CAR T-cell therapy

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http://www.imi.si/

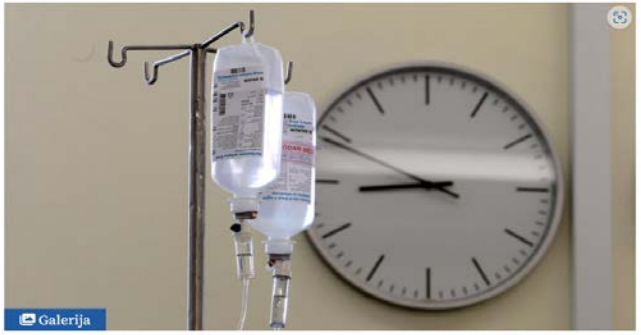
Univerza v Ljubljani

Uncle plasmacyTom's cabin

- The establishment of therapy with CAR-T cells began in Slovenia with a high-profile social action. The start was an article in the newspaper "Delo" ("Koča strica plasmacitoma - Uncle plasmacytoma's cabin") by the composer Drago Ivanuša, who suffered from plasmacytoma.
- The article was followed by an initiative of the Slovenian Association of Patients with Lymphoma and Leukemia to collect donations for the purchase of the Prodigy device from the company Miltenyi (in the value of EUR 500,000), which would enable the establishment of CAR-T cell therapy at the Clinical Department of Hematology at the University Clinical Center (UKC) Ljubljana.

Koča strica plazmocitoma

Skladatelj Drago Ivanuša: Presaditev kostnega mozga je generacijam bolnikov, tudi meni dvakrat, predvsem življenje podaljševala, CAR-T bi jih reševal.



Dogovor, da bo nabavljena naprava, ki je potrebna za izvajanje CAR-T, je ostal neizpolnjen. Odgovorov od tistih, ki odločajo in so zadevo ustavili, je malo ali nič. Bolniki pa z odprtimi usti v šoku opazujemo, kako se naše upanje razblinja v času. FOTO: Matej Družnik

Drago Ivanuša

6. 2. 2021 | 06:00

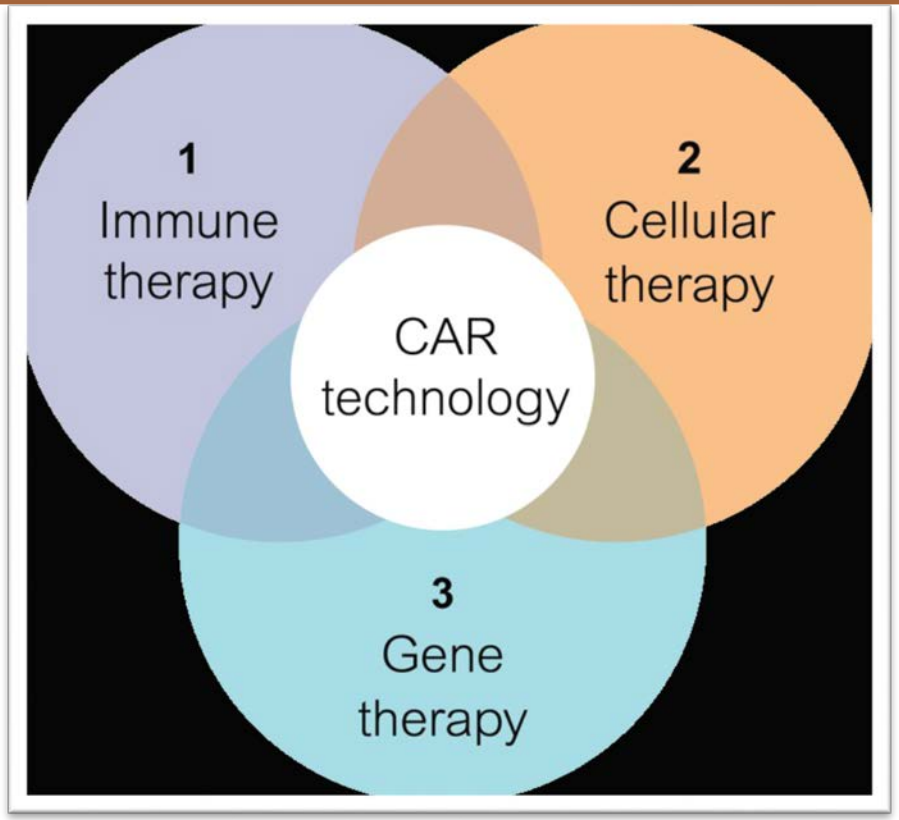
A+ A-

Donations for the purchase of the Prodigy device

- In March 2021, the campaign managed to collect over 770,000 EUR, after the purchase of the device, a donor appeared who bought another device for CAR-T production.
- During the donation campaign, I was called by Prof. dr. Samo Zver, head of the UKC Clinical Department of Hematology (KOH), to participate in the campaign as head of the Immunology Department at the Faculty of Medicine.
- After both devices were purchased, it became clear that it would be a long journey from the purchased device to the production of CAR-T and to CAR-T cell therapy.

CAR T cells are at the intersection of 3 Innovative Technologies

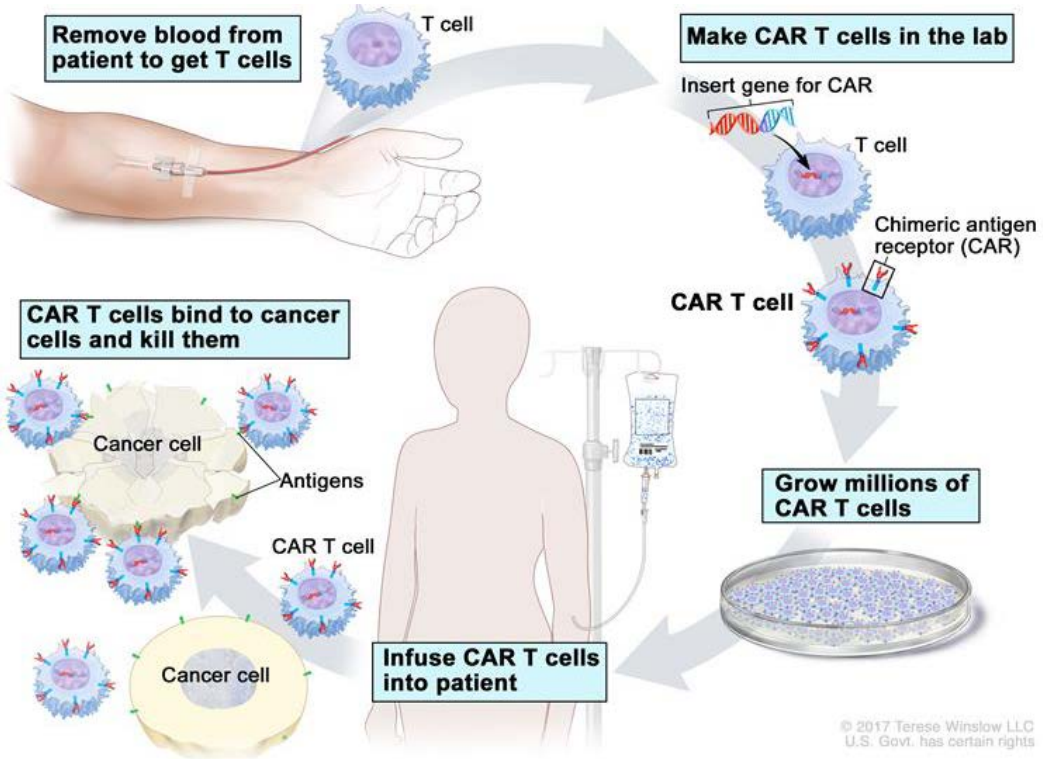
1. Immune therapy
Control and influence an individual's own immune system (monoclonals)
2. Cellular therapy
CAR T Cells Produced From the Patient's Own Cells (Stem cell transpl.)
3. Gene therapy
Inserting genes into a patient's cells, causing them to express a new CAR protein



Porter DL et al. J Cancer. 2019;2(1):331-332

CAR T-Cell Treatment Is Individualized

CAR T Cells Produced From the Patient's Own Cells



Current CAR T-cell therapies are engineered for each individual recipient

To create CAR T cells, T cells must be collected from the patient's tissue, tumor, or blood

T cells are then processed in manufacturer's lab to add an engineered antibody that is specific to the disease target

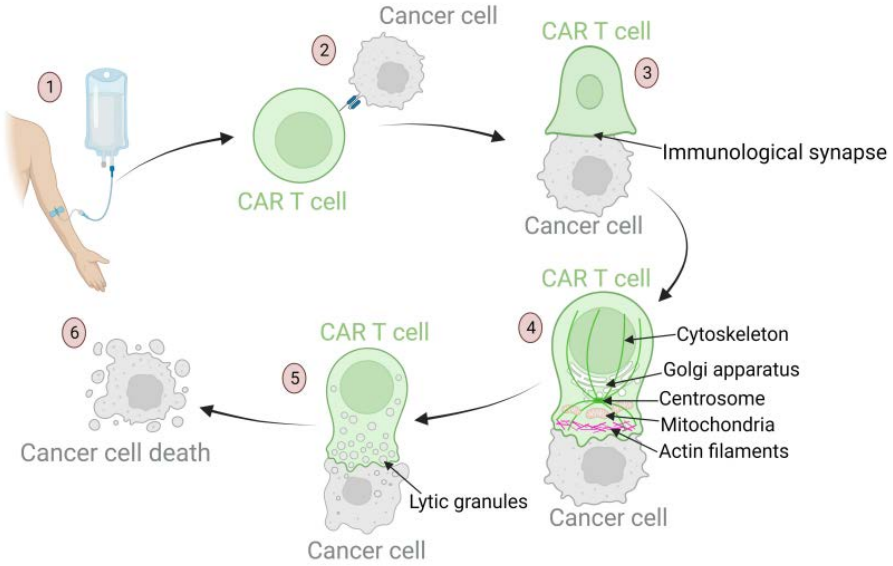
NIH. CAR-T cell therapy. www.cancer.gov/publications/dictionaries/cancer-terms/def/car-t-cell-therapy. Accessed October 7, 2019..

Definition of CAR T-cell therapy

CAR-T cells - autologous T cells engineered to express a surface receptor to target a specific (auto)antigen.

Current CAR T-cell therapies DO NOT recognise and attack tumor-specific antigens but kill specifically defined patient‘s own cell type

Aim – depletion of (auto)antigen positive cells, e.g. CD19-targeting CAR-T kill patient‘s own B cells expressing CD19 (pathogenic and healthy cells); in future non-depleting CAR Treg for autoimmunity?

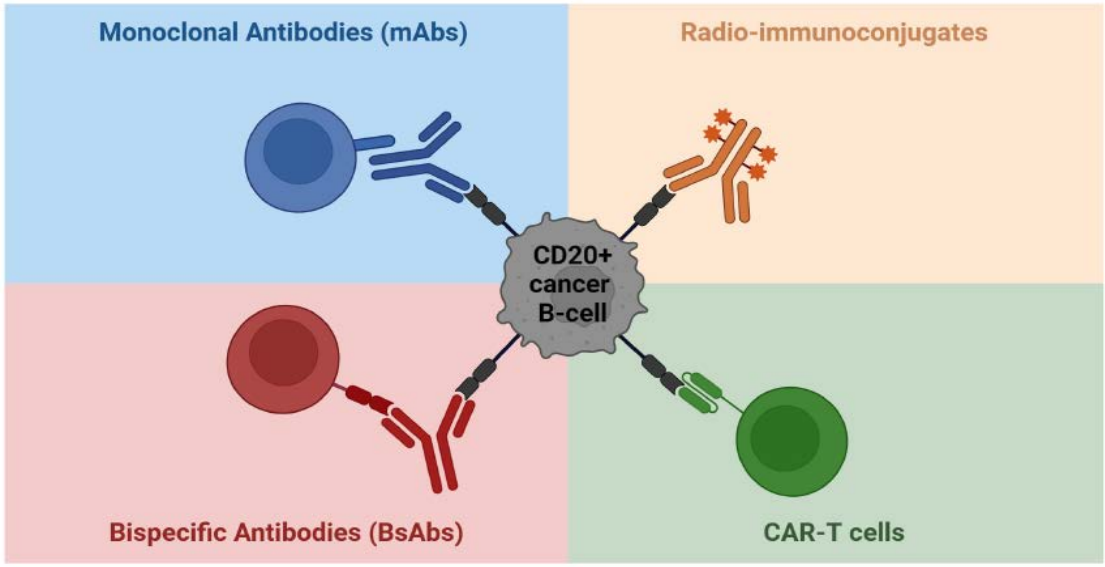


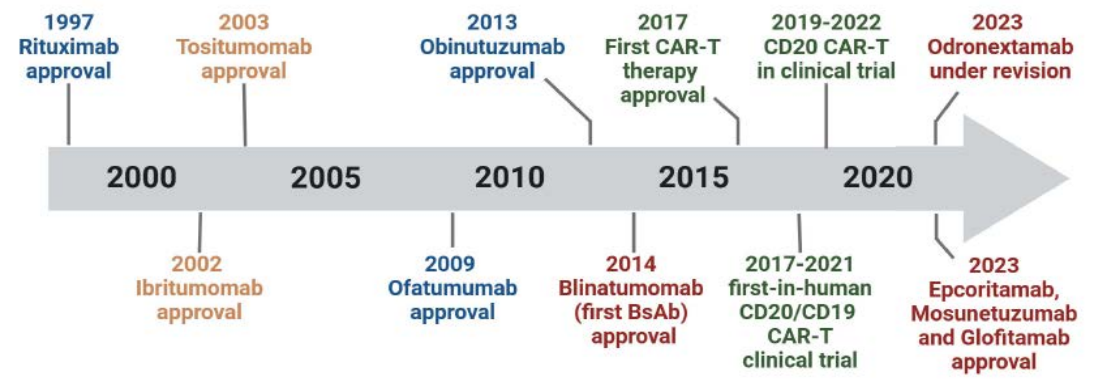
Levstek L, Janžič L, Ihan A, Kopitar AN. Front. Immunol. 2024;15:1378944

CAR T-cell therapy vs. antibody therapies vs. bispecific T-cell engager (BiTE) therapies

CAR T-cell therapy is similar to existing antibody therapies (e.g., Rituximab - a chimeric (mouse/human) monoclonal antibody against the protein CD20. Similar mechanism of action because they both have antigen targets on B cells and cause B-cell depletion.
CAR-T: long-term depletion (years) with single-dose; tissue-hidden cells; complement-independent lysis

Also can be compared to bispecific T-cell engager (BiTE) therapies (e.g., blinatumomab – CD19/CD3) Both therapies rely on the engagement of T cells with malignant cells Adverse events can be similar to those associated with CAR T cells.
CAR-T: long-term depletion (years) with single-dose





Dabkowska A, Domka K and Firczuk M 2024; Front. Immunol. 15:1363102

Advantages of CAR T-Cell Therapy

CAR T cells retain long-term "graft"-versus-tumor effect

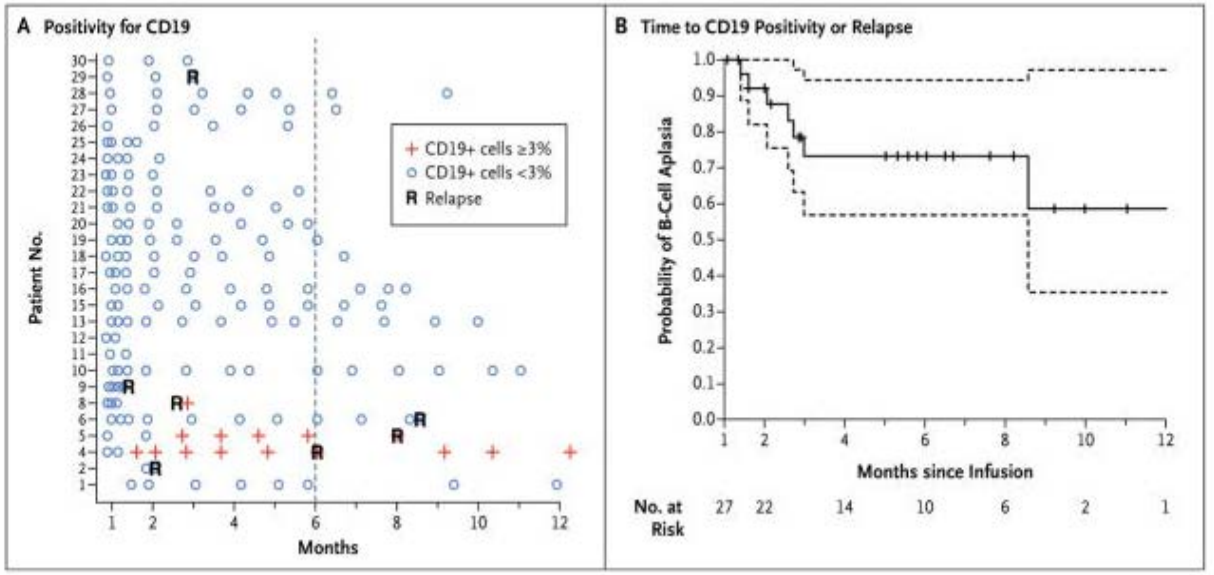
HLA-independent antigen recognition, therefore universal application

Rapid generation of tumor specific T-cells

Active in both CD4+ and CD8+ T-cells

Because it utilizes the patient's own cells, there are no graft-versus-host complications

Target antigens include proteins, carbohydrates, and glycolipids



B-cell aplasia can be used as a measure of CAR-T (CD19) function
B-cell aplasia occurred in all the patients who had a response and persisted for up to 1 year after CTL019 cells were no longer detectable by means of flow cytometry.

With a follow-up period 24 months 90% rate of complete remission among patients who received CTL019 for ALL that was relapsed or refractory. Relapses were associated with either lack of CTL019 persistence or CD19 escape variants.

N Engl J Med. 2014 Oct 16; 371(16): 1507–1517

Advantages of CAR T-Cell Therapy

CAR T cells retain long-term “graft”-versus-tumor effect

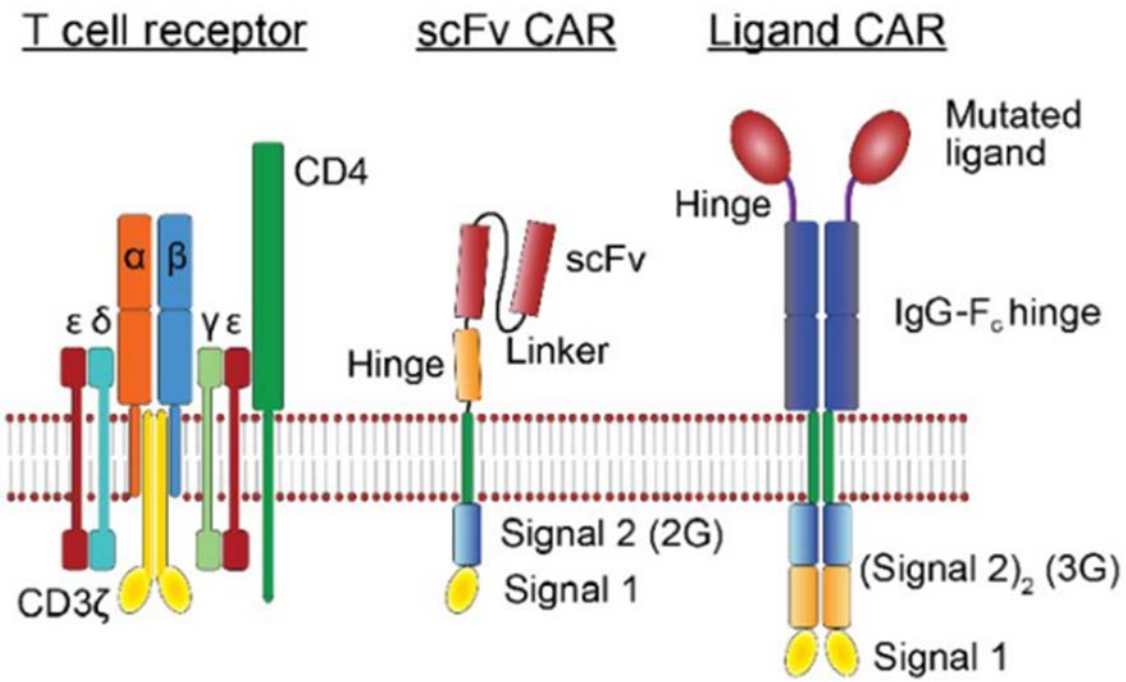
HLA-independent antigen recognition, therefore universal application (but still prepared for each individual recipient)

Rapid generation of tumor specific T-cells (2-4 weeks)

Active in both CD4+ and CD8+ T-cells (future specific CD4, CD8, NK, Treg…)

Because it utilizes the patient’s own cells, there are no graft-versus-host complications

Target antigens include proteins, carbohydrates, and glycolipids



Mitra A, Barua A, Huang L(2023) Front. Immunol. 14:1188049

After purchasing the Prodigy device, UKC asked the Faculty of Medicine if it could organize the production of CAR-T

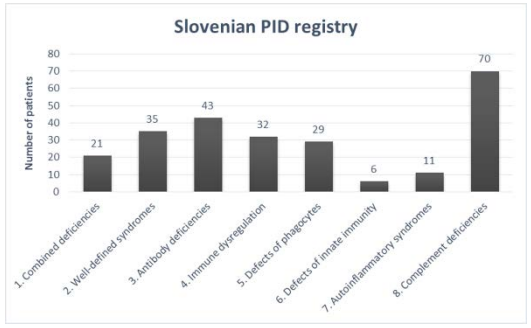
- The Institute of Microbiology and Immunology is the central, largest Slovenian institution for microbiological and immunological diagnostics
- Over 600 types of investigations
- 320 000 samples / year
- 615,000 investigations / year
- 180 employees (15 medical doctors, 36 Ph.D.)
- 7 professors

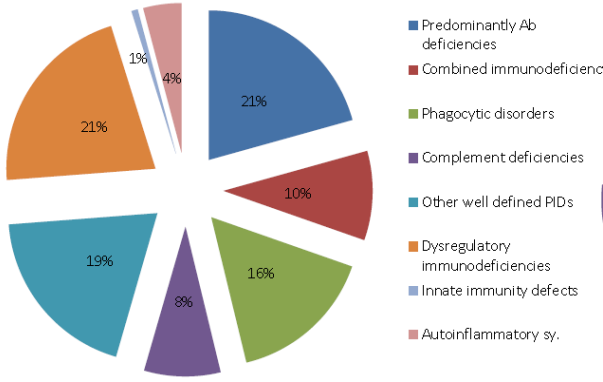
http://www.imi.si/

Slovenian PID team

260 registered PID patients, 51 different disease entities, 144 (55%) of Pts according to genetic diagnosis

Department of Allergology, Rheumatology and clinical Immunology, University Children's Hospital Ljubljana





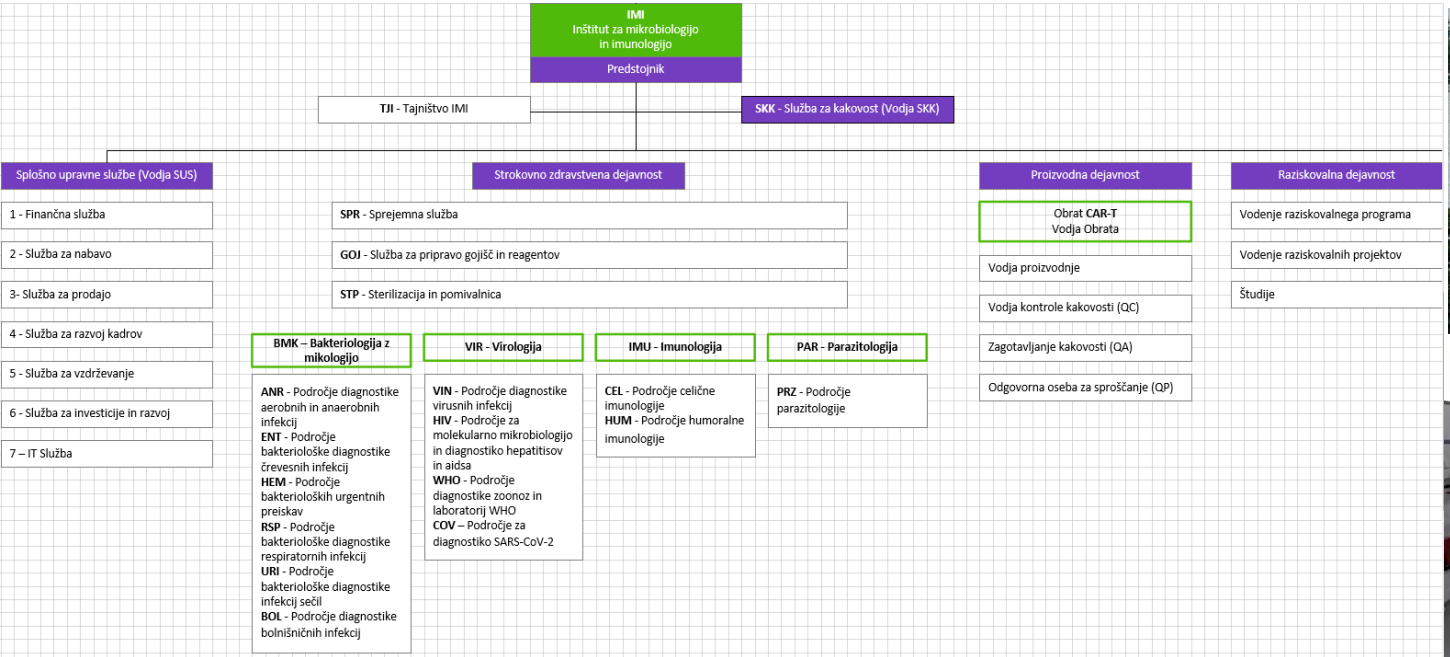
Immunology laboratory – Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana Medical faculty Ljubljana

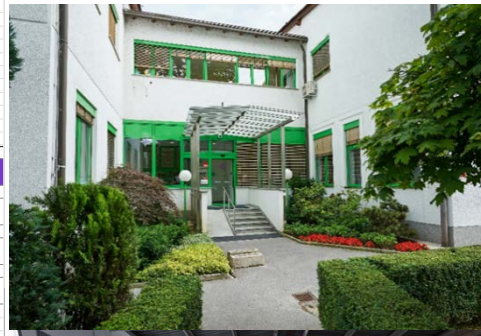
Hematopoietic stem cell transplantation team Department of University Children's Hospital Ljubljana

Diagnosis	Age at HSCT
XLP	4 yrs
XLP	3,5 yrs
SCID - OMENN syndrome (RAG1)	3 months
XCGD	5 yrs
XCGD	25 yrs
SCID - Hypomorphic Rag1 deficiency	4 yrs
Osteopetrosis	10 months
APDS (PI3Kδ)	8,5 yrs
AR CGD	21,5 yrs
fHLH	1,5 yrs
SCID - OMENN syndrome	17 months
SCID - JAK3	9 months
XCGD	19,5 yrs
SCID (CD3E) - sibling A	12 months
SCID - OMENN syndrome (RAG1)	5 months
MALT 1 deficiency - sibling A	6,5 yrs
MALT 1 deficiency - sibling B	4,5 yrs
unknown CID, Monosomy 7	5,5 yrs
XCGD	7 yrs
XCGD	3,5 yrs
SCID (CD3E) - sibling B	4 months

UKC and the Faculty of Medicine signed an agreement on cooperation in the production of CAR-T cells

- The Institute of Microbiology and Immunology (IMI) at the Faculty of Medicine decided to establish a GMP-compliant ATMP center for the production of CAR T cells in order to meet the demand for CAR T cell therapy in Slovenia.
- In cooperation with the Clinical Department of Hematology of the University Clinical Center in Ljubljana, IMI is establishing the production of anti-CD19 CAR T-cells for the treatment of acute lymphoblastic leukemia (B-ALL) and large B-cell lymphomas.
- The plant is built modularly (172 m2 clean rooms in a 600 m2 production hall).



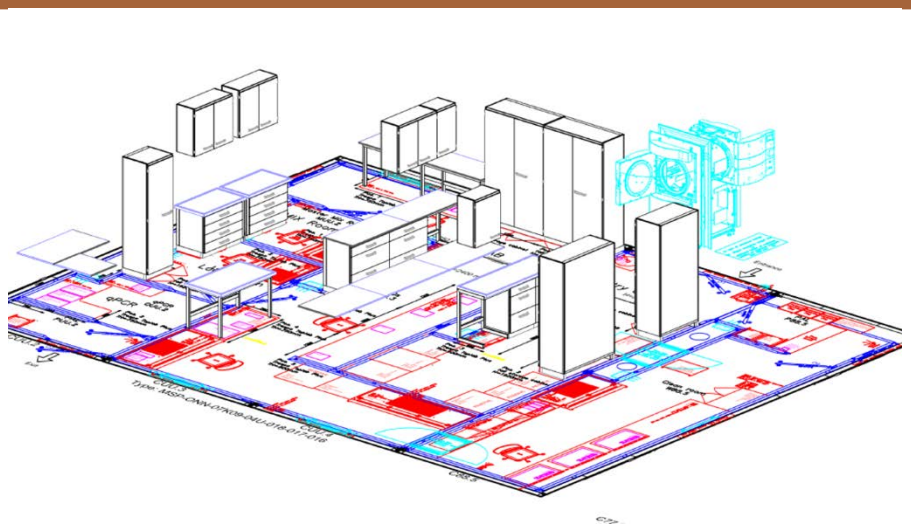


Most important – a new dedicated team!

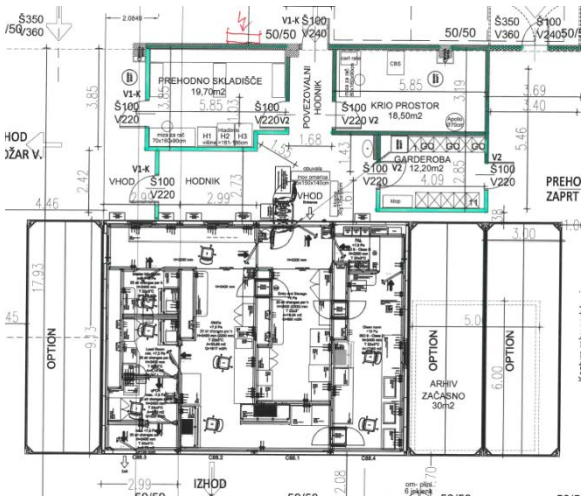
- We fully engaged three young but already proven researchers to fully dedicate themselves to establishing a CAR-T production unit.
- As an external collaborator, we contracted an expert for the registration of pharmaceutical products
- We established a permanent dialogue with the Slovenian Medicines Agency (JAZMP). This helped us a lot in obtaining the necessary permits (GMP certificate, permit to work with genetically modified organisms, permit to conduct a clinical study and clinical exemptions).
- We have established a permanent dialogue with the Ministry of Health and the Health Insurance Institute (ZZZS). Through the application of a new treatment to the Health Council, we secured funding for CAR-T therapies from ZZZS.

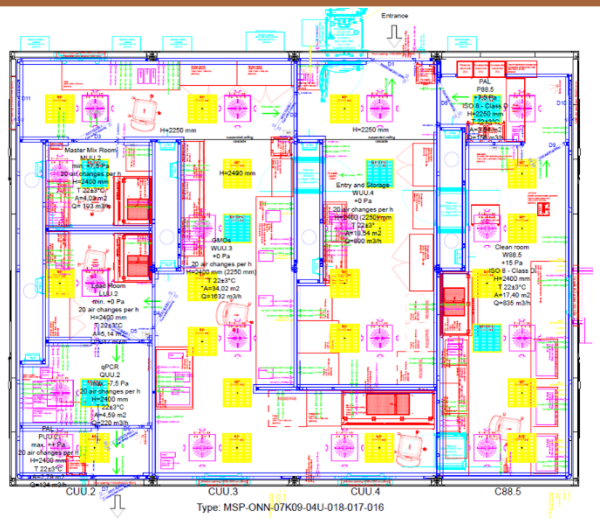
Due to the great interest of the public, all stakeholders were very responsive

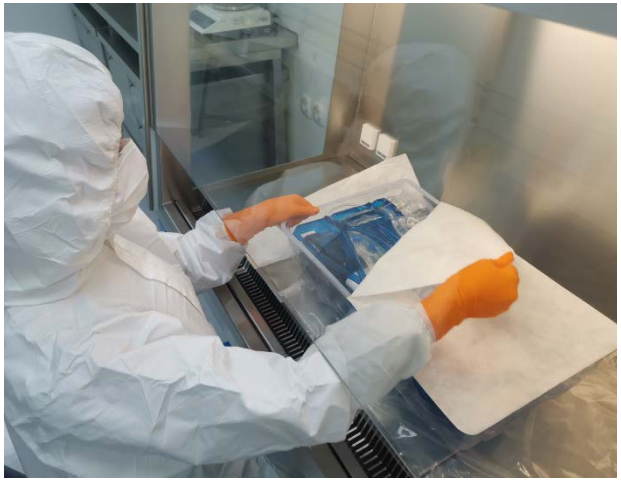
Modularly constructed clean rooms



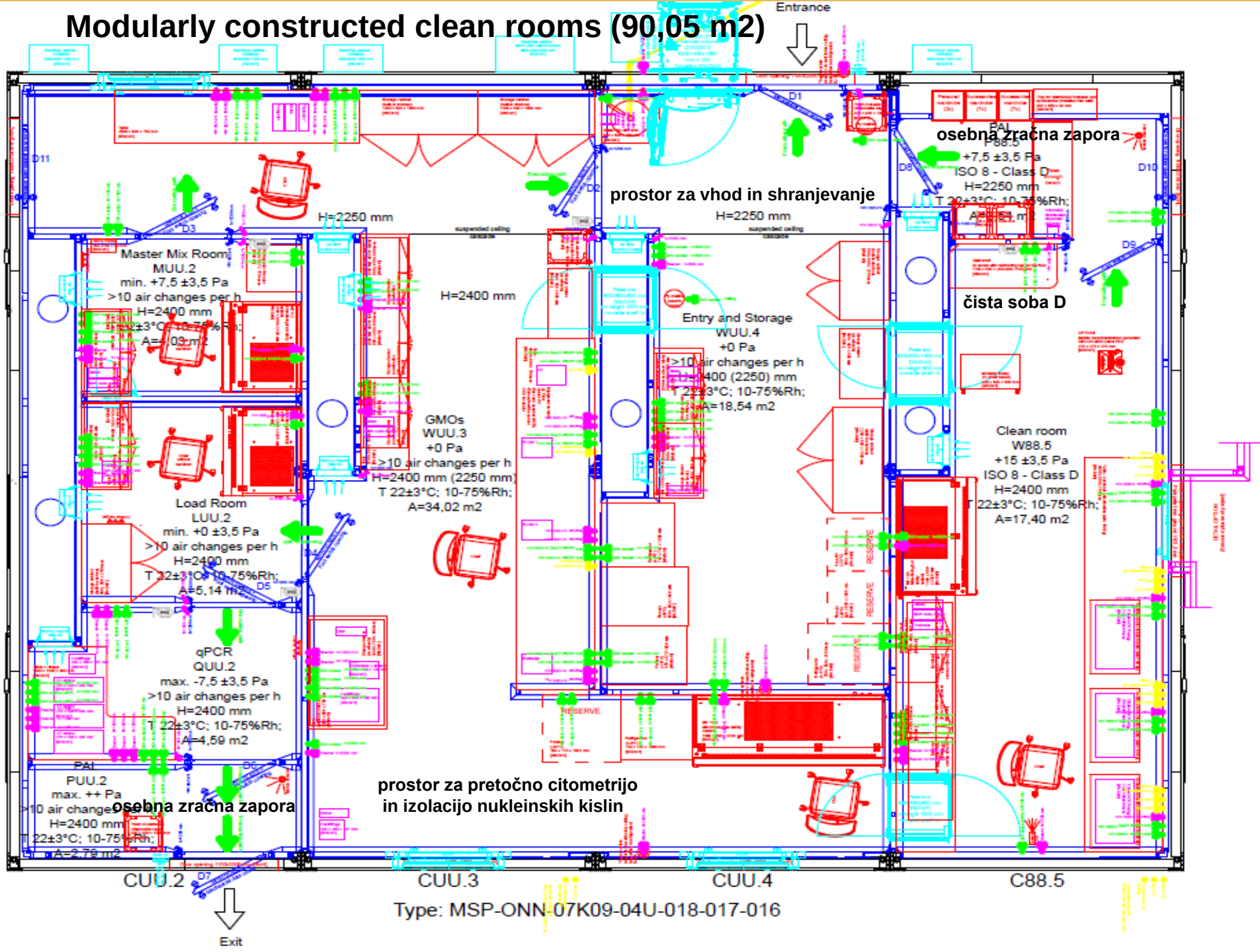
CAR-T production unit

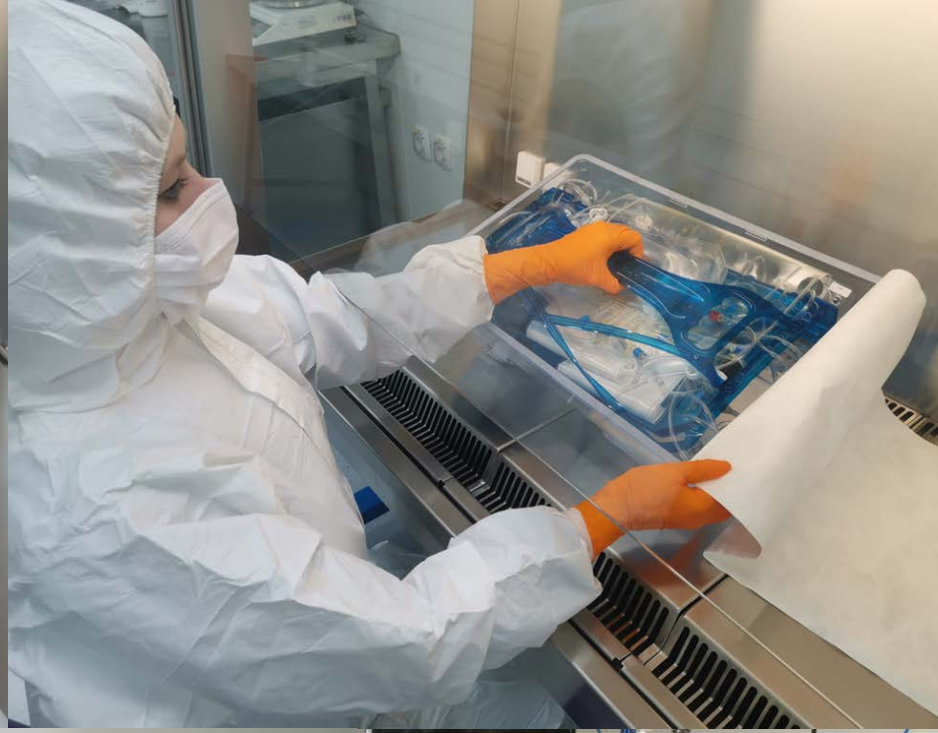


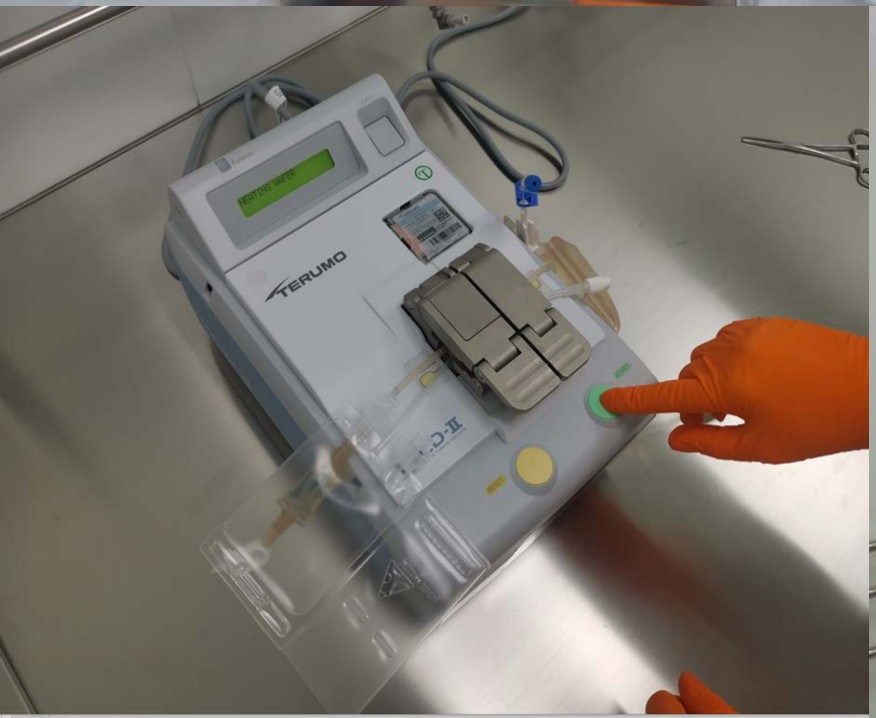




Modularly constructed clean rooms (90,05 m2)



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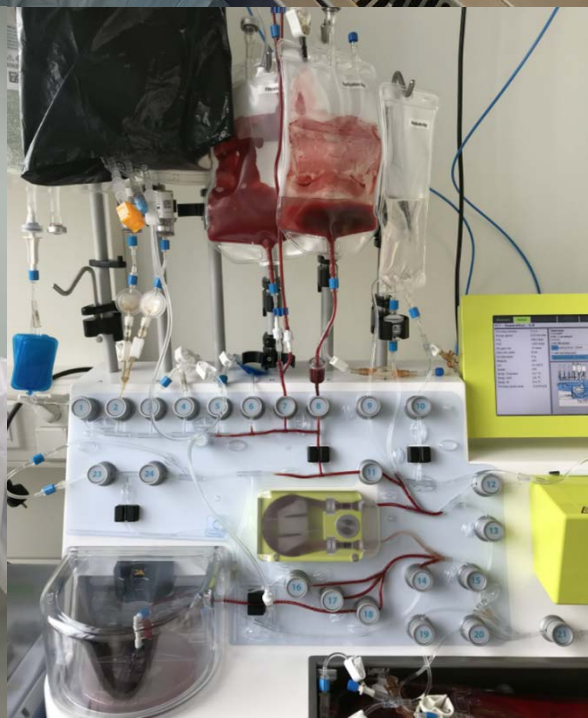


Tasks for establishing the conditions for starting therapy: clean rooms, certificates, training

- establishment of clean rooms of class D (ISO8), which also meet the conditions for working with GMOs of the 2nd safety class,
- establishment of a laboratory for quality control of CAR-T production,
- introduction of manufacturing CAR T cell therapy and quality control,
- obtaining permission to use products containing GMOs
- obtaining permission for the preparation of non-routinely prepared medicines for advanced treatment by JAZMP
- training staff to work with CliniMACS Prodigy
- staff training to work with the MACSQuant Analyzer
- qPCR validation for quality control

Agency For Medicinal Products And Medical Devices Of The Republic Of Slovenia

CERTIFICATE NUMBER: *450-11/2024-1*

CERTIFICATE OF GMP COMPLIANCE OF A MANUFACTURER [1, 2]

Part 1

Issued following an inspection in accordance with
Art. 63 of Regulation (EU) 536/2014 as amended

The competent authority of Slovenia confirms the following:
The manufacturer: ***University of Ljubljana Faculty of Medicine***
Site address: ***Dolenjska Cesta 242, Ljubljana, 1000, Slovenia, GPS: 46.037980, 14.519123***
OMS Organisation Id. / OMS Location Id.: ***ORG-100051370 / LOC-100091433***

Has been inspected under the national inspection programme in connection with manufacturing authorisation no. ***800-5/2024-17*** in accordance with Art. 61 of Regulation (EU) No 536/2014.

From the knowledge gained during inspection of this manufacturer, the latest of which was conducted on ***2024-05-09***, it is considered that it complies with:

- The principles and guidelines of Good Manufacturing Practice laid down in Directive (EU) 2017/1572 and/or Commission Delegated Regulation (EU) 2017/1569, as reflected by the product categories stated in Part 2.[3]

This certificate reflects the status of the manufacturing site at the time of the inspection noted above and should not be relied upon to reflect the compliance status if more than three years have elapsed since the date of that inspection. However, this period of validity may be reduced or extended using regulatory risk management principles by an entry in the Restrictions or Clarifying remarks field. Updates to restrictions or clarifying remarks can be identified through the EudraGMDP website (http://eudragmdp.ema.europa.eu/).
This certificate is valid only when presented with all pages and both Parts 1 and 2.
The authenticity of this certificate may be verified in EudraGMDP. If it does not appear, please contact the issuing authority.

[1] *The certificate referred to in paragraph Art. 15 of Directive 2001/20/ECis also applicable to importers.*
[2] *Guidance on the interpretation of this template can be found in the Interpretation of the Union format for GMP certificate.*
[3] *These requirements fulfil the GMP recommendations of WHO.*

Cooperation, research

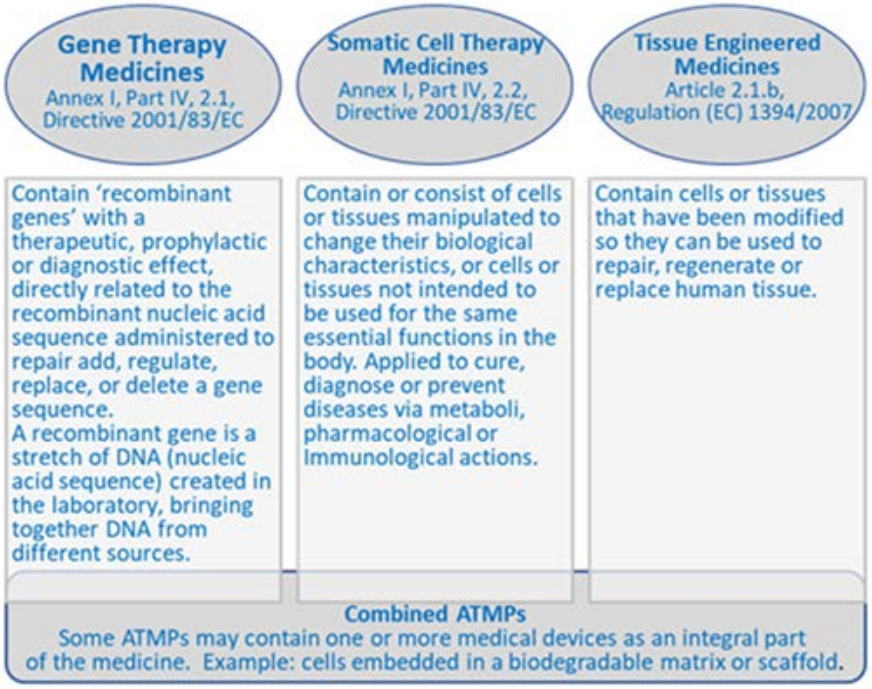
- We have established cooperation with centers for CAR-T therapy in Frankfurt and Barcelona. Our young colleagues went there several times for training, which helped them a lot in establishing the center in Ljubljana.
- We have established a consulting relationship with an experienced expert in CAR-T therapy -the involvement of Dr. Halvard Boenig and his team from Goethe University Frankfurt am Main enriched the project with expertise in the development and optimization of CAR T cell production processes and therapy.
- We established a research collaboration with Professor Roman Jerala, who heads the new center for gene and cell therapy technologies. We cooperate with him in several national and European projects.

Communication with the public is key!

- The project was born as a public initiative (donation). Due to the commitment of the public, it was much more effective to conduct discussions with the Ministry of Health and the health insurance company. The willingness of the management of the Faculty of Medicine and the University Clinical Center to cooperate was also greater due to public interest.
- It is a fortunate circumstance that Prof. dr. Zver has a great reputation and influence in the public, so his occasional public "interventions" (newspaper articles, press conferences) were enough to sustain the willingness of all stakeholders to help the development of CAR-T therapy.
- We therefore did not need to engage PR agencies, but this is not a general recipe.

A welcome side effect: ATMP legislation

- Apart from us, also our state drug agency JAZMP, working with us encountered many aspects of the practical application of the legislation governing ATMP for the first time.
- In June 2024, the Ministry of Health and JAZMP gave the initiative to jointly formulate ATMP regulations. From June to November, we met regularly to formulate rules that would be useful in facilitating the introduction of ATMP into clinical practice.



The regulation introduces the possibility of using ATMP as a Hospital Exemption

- The principle of hospital exemption (HE) allows for the use of an ATMP without a marketing authorization under certain circumstances. This only applies in a hospital setting on a non-routine basis for an individual patient and when no centrally authorized treatment or clinical trial is available.

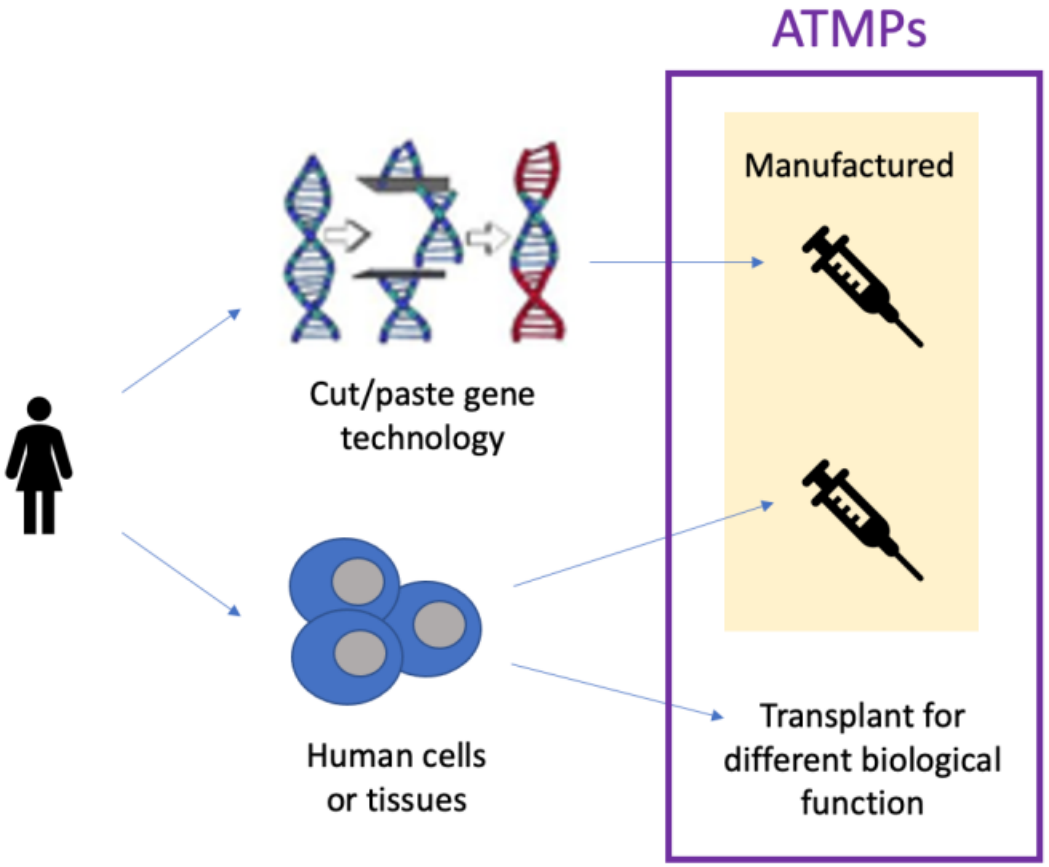
- Basic recommendations: Limit the use of HE to situations when there are no centrally authorized ATMPs available; The principles of long-term follow-up; Need for publicly available information about HE product at EU and/or national levels (use and safety/efficacy profile).

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Advanced therapy medicinal products

Advanced therapy medicinal products (ATMPs) are medicines for human use that are based on genes, tissues or cells.

ATMPs – new Pharmaceuticals using human cells and/or genes



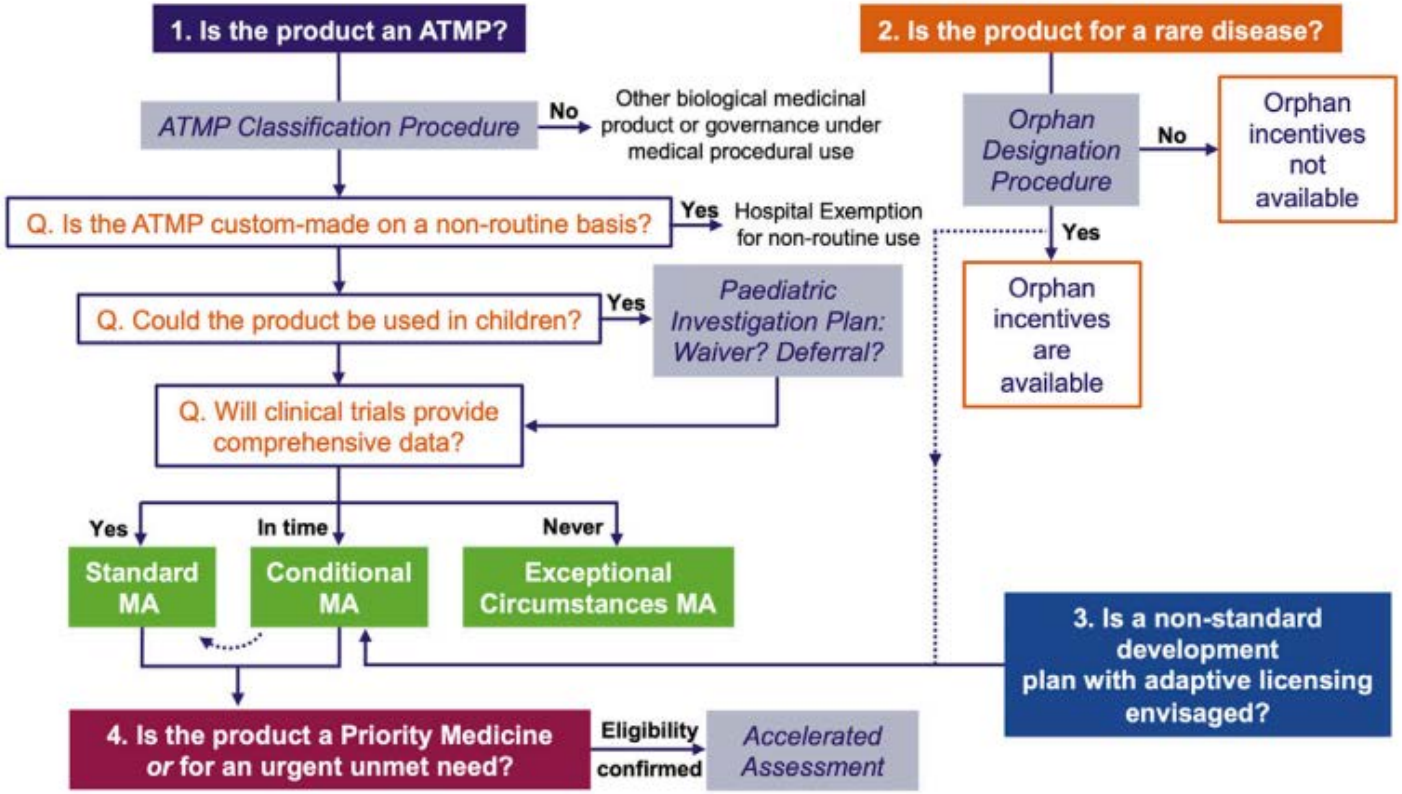
What can ATMPs do?

- Correct patient DNA
- Treat disease by adding mRNA
- Add gene corrected cells
- Instruct the body to kill defective cells eg. CAR T
- Replace diseased cells/tissues
- Treat/prevent disease by adding cells

Heather Main, The Niche, ipscell.com

EU Regulatory Pathways for ATMPs: Standard, Accelerated and Adaptive Pathways to Marketing Authorisation

Giulia Detela[1] and Anthony Lodge[2]



Thank You!!

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