Development of an advanced therapy medicinal product (ATMP) for treatment of cancer at UGent / UZGent

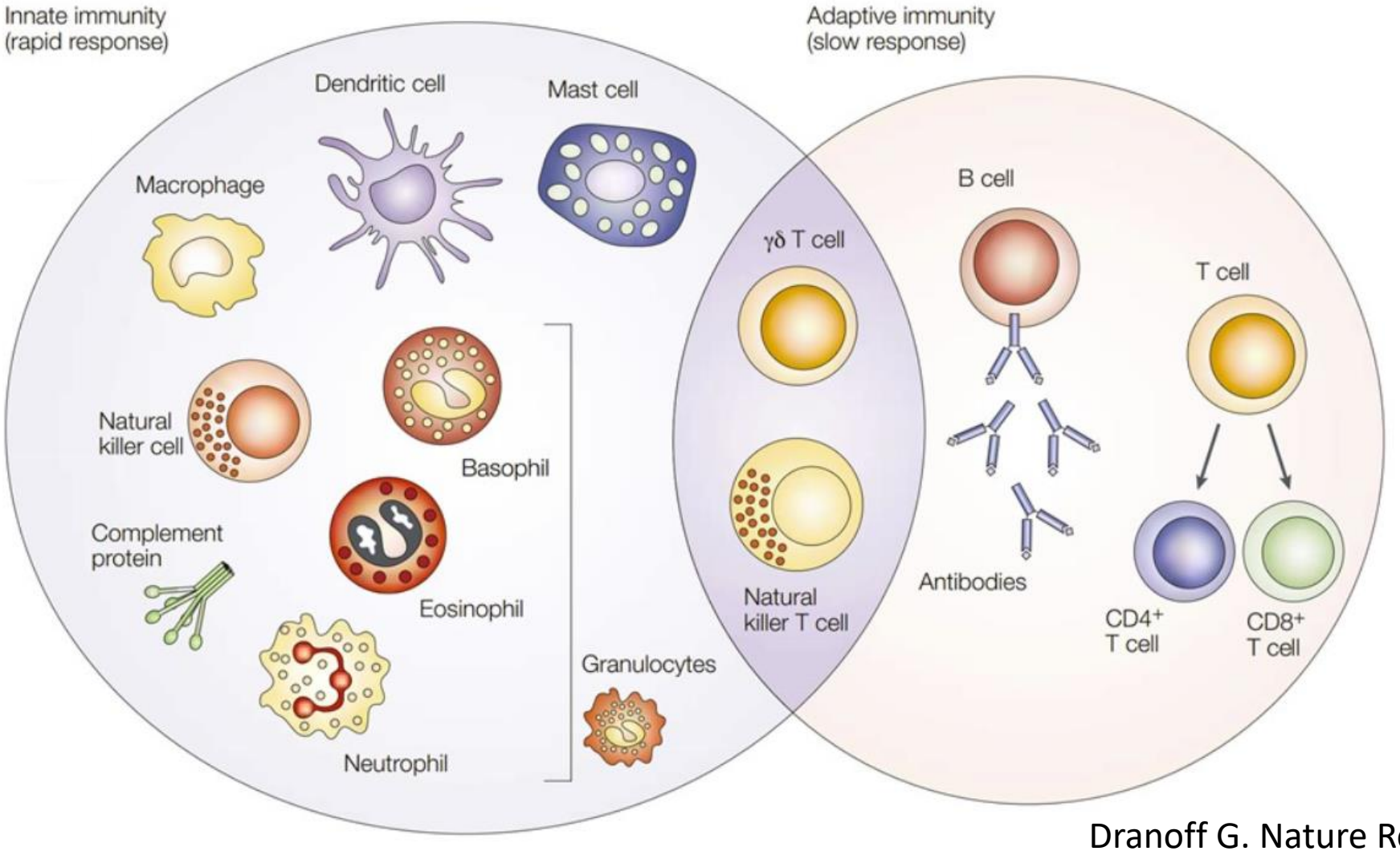
From preclinical stage to a first-in human clinical trial

Joline Ingels
PhD Fellow
Lab of prof. Bart Vandekerckhove

GHENT UNIVERSITY

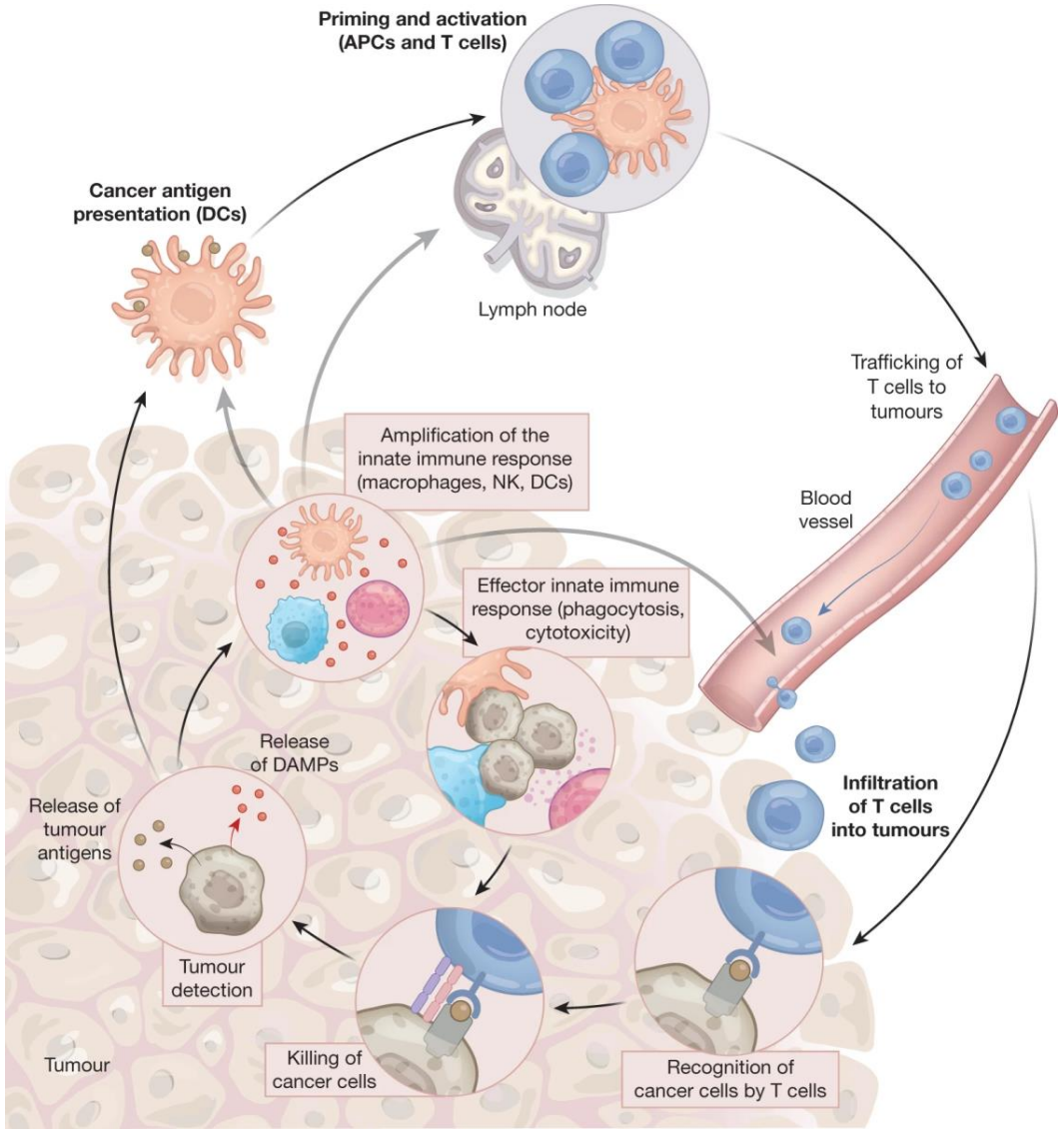
UZ GENT

The human immune system

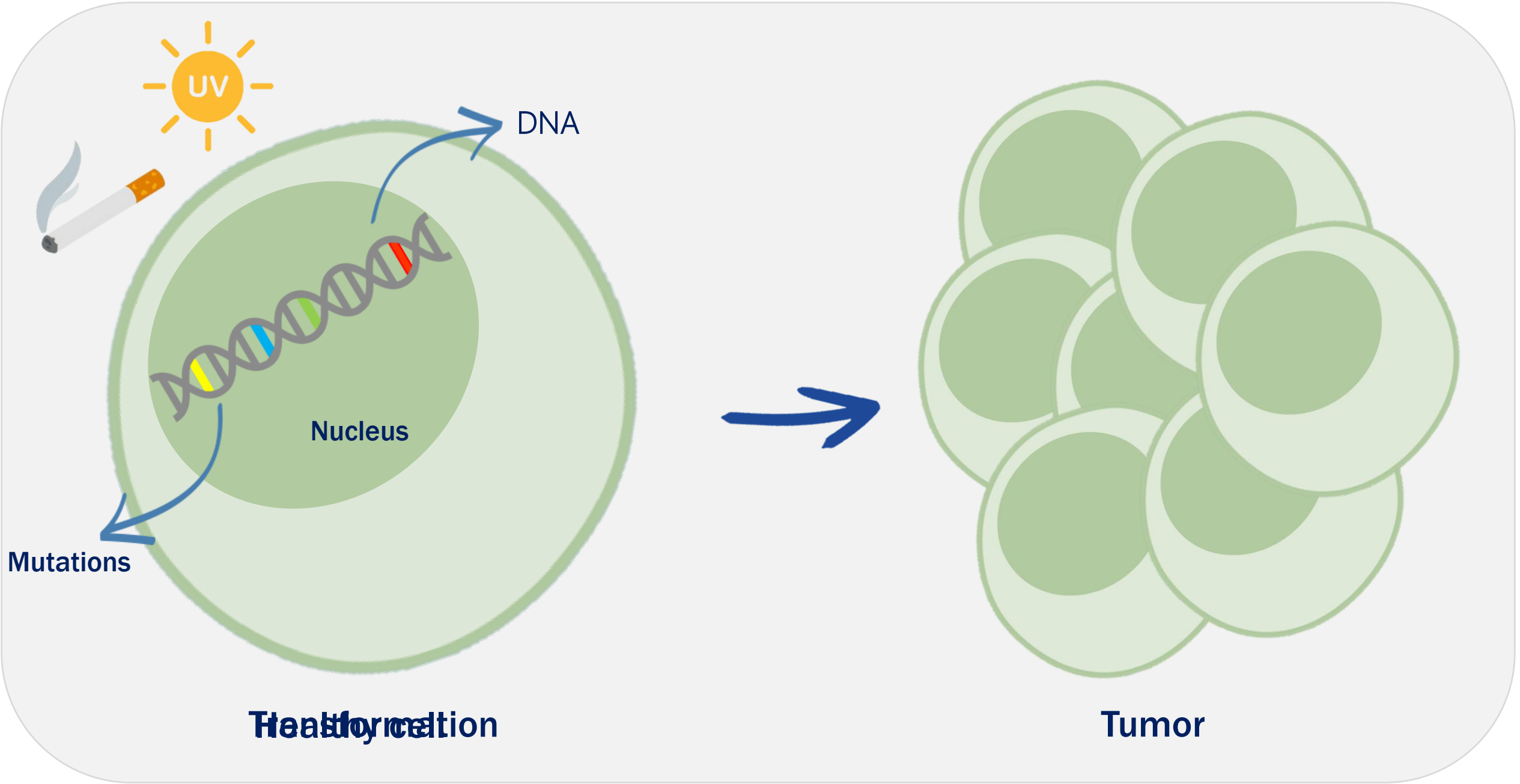


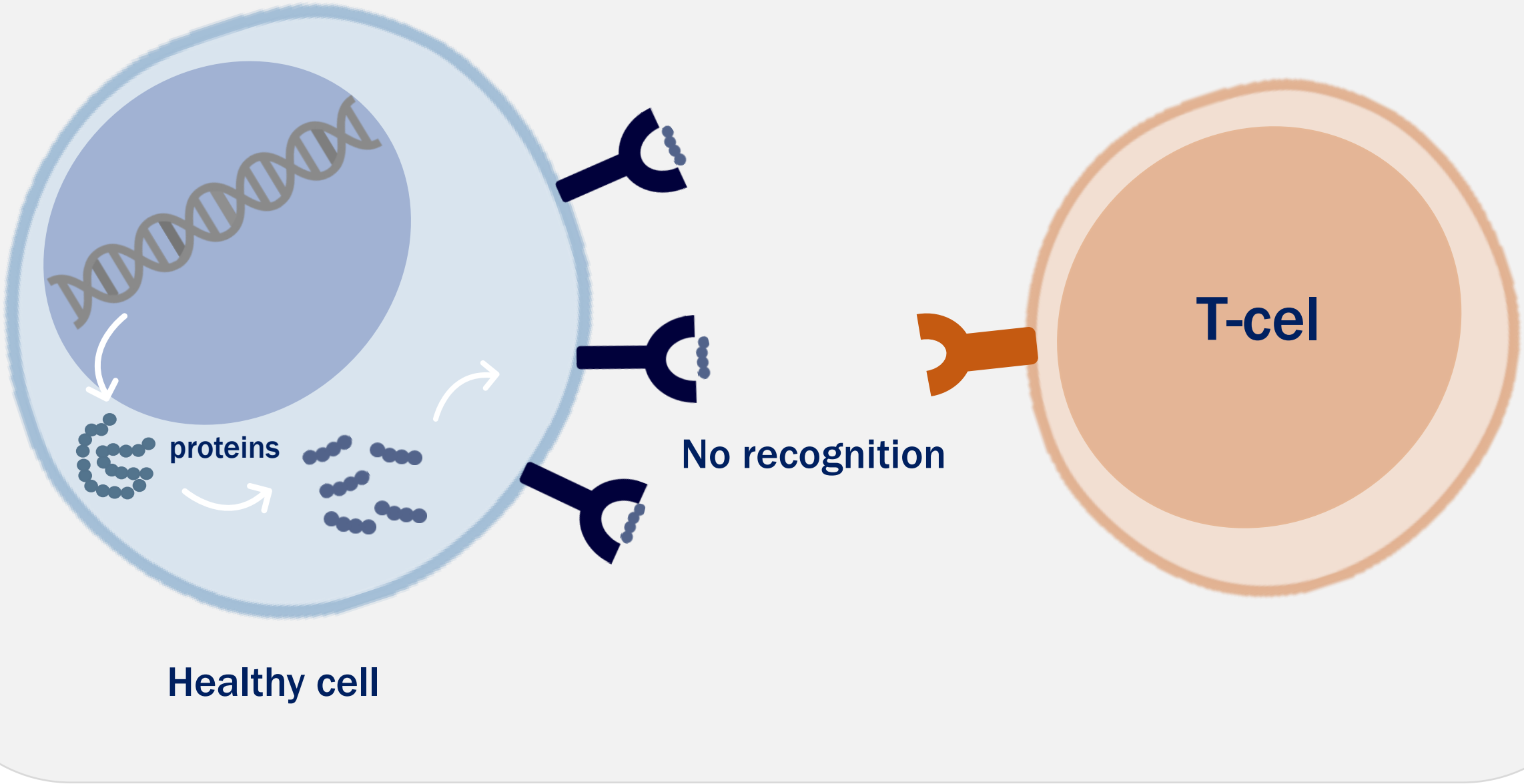
Dranoff G. Nature Rev Cancer 2004

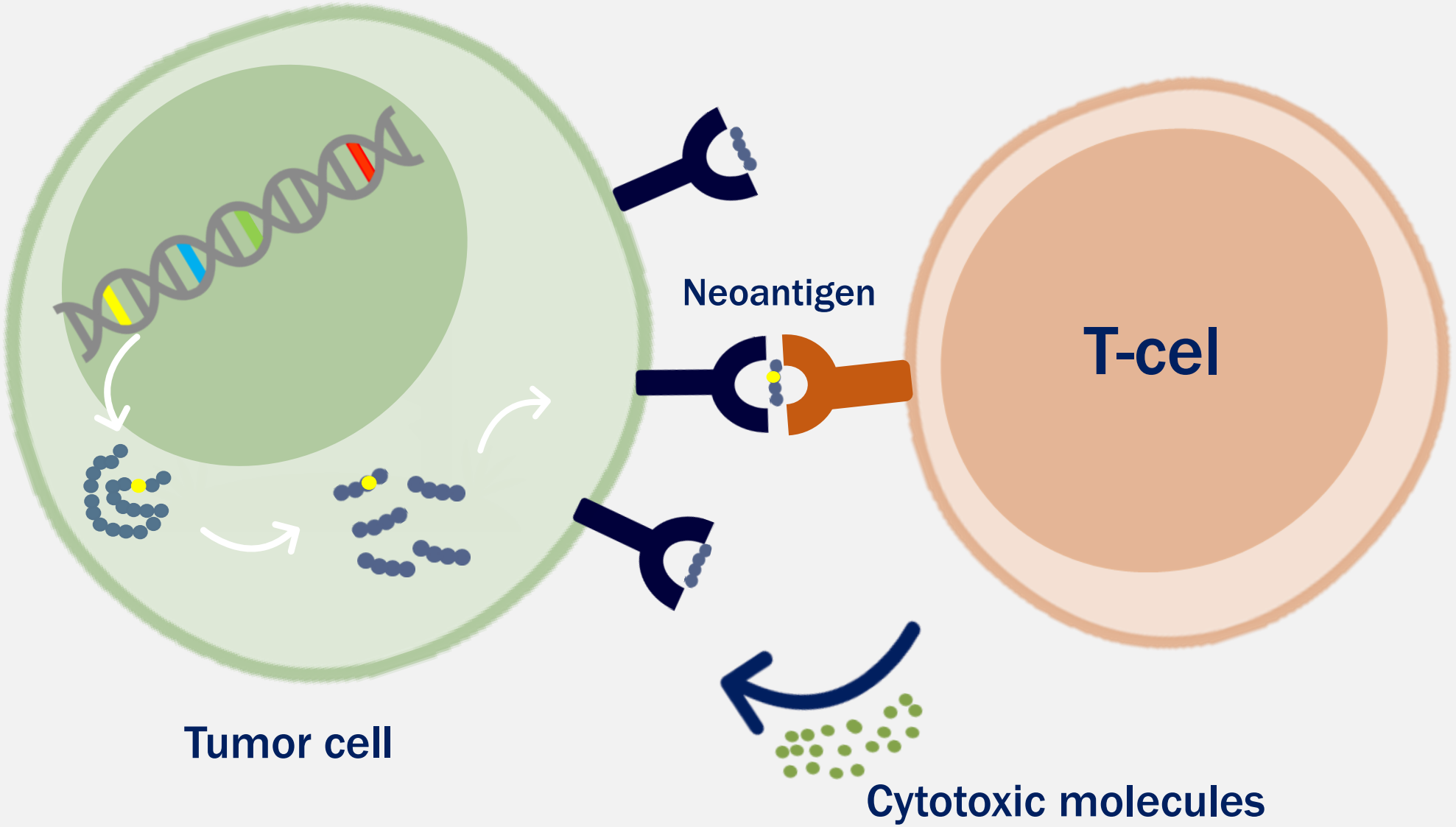
The role of the immune system in cancer: DCs and T cells as central players



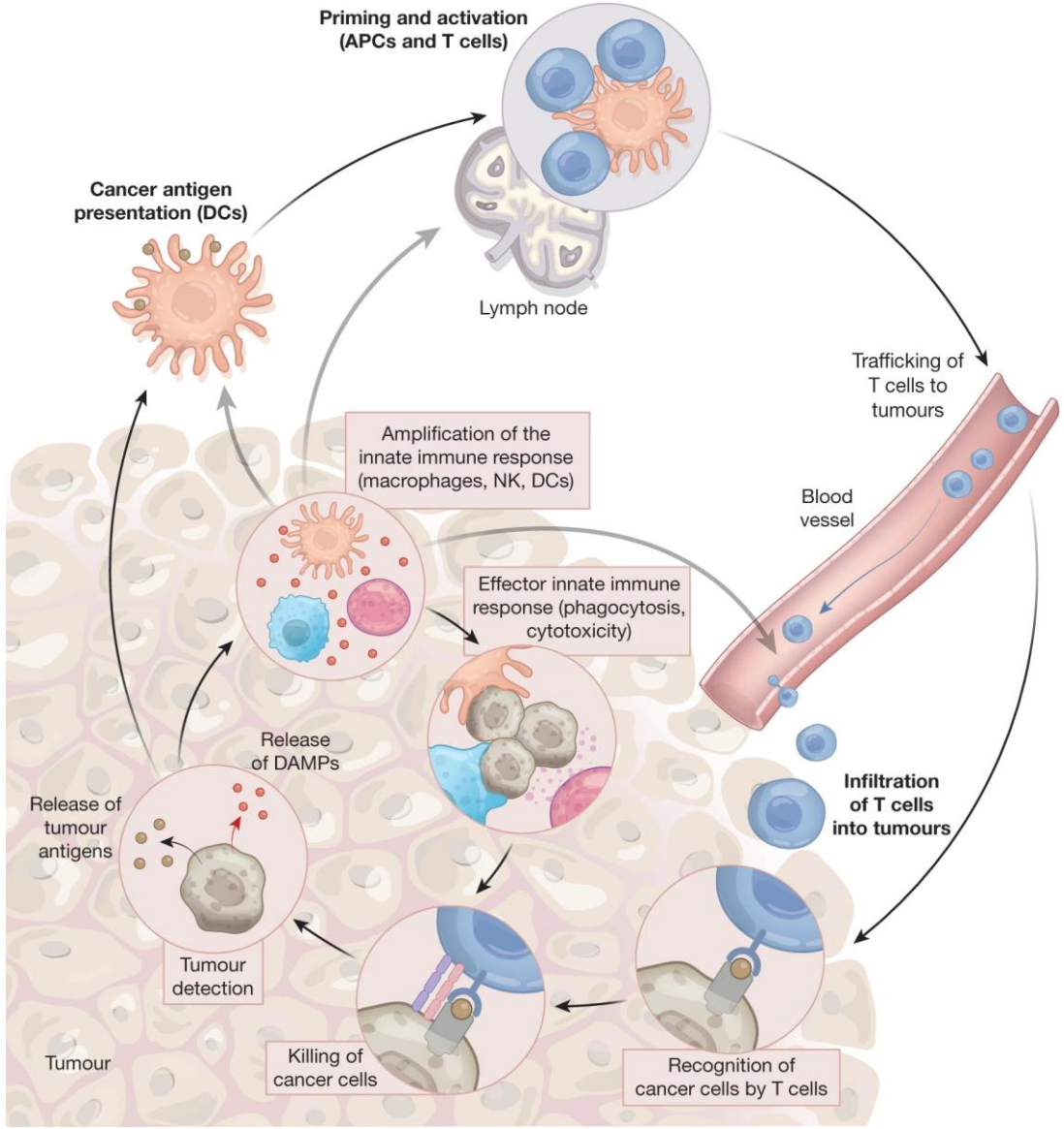
Demaria *et al.* Nature 2019


UV
DNA
Nucleus
Mutations
Tumor


proteins
No recognition
T-cel
Healthy cell


Neoantigen
T-cel
Tumor cell
Cytotoxic molecules

The role of the immune system in cancer



Demaria *et al.* Nature 2019

MIDRIX: MPLA-Interferon-activated dendritic cell vaXine

Prof. Dr. Karim Vermaelen

Cytotherapy, 2018; 20: 1164–1181

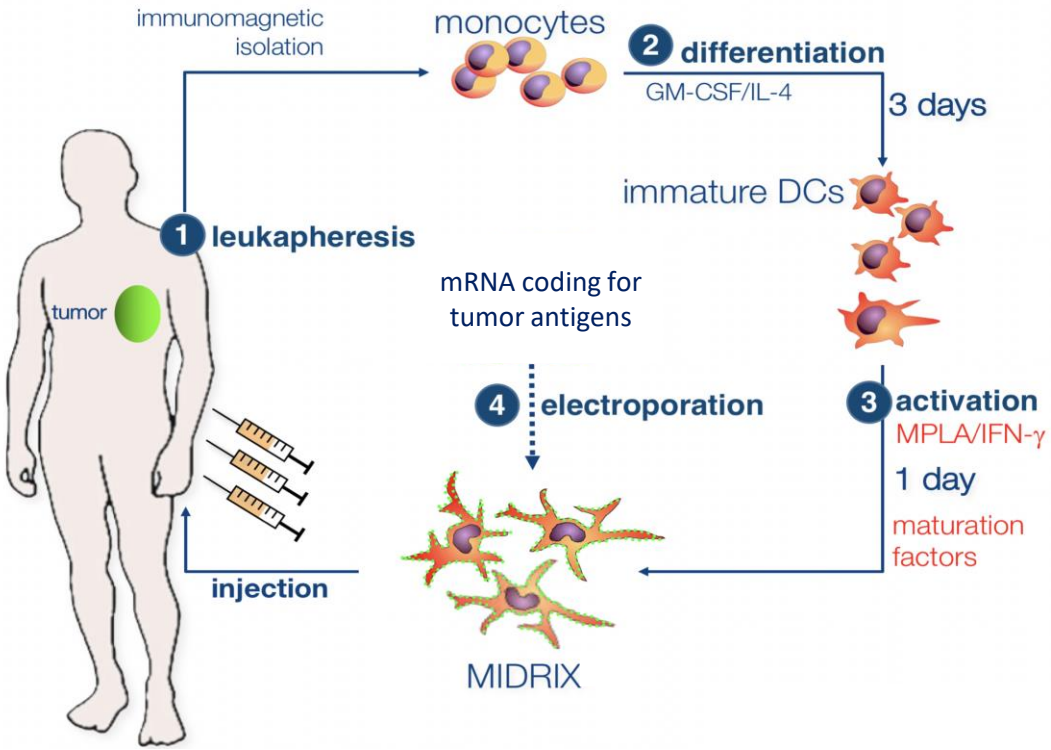
International Society for Cellular Therapy
ISCT

An accelerated, clinical-grade protocol to generate high yields of type 1-polarizing messenger RNA–loaded dendritic cells for cancer vaccination

E. BRABANTS[1], K. HEYNS[1], S. De Smet[2], P. DEVREKER[2], J. INGELS[2], N. De Cabooter[1,3], V. DEBACKER[1,3], M. DULLAERS[1,3], J.P. VAN MEERBEECK[4], B. VANDEKERCKHOVE[2] & K.Y. VERMAELEN[1]

UNIVERSITEIT GENT

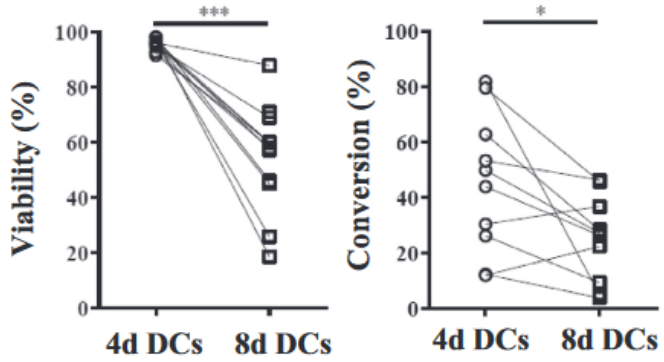
4-day protocol



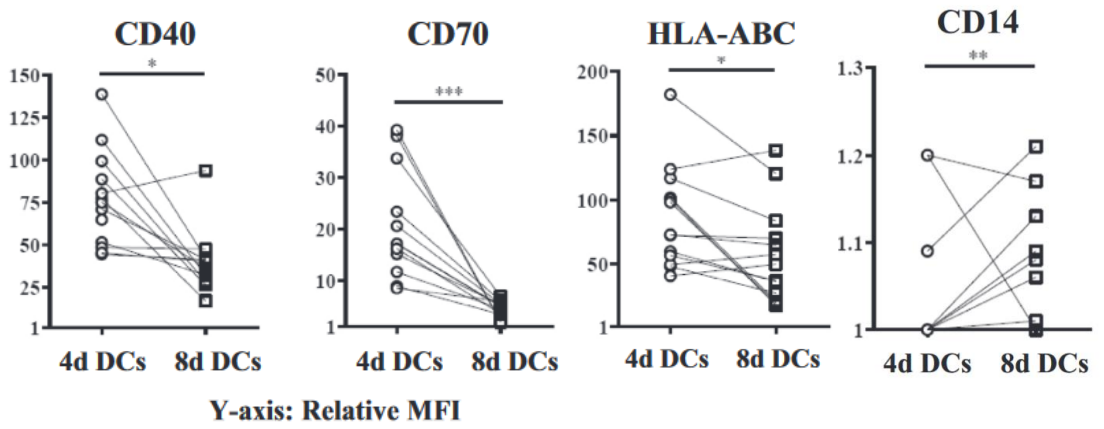
MIDRIX: MPLA-Interferon-activated dendritic cell vaXine

Superior to classical 8-day culture DCs in terms of

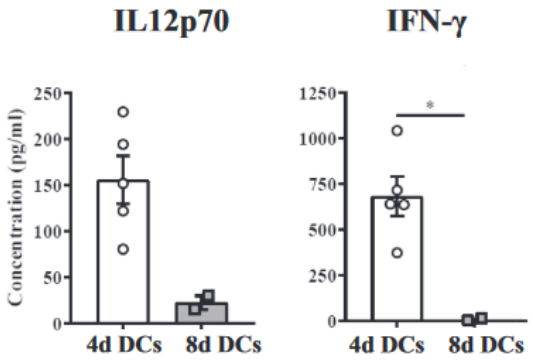
Yield and viability



Phenotype

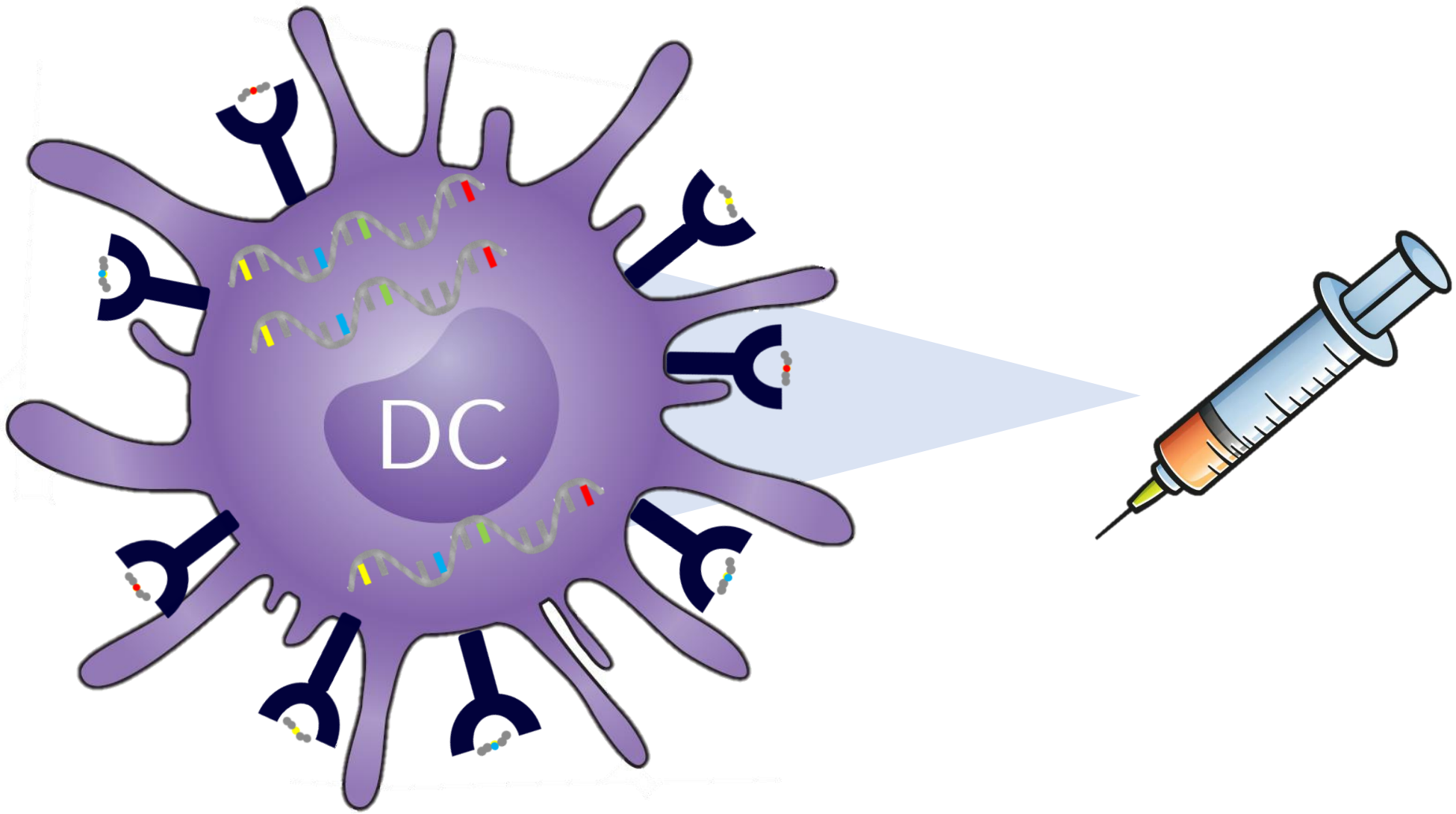


Functionality

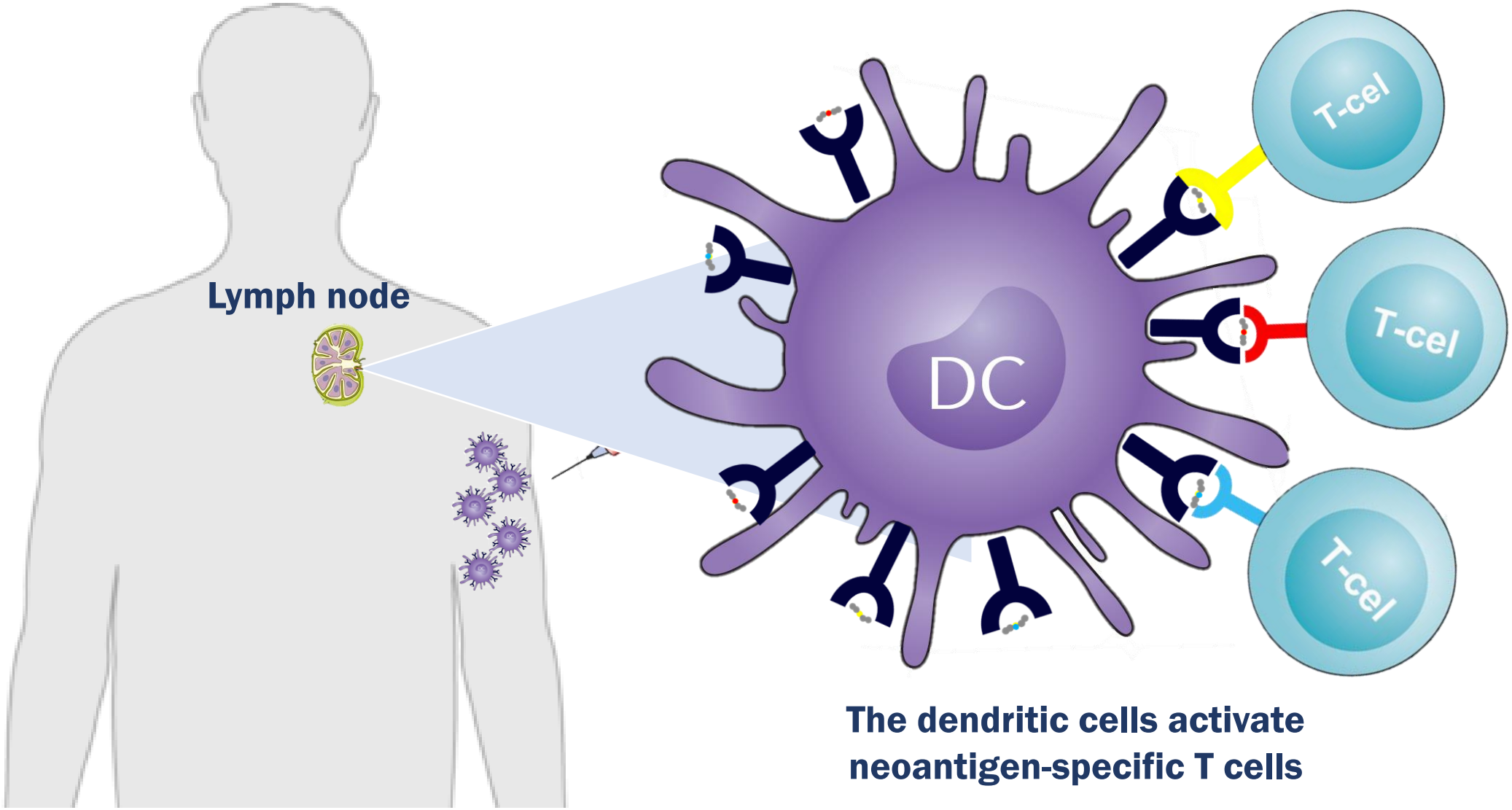


Brabants E et al. Cytotherapy 2018.

Mode of action

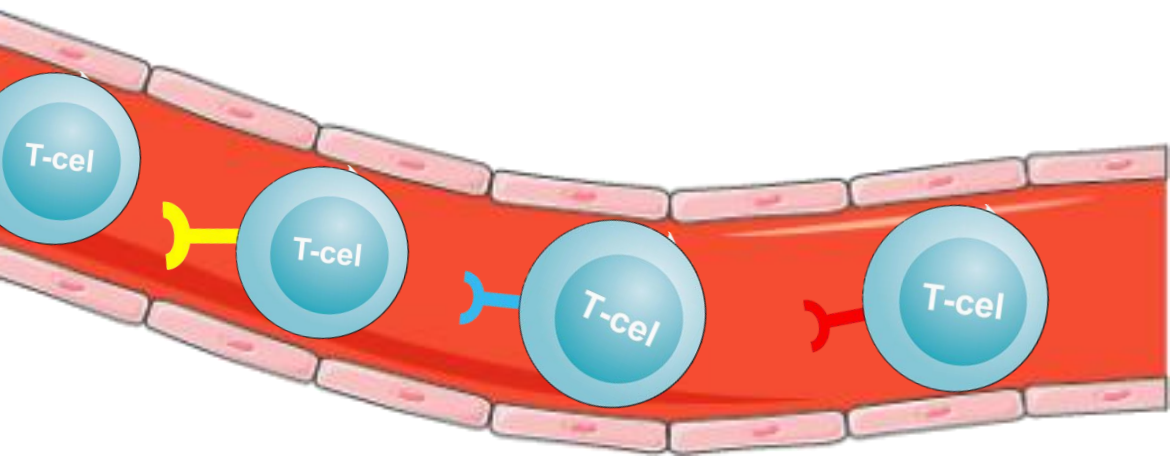


Mode of action



The dendritic cells activate neoantigen-specific T cells

Mode of action

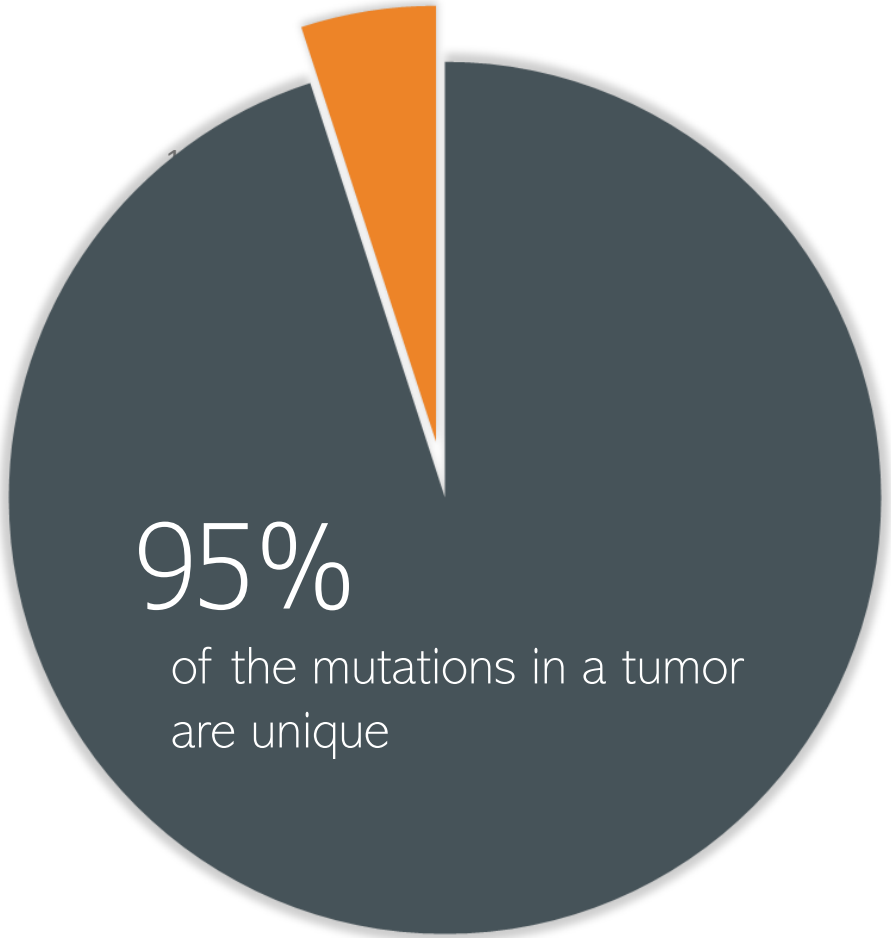


The activated T cells traffic to the tumor where they will kill tumor cells presenting neoantigens

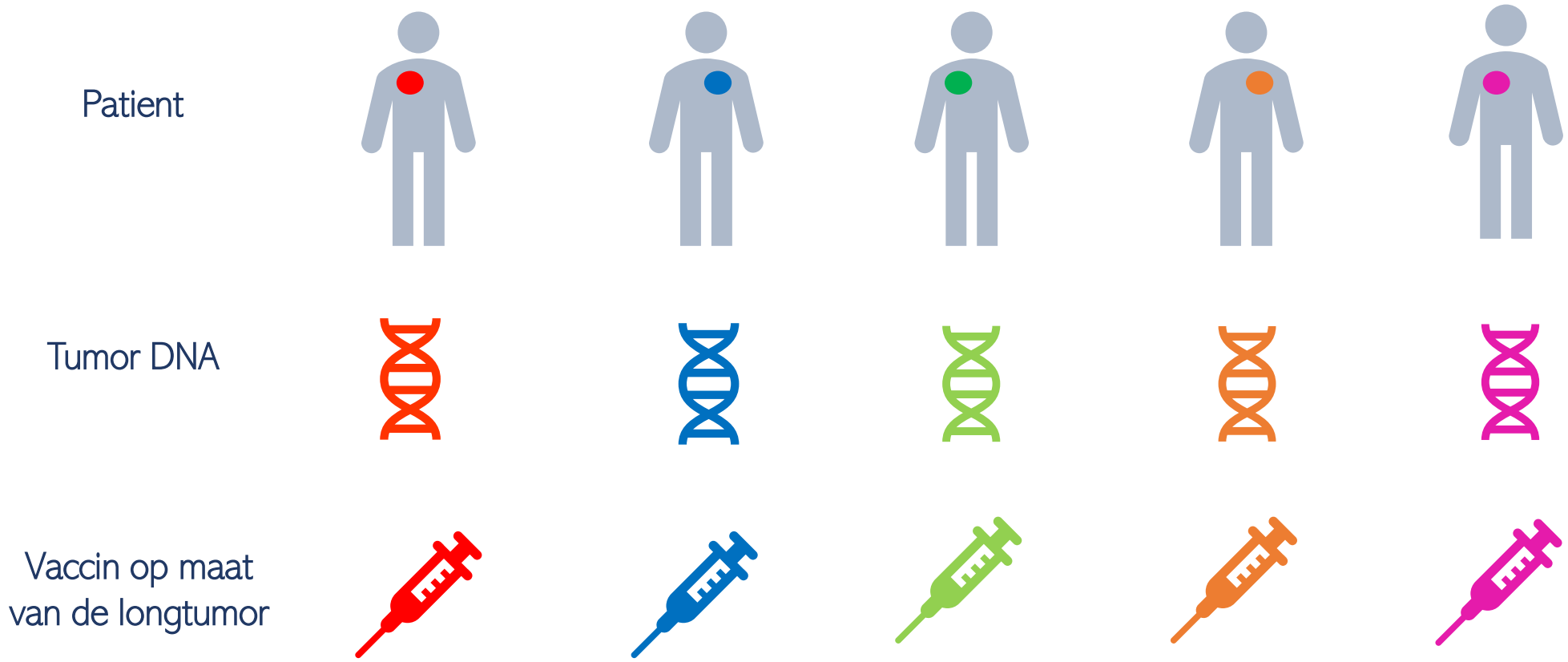
Neoantigens as tumor targets

NEOANTIGENS

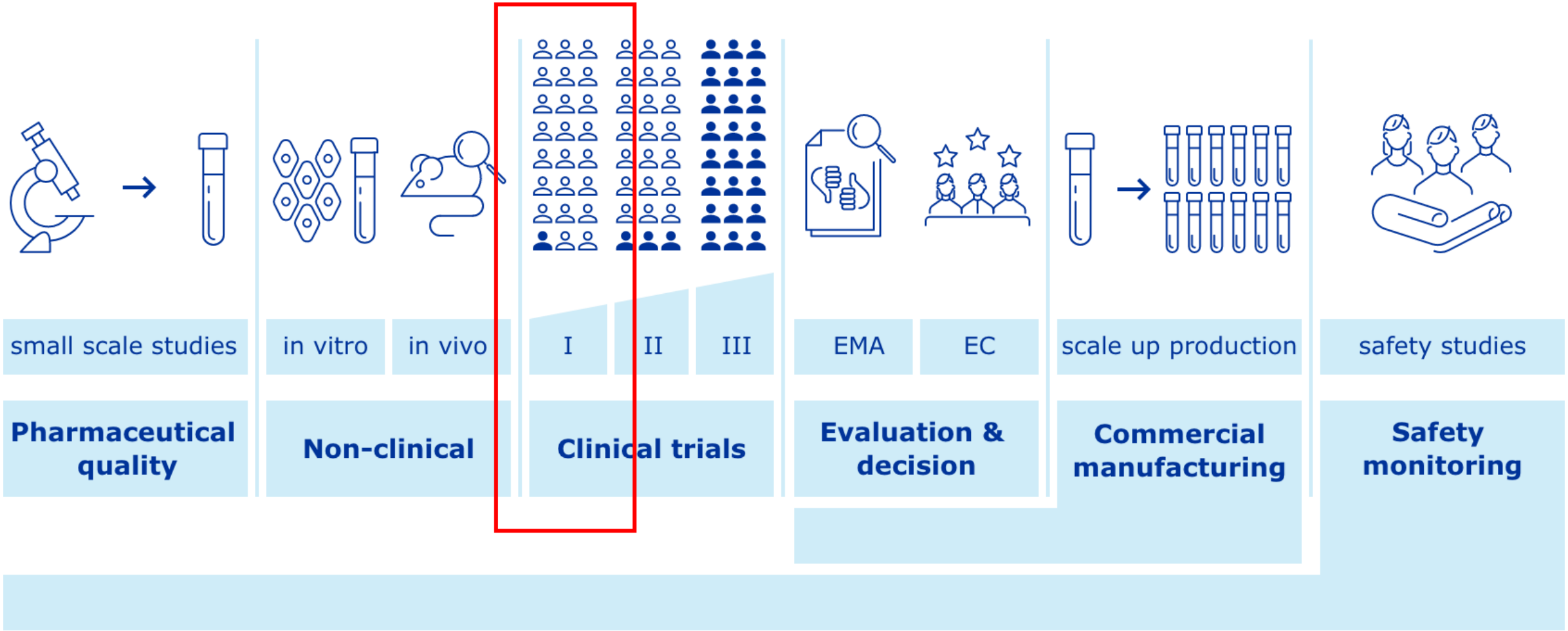
Neoantigens are mostly unique for a patient!



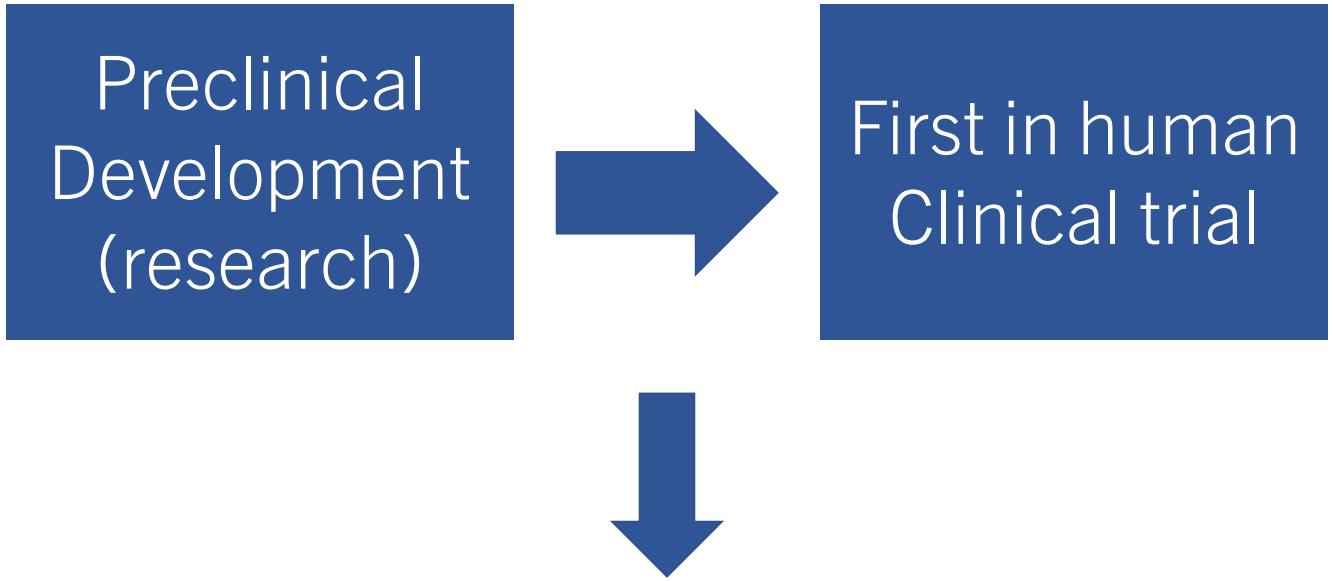
Personalized therapy: for each patient an unique vaccine is designed and produced



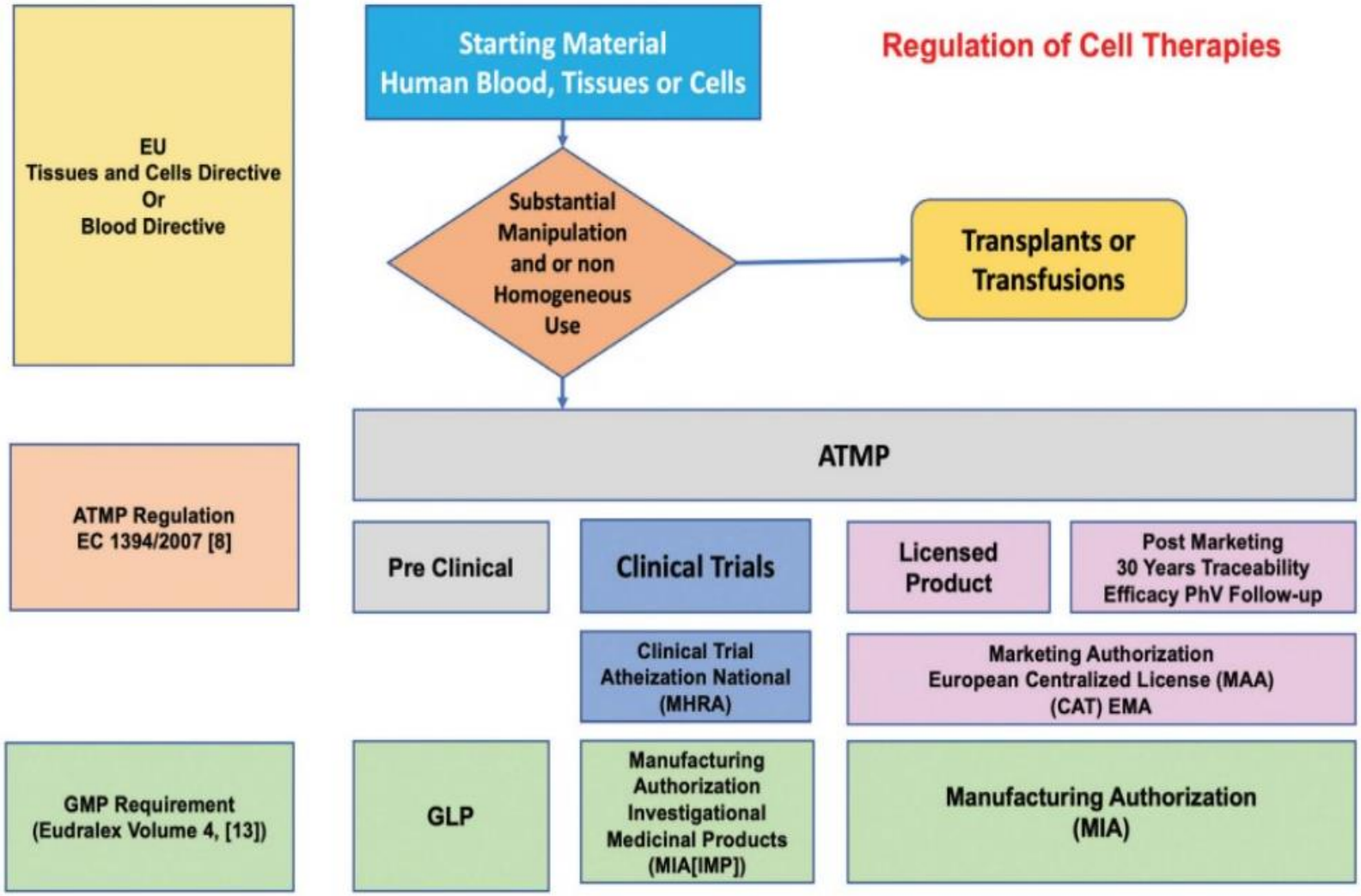
From research to the clinic



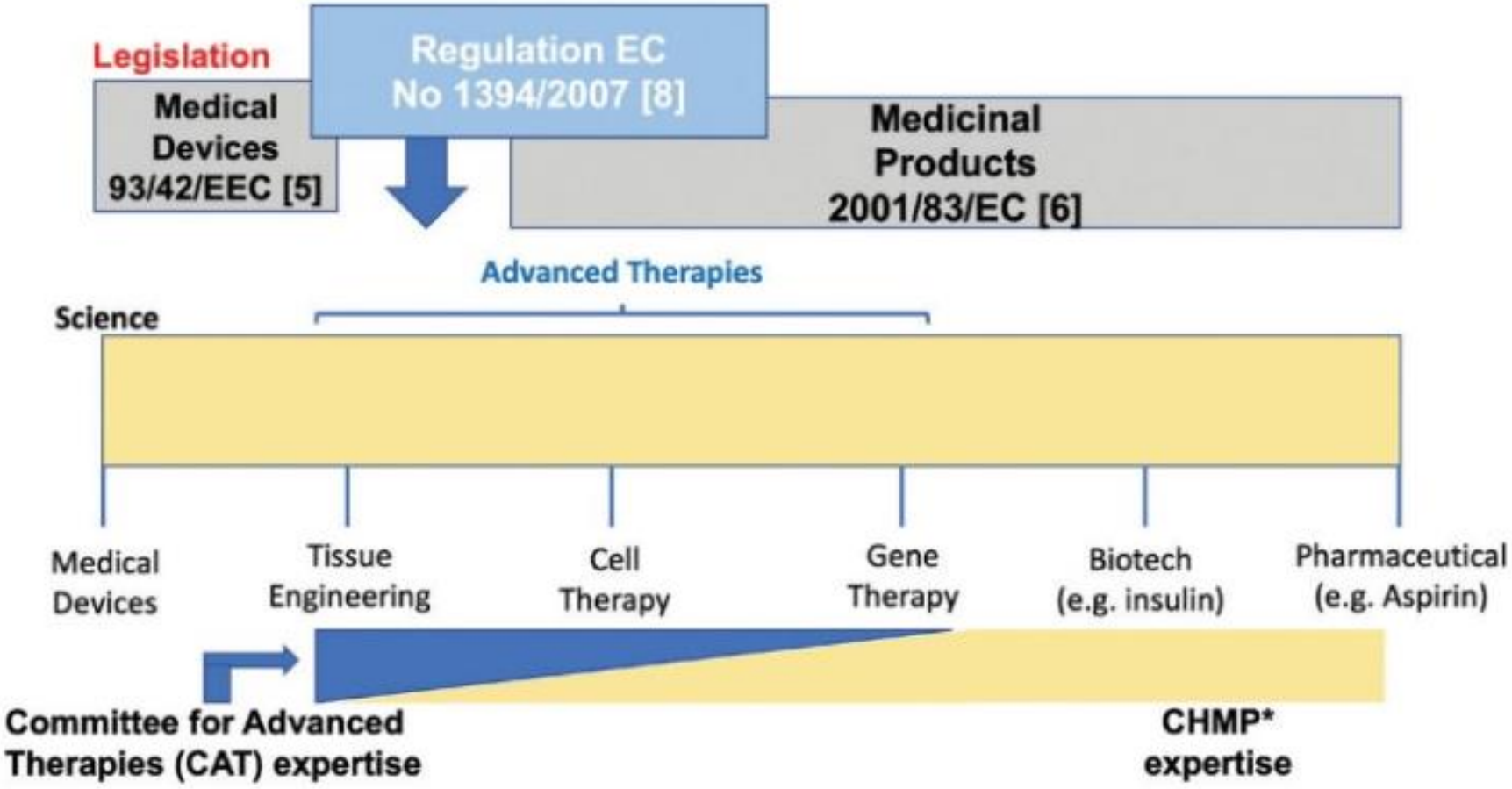
From bench to bedside



Advanced therapy medicinal products (ATMPs)



European legislation of ATMPs



ATMP classifications

Gene therapy medicines

Somatic cell therapy medicines

Tissue-engineered medicines

Combined ATMP

Good manufacturing practices

Good manufacturing practice (GMP)

= describes the minimum standard that a medicines manufacturer must meet in their production processes.

-> mandatory for authorized and investigational product

GMP requires that medicines:

- are of consistent high quality;
- are appropriate for their intended use;
- meet the requirements of the marketing authorisation or clinical trial authorisation.

Eudralex Volume 4: guidance for the interpretation of the principles and guidelines

of GMP for medicinal products for human and veterinary use
-> Part IV - GMP requirements for Advanced Therapy Medicinal Products

GMP requirements for ATMPs (Part IV): risk-based approach

Part IV - GMP requirements for Advanced Therapy Medicinal Products

- Variable starting/raw materials
- Complex production procedures

➔ Emphasis on risk-based approach

Mission

GMP unit Cell Therapy
GENT

To facilitate moving research “from bench to bedside” at the U(Z) Gent by providing expertise, equipment and a licensed facility for the production of cell and gene therapeutics to investigators.

GMP unit Cell Therapy @ UZ Ghent

iATMP	Funding	Status
TRACE CMV-EBV-Adeno-specific (donor) T cells	T Kerre. Horizon2020	Recruting 2 products produced
MIDRIX4LUNG 4 CTAG loaded autologous dencritic cells	K Vermaelen KOTK	Closed 6 product produced
MIDRIX-NEO Neoantigen loaded autologous dendritic cells	B Vandekerckhove KOTK	Data collection 6 products produced
GALSOME-NEO Neoantigen lipid nanoparticles	B Vandekerckhove KOTK	Validation studies-Toxicity studies
MIDRIX4LUNG follow up study CTAG loaded autologous dendritic cells	K Vermaelen KOTK-IOF	Validation studies
SVF Stromal vascular fraction	P.Blondeel-B Depypere. IOF	Validation studies
MIDRIX-NEO follow up study (Prodigy-Stage IV lung cancer)	B Vandekerckhove KOTK	Not started yet
Vaccines (Covid,..)	Ziphius vaccines	Engineering batch
Lentiviral particles for gene and somatic cell therapy	B+L Vandekerckhove Innovatiefonds UZ Gent	Coming soon

GMP unit Cell Therapy

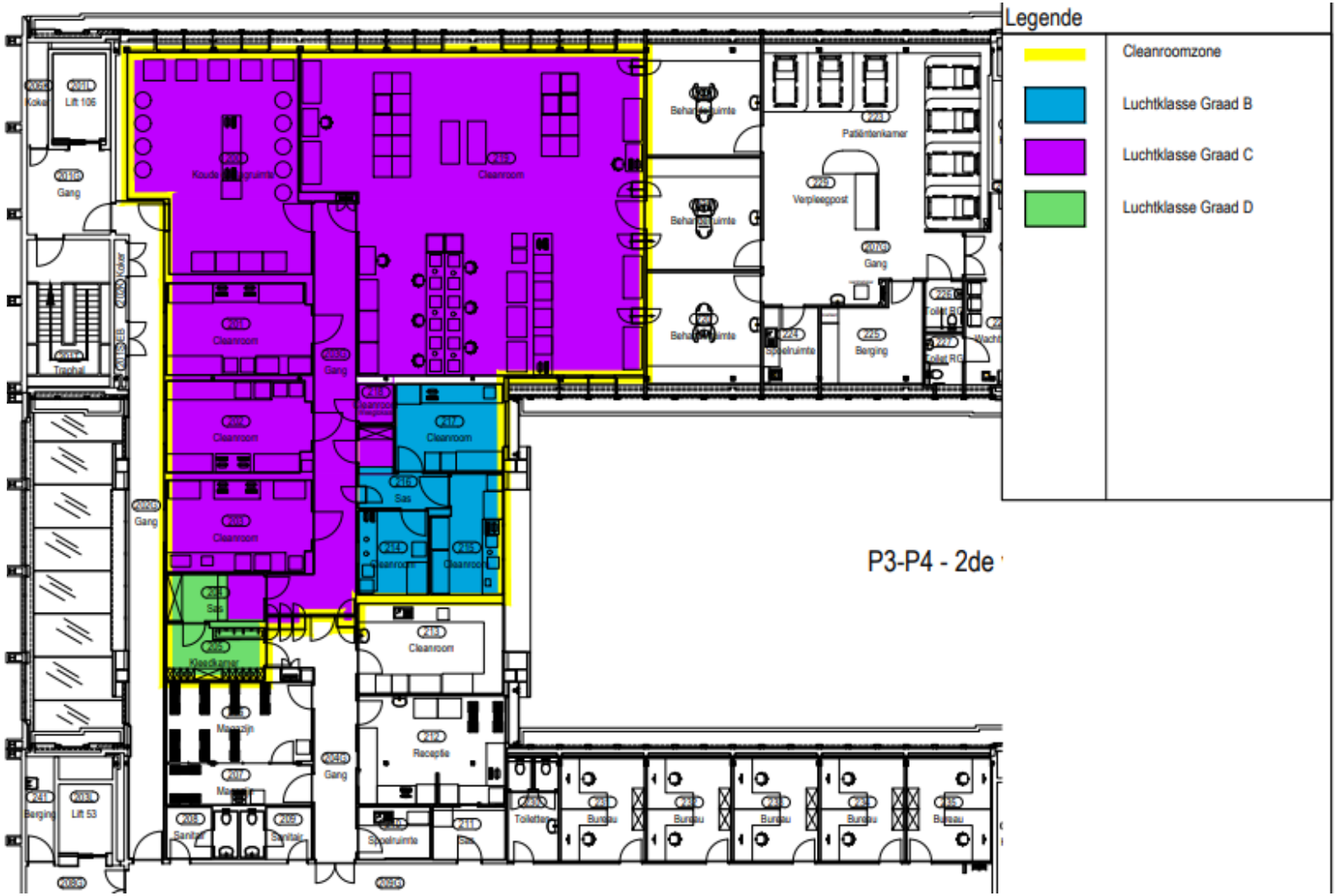
The team of GMP unit Cell Therapy

Map of the clean room facility @ UZ Ghent

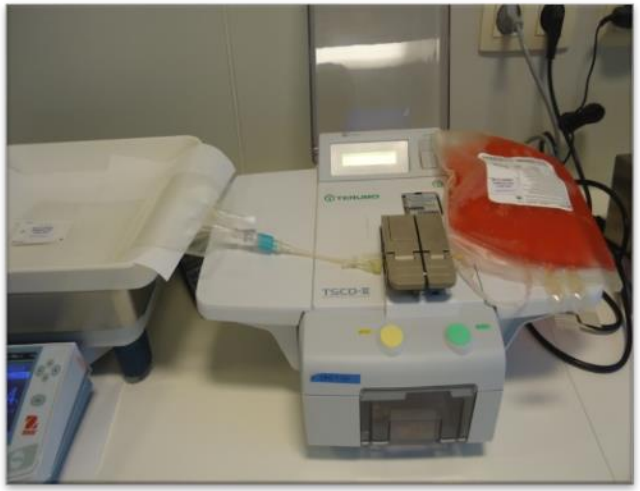
EudraLex
The Rules Governing Medicinal Products in the European Union

Volume 4
EU Guidelines to
Good Manufacturing Practice
Medicinal Products for Human and Veterinary Use

Annex 1
Manufacture of Sterile Medicinal Products
(corrected version)



Aseptic processing



Mediafill = aseptic process simulations

Periodic validation of aseptic manufacturing steps including all equipment used:

Mediafill testing

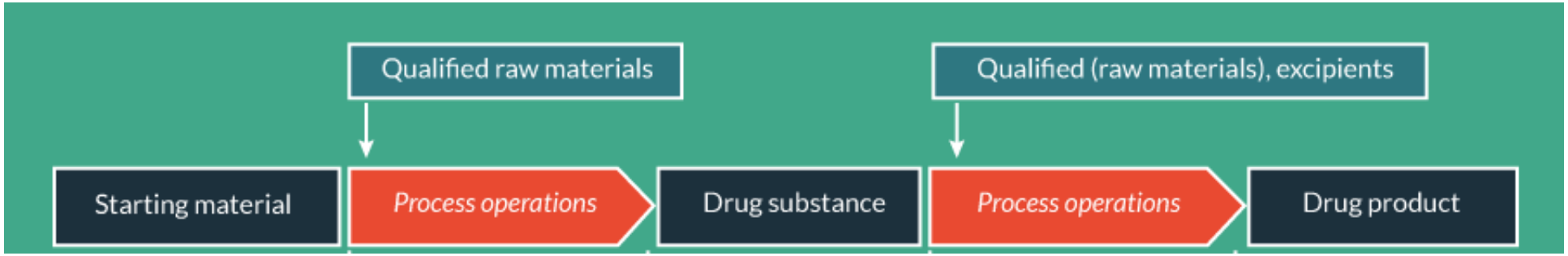
= simulation of process with microbial growth medium

IMPD

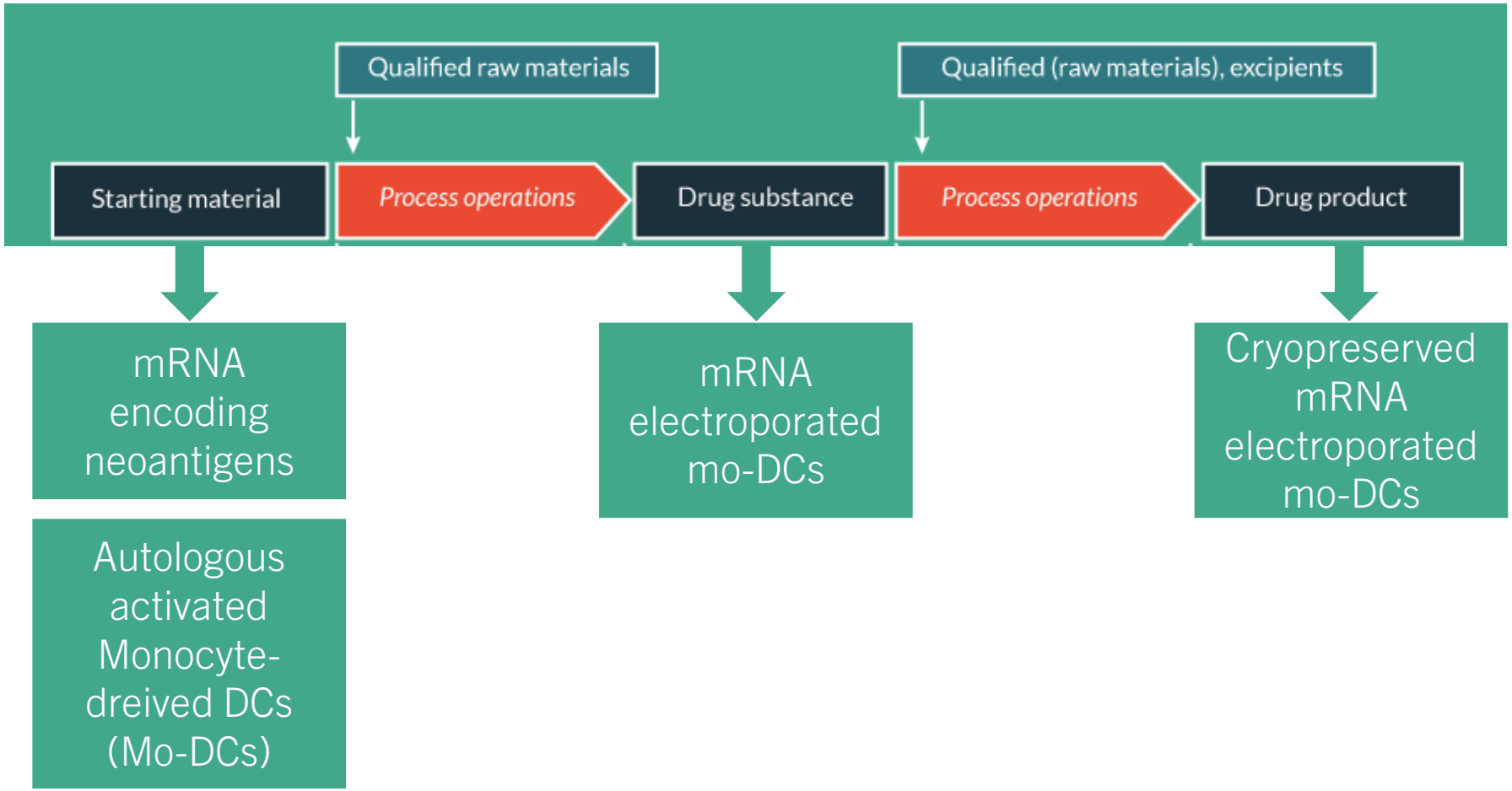
For a clinical trial application,

an Investigational Medicinal Product Dossier (IMPD) is required

Definition of starting materials – Drug Substance – Drug product



Starting materials – Drug Substance – Drug Product



Production of starting materials

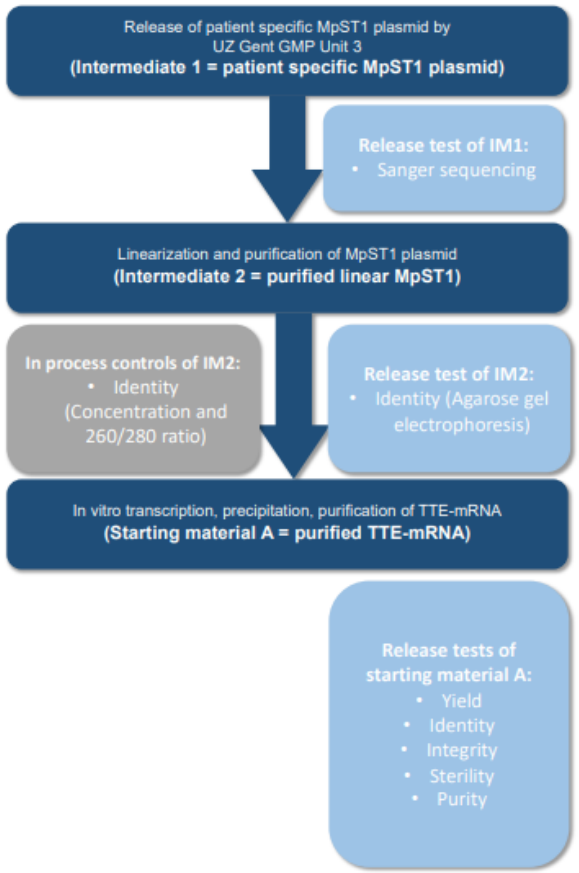
Neoantigen-encoding mRNA

Contents lists available at ScienceDirect
CYTOTHERAPY
journal homepage: www.isct-cytotherapy.org
International Society ISCT Cell & Gene Therapy®
ELSEVIER

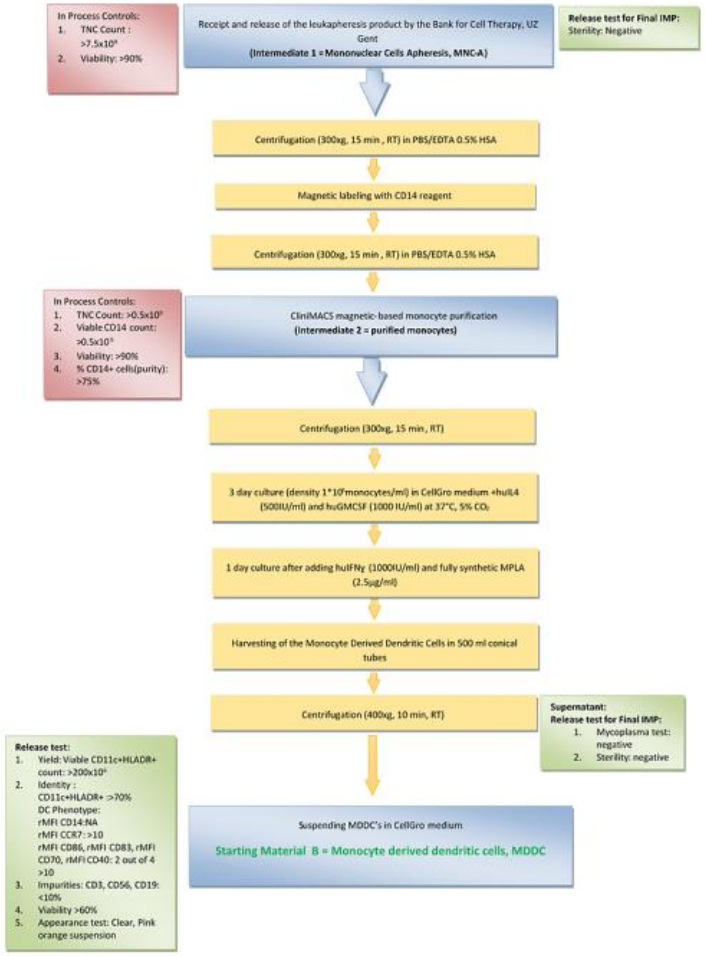
FULL-LENGTH ARTICLE
Manufacturing

Small-scale manufacturing of neoantigen-encoding messenger RNA for early-phase clinical trials

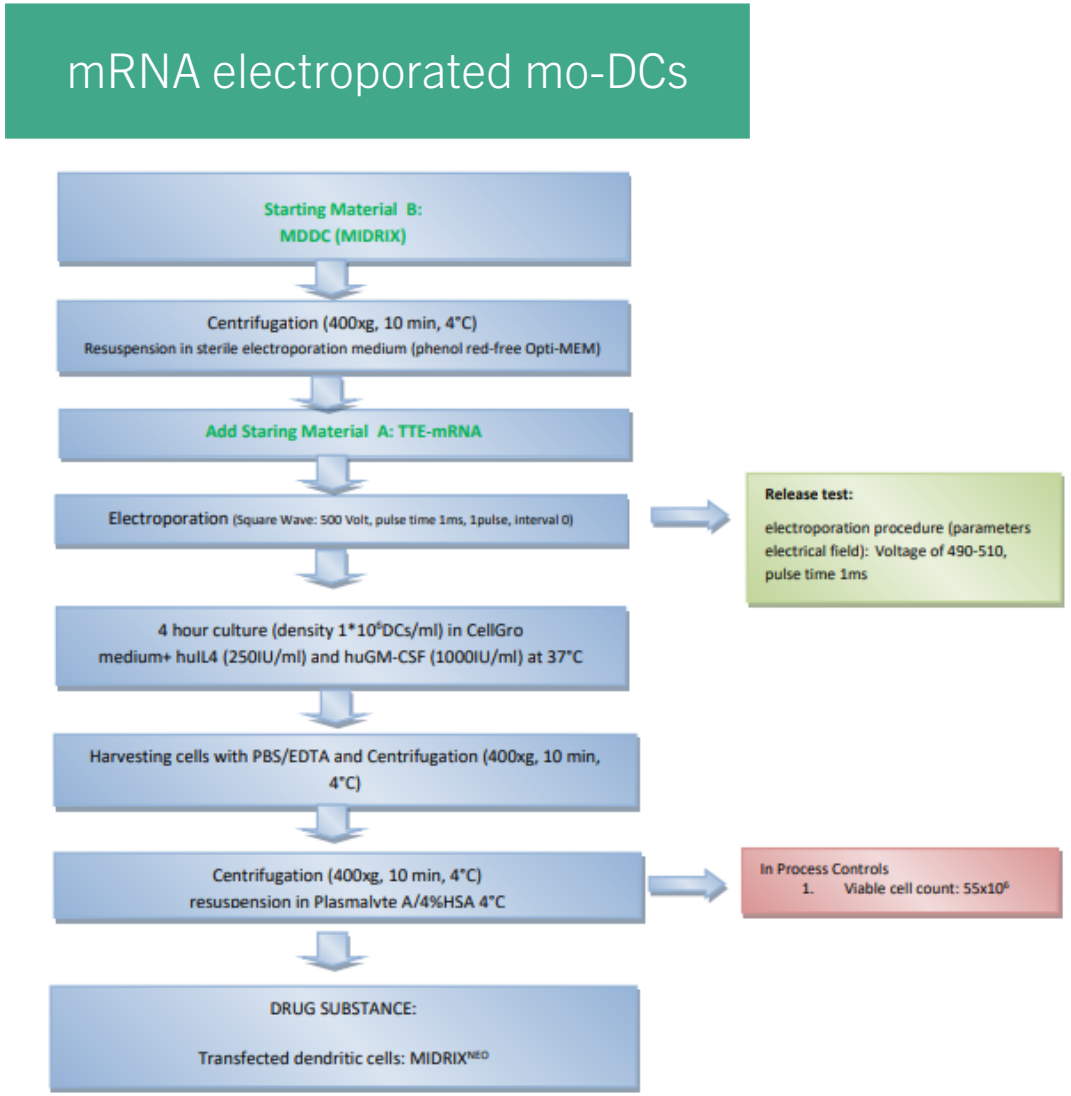
Joline Ingels[1,2], Laurenz De Cock[2,3], Rupert L. Mayer[3,4], Pam Devreker[3], Karin Weening[1], Kelly Heyns[6], Nele Lootens[5], Saskia De Smet[5], Marieke Brusseel[5], Stijn De Munter[1,2], Melissa Pille[1], Lore Billiet[1], Glenn Goetgeluk[1,2], Sarah Bonte[2,7], Hanne Jansen[1], Sandra Van Lint[2,6], Georges Leclercq[1], Tom Taghon[1], Björn Menten[2,3], Karim Vermaelen[2,6], Francis Impens[2,3,4], Bart Vandekerckhove[1,2,5,*]



Autologous activated Monocyte-dreived DCs (Mo-DCs)



Drug Substance



Drug Product

Cryopreserved mRNA electroporated mo-DCs

DRUG SUBSTANCE:MIDRIX^{NEO}

Transfected dendritic cells in Plasmalyte A/4%HSA 4°C

↓

Centrifugation (400xg, 10 min, 4°C)
resuspension in Plasmalyte A/4%HSA 4°C

↓

Add HES/DMSO to a final viable cell concentration of 50*10^6/ml → In Process Controls
1. Double check calculation for final cell dose: 50x10^6 viable cells/ml

↓

Sterile Filling 100µl/ vial (cell dose 5*10^6/vial)

↓

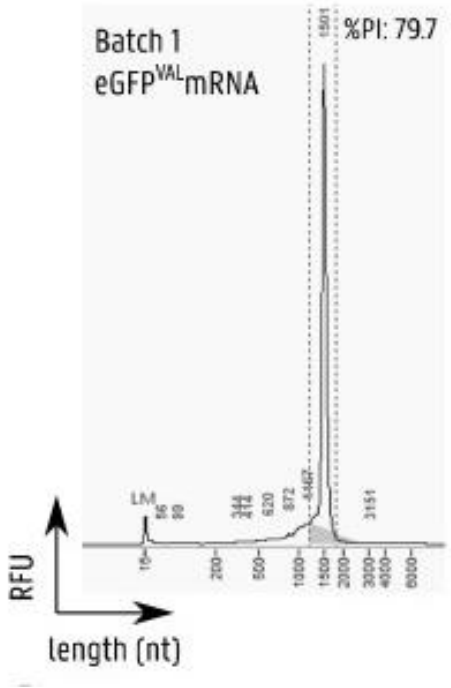
Cryopreservation and storage <-135°C
IMP: MIDRIX^{NEO} → **Release tests:**

1. Visual control of the vials
2. Endotoxin: <125EU/ml
3. Sterility testing: negative
4. viable cell count: >3x10^6/vial
5. Identity : CD11c+HLADR+ :>70%
   DC Phenotype:
   rMFI CD14:NA
   rMFI CCR7: >10
   rMFI CD86, rMFI CD83, rMFI CD70,
   rMFI CD40: 2 out of 4 >10
6. Impurities: CD3, CD56, CD19: <10%
7. IP 10 production: positive

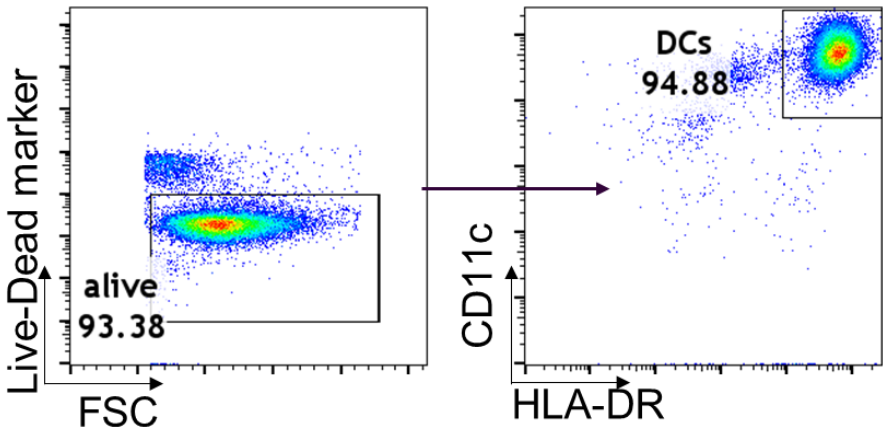
Reference samples:
1. Endotoxin and sterility
2. Viable Cell count

Quality controls: purity testing

Purity testing on Starting material (mRNA)



Purity testing on Drug Product



Representative example of flowcytometry analysis of HLA-DR^{+}CD11c^{+} DCs

IMPD

- Characterization and description of impurities
- Manufacturing processes and validations
- Quality control
  -> Analytical procedures
  -> Specifications
  -> Validation of analytical procedures
- Stability data
- Non-clinical pharmacology and toxicology
- Data from previous clinical trials

Clinical trial application (CTA)

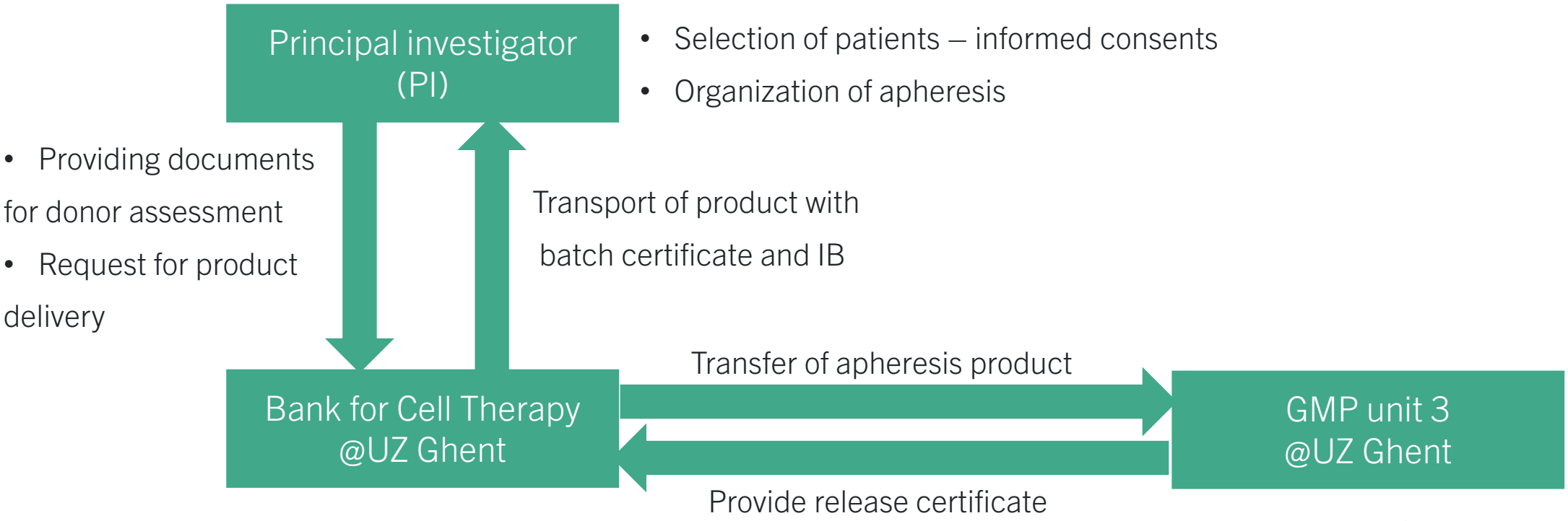
Submission to FAGG

- Cover letter
- Clinical trial protocol

(trial design, study endpoints,
selection criteria, follow up…)

- Investigators Brochure
- IMPD
- Approval of the Ethical Committee
- Label for each IMP
- Scientific advice, if applicable
- Non-clinical pharmacology and toxicology
- Data from previous clinical trials

Responsibilities of the parties involved in the clinical trial

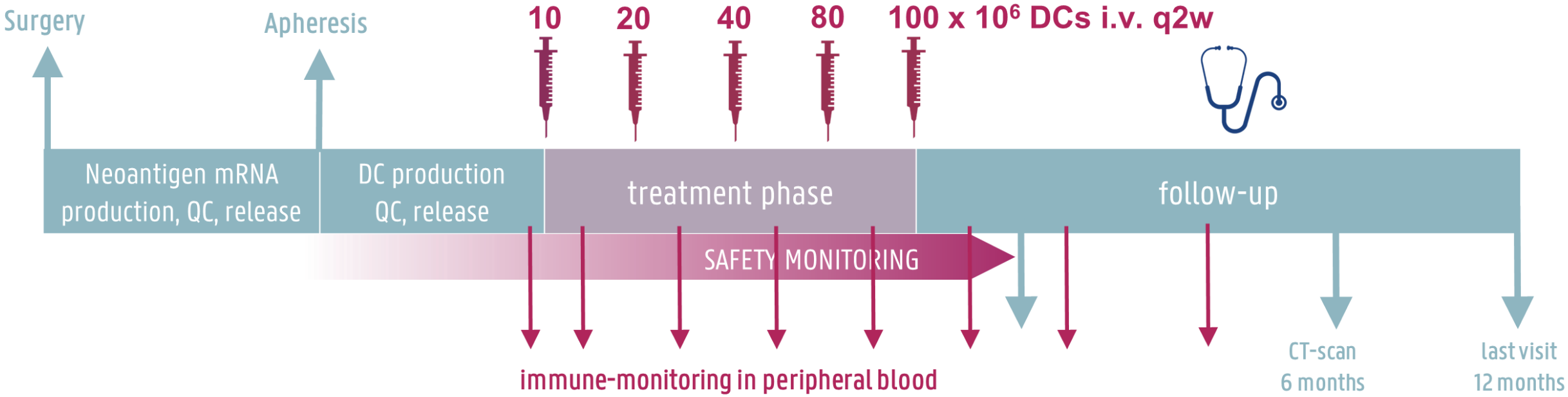


Clinical trial design (NCT04078269)

target #patients n=6
- NSCLC resectable stage IA-IIIA
- adjuvant chemo-therapy allowed

design
- phase 1a, monocentric, single-arm
- open-label first-in-human
- **intra-patient dose escalation**

endpoints
- primary: safety/toxicity
- secondary: feasibility, immune responses, relapse-free survival



Ingels J, De Cock L, *et al.* Submitted to Cell Reports Medicine

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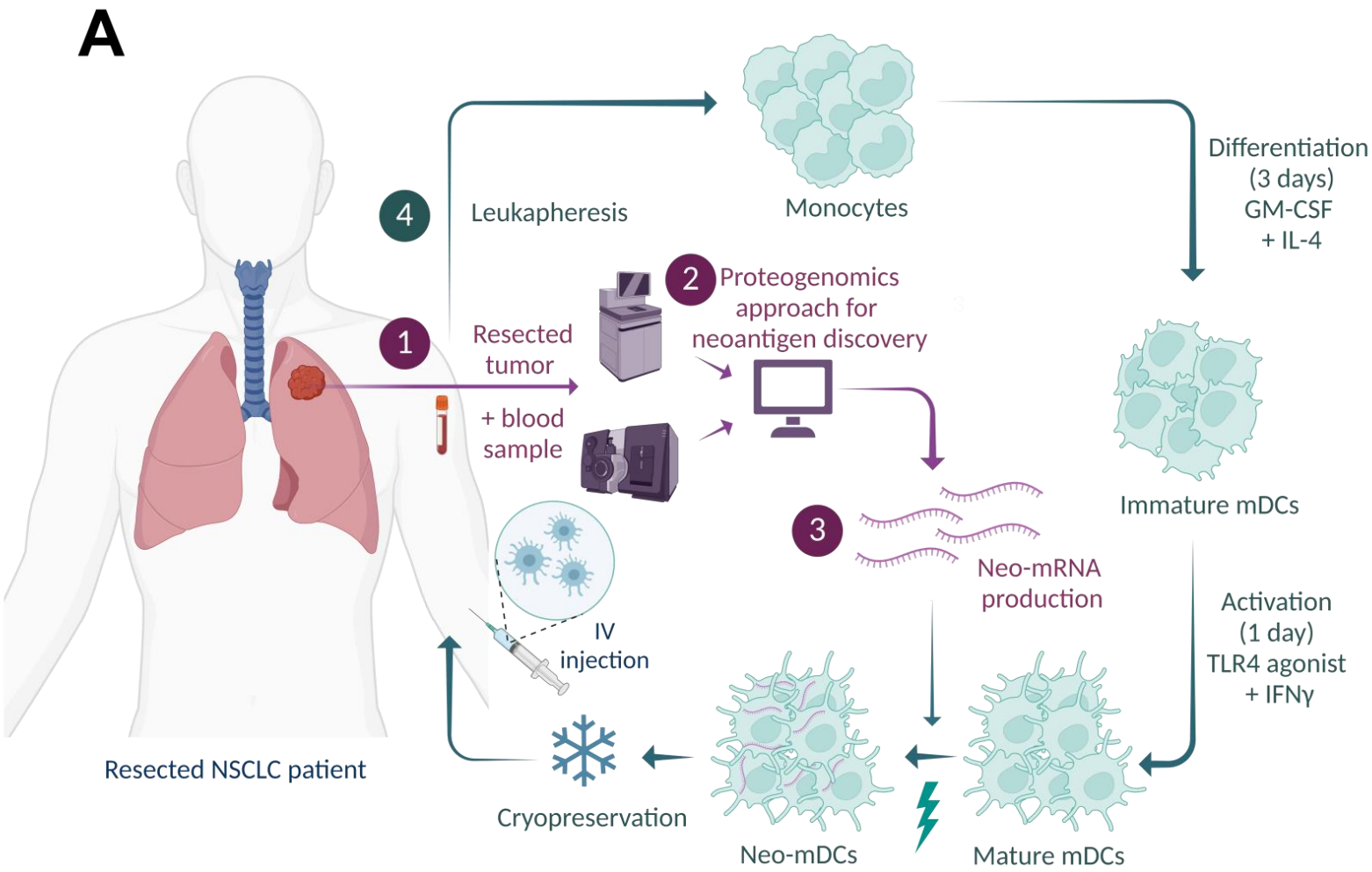
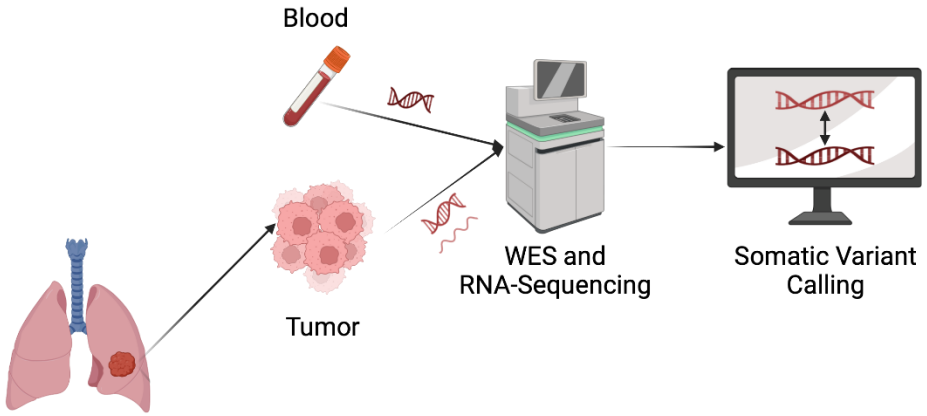
MIDRIX^{NEO} production process

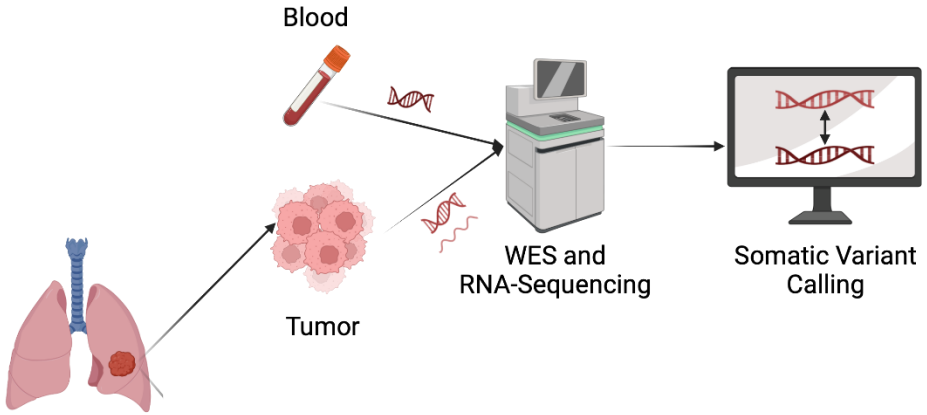


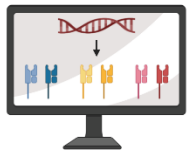
The neoantigen selection process

Laurenz De Cock



HLA typing





Blood sample (DNA)

HLA-A		HLA-B		HLA-C	
A*03:01	A*11:01	B*07:02	B*55:01	C*03:03	C*07:02

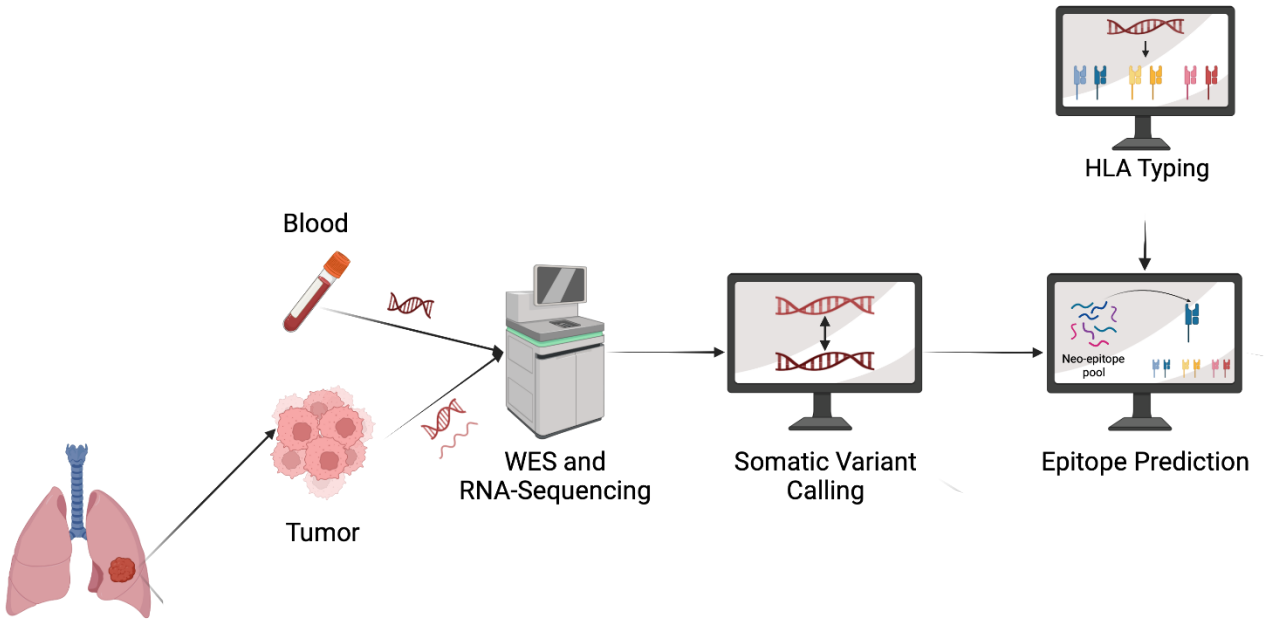
Tumor sample (DNA)

HLA-A		HLA-B		HLA-C	
A*03:01✓	A*11:01✓	B*07:02✓	B*55:01✓	C*07:02✓	C*03:03✓

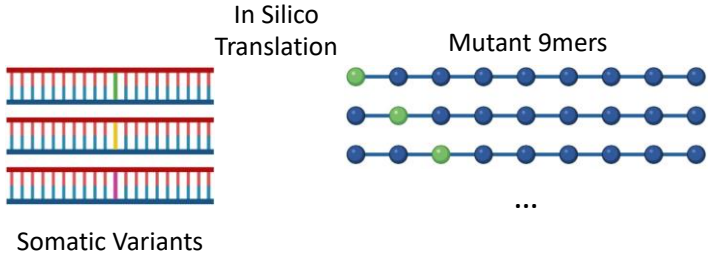
Tumor sample (RNA)

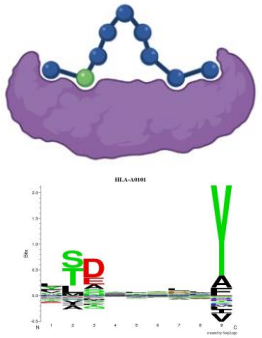
HLA-A		HLA-B		HLA-C	
A*11:01✓	A*03:01✓	B*55:01✓	B*07:02✓	C*07:02✓	C*03:03✓

Epitope prediction



Neural-network based prediction algorithms

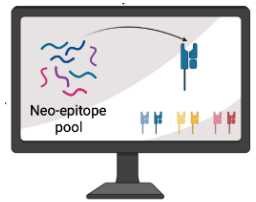




Binding Prediction

Neoantigen selection

Laurenz De Cock



Epitope Prediction

Clonality

Binding Affinity (Rank)

Transcript and Variant Expression

Overlap Immunopeptidomics

Intersection netMHCpan and HLAthena

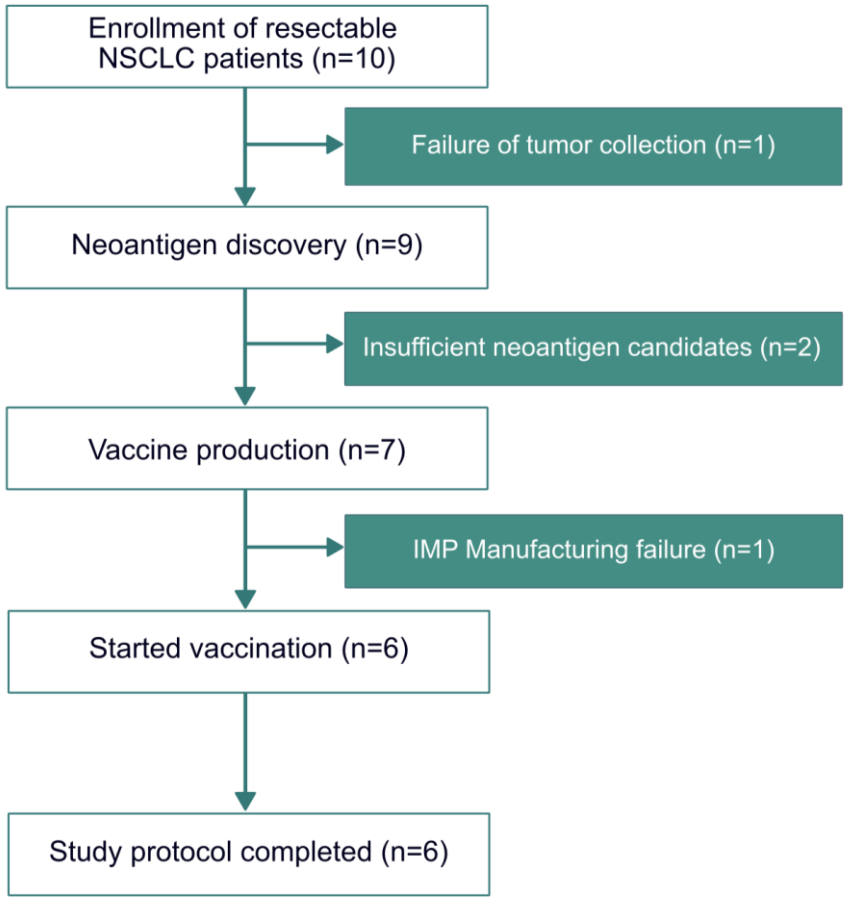
Location in Peptide

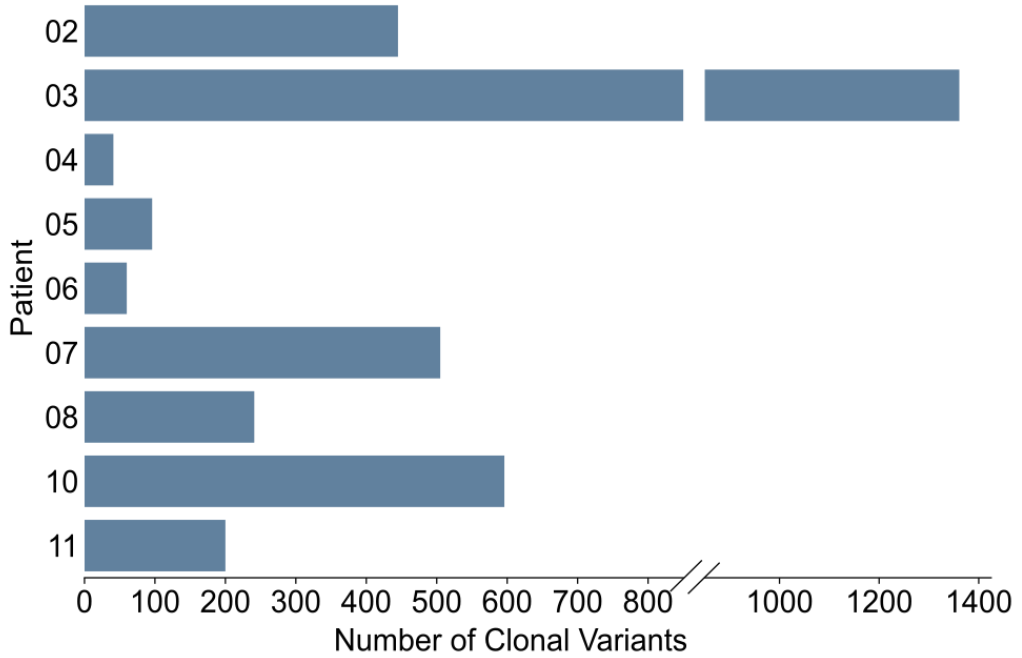
Binding Affinity WT epitope

4 to 6 Selected Neo-epitopes

6 patients were vaccinated and completed the study protocol

D





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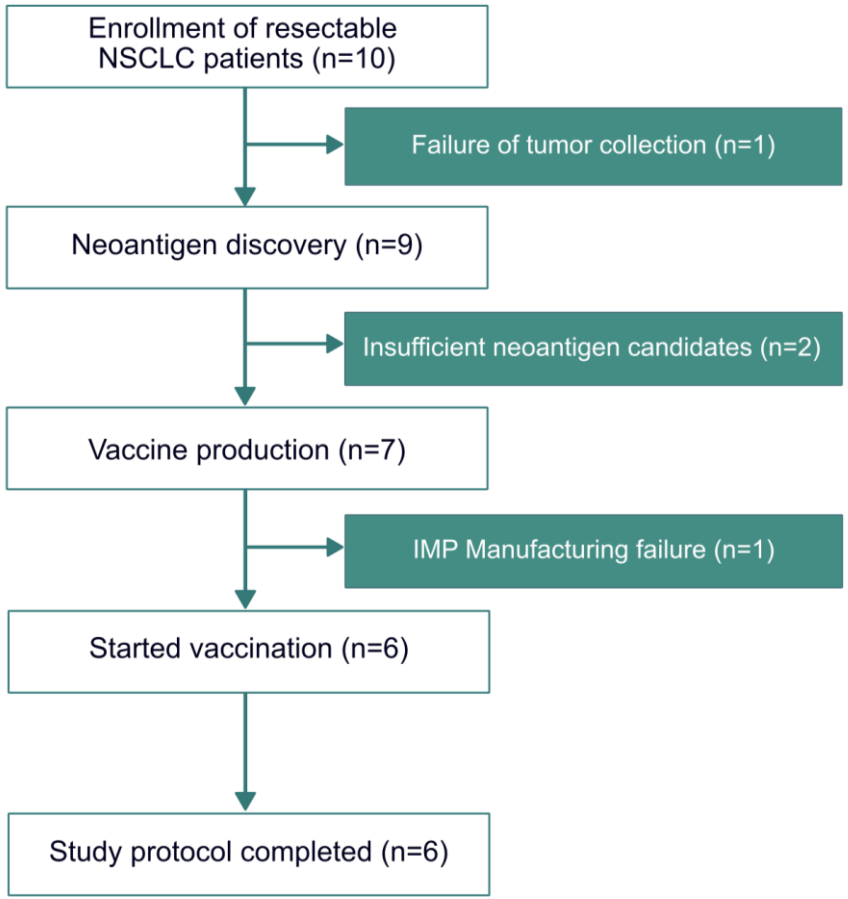

IMP doses administered per patient

	02	03	05	08	10	11
10 x 10^6	x	x	x	x	x	x
20 x 10^6	x	x	x	x	x	x
40 x 10^6	x	x	x	x	x	x
80 x 10^6	x	x	/	x	x	x
100 x 10^6	/	x	/	x	x	/

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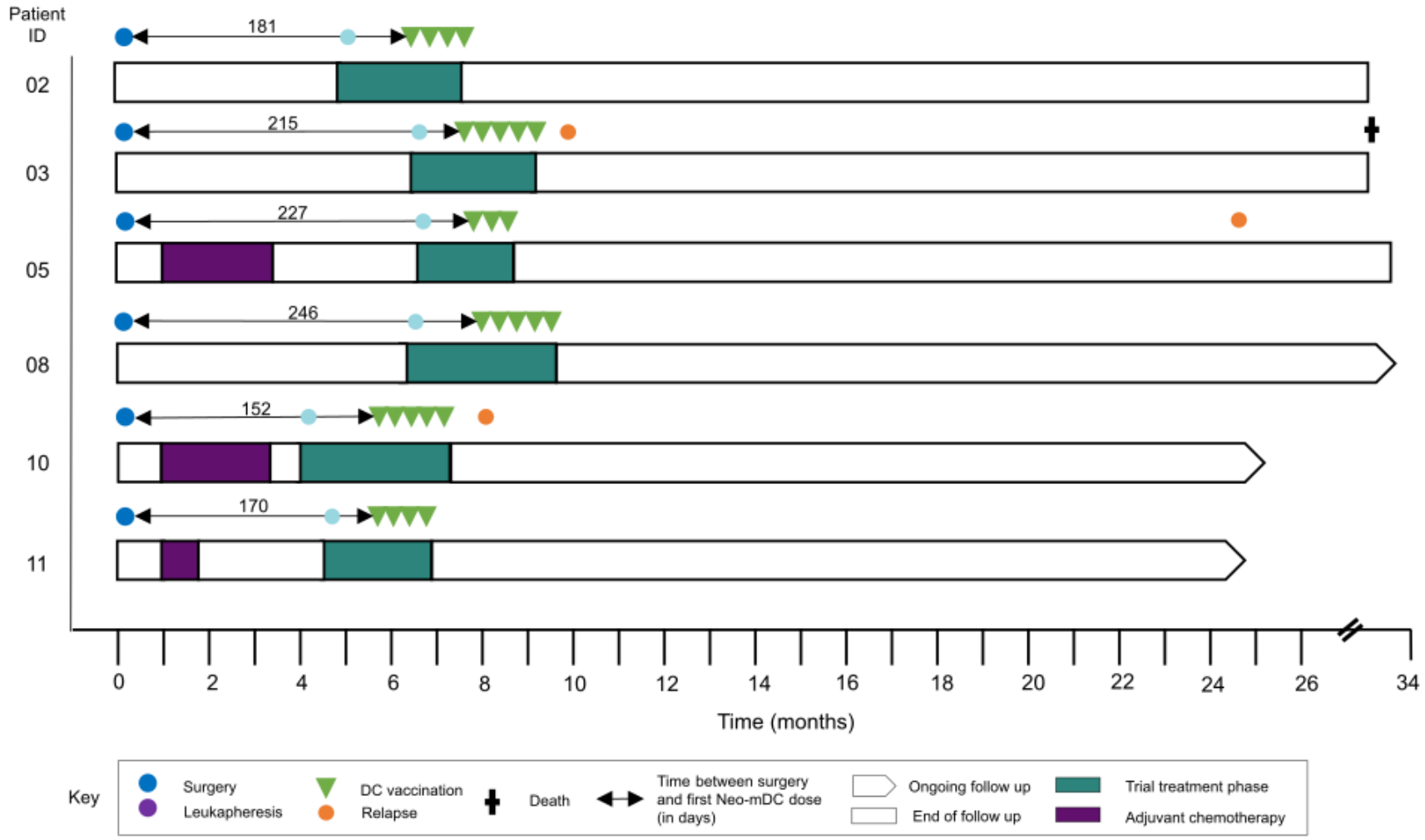
Clinical course of vaccinated patients



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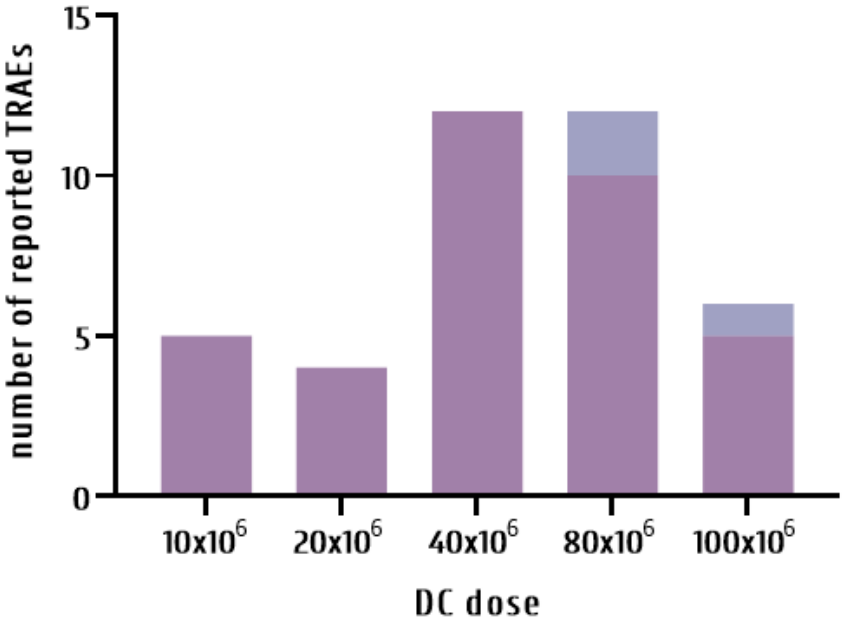
The vaccine was well-tolerated

Dieter Stevens



- No Grade 3-4 events
- Most common TRAEs:
  Flu-like symptoms, fatigue, headache and chills
- Maximum tolerated dose not reached

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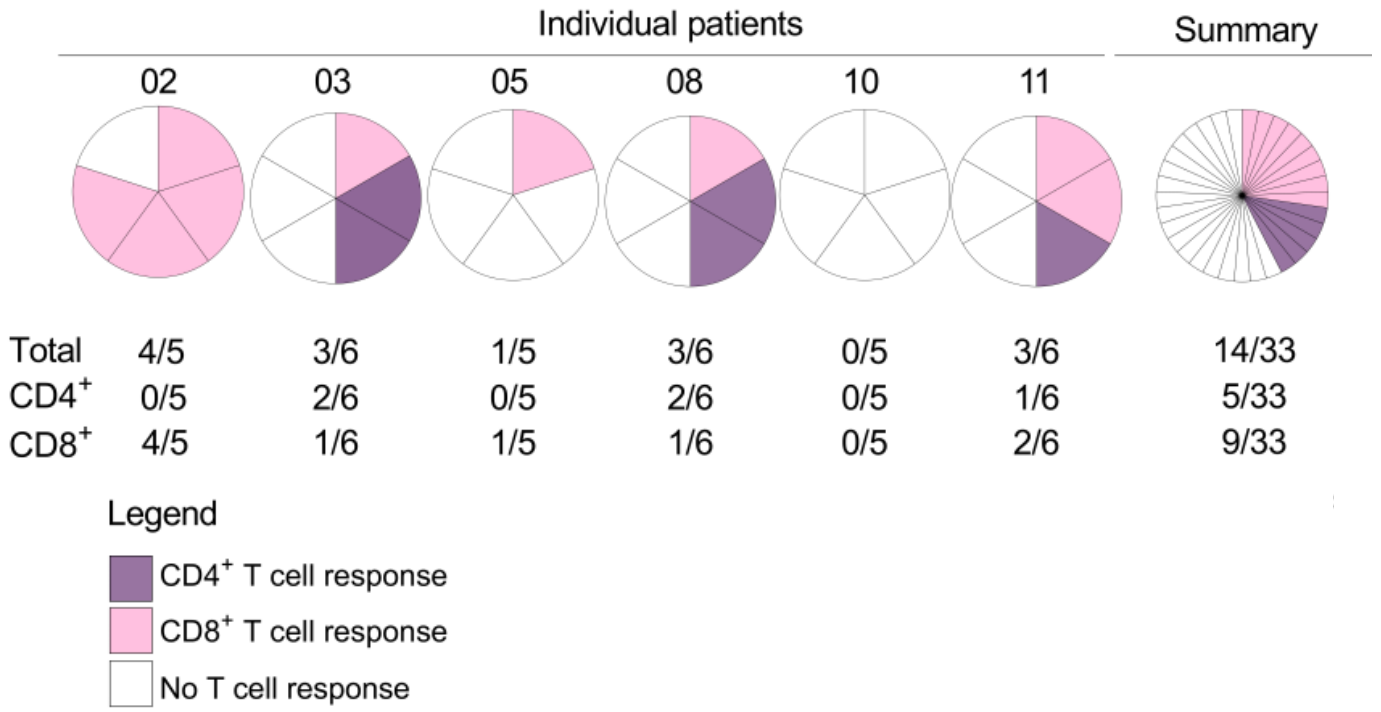
Systemic T cell responses were observed in 5 of 6 vaccinated patients



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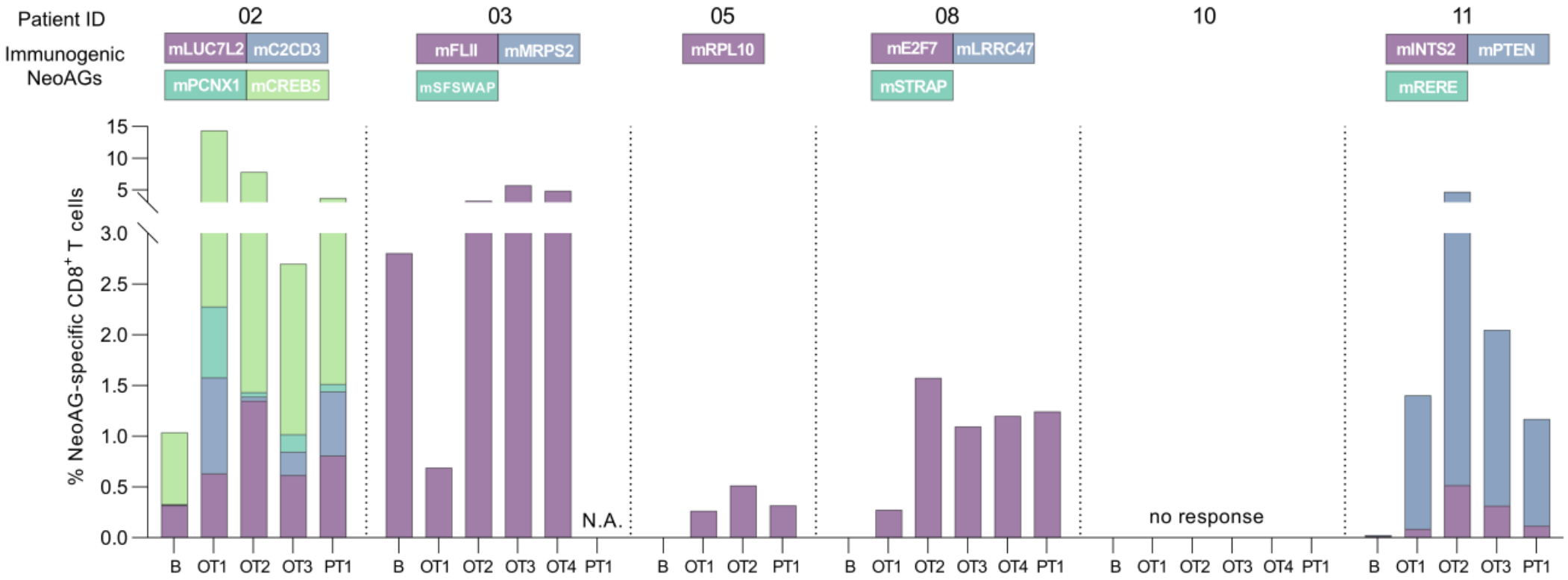
T cell responses were observed after minimal vaccine doses

CD8⁺ T cell responses



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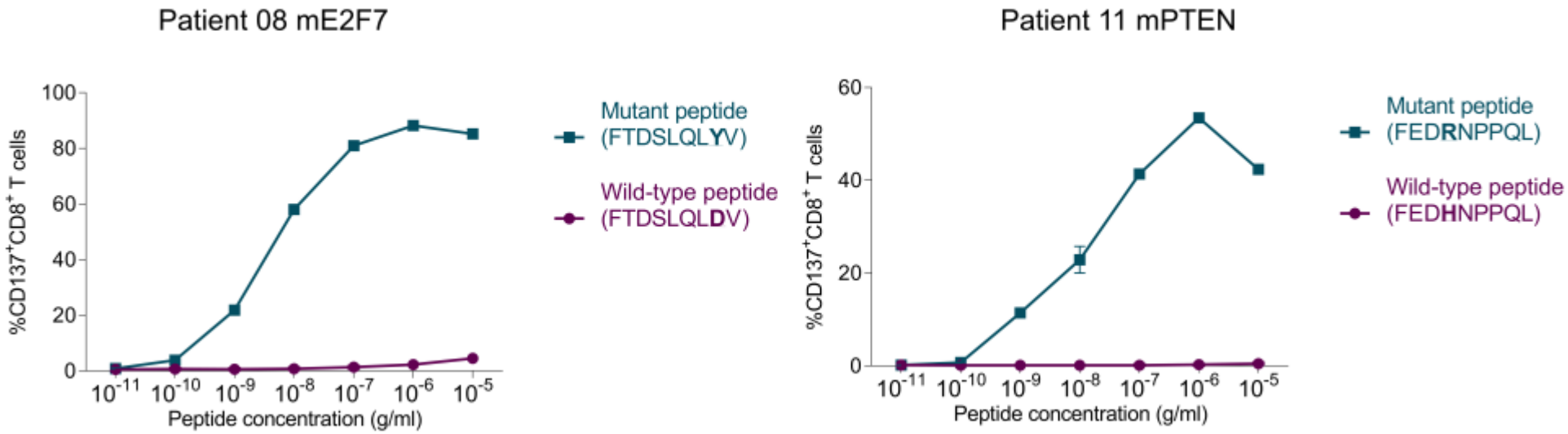
T cells responses are exquisitely specific for the mutant antigens



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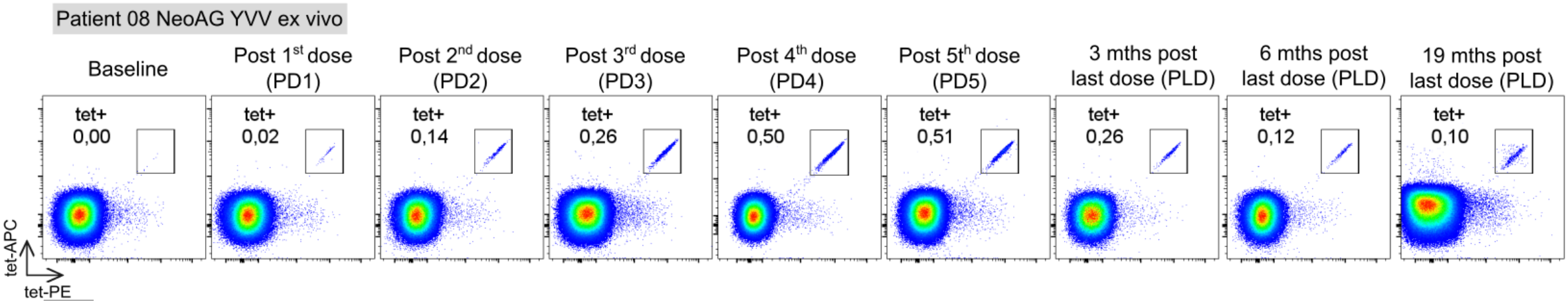
Vaccination induced high frequencies of neoantigen-specific T cells that persist for at least 1.5 year



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Conclusions

- This phase 1a clinical trial in NSCLC patients demonstrates for the first time the **manufacturing feasibility** and **excellent safety profile** of an autologous dendritic cell vaccine targeting patient-individual tumor mutanome-derived neoantigens (Neo-mDC)

- The vaccine could evoke **de novo neoantigen-specific T-cells**, detected systemically already after the **lowest dose level**, and **persisting up to 1.5 years** after the last dose

- MIDRIX^{NEO} is a promising adjuvant therapy for resected, early stage NSCLC patients

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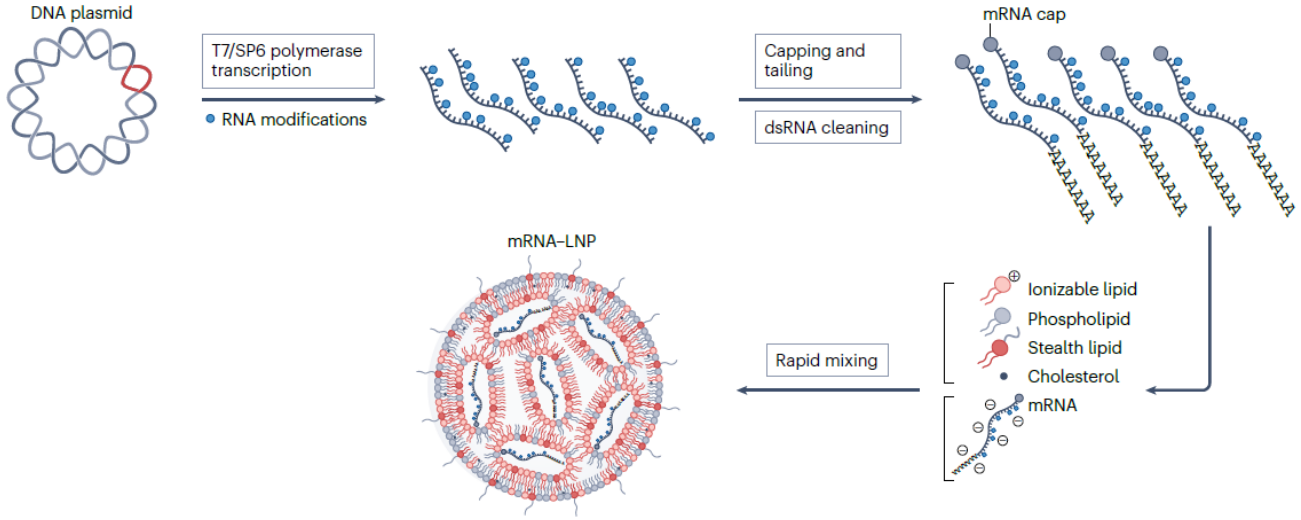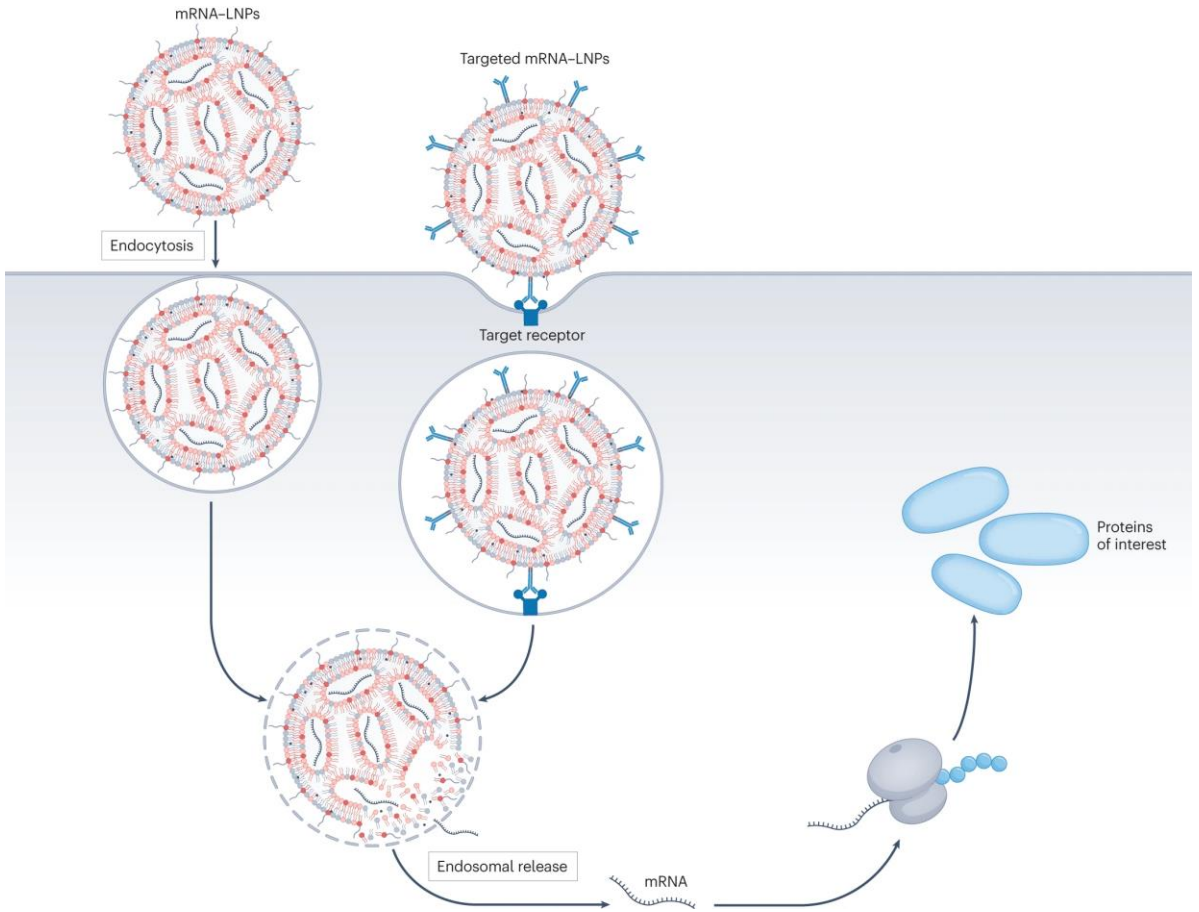
mRNA encapsulated in lipid nanoparticles