14th International Donor Registry Conference & WMDA Meetings
25 – 29 June, 2024 – Cape Town, South Africa
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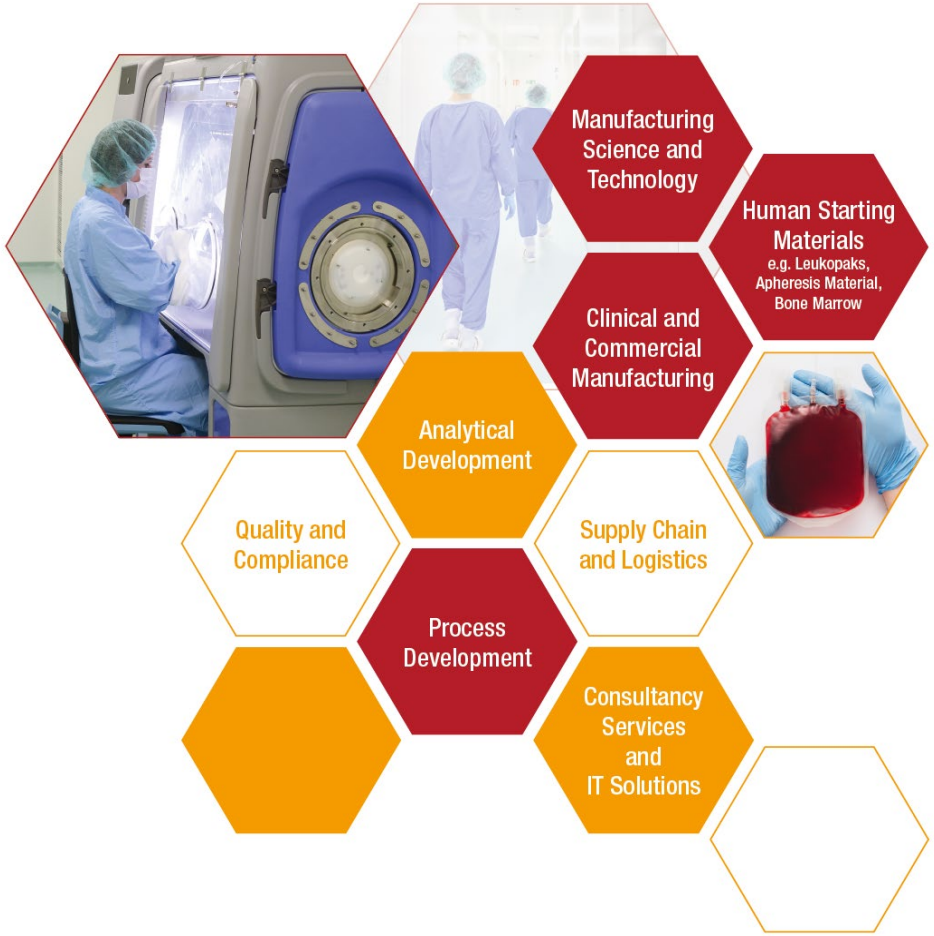
Carla Kreissig

Challenges in allogeneic CAR-T Manufacturing using Viral Vectors and LNPs

Cellex Group



Cellex Medical Services (CMS)

- Starting material supplier
- Cell collections for R&D, clinical trials and commercial use
- Leukopaks, mobilized cells, bone marrow, whole blood
- Small donor data base

Cellex Manufacturing Transports & Logistics (CMT)

- CDMO for clinical and commercial ATMP manufacturing
- Manufacturing license for > 10 ATMPs
- 1300 m² cleanrooms, QC laboratories and cryostorage
- Complex logistics network and adaptable transport solutions

CAR-T cell therapy landscap

Commercial CAR-T cell therapies

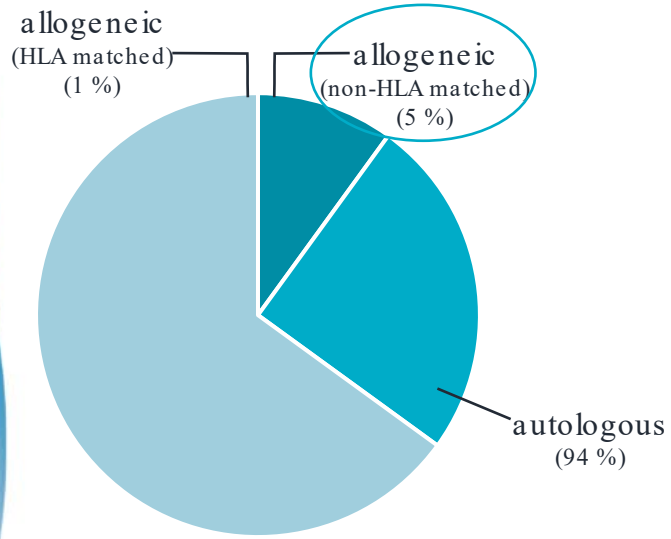
Product	CAR-T therapy	MAH	Target antigen	Indication	FDA / EMA approval	Approach
Kymriah™	Tisagenlecleucel	Novartis	CD19	r/r ALL, r/r DLBCL	2017 / 2018	Autologous
Yescarta™	Axicabtagene ciloleucel	Kite Pharma	CD19	certain types of r/r LBCL	2017 / 2018	Autologous
Tecartus™	Brexucabtagene autoleucel	Kite Pharma	CD19	r/r MCL[1)], rr ALL[2)]	2020 / 2021	Autologous
Breyanzi™	Lisocabtagene maraleucel	BMS	CD19	r/r LBCL	2021 / 2022	Autologous
Abecma™	Idecabtagene vicleucel	BMS	BCMA	r/r multiple myeloma	2021 / 2021	Autologous
Carvykti™	Ciltacabtagene autoleucel	Janssen-Cilag	BCMA	Multiple myeloma	2022 / 2022	Autologous

r/r = relapsed/refractory; ALL = acute lymphatic leukemia; (D)LBCL = (diffuse) large B cell lymphoma; MCL = mantle-cell lymphoma

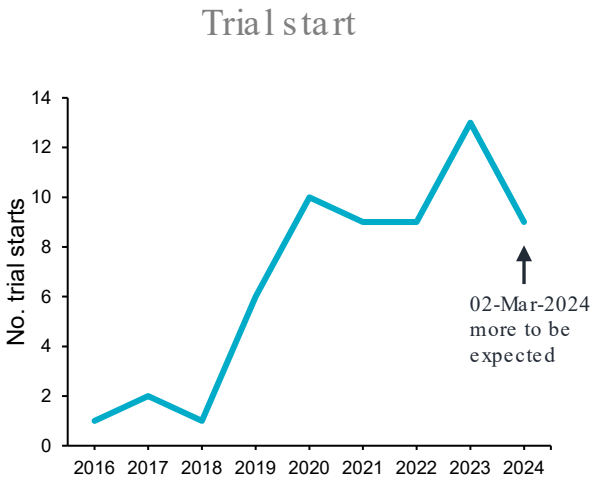
- All commercial CAR-T cell therapies are autologous approaches
- All commercial CAR-T cell therapies directed to treat r/r haematological malignancies
- List prices (WACs, USD, march 2023): $ 424,000 - $ 544,000

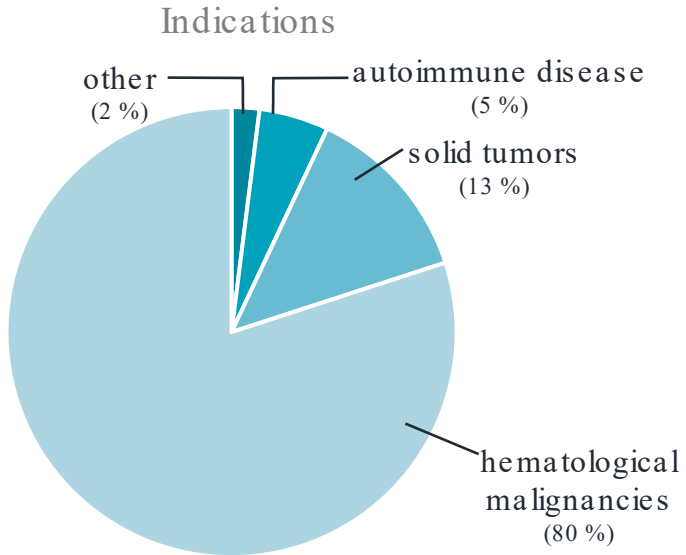
CAR-T cell therapy landscape

Interventional clinical trials*



Allogeneic CAR-T (non-HLA matched)

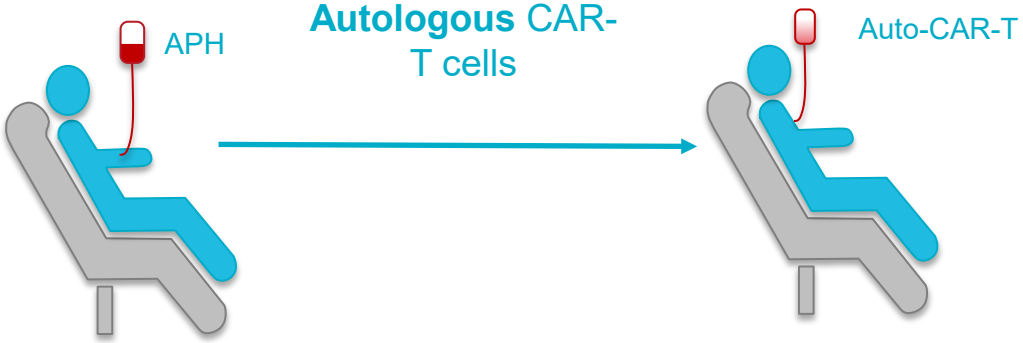




- Field of allogeneic CAR-T therapies is rapidly growing, main indications so far hematological malignancies

*(clinicaltrials.gov, 02-Mar-2024)

Autologous CAR-T cell therapy

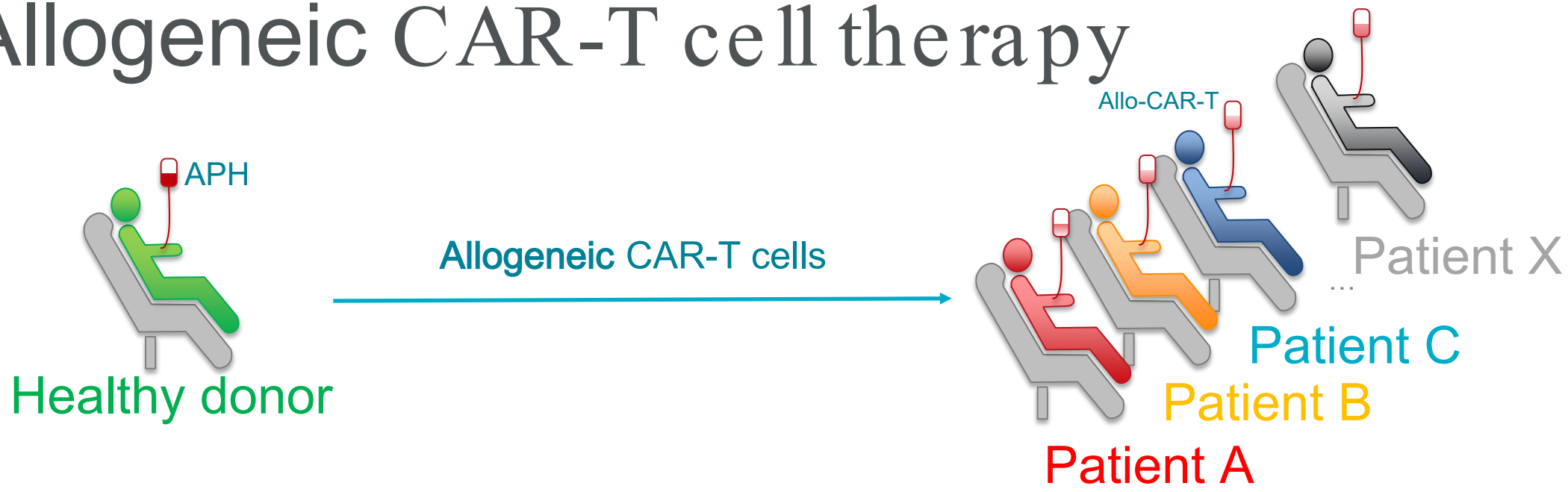


- Manufacturing of CAR-T cells for autologous treatment uses patient starting material
- Production of individual drug products:
  a single cell donation/a single production is used to treat one patient

- Pro:
  - Availability of starting material (high motivation for donation)
  - No graft versus host (GvHD) or host versus graft reaction (allo-rejection)
  - Moderate genetic modification needed

- Contra:
  - Apheresis of patients needed
  - Potentially impaired cellular fitness
  - Time-consuming production, delay in treatment
  - High costs per patient

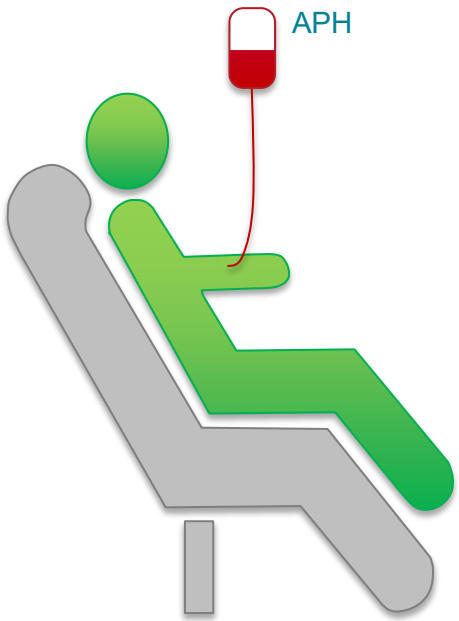
Allogeneic CAR-T cell therapy



- Manufacturing of CAR-T cells for allogeneic treatment uses healthy donor starting material
- Production of Off-the-shelf drug products:
- a single cell donation/ a single production may be used to treat several patients

- Pro:
  - No need for patient apheresis
  - Improved cellular fitness
  - Immediate treatment of patients possible
  - Reduced production cost per patient
- Contra:
  - Suitable donor needed
  - Extensive genetic modification, possibly cell depletion needed
  - Logistics and storage space required

Challenges – Starting Material

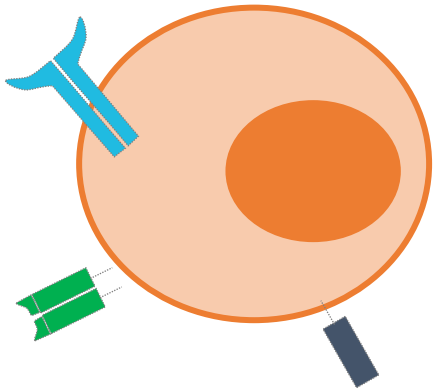


Healthy donor

- Donor selection criteria: HLA, KIR epitopes, sex, age, nationality/location,…
- Donor availability might be strongly reduced depending on choosen donor selection criteria!
  - RnD donation databanks not sufficient, need for cooperation with bone marrow donation registries
- GMP compliant collection required
- Time-consuming donor search, pre-screening and donation coordination
  - Decoupling of donation and production by establishing production from frozen intermediate

Challenges - Production

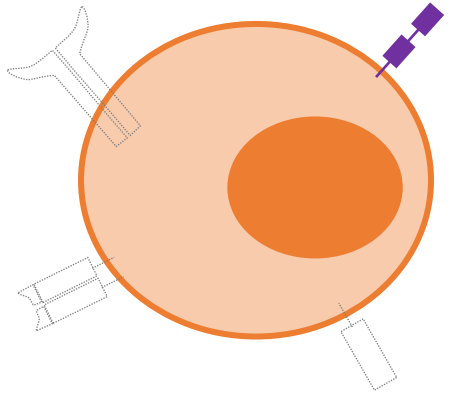
Extensive genetic modifications needed to prevent GvHD and allo-rejection



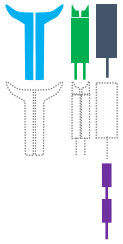
Healthy donor T cell

Genetic modifications

1) Transduction with CAR
2) Deletion of immune rejection relevant proteins



CAR T cell
for allogeneic infusion

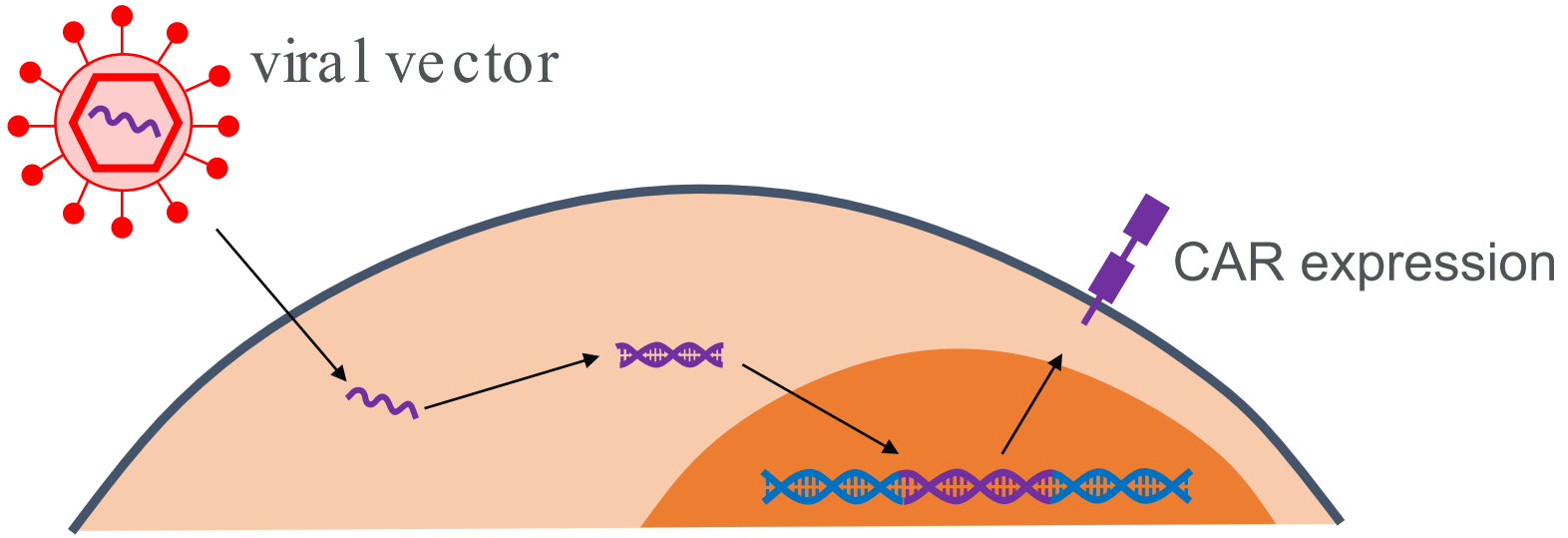


Expressed rejection relevant surface proteins

Deleted rejection relevant surface proteins

CAR

Challenges – Genetic modifications



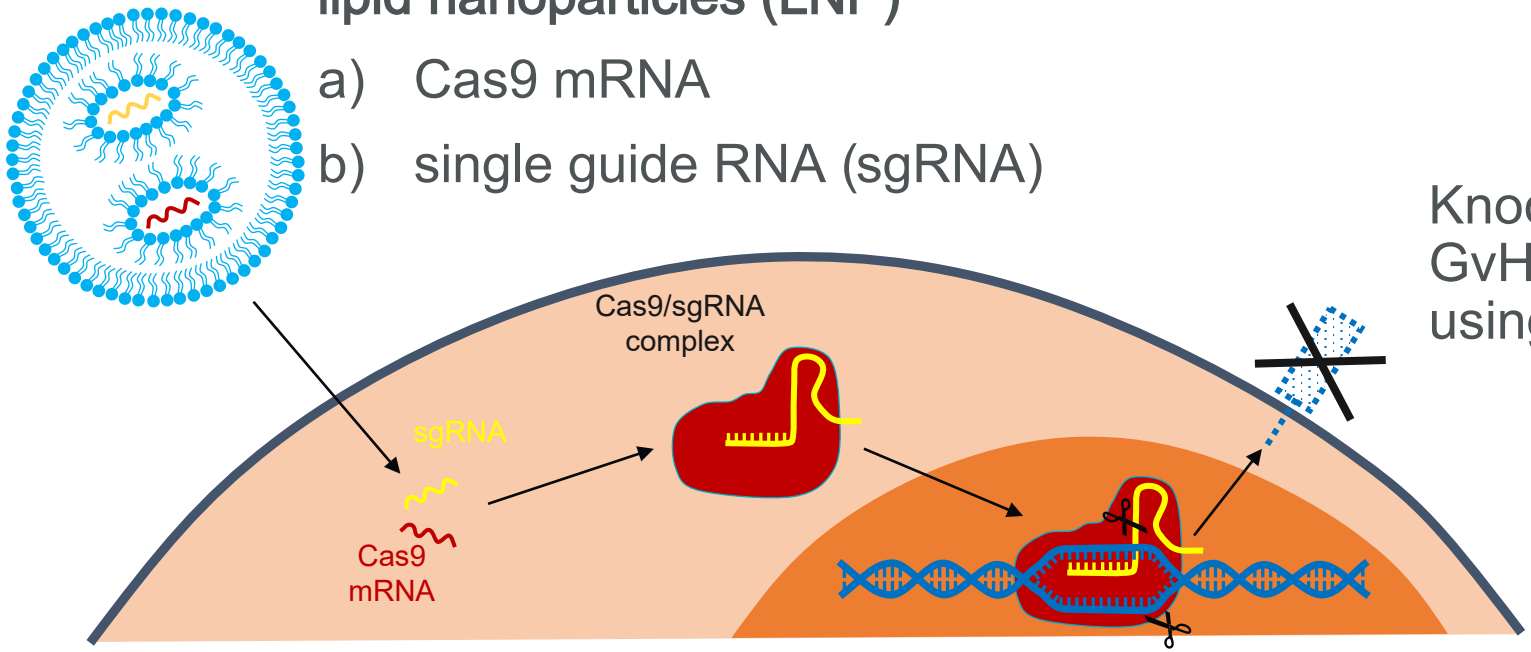
Challenges identical to autologous CAR T cell production
(viral vector system, transduction efficiency, VCN,…)

Challenges – Genetic modifications

lipid nanoparticles (LNP)

a) Cas9 mRNA

b) single guide RNA (sgRNA)



Knockout of different rejection/ GvHD relevant surface proteins using different LNPs

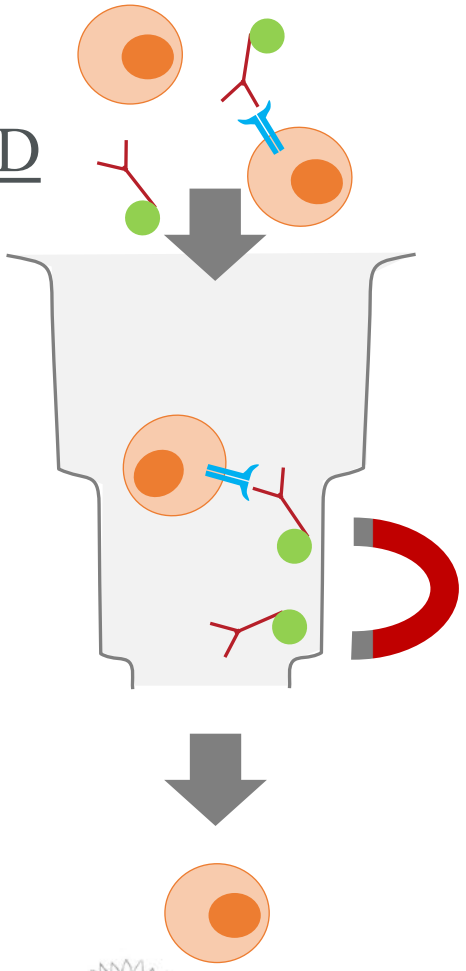
- Multiple edits might be required
- Main Challenges:
  - increase knockout efficiency / limit residual surface molecule expression
  - preserving high viability and strong expansion
  - limiting genotoxicity

Challenges - Depletion

Depletion of cells not genetically modified in order to reduce GvHD

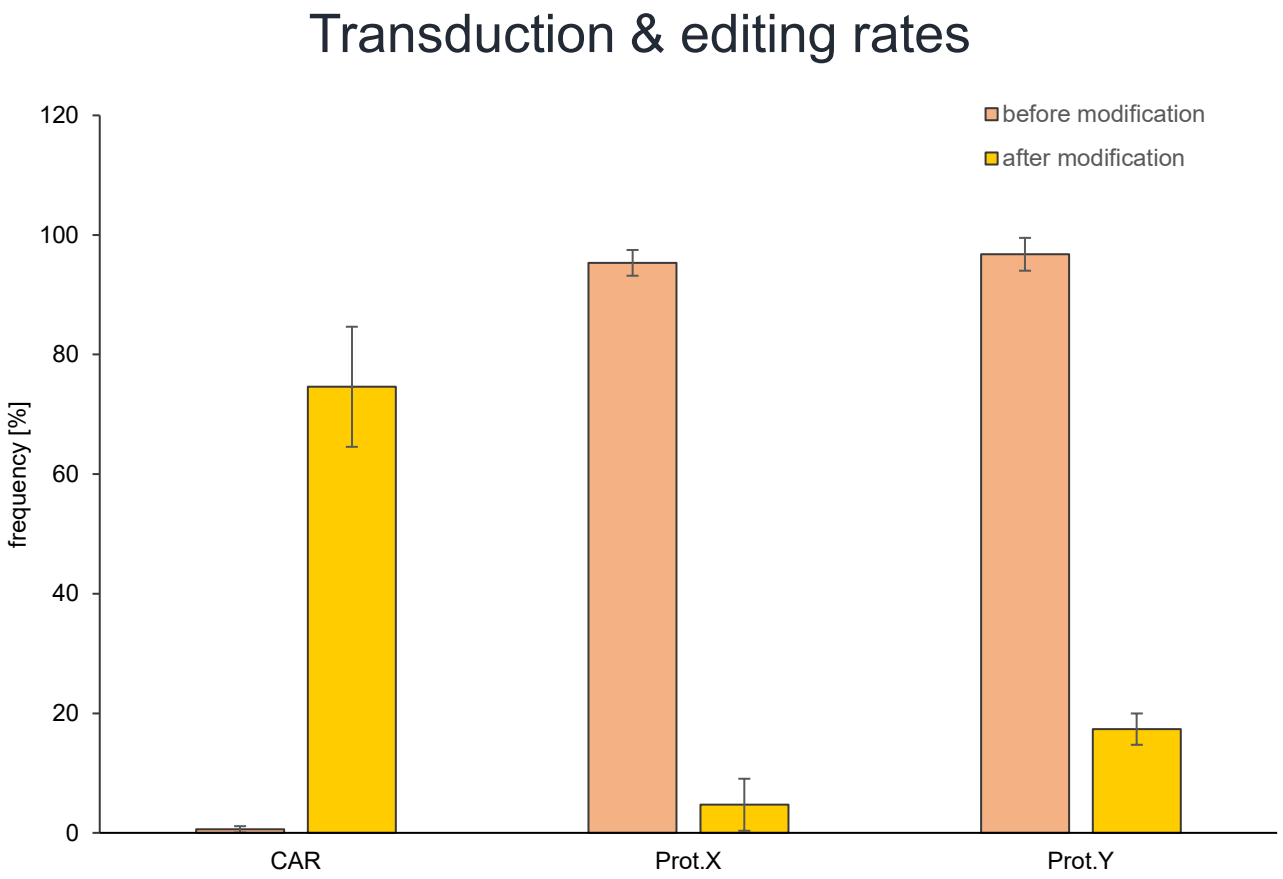
- Establish depletion process for genetically modified cells
  - Depletion target?
  - Depletion time point?
  - Depletion efficiency?

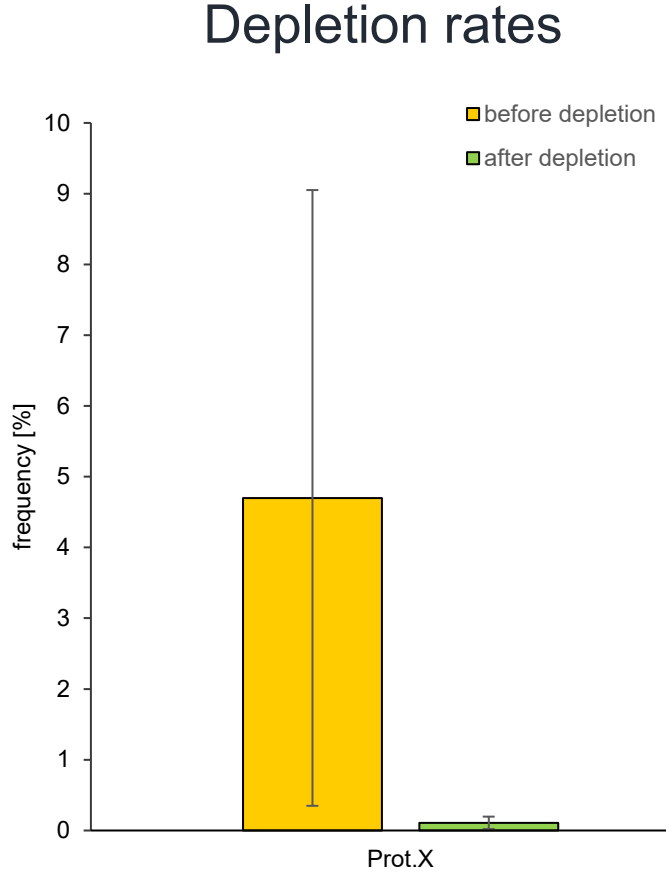
- Increase depletion efficiency while minimizing cell loss!



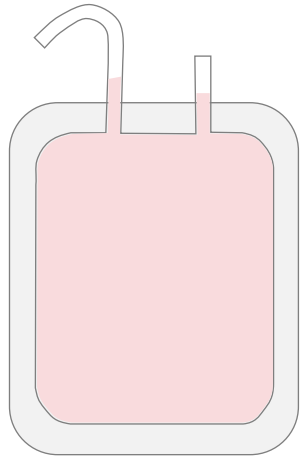
Transduction, editing and depletion rates

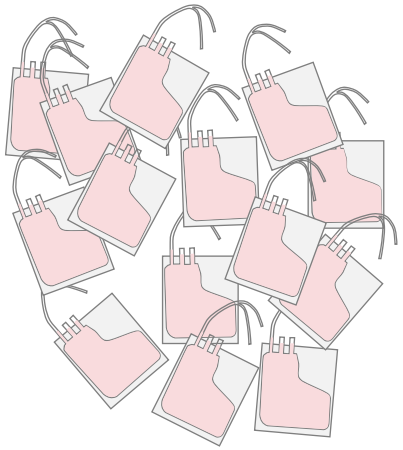
Case study CMT allogeneic CAR-T project





Challenges – Fill & Finish





<u>Allogeneic CAR-T cell production aims at maximizing batch size</u>

- Cryopreserved drug products:
- Limited time for fill & finish due to limited DMSO contact time
- Fast fill & finish solutions needed to increase number of products
- Manual fill & finish vs. automated solutions
- Feasibility will depend on
  - volumes to be filled and containers (bags, vials,…)
  - sensitivity of the final product

Challenges – Quality control

Establishment and validation of assays to analyze critical quality attributes

Testing autologous CAR-T cells

Critical quality attribute	Analysis of
Safety	Sterility
	Mycroplasma
	Endotoxin
	Vextor copy number
Content	T cell content
	CAR-T cell content
Purity	Contaminating cell content
Potency	Cytoxicity
	Cytokine release

For allogeneic CAR-T cells, further assays have to be established and validated:

- Residual expression of edited proteins
- Translocation analysis
- Karyotype analysis
- Genotoxicity analysis
- …

Challenges – Logistics

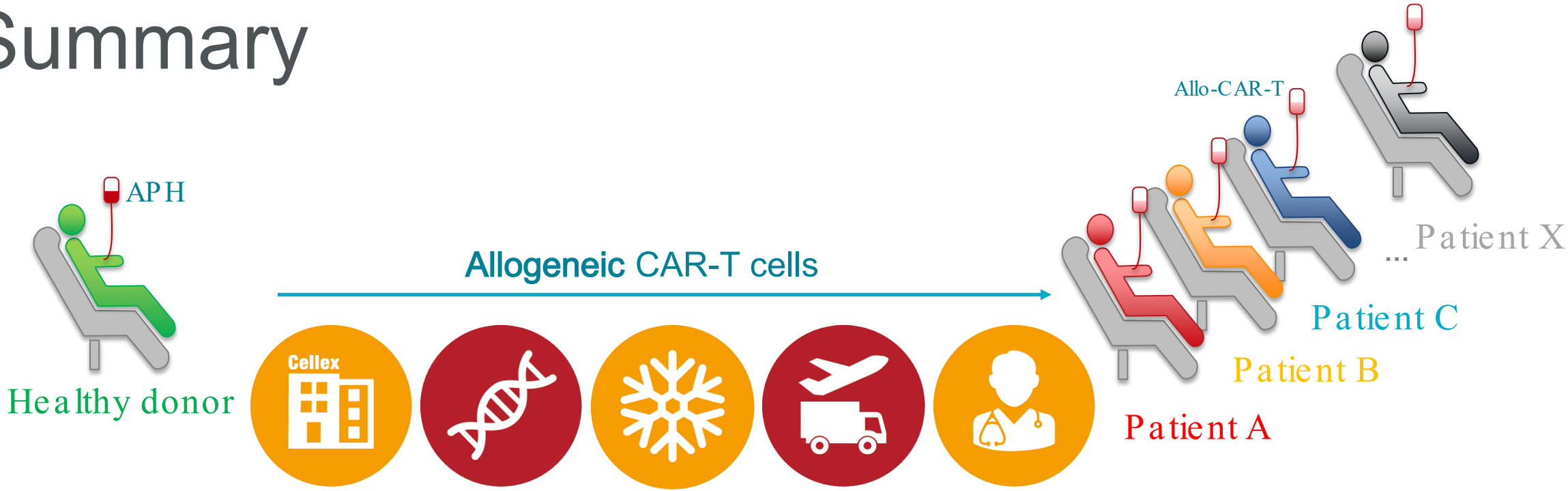
1) Cryostorage

- Allogeneic CAR-T cell production aims at maximizing batch size
- Need for increased cryostorage capacity

2) Patient supply

- As off-the-shelf therapy, patient treatment coordination and product delivery need to be fast and efficient
- Logistics for (worldwide) transport of cryopreserved drug product transport, coordination with trial sites and principal investigators

Summary



- Allogeneic CAR-T cells as promising and fast growing therapy approaches
- Successful establishment of allogeneic CAR-T cell therapy is associated with considerable challenges, such as starting material, production, quality control, storage as well as logistics

Thank you.

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