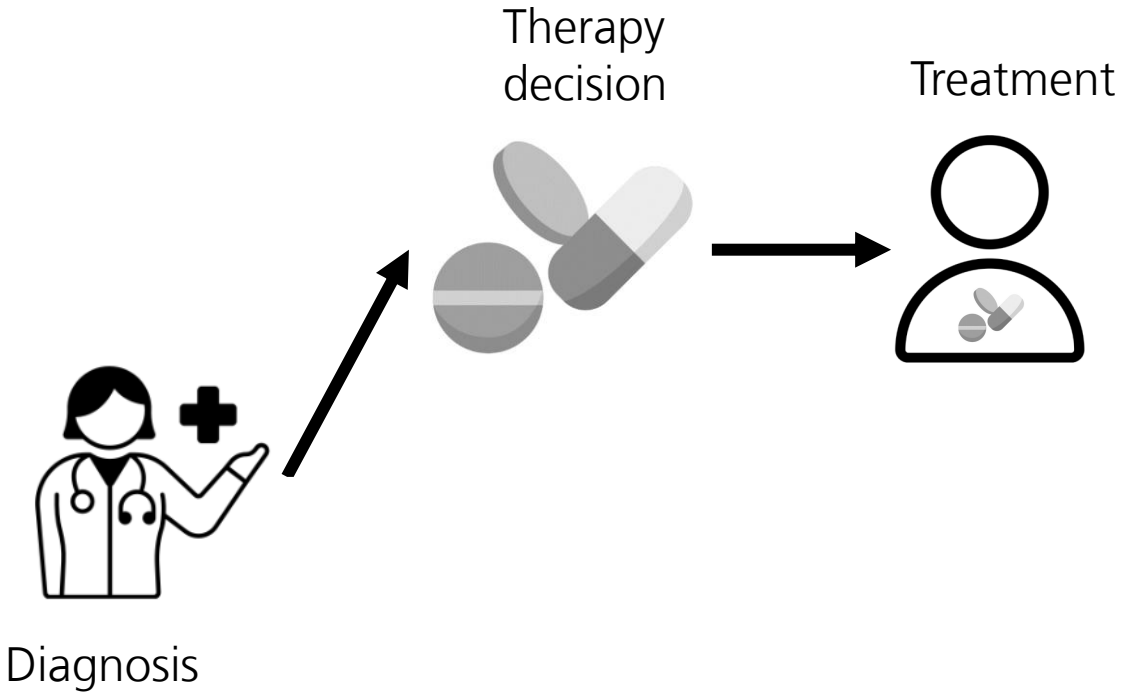
Adoptive Cellular Immunotherapies

Diagnosis

Living Drugs compared to Conventional Drugs

Conventional Drugs

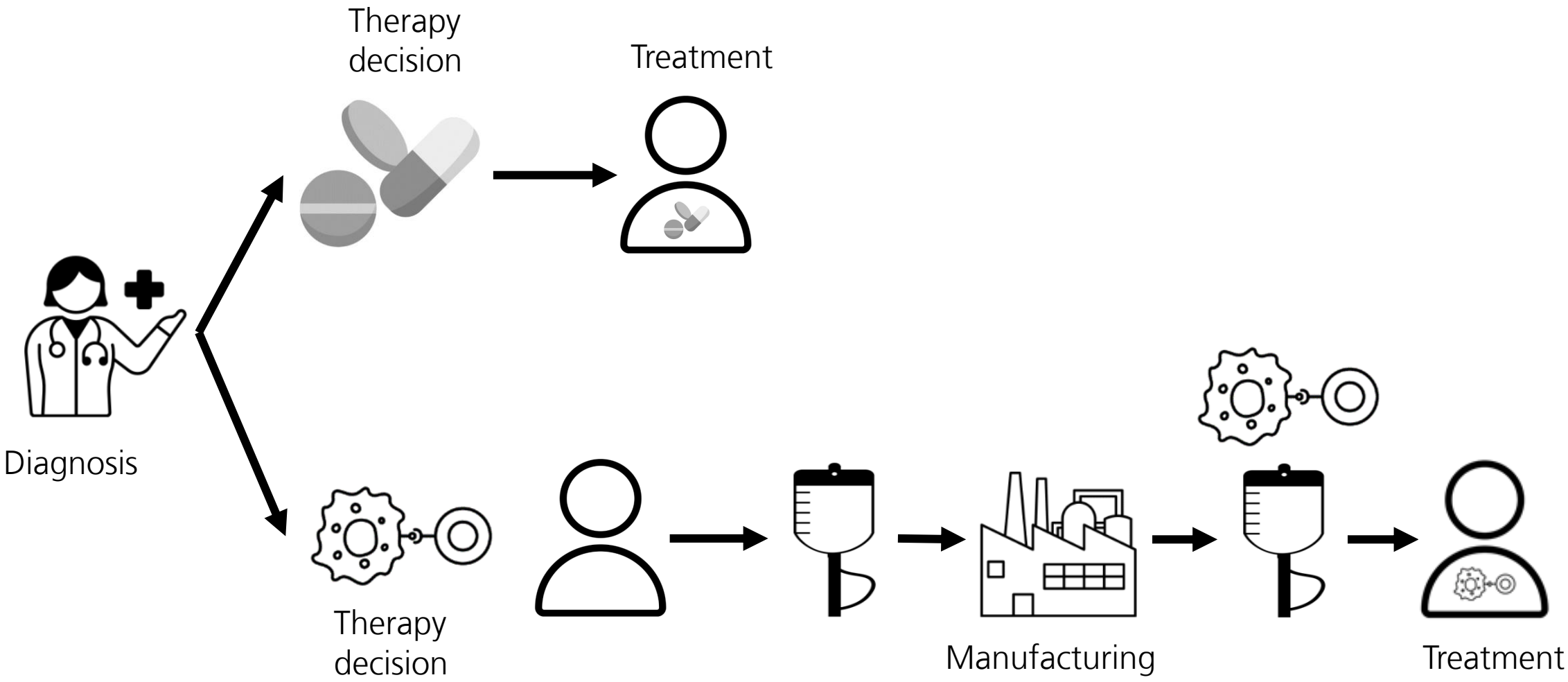
Adoptive Cellular Immunotherapies



Living Drugs compared to Conventional Drugs

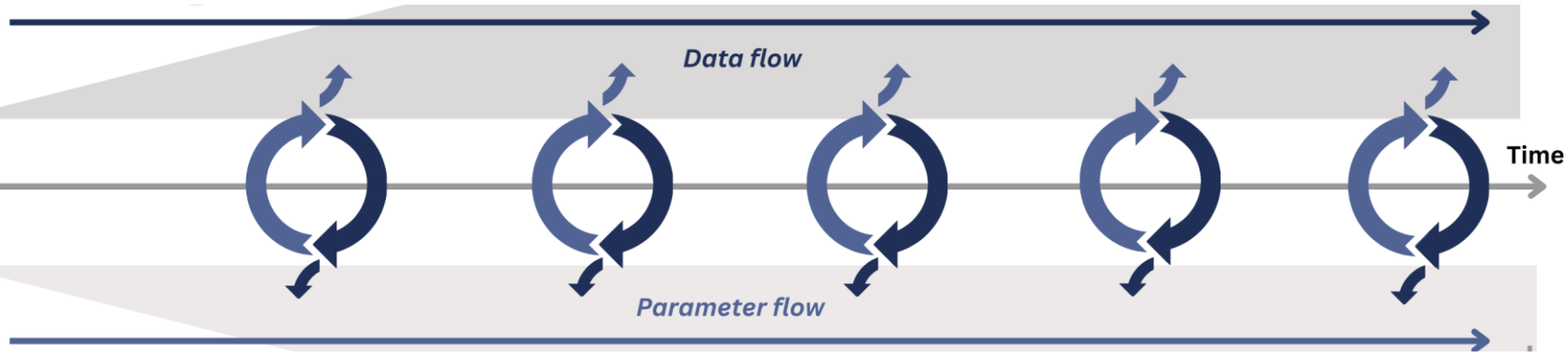
Conventional Drugs

Adoptive Cellular Immunotherapies



Real world

Different data types & categories



Bidirectional interactions
Types, categories, decision making, resolution, fidelity

Virtual representation

Different parameter & model types

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Weirauch et al. Biomedical Virtual Twins for Living Drugs: Design Specifications for Virtual Twins in Engineered Adoptive Cellular Immunotherapies. NPJ Digital Medicine. 2025.

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Real world

Different data types & categories

Patient's path

Leukapheresis → Bridging therapy → Lymphodepletion

Data flow

Bidirectional interactions
Types, categories, decision making, resolution, fidelity

Time

Parameter flow

Virtual representation

Different parameter & model types

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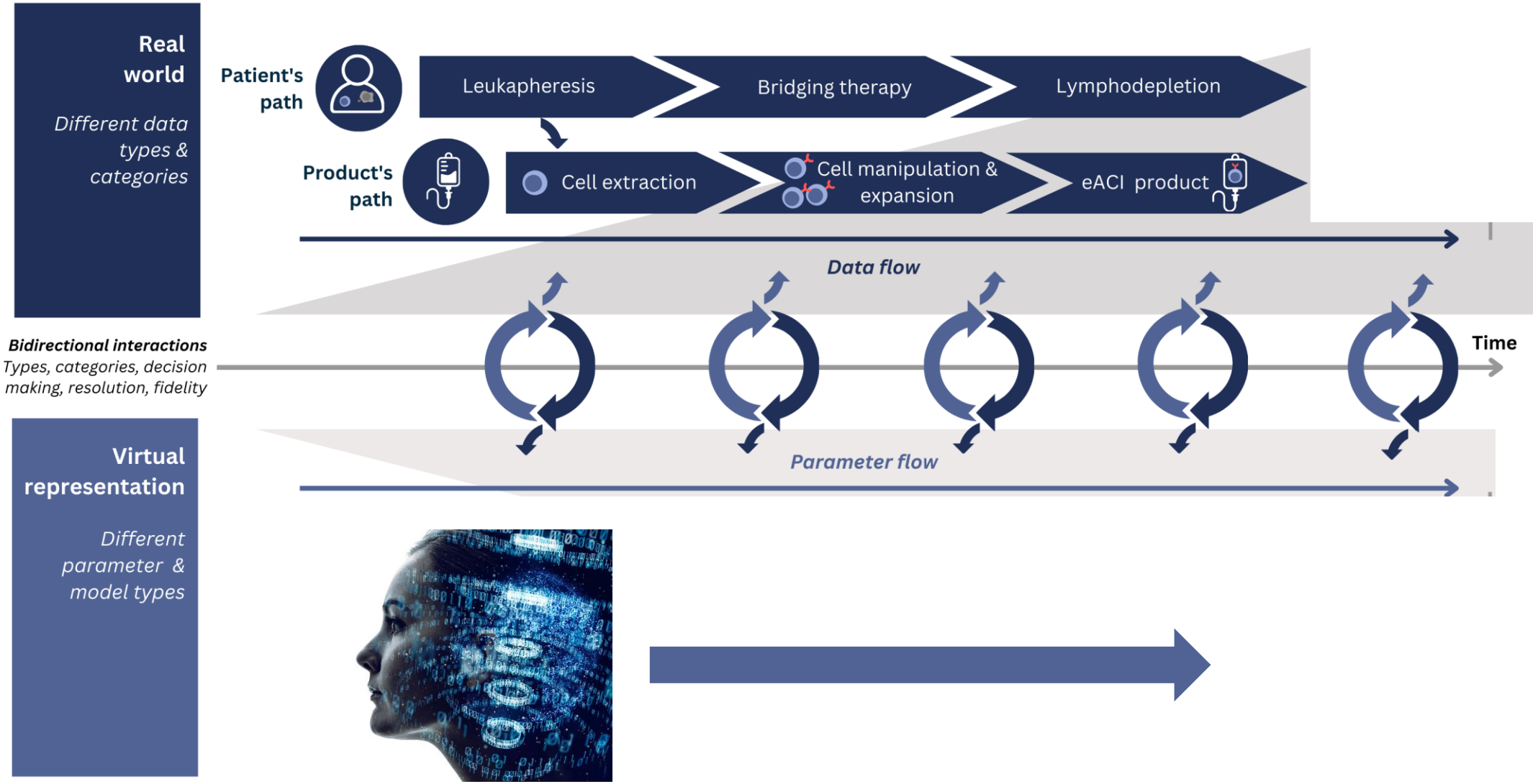

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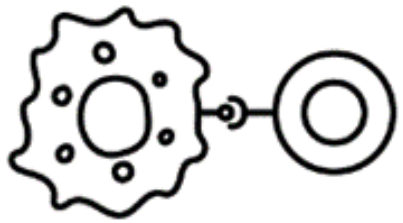
Living Drugs compared to Conventional Drugs

CAR T cells are living therapies with the unique feature of being a biological system.



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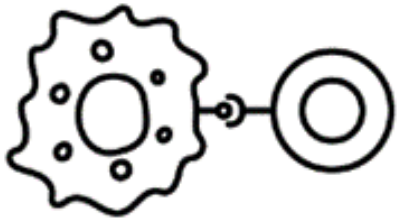
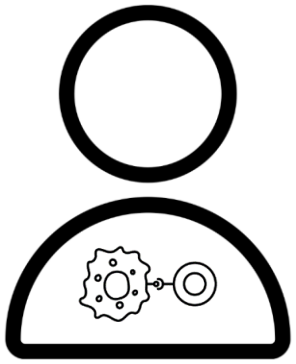
CAR T cells interact with a living system, that is the patient.





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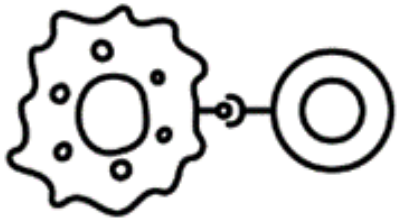
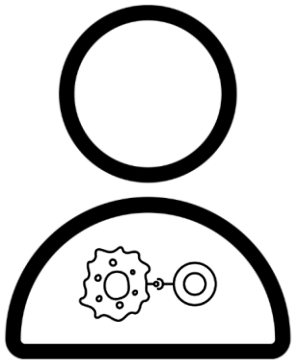
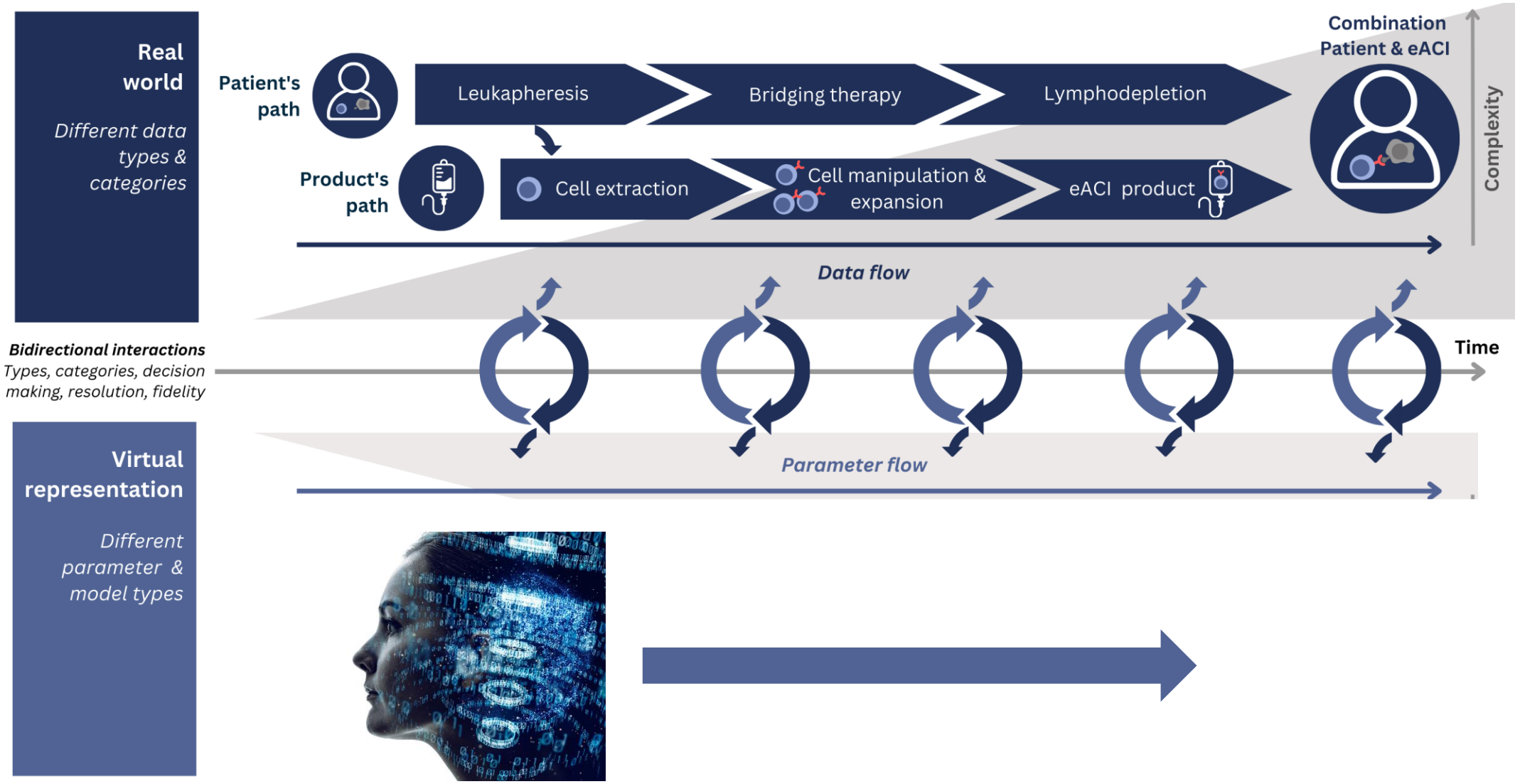
CAR T cells interact with a living system, that is the patient.

Life-long monitoring required?







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Minimum data categories required in observations of the real-world instance

First, **longitudinal multiomics at the single-cell** level are needed to **measure intra- and intercellular processes** influencing, for example, T cell activation, expansion, exhaustion, genotoxicity, on-target/off-tumor binding, immunosuppressive environment, or imbalances in (CAR) T cell clones.

1

2

Second, **longitudinal observations along the organ and body scale**, such as CAR T cell expansion and persistence, response to treatment, comorbidities, and side effects, are required.

Third, **integration of patient-reported outcomes in combination with socio-economic factors** like gender, income, education, and geographic location enhances eACI-VT simulations and validation, prevents bias, and increases predictive accuracy.

3

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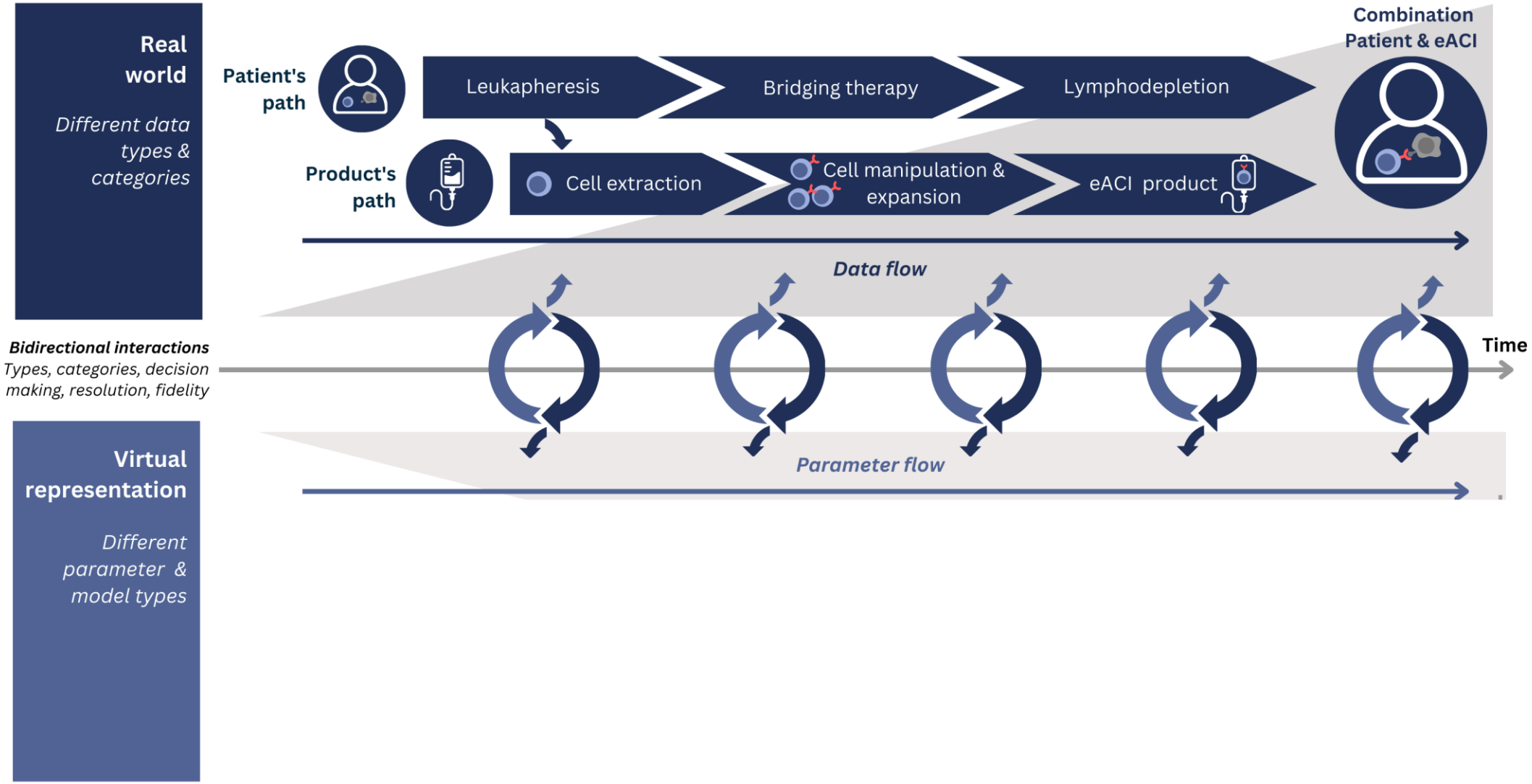

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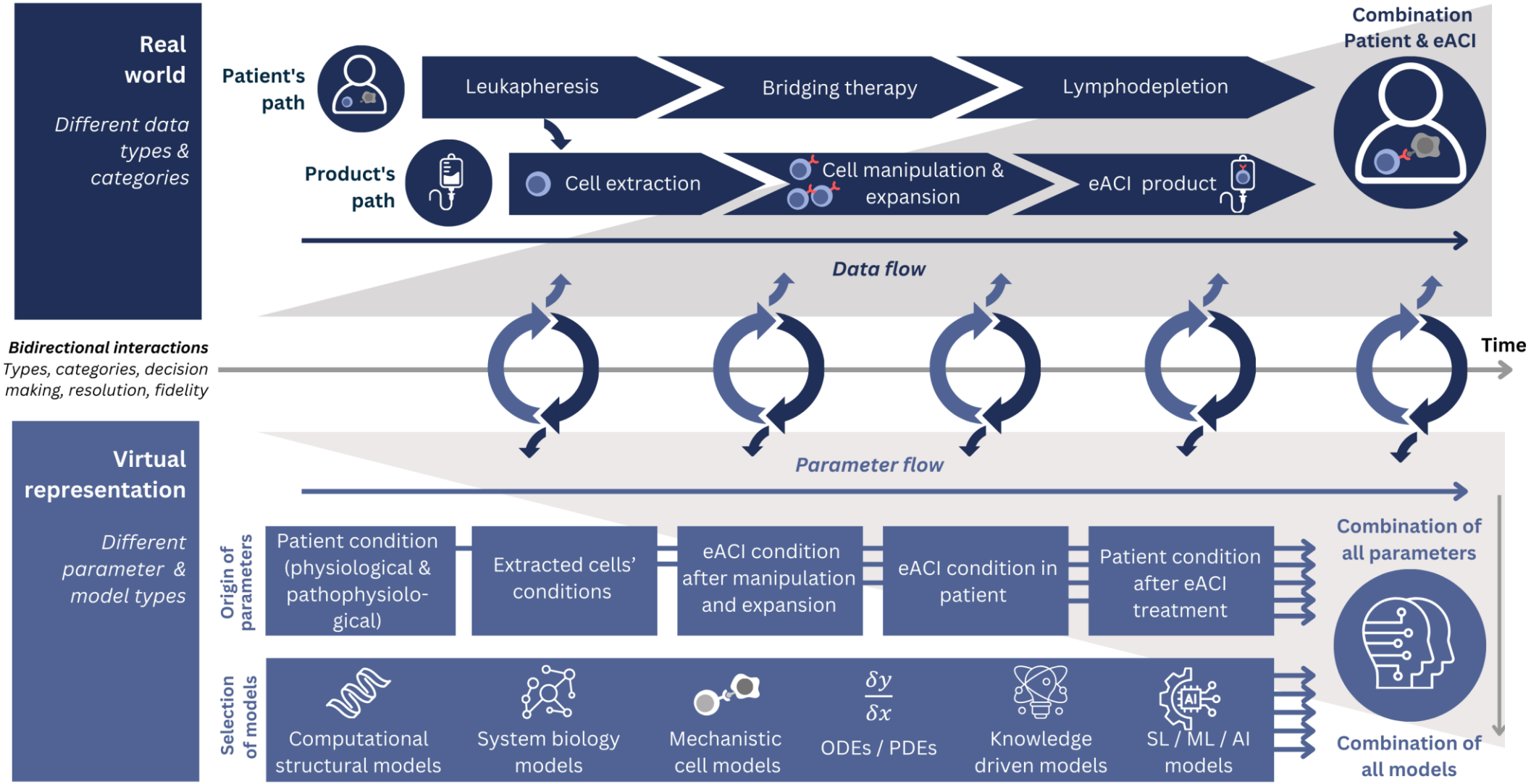

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Multi-scale VTs are essential in CAR T cell therapies

Devices for data collection

- IOT Internet of Things
- CPS Cyber-Physical System
- Electronic health records
- (Genomic) lab test data (incl. single-cell multiomics)
- Wearables
- Digital health application

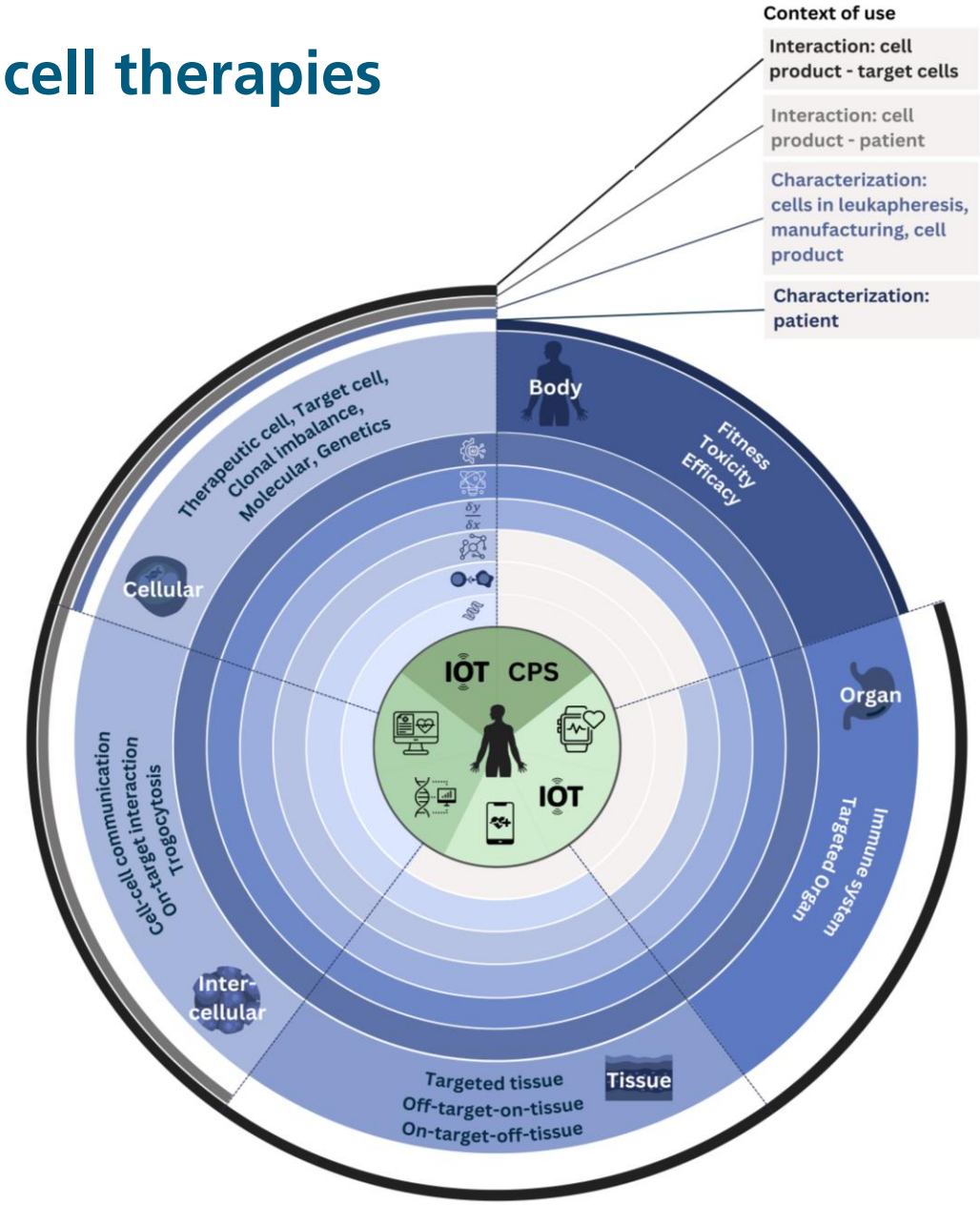
Type of model

- SL / ML / AI
- Knowledge driven models
- Systems biology models
- Mechanistic cell models
- $\frac{\delta y}{\delta x}$ ODE, PDE
- Computational structural biology

Devices during manufacturing

Devices during inpatient care

Smart devices during outpatient care



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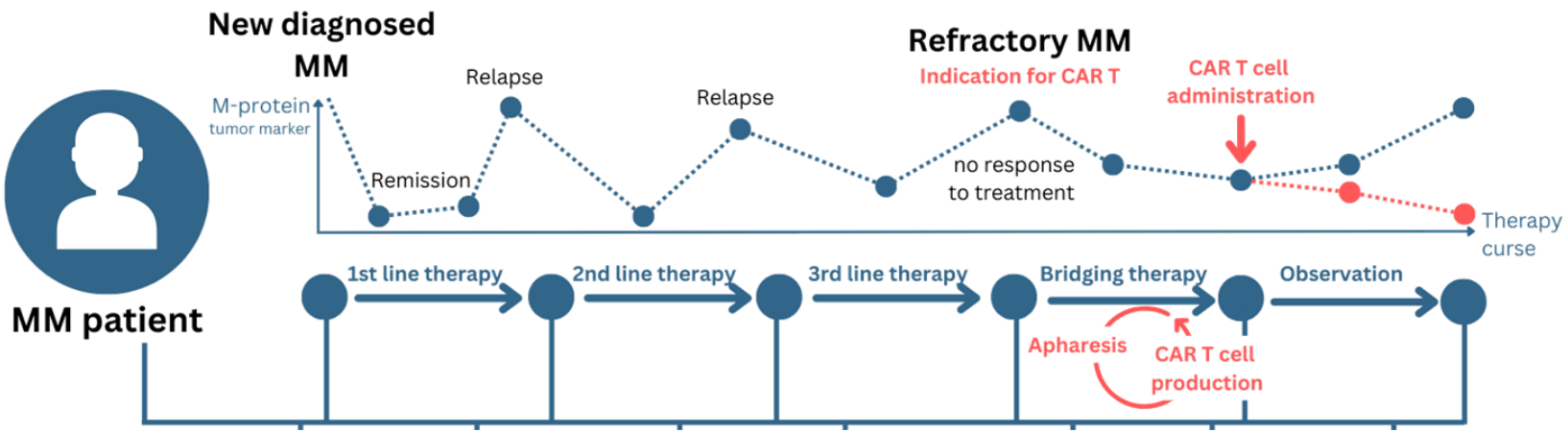
Towards a VT for Multiple Myeloma patients eligible for CAR T cell treatment

Multiple Myeloma



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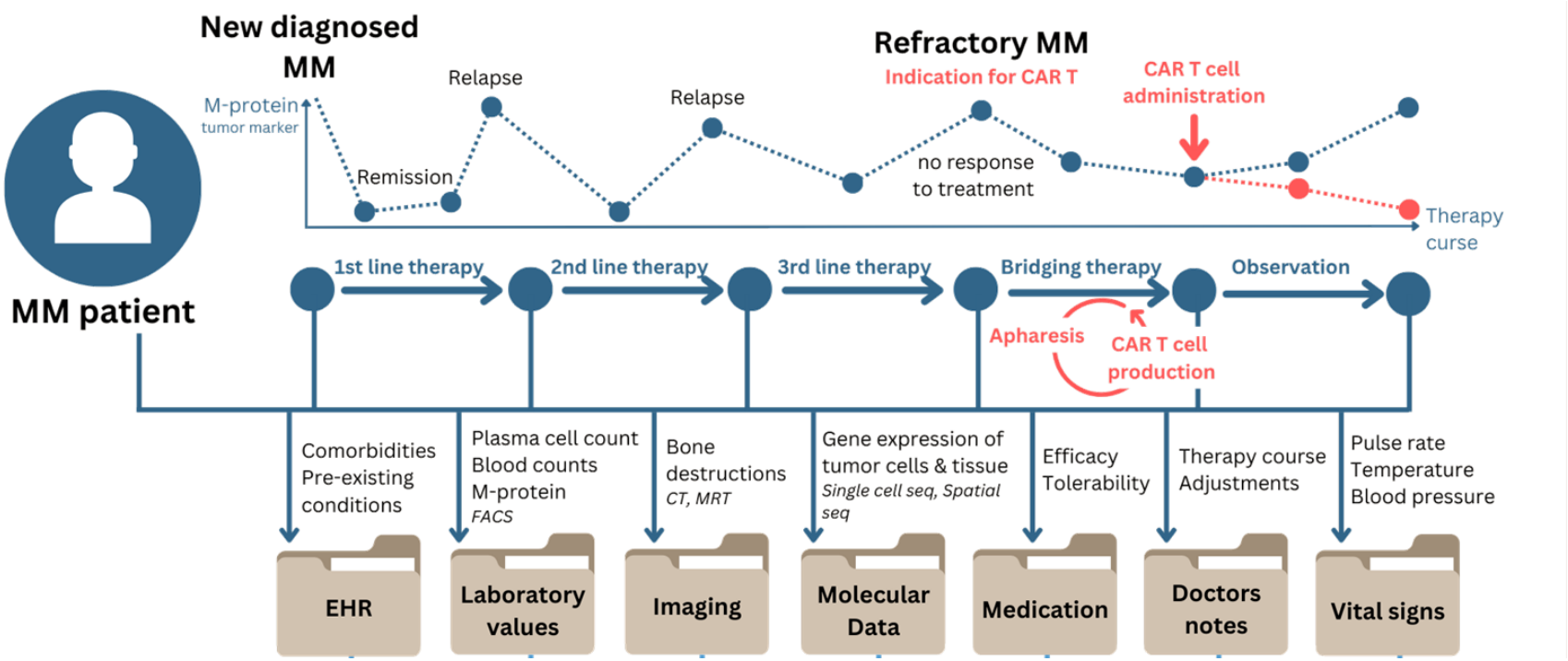
Multiple Myeloma



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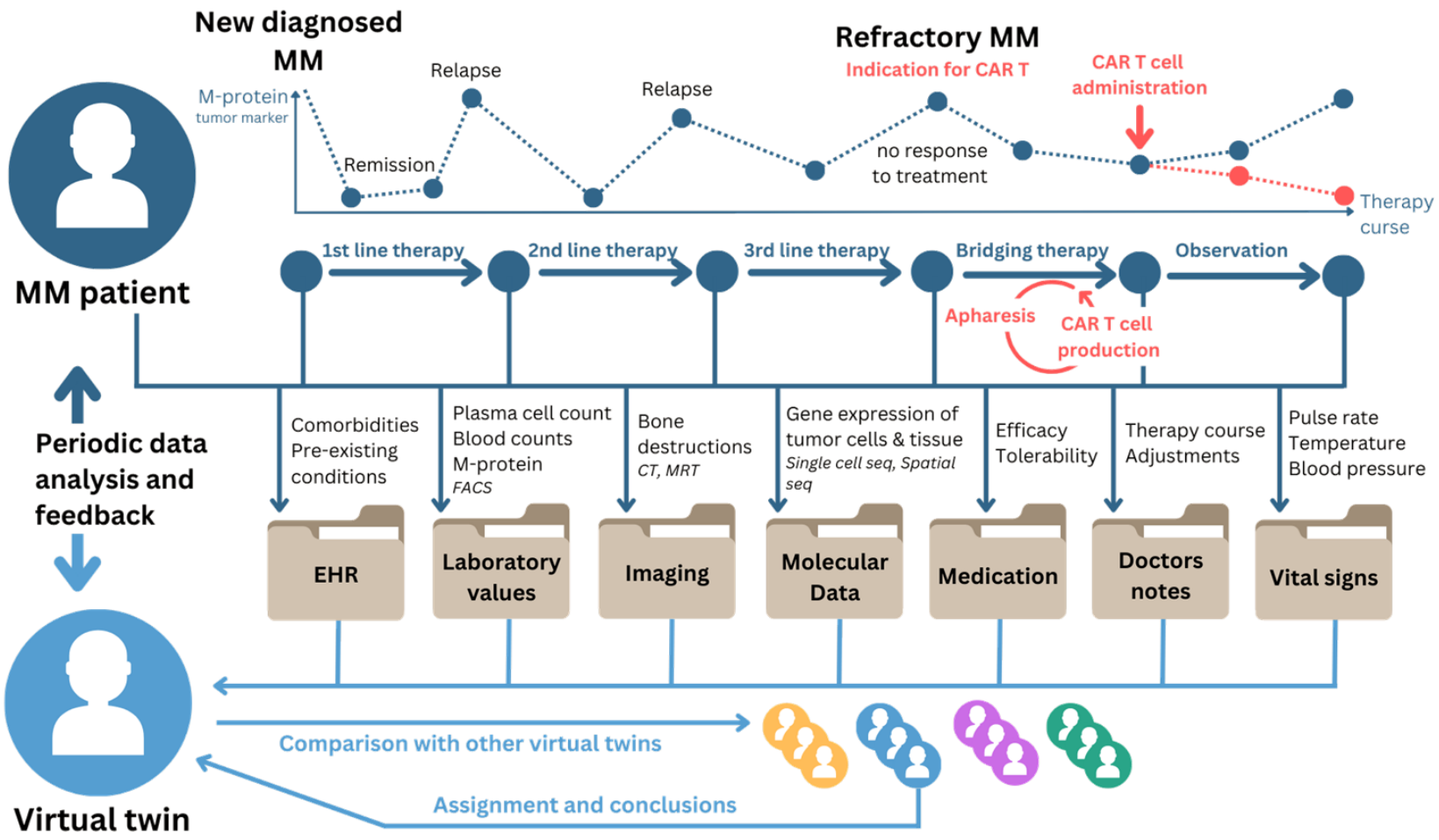
Multiple Myeloma



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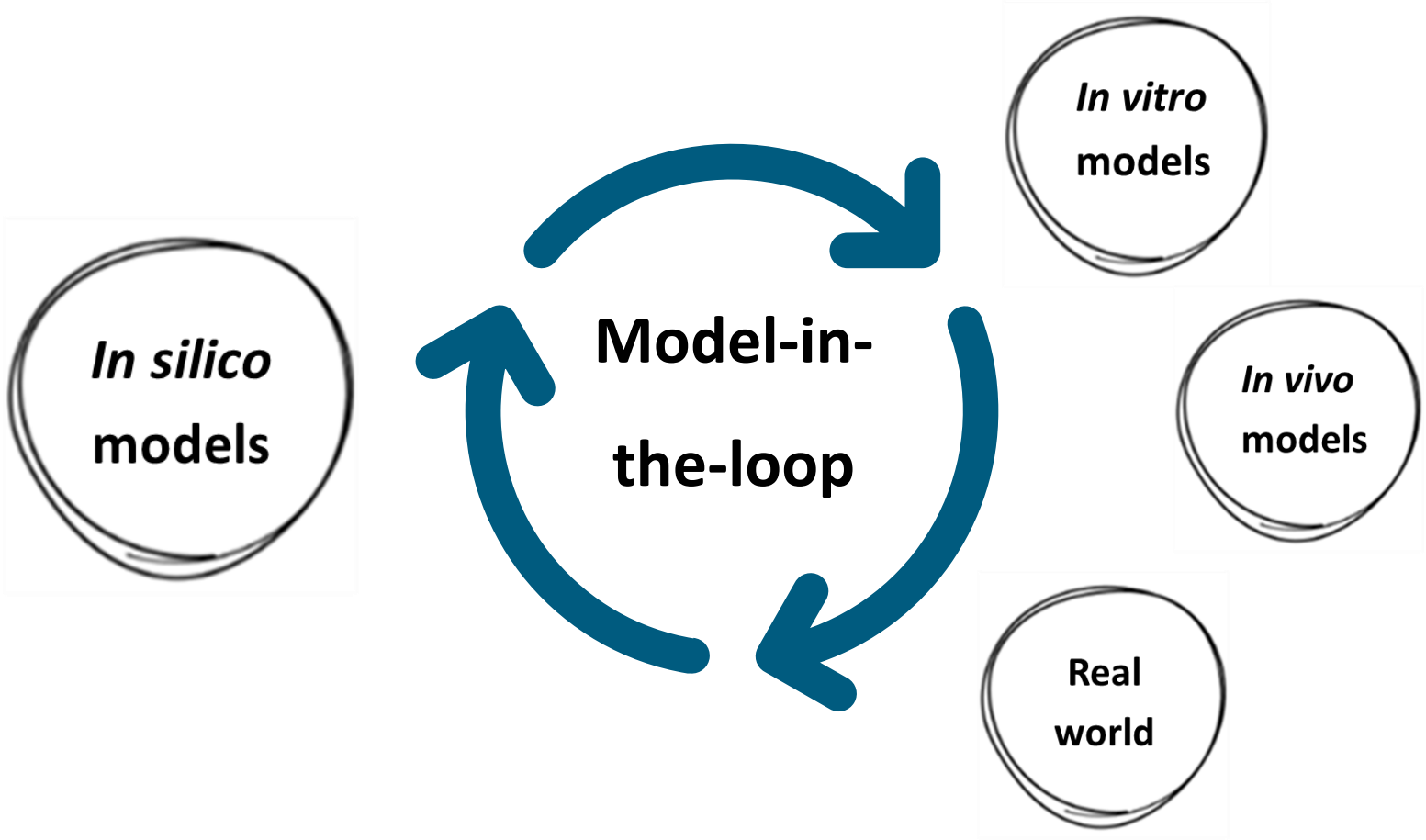
Approach to implementation



Weirauch et al. Design Specifications for Biomedical Virtual Twins in Engineered Adoptive Cellular Immunotherapies. npj Digital Medicine 2025.

In vitro models include NAMs (new approach methodologies)

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Results – RWE

RWE = Real-world evidence

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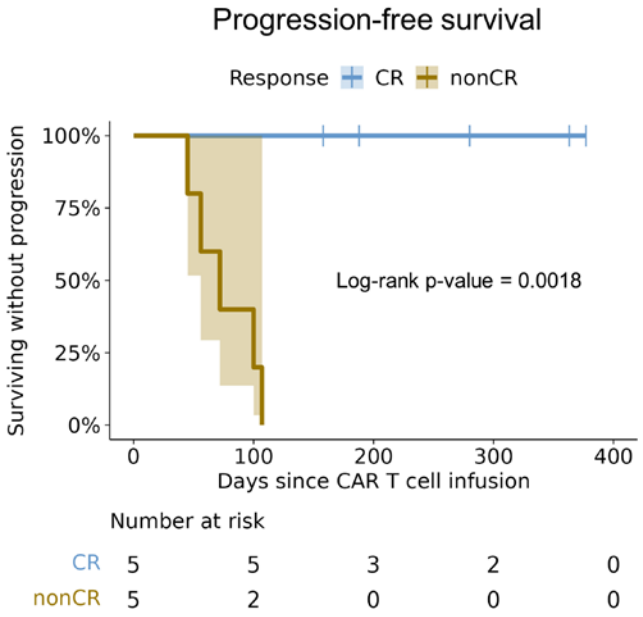
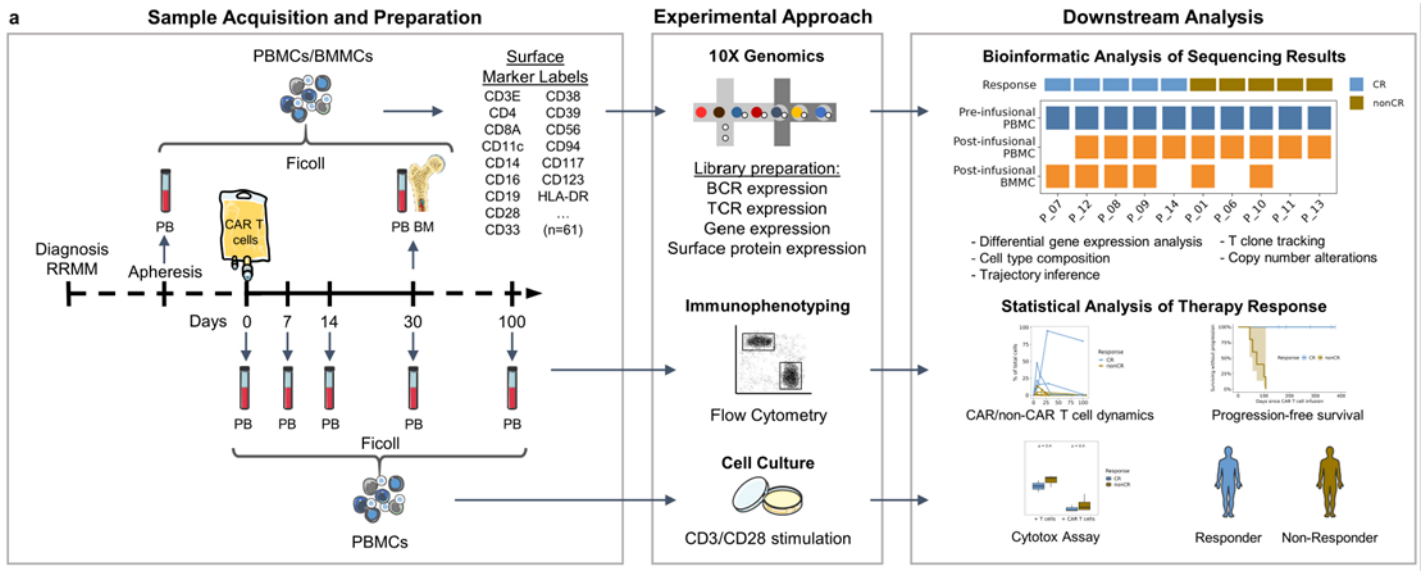
In search for biomarkers using multi-omics: Multiple Myeloma, anti-BCMA CAR-T

Real world





Missing predictive markers

Rade M, … Koehl U, … Reiche K*, Vucinic V* and Merz M*. Nature Cancer. 2024

CR = Complete response
nonCR = non Complete response

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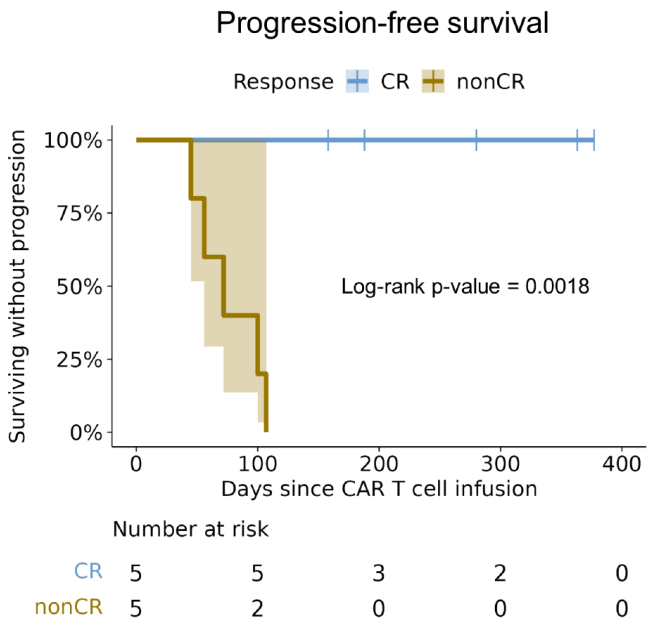
Predictive markers for treatment response

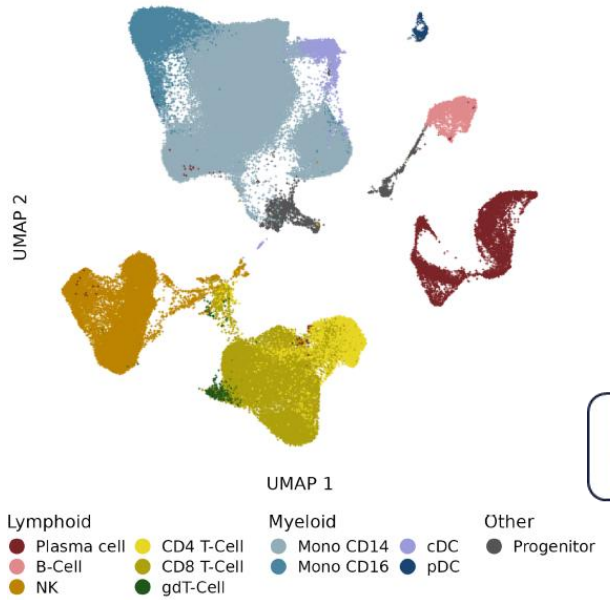
N = 10 anti-BCMA CAR T patients with RRMM (n=2 cilta-cel, n=8 ide-cel)

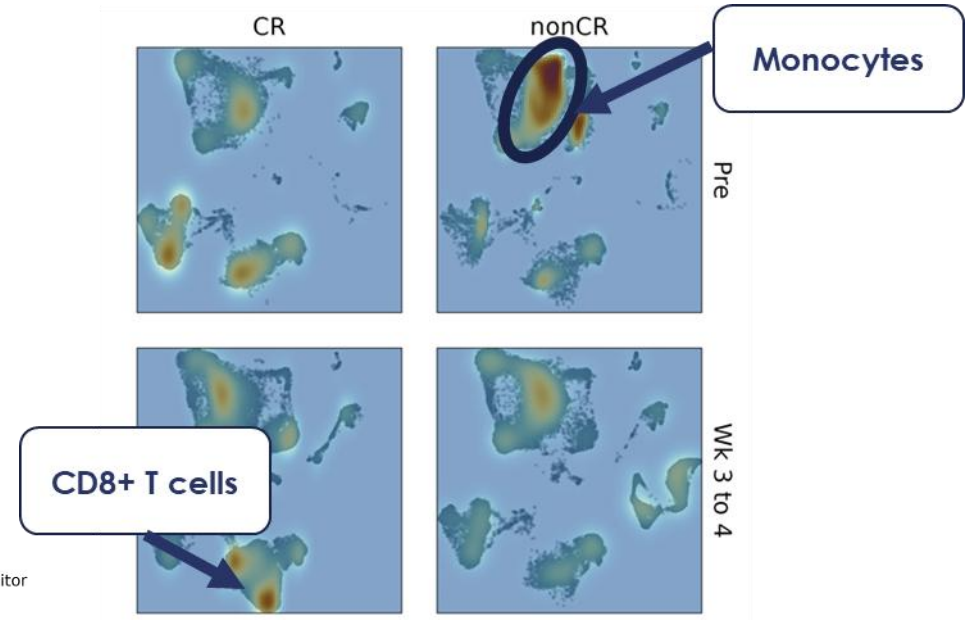
Real world

Kaplan-Meier curve



PB/BM MC at the timepoint of apharesis and 30 days post CAR T cell therapy





Differences between CR and nonCR (Wilcoxon, p-value<0.1)

To date, still missing predictive marker

→ Differences between CR and nonCR could already be identified at the time of leukapheresis

CR = Complete response
nonCR = non Complete response

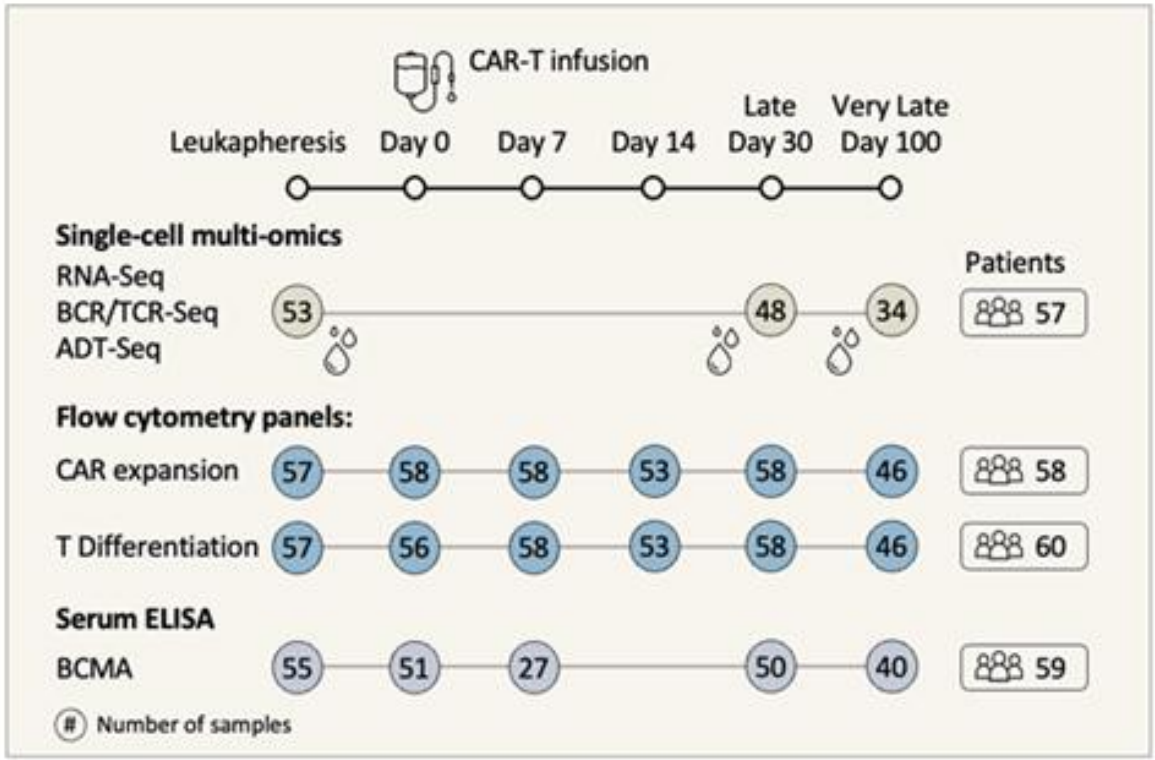
PB/KBM MC = Mononuclear cells from peripheral blood or bone marrow

Rade M et al. Nature Cancer 2024

Extension to larger cohort

Real world

Longitudinal single cell landscape of patients treated with anti-BCMA CAR T cells :
Single-cell multiomics of 123 PBMC samples **across 57 MM patients and 3 time points**



Rade et al. A longitudinal single-cell atlas to predict outcome and toxicity after BCMA-directed CAR T cell therapy in multiple myeloma. In review.

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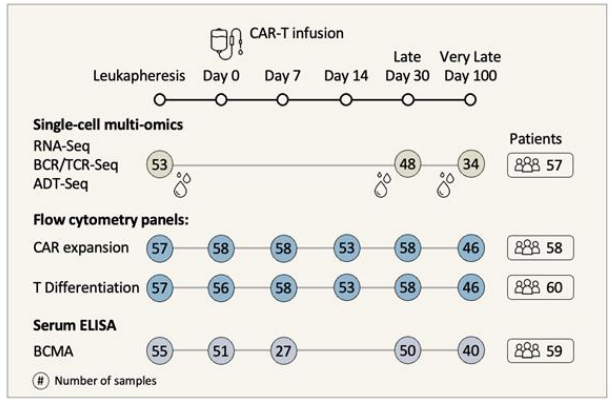
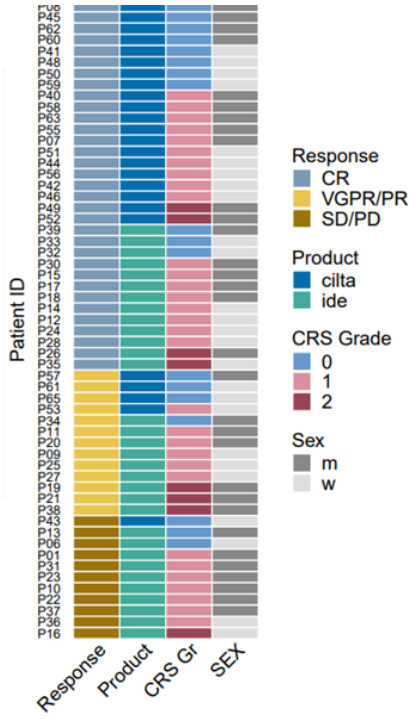
Extension to larger cohort

Real world

Longitudinal single cell landscape of patients treated with anti-BCMA CAR T cells :
Single-cell multiomics of 123 PBMC samples **across 57 MM patients and 3 time points**

Experimental design and patient cohort

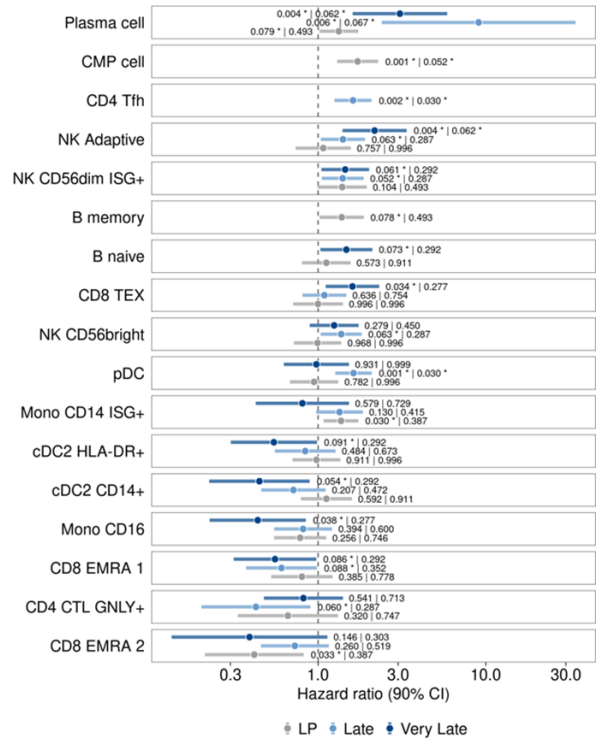




CAR T cell phenotype

after treatment (day 30)

Cell subtype proportion vs. treatment response



Rade et al. A longitudinal single-cell atlas to predict outcome and toxicity after BCMA-directed CAR T cell therapy in multiple myeloma. In review.

Please do not post! See preprint: A longitudinal single-cell atlas to predict outcome and toxicity after BCMA-directed CAR T cell therapy in multiple myeloma | Research Square

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CERTAINTY – Predicting Progression Events in Multiple Myeloma from Routine Blood Work

Real world

NN Model

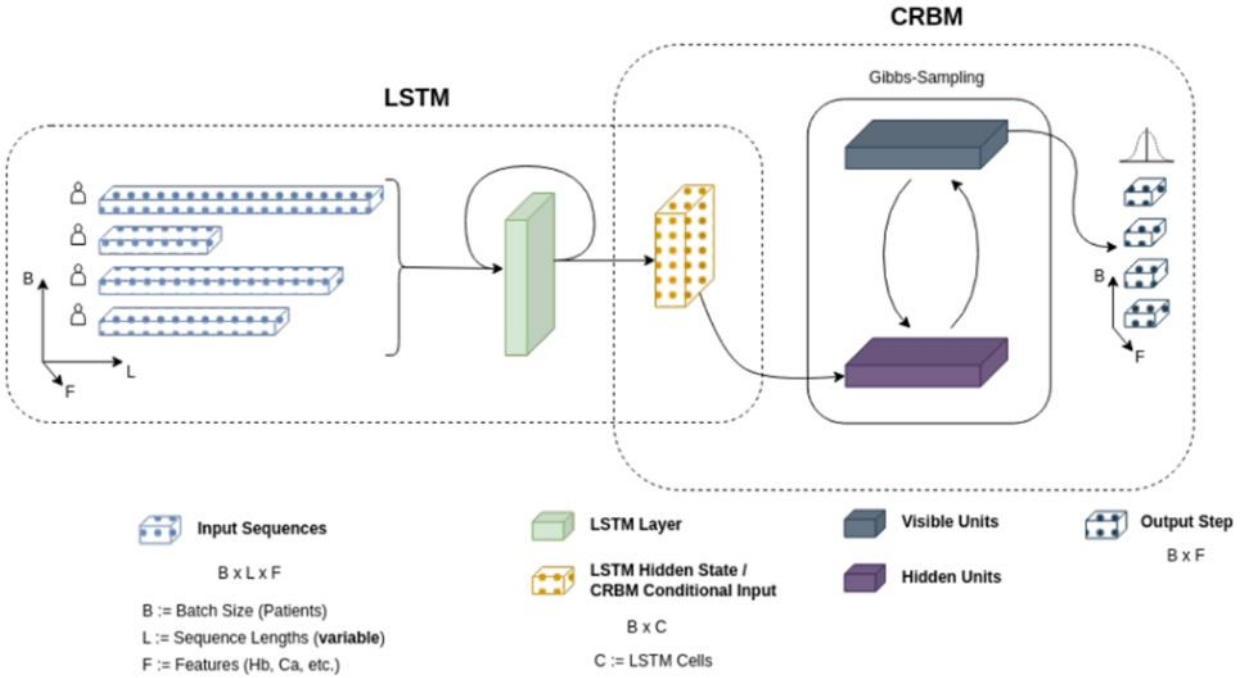

Figure 7: Architecture diagram of the forecasting model. The LSTM summarizes all observations of a patient's sequential blood work within its internal state. The CRBM then generates probabilites of possible outcomes conditioned on the patient history encoded by the LSTM through a Gibbs-sampling algorithm.

Individual predictions for patients

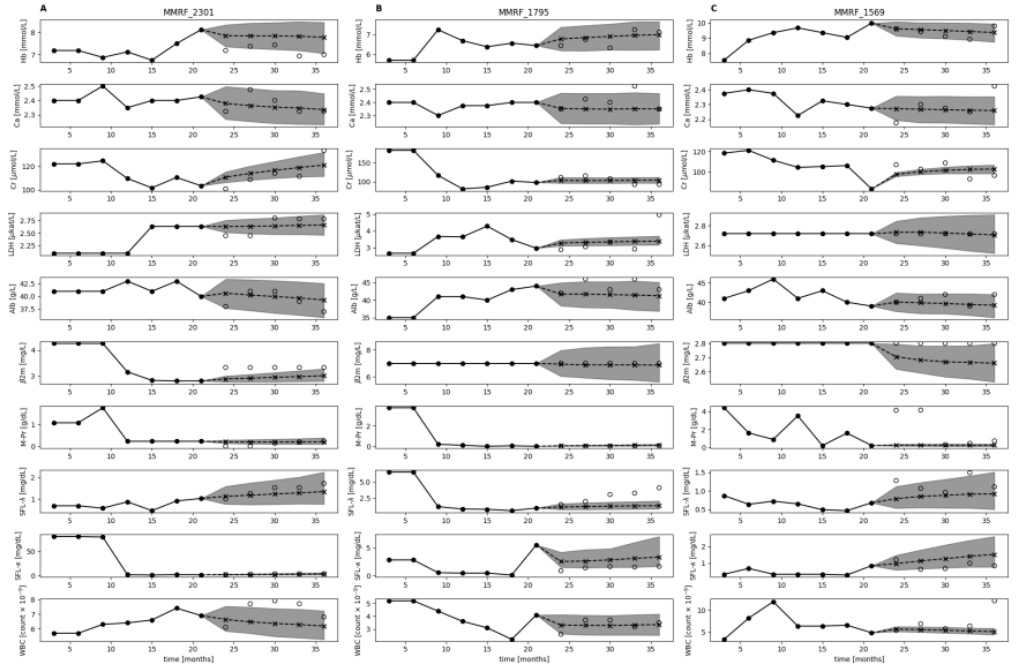

Figure 2: Forecasted patient trajectories of three individual patients undergoing Bortezomib- (**A**), Carfilzomib- (**B**) and IMIDs-based (**C**) treatments. The forecasting model was provided with the initial seven follow-ups, corresponding to 21 months of clinical data, to forecast the subsequent five follow-ups, covering an additional 15 months. Dashed lines and crosses show forecasts, circles show actual observations. Grey sleeves indicate the 95% confidence interval of the distribution of forecasted trajectories.

Ferle et al. Predicting Progression Events in Multiple Myeloma from Routine Blood Work. NPJ Dig Med 2025

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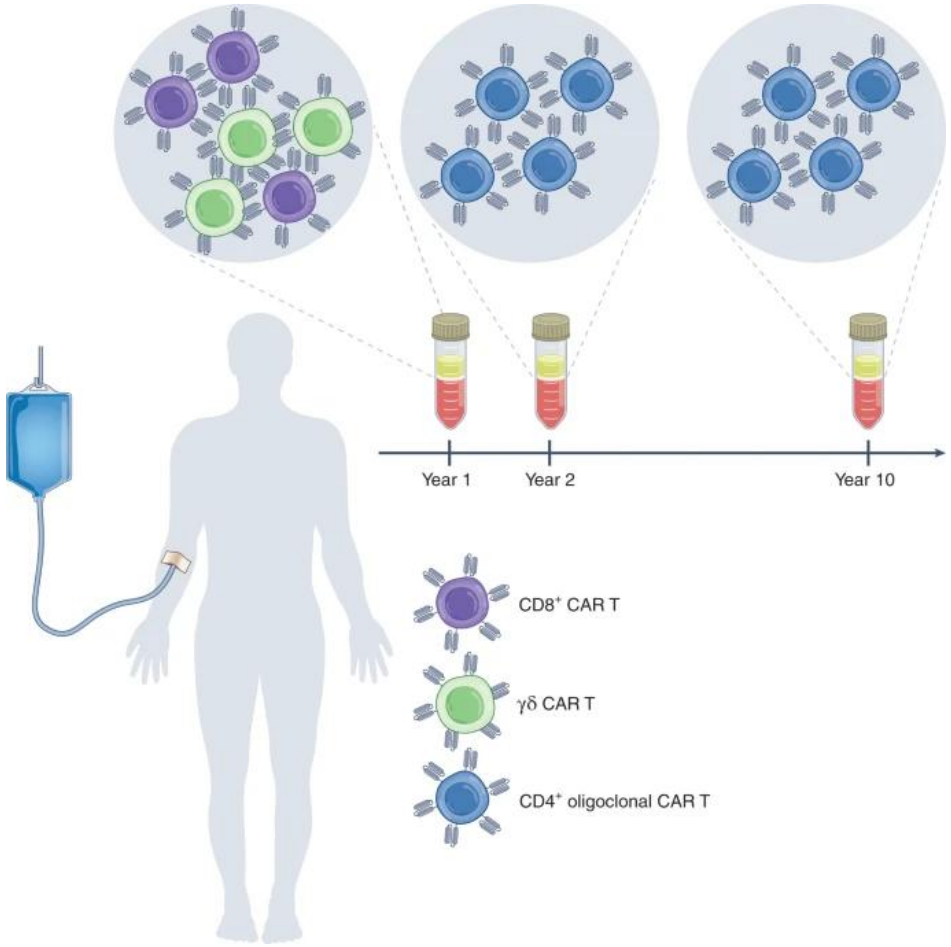
CAR T Cell Therapies

Long term persistence of CD4+ CAR T cell clones



Anti-CD19, n=2, Melenhorst et al.

Decade long persistence of CD4+ CAR T cell clones with cytotoxic, proliferating and metabolically active phenotypes

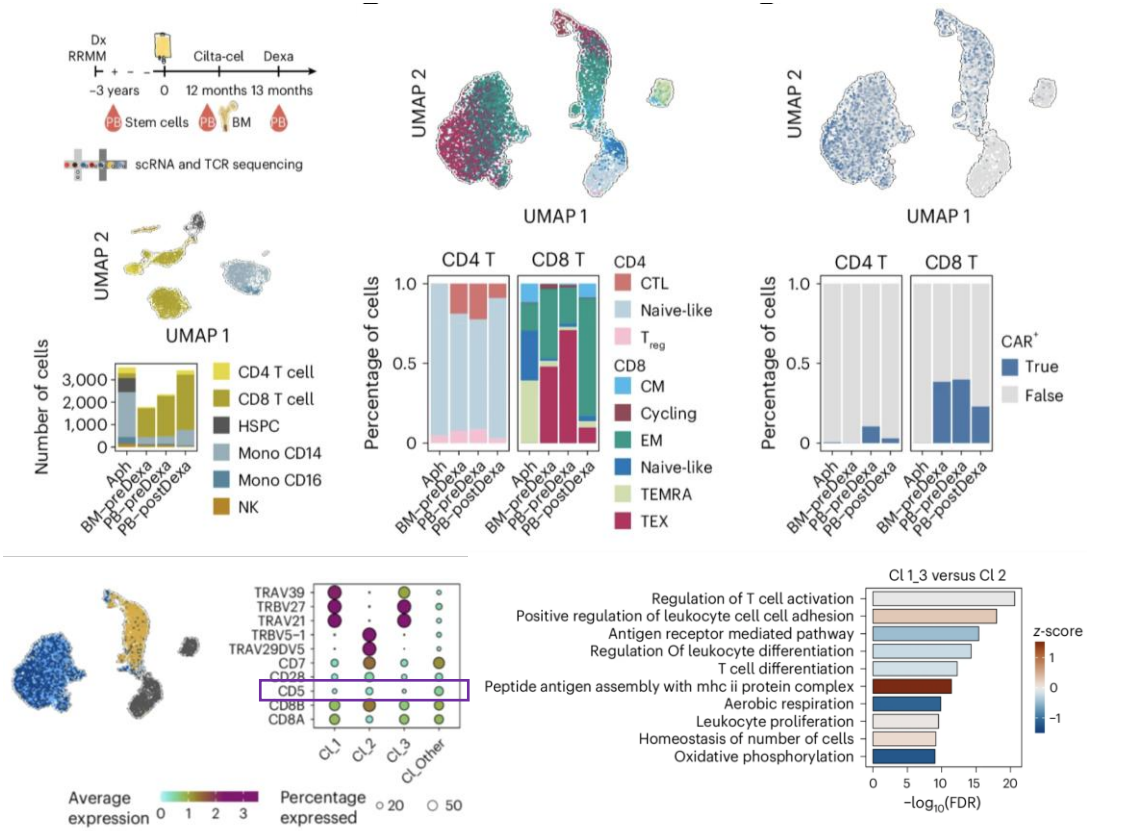
→ Long term response **AND side effects?**

Maus. A decade of CAR T Cell evolution. Nature Cancer. Comment on Melenhorst et al. Decade-long leukaemia remissions with persistence of CD4+ CAR T cells. 2022

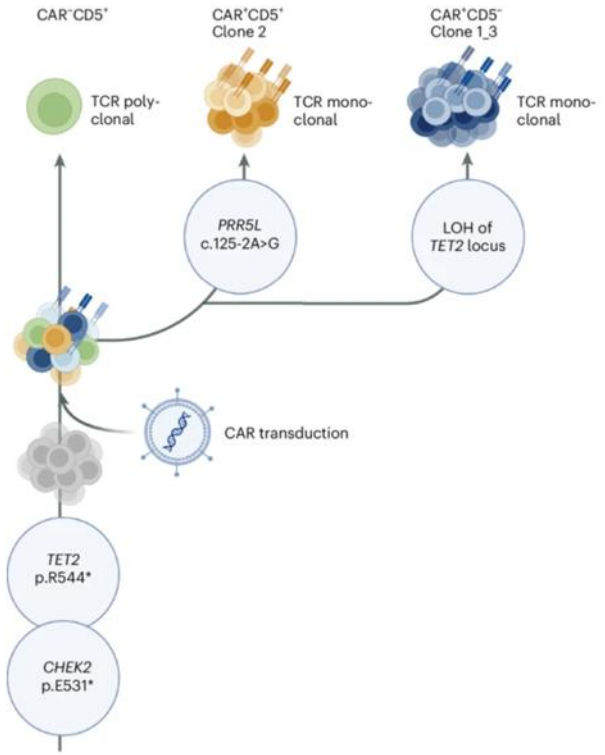
Rare adverse effects – development of CAR-T cell neoplasia

Real world

Single cell transcriptomic analyses



Result



- Patient diagnosed with RRMM received **cilta-cel** and was treated with **talquetamab** upon relapse
- At month 9 after CAR-T infusion, he presented with **cutaneous and intestinal lesions** compatible with T cell lymphoma
- **Multiomics** revealed pre-existing *TET2* and *CHEK2* lesions and mutational phylogeny of CAR+ T cell neoplasia

RRMM = Relapsed/Refractory Multiple Myeloma | CAR = Chimeric Antigen Receptor

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Annual report 2024 (EMA): Important new safety advice issued in 2024 included: CAR T-cell medicines, recommendation on the need for life-long monitoring of secondary malignancies in patients treated with these medicines.

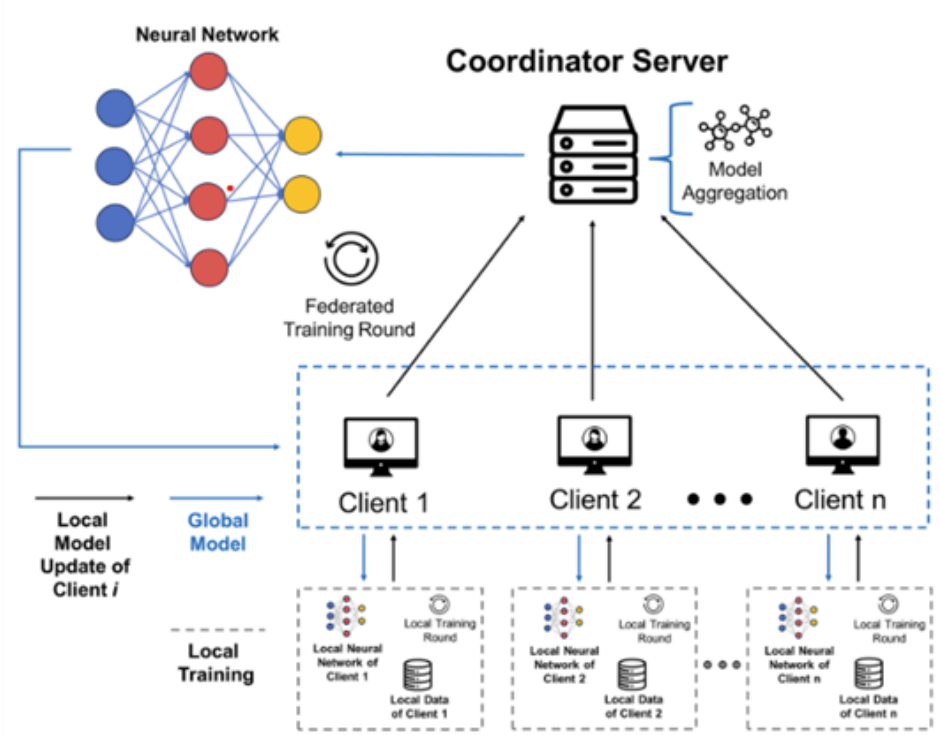
https://www.ema.europa.eu/en/documents/annual-report/2024-annual-report-european-medicines-agency_en.pdf

Basis for Sample and Data Access

Optimized contracting process with Fraunhofer

Real world

Federated Learning Network for RWD in CAR T cell treatment



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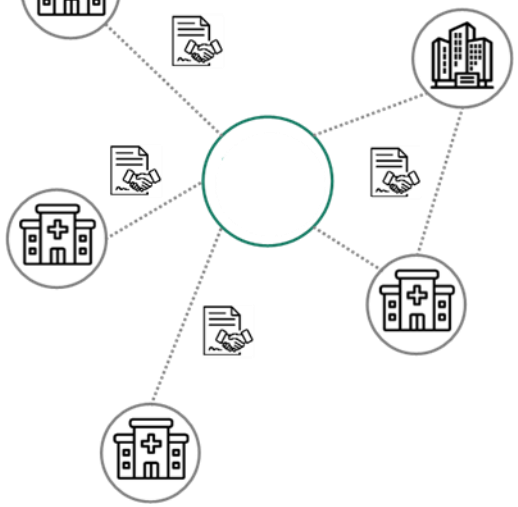
Flexible and goal-oriented legal contract management

Data management plans

GDPR-conform processing



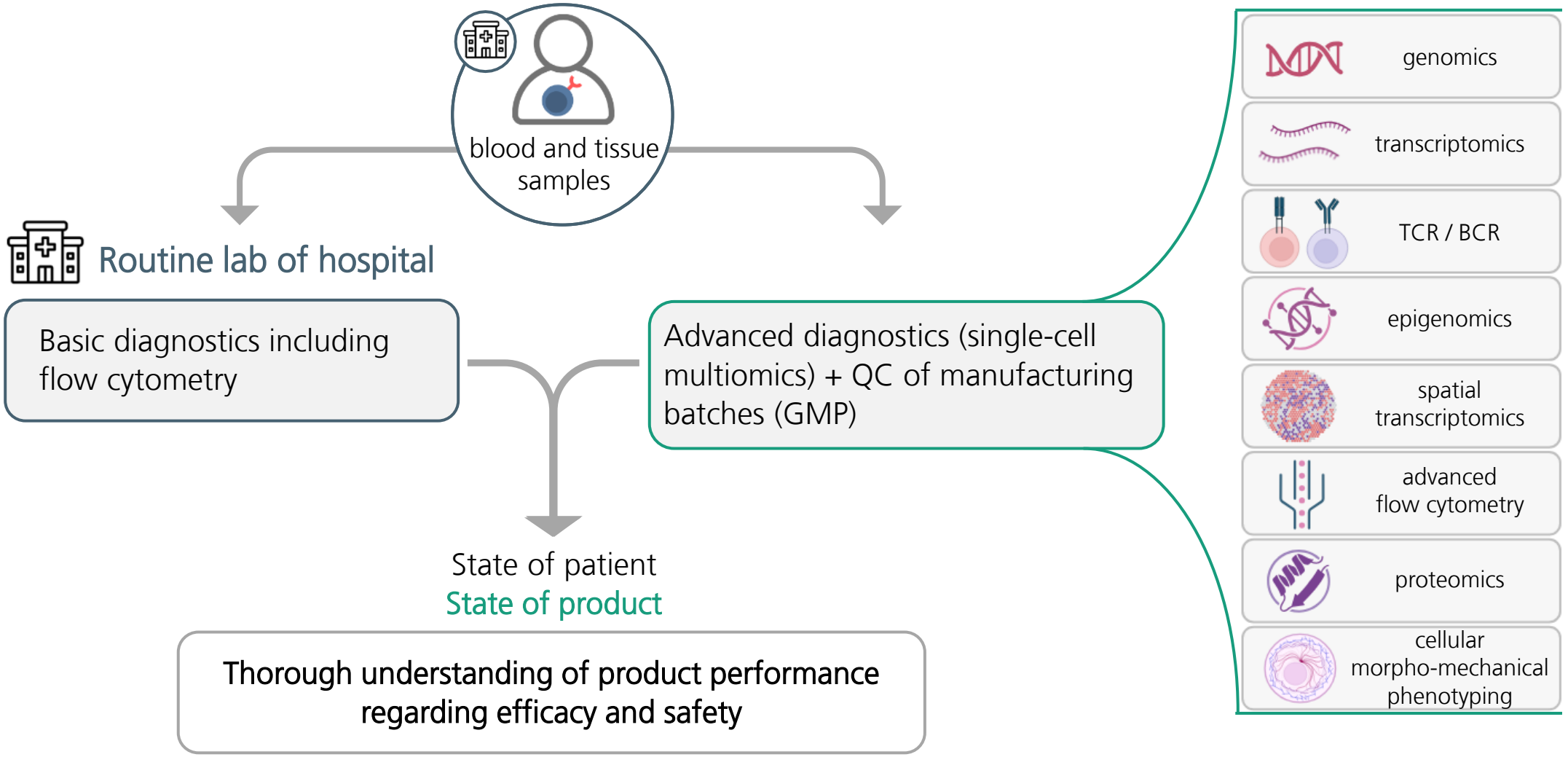
GDPR = General Data Protection Regulation

RWD = Real-World Data

Workflow for Enhanced Diagnostics in CAR T cell treatment

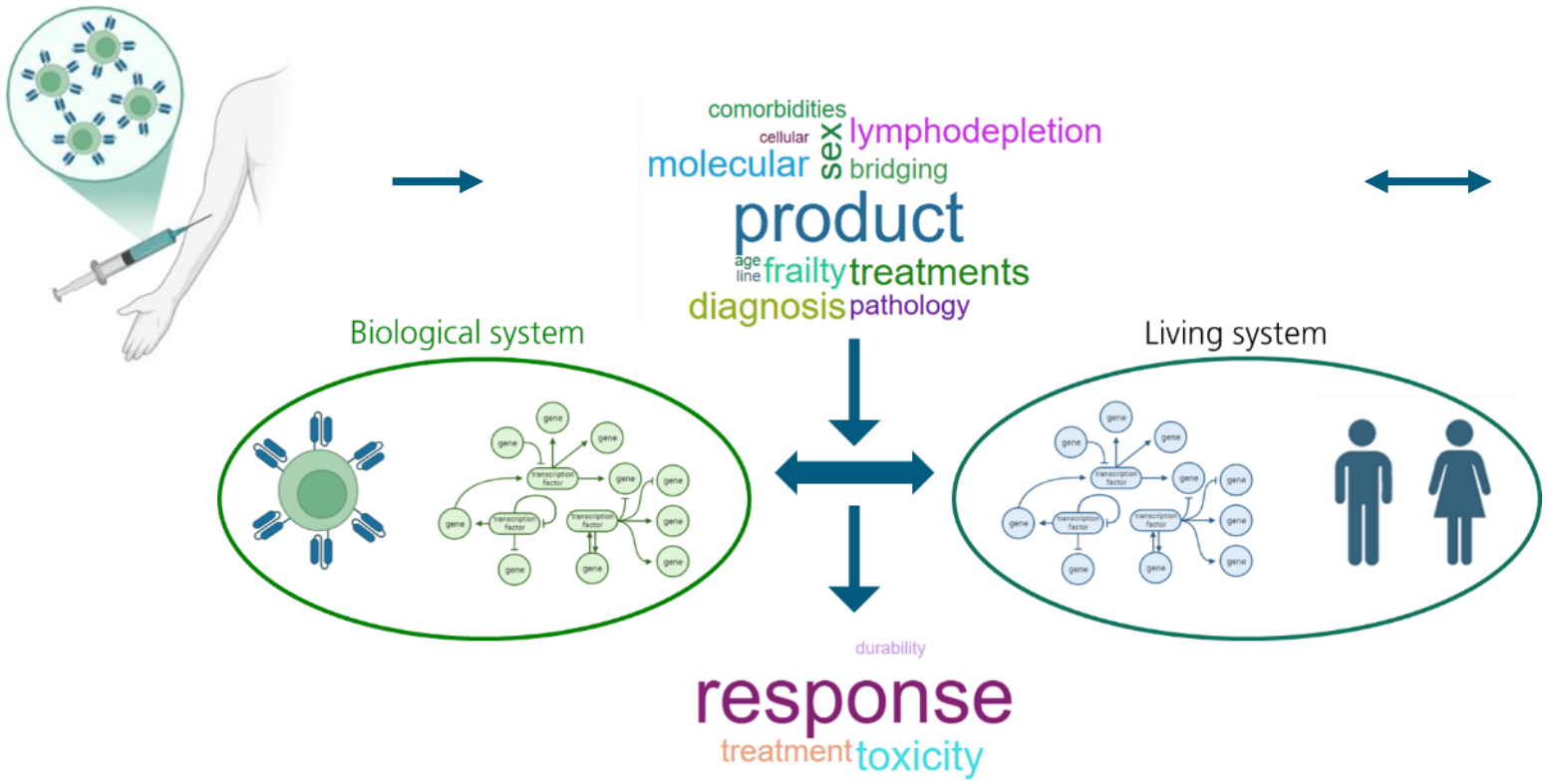
Characterization of patients and products in clinical routine or in clinical studies

Real world

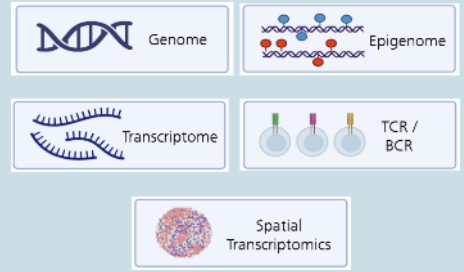


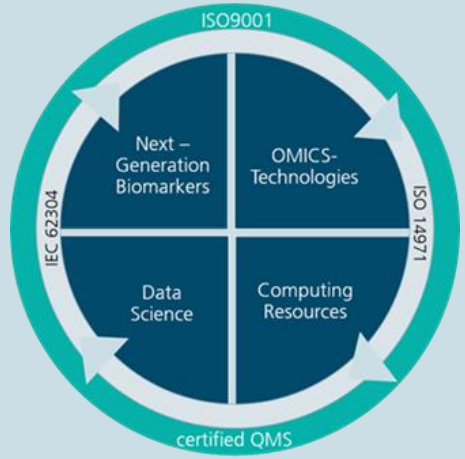
TCR: T cell receptor | BCR: B cell receptor

Workflow for Enhanced Diagnostics in CAR T cell treatment



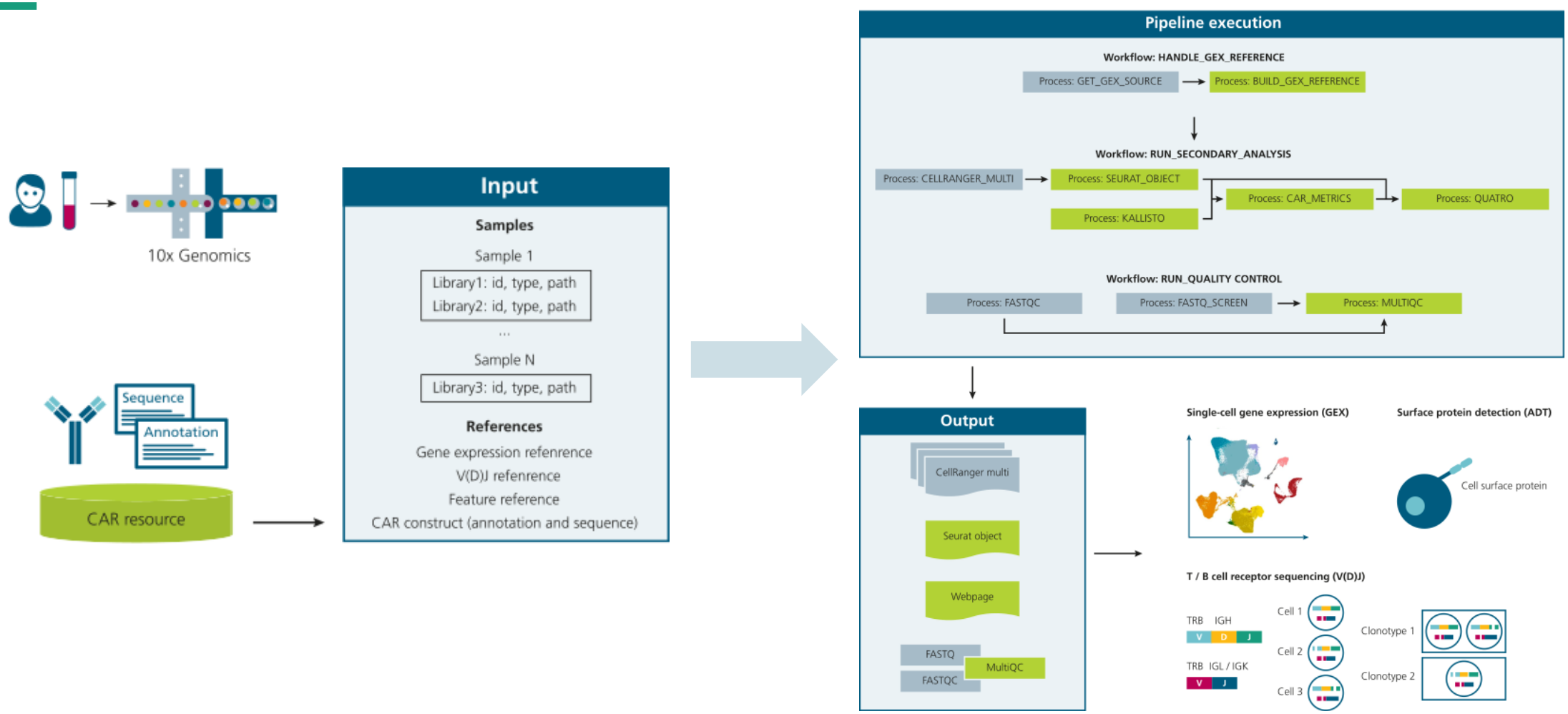
Trusted Single-cell Multiomics





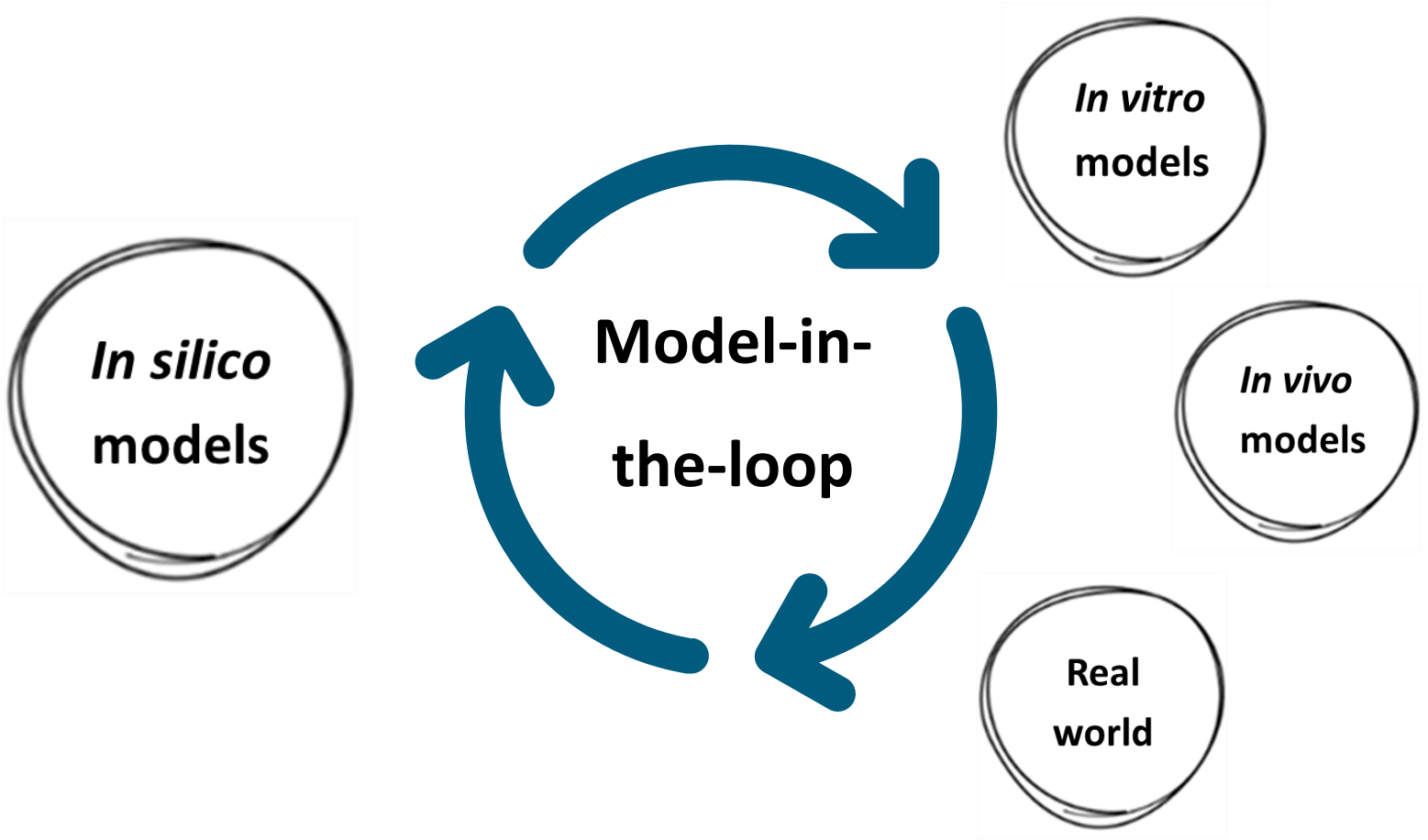
Biomedical AI and Software Engineering

CERTOMICS is a Nextflow-based pipeline offering enhanced CERTainty in immunophenotyping and data interpretation, tailored for single-cell multiOMICS profiling of adoptive cellular immunotherapies



GitHub - fraunhofer-izi/CERTOMICS: A Nextflow-based pipeline tailored for single-cell multiomics profiling of adoptive cellular immunotherapies.

Approach to implementation

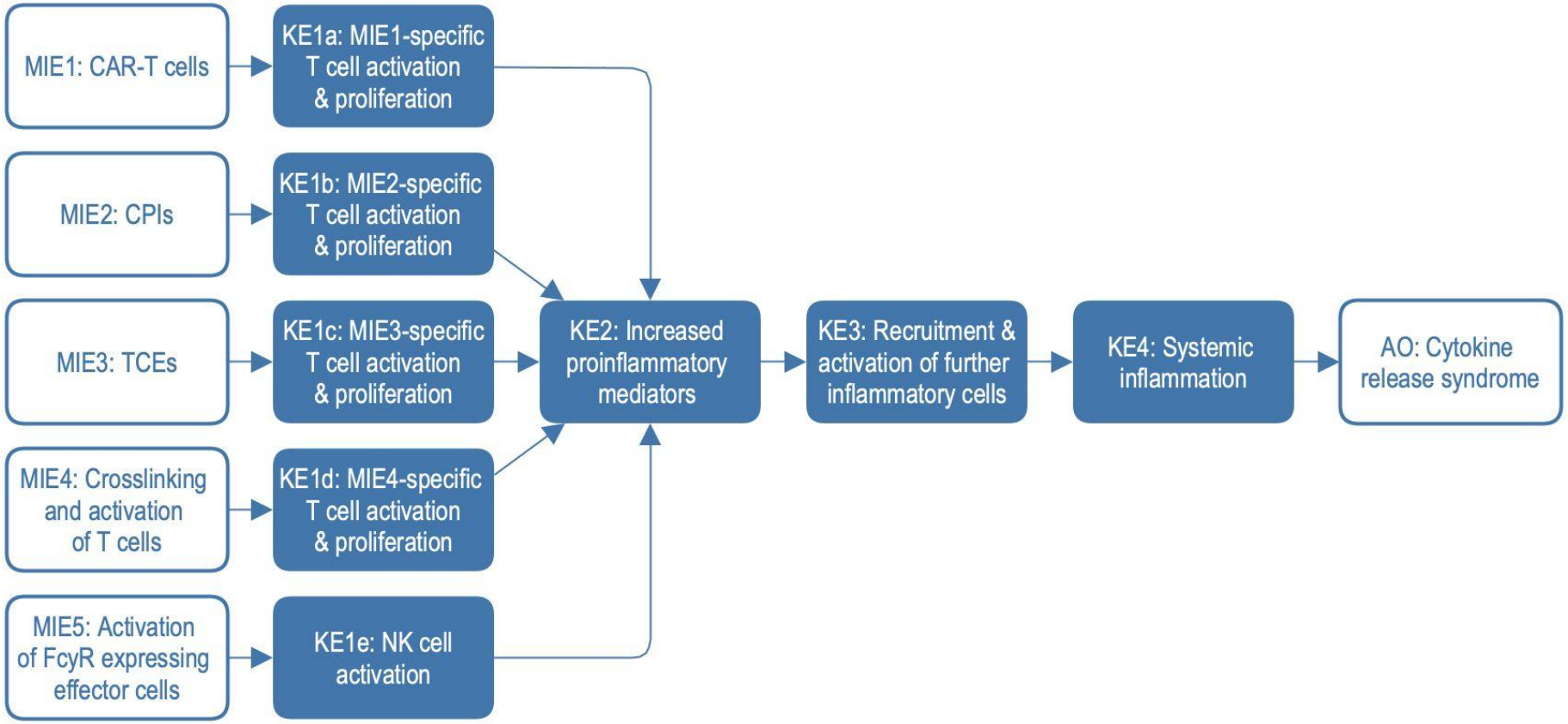


In vitro models include NAMs (new approach methodologies)

Results – Models for predicting toxicity of CAR T cells (AOPs)

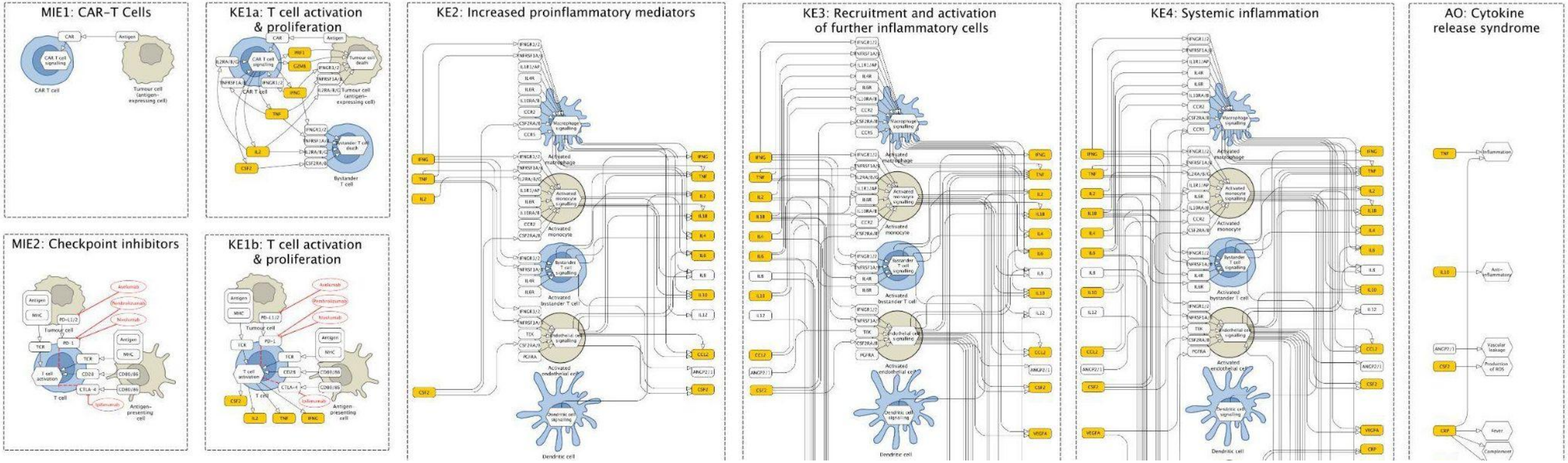
RWE = Real-world evidence | AOP = Adverse Outcome Pathways

Results – Models for predicting toxicity of CAR T cells (AOPs)



RWE = Real-world evidence | AOP = Adverse Outcome Pathways

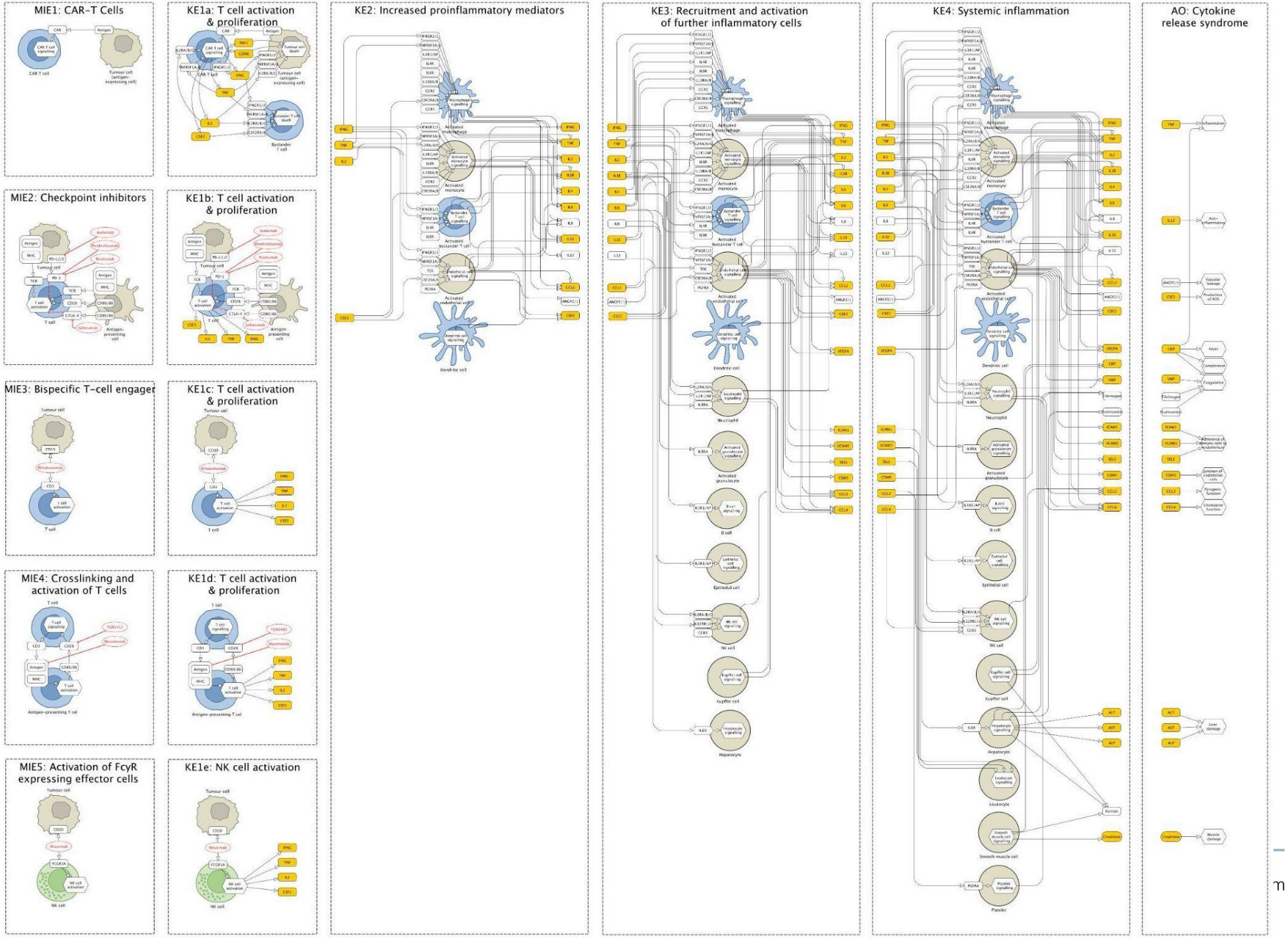
An explorable model of an adverse outcome pathway of cytokine release syndrome



Alb, Reiche et al. Novel strategies to assess cytokine release mediated by chimeric antigen receptor T cells based on the adverse outcome pathway concept. Journal of Immunotoxicol. 2024.

Mazein et al Using interactive platforms to encode, manage and explore immune-related adverse outcome pathways. Journal of Immunotoxicology 2024

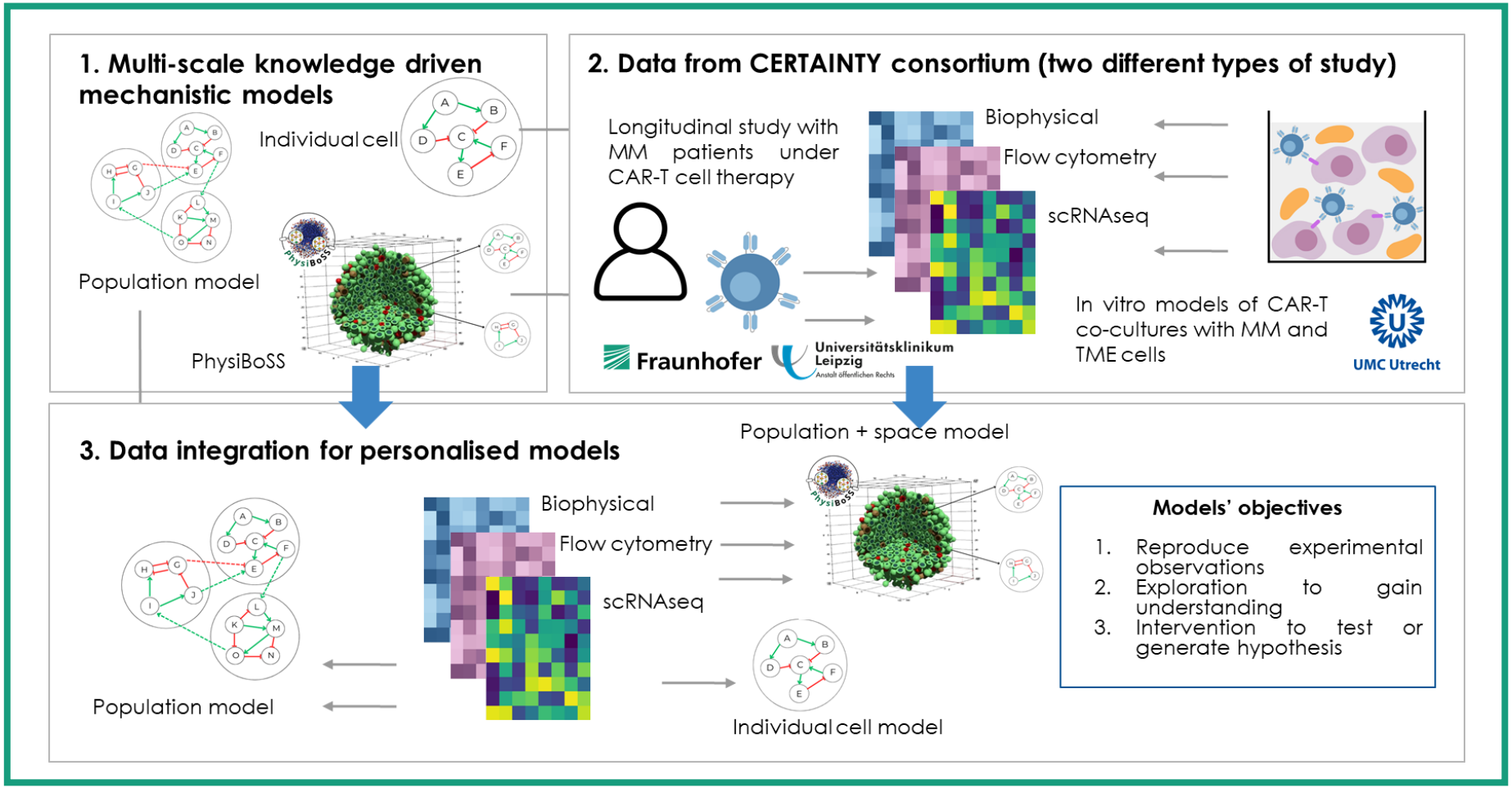
Mazein et al. An explorable model of an adverse outcome pathway of cytokine release syndrome related to the administration of immunomodulatory biotherapeutics and cellular therapies. Front Immunol. 2025.



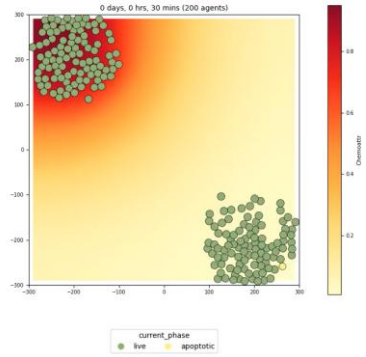
Mazein et al. *An explorable model of an adverse outcome pathway of cytokine release syndrome related to the administration of immunomodulatory biotherapeutics and cellular therapies.*

Front Immunol. 2025.

Results – Mechanistic Cell Models



Prof. Laurence Calzone & Sophia Orozco Ruiz (Institut Curie)



Summary

Federated virtual twin: Completely integrated and interoperable clinical decision support system available for patients who meet the criteria for cellular immunotherapies.

Agent-based cell models: Enhanced models that combine multiple levels of omics data and allow for the simulation of cell population dynamics of (modified) immune cells.

Multi-modal single-cell and spatial transcriptomics: Improved companion diagnostic tools for CAR T cell therapies.

Stakeholder engagement in the co-creation of the virtual twin: Perspectives from stakeholders as well as social and socio-economic factors taken into consideration in the implementation process.

https://youtu.be/jlEGdrS3Rzs

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ACKNOWLEDGEMENTS

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Prof. Maximilian Merz
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Dr. Ronald Weiß
PD Vladan Vucinic
Prof. Thomas Neumuth
Dr. Stefan Franke
Prof. Manfred Kunz
Prof. Jens Meiler
Prof. Clara Schoeder
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Prof. Zsolt Sebestyen

Frankfurt University
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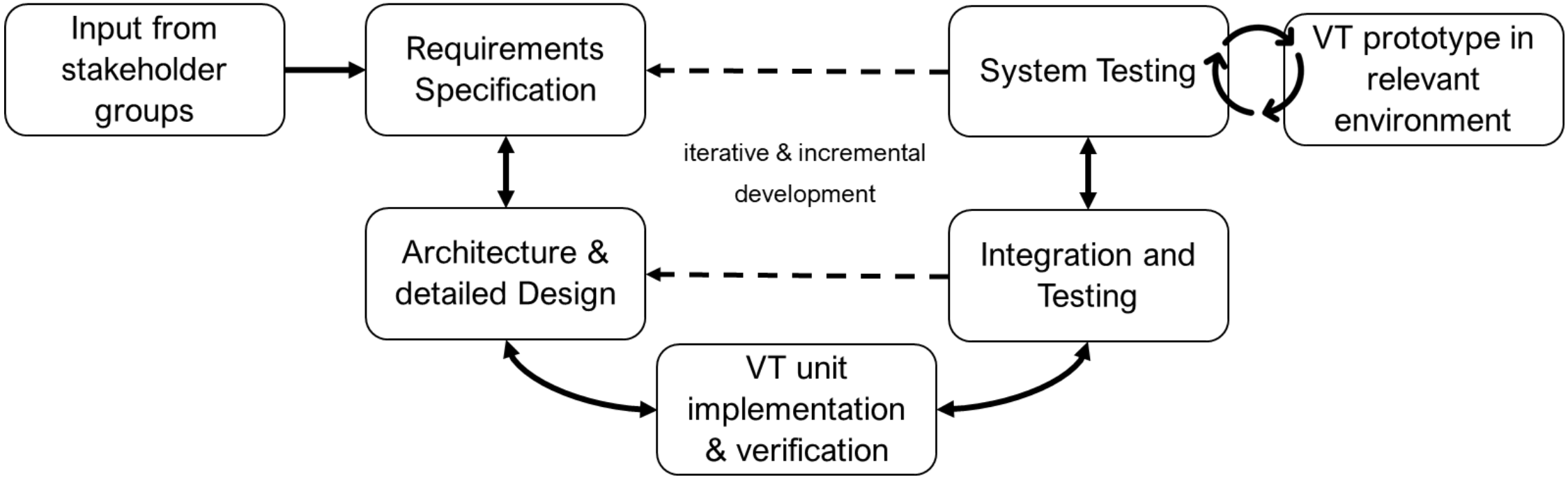
Uni Luxembourg
Prof. Reinhard Schneider
Dr. Alexander Mazein
Dr. Marek Ostaszewski

Institut Curie
Prof. Laurence Calzone
Sophia Orozco-Ruiz

RDA IDT Working group
Prof. Anna Niarakis
Athina Papadopoulou

… and many others

Co-creation



…. ensuring co-creation, rapid prototyping to receive fast feedback from feasibility studies.

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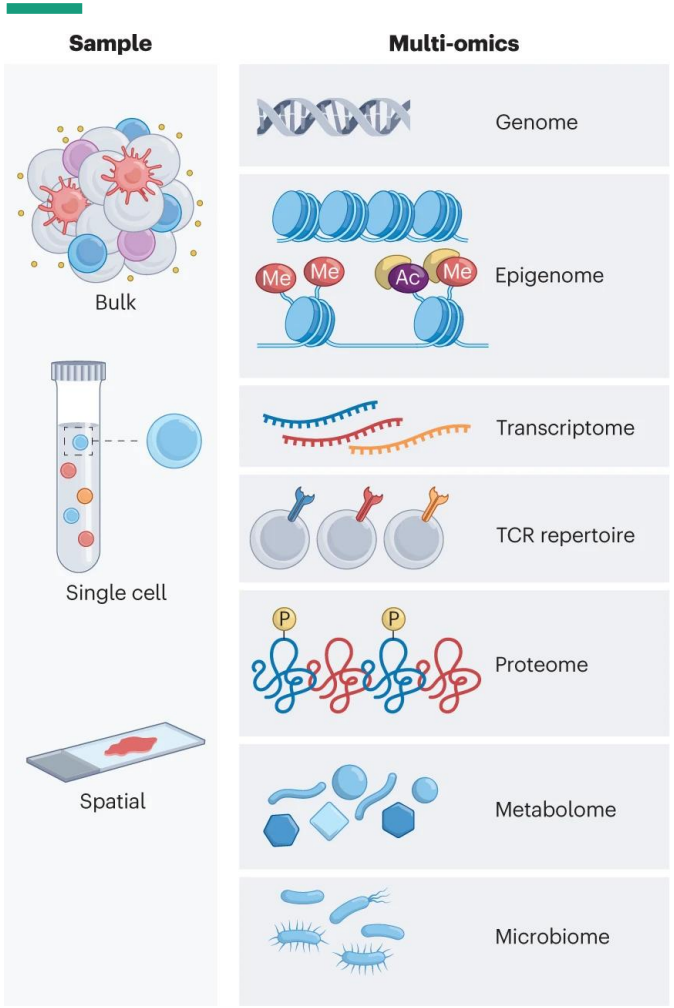
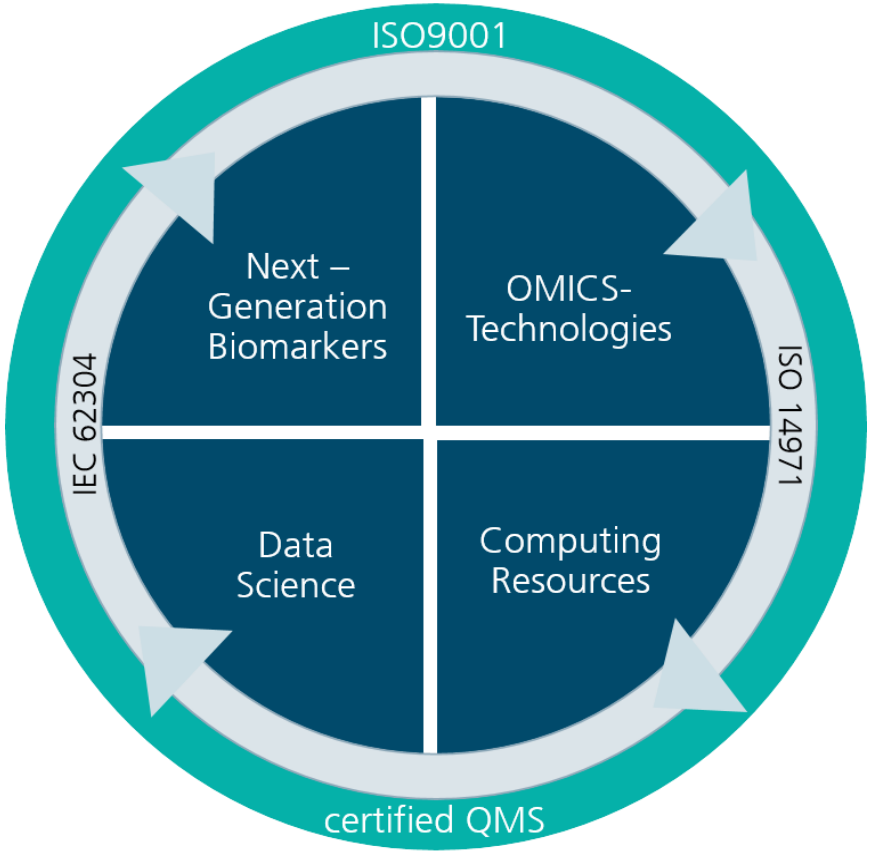
Our Approach towards Enhanced Diagnostics for Cell and Gene Therapies



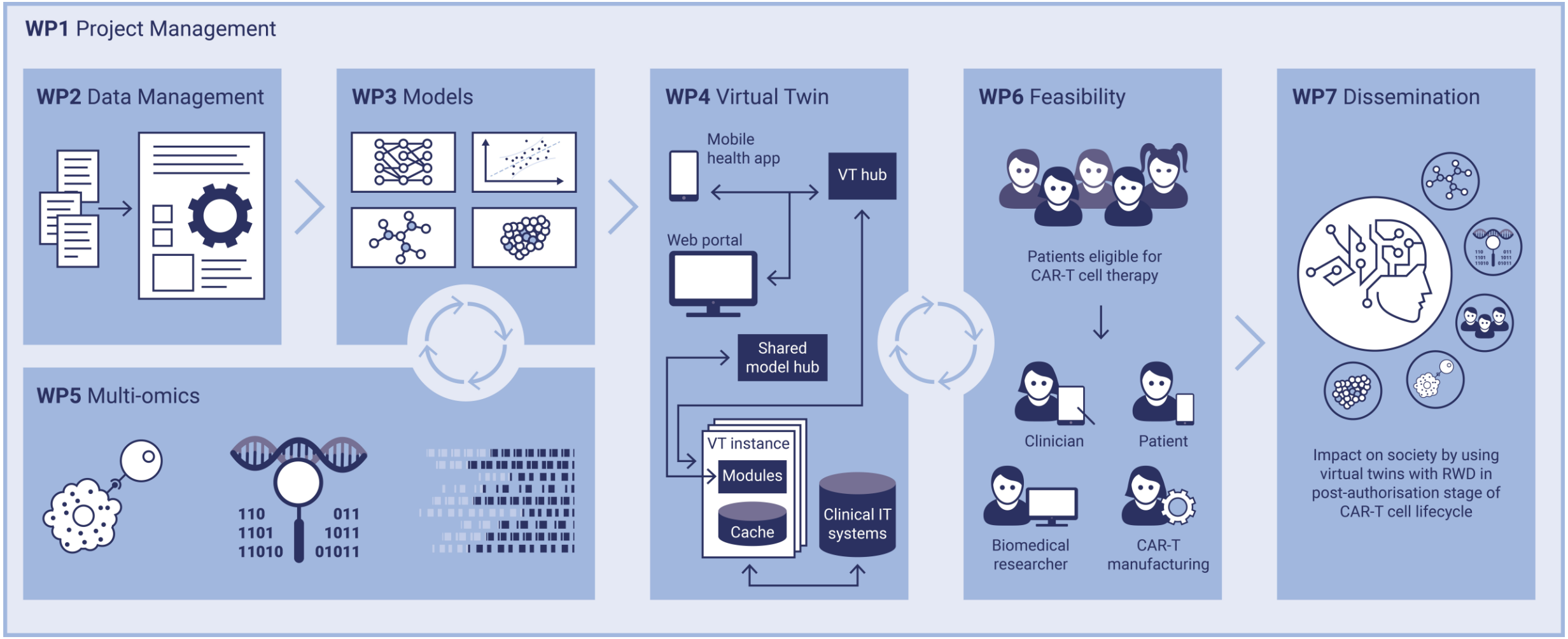


Yang et al. Advancing CAR T cell therapy through the use of multidimensional omics data. Nat. Rev. Clin. Oncol, 2023.

MolDx = Molecular Diagnostics

IVD = In-vitro diagnostics

How we will make the Virtual Twin



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Collaborations we build on

Data providers

Clinicians

Patient representatives

Legal experts

Computational biologists

Data management

Model creators

Virtual twin architects

Multi-omics data generation

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