Federal Institute for Vaccines
and Biomedicines

Paul-Ehrlich-Institut

The gene therapy boom: from viral vectors and cell targeting to lipid nanoparticles

Christian J Buchholz

8th International Symposium on Phospholipids in Pharmaceutical Research, Heidelberg, 9.9.2024

Disclaimer

The views expressed in this presentation are the personal views of the presenter. They shall not be understood or cited as opinions of the Paul-Ehrlich-Institut. The presenter has not received any funding or grants from companies or from associations representing companies.

Disclosures

All past and ongoing research activities are supported by institutional or competitive grants.
No funding from pharmaceutical industry.

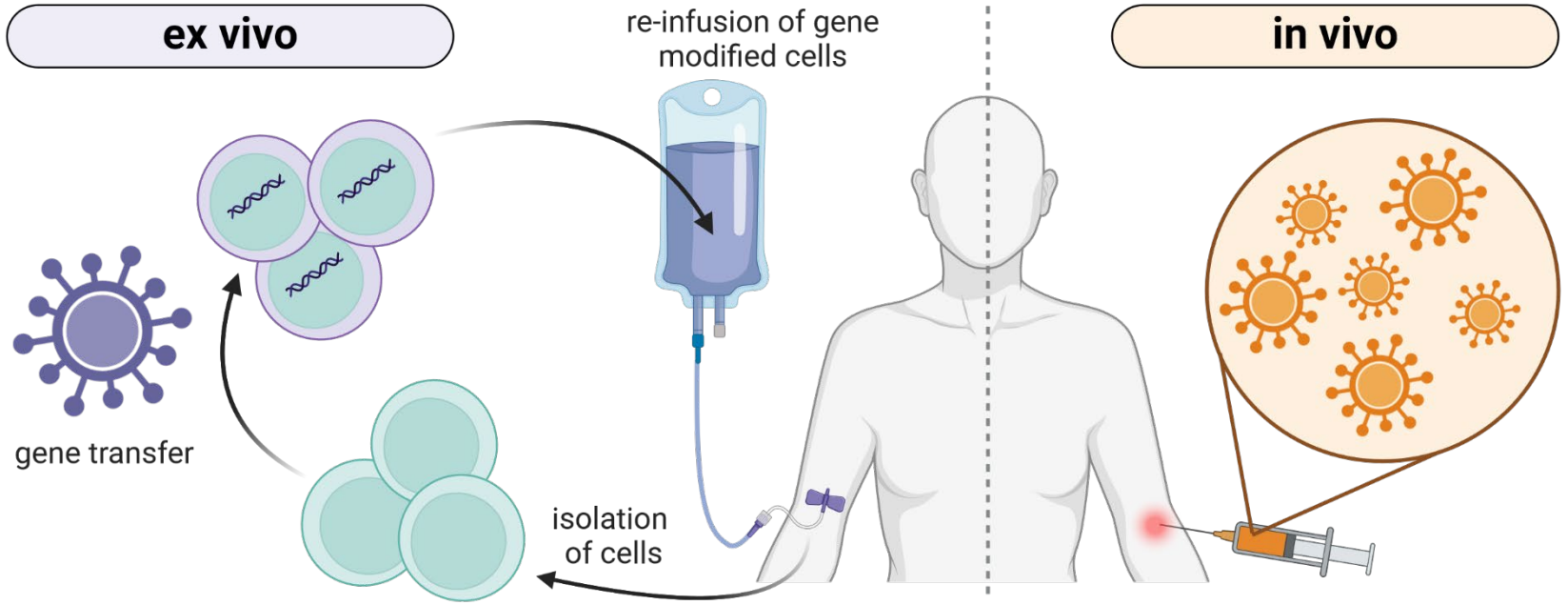
Inventor on filed and granted patents.

Paul-Ehrlich-Institut:
Federal Institute for Vaccines and Biomedicals

- Marketing Authorisation
- Approval of Clinical Trials
- Pharmacovigilance
- Inspections
- Batch release
- Research in related areas

- **Intro to gene therapy and vectors**
- **CAR T cells generated directly in vivo**
- **DARPins and DART-AAVs**
  (designed ankyrin repeat protein targeted)
- **RNA-LNPs**

Gene therapy medicinal products on the Market (US/EU)



Tecartus	CAR-T (MCL)
Yescarta	CAR-T (NHL)
Kymriah	CAR-T (ALL, NHL)
Abecma	CAR-T (MM)
Breyanzi	CAR-T (NHL)
Carvykti	CAR-T (MM)

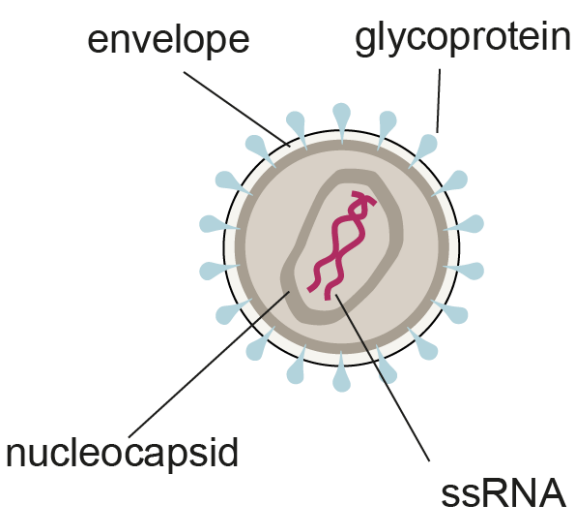
Libmeldy	CD34+ cells (MLD)
Strimvelis	CD34+ cells (ADA-SCID)
Casgevy	CD34+ cells (b-THAL, SCD)
Zynteglo*	CD34+ cells (b-THAL)
Skysone*	CD34+ cells (CALD)

Luxturna	AAV (RP, LCA), eye
Zolgensma	AAV (SMA), motoneurons
Upstaza	AAV (AADC), CNS
Roctavian	AAV (Hem A), liver
Hemgenix	AAV (Hem B), liver
Imlygic	Oncolytic herpesvirus, melanom
Beqvez	AAV (Hem B), liver
Durveqtix	AAV (Hem B), liver

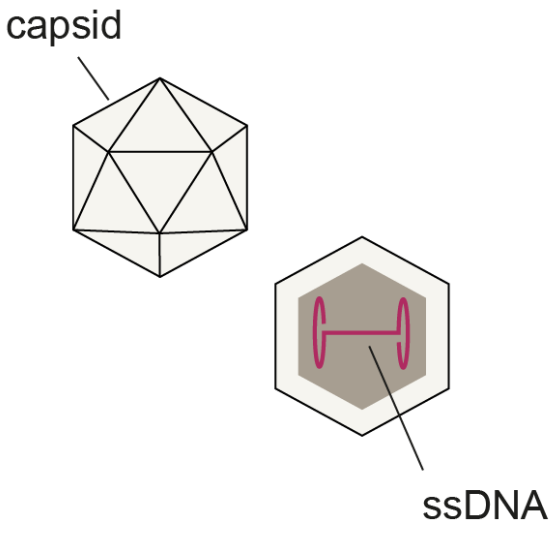
* withdrawn in EU

Gene transfer vectors

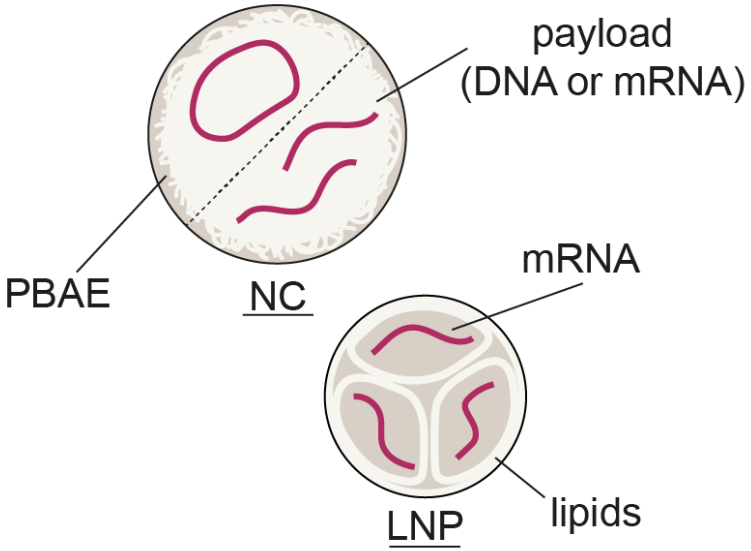
Lentiviral vector



AAV vector

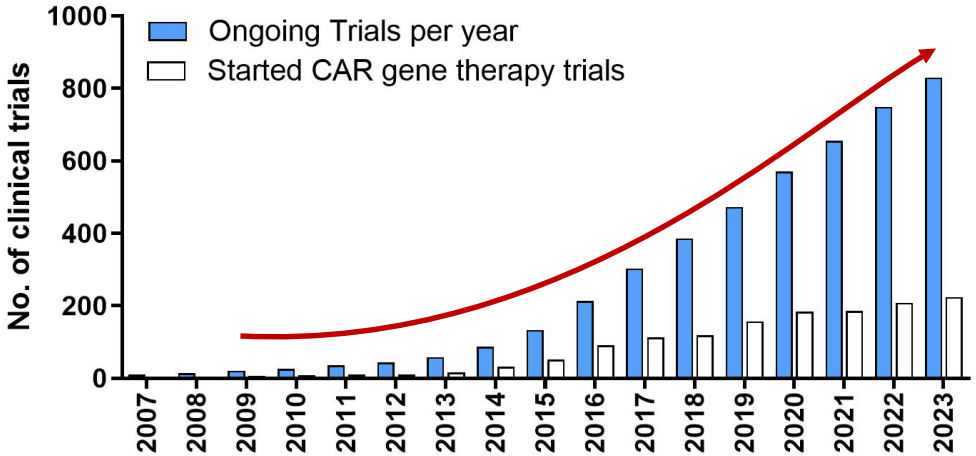


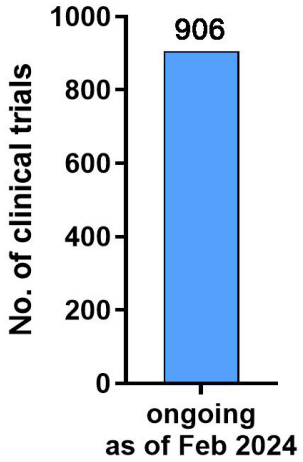
Nonviral vector

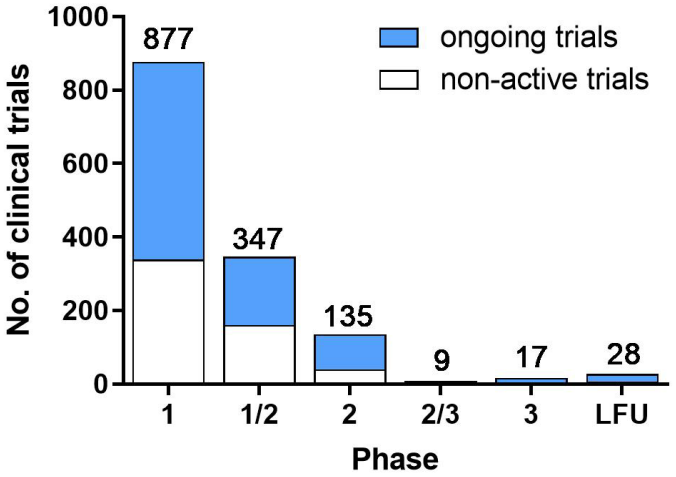


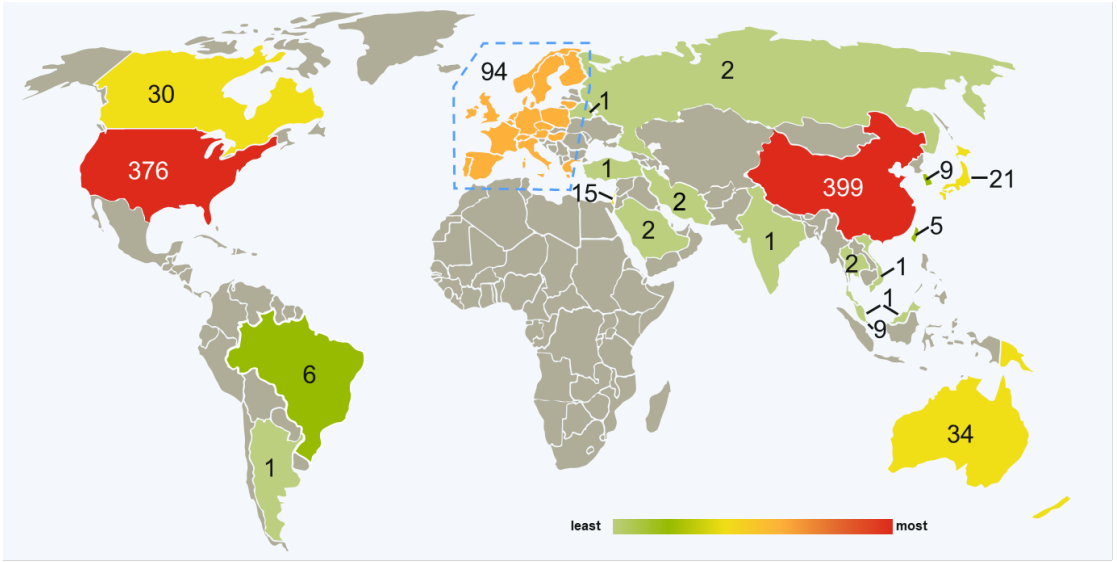
Protein expression	permanent	Transient/permanent	transient
Target cells	Stem cells/mit. active	Differentiated cells	Immunsystem, others
Applications	genetic disease/cancer	genetic disease	immunotherapy, genetic disease
Manufacturing	Biological	Biological	Synthetic

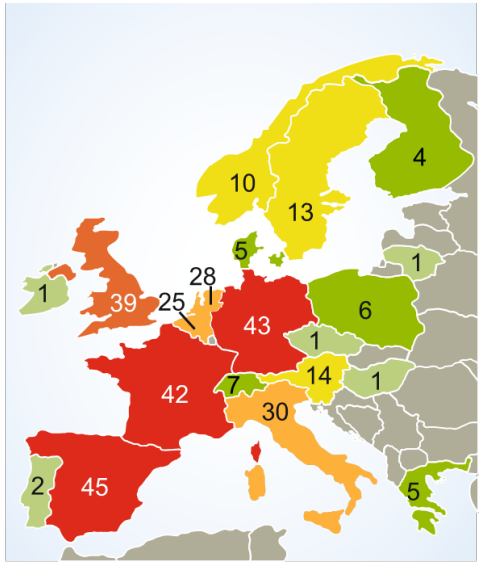
CAR T cell studies world-wide





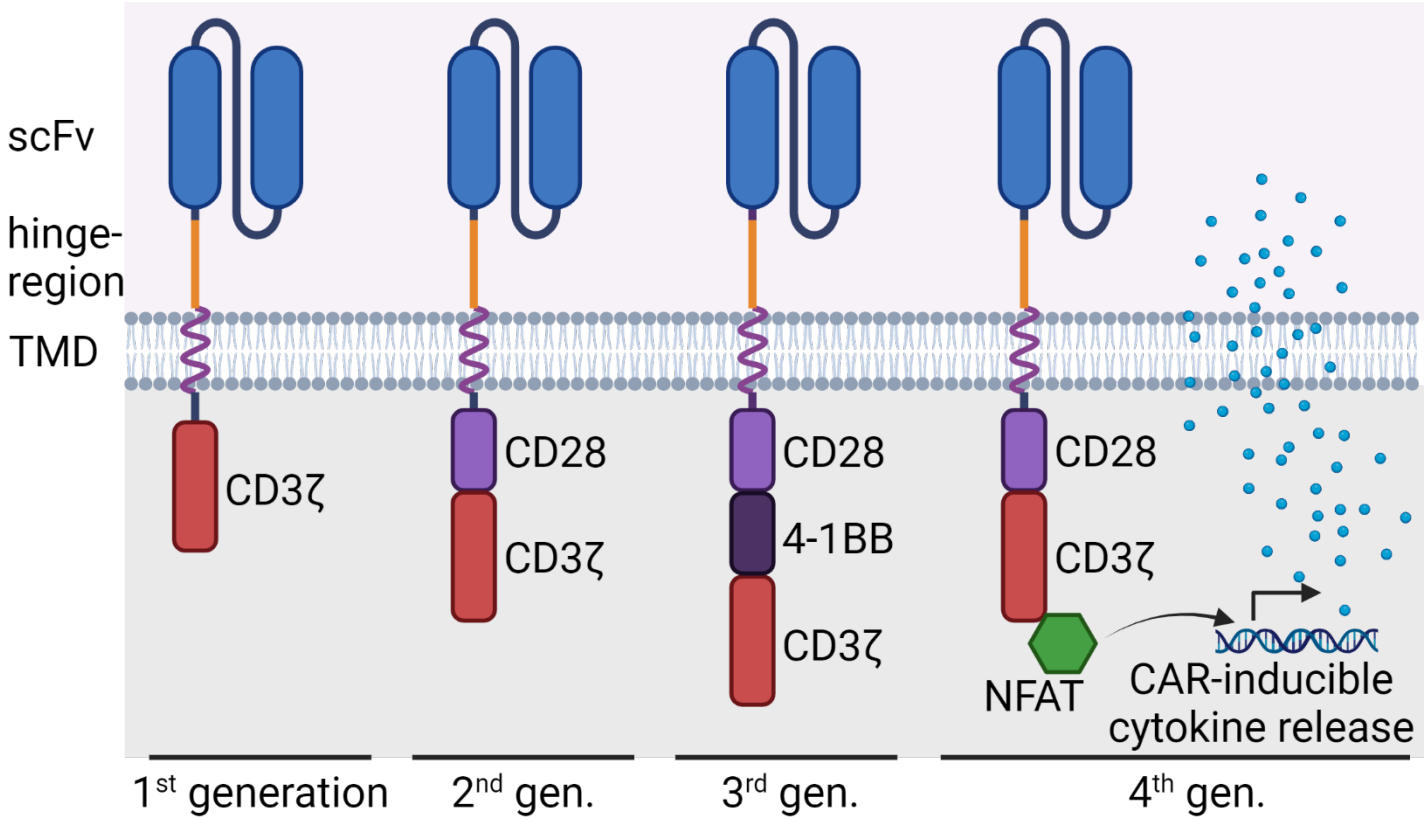


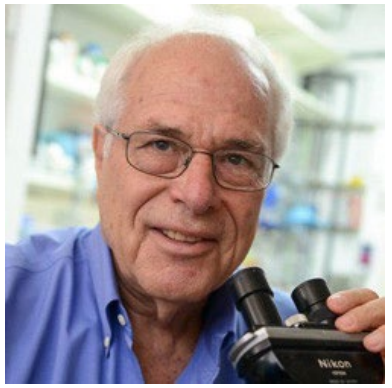




Adapted from Hartmann et al 2017
Michels et al., 2020

Chimeric antigen receptors (CARs)

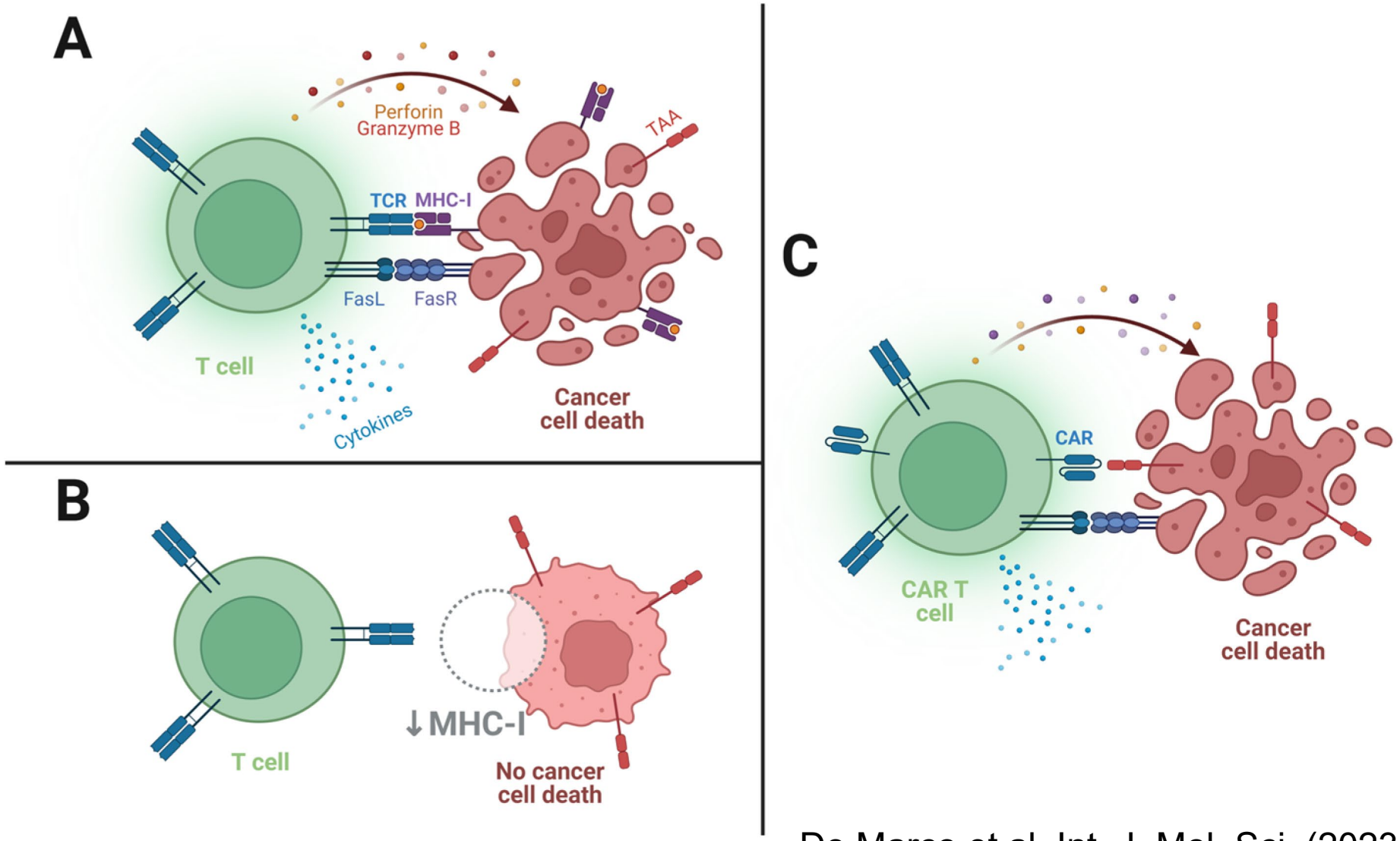




Zelig Eshhar

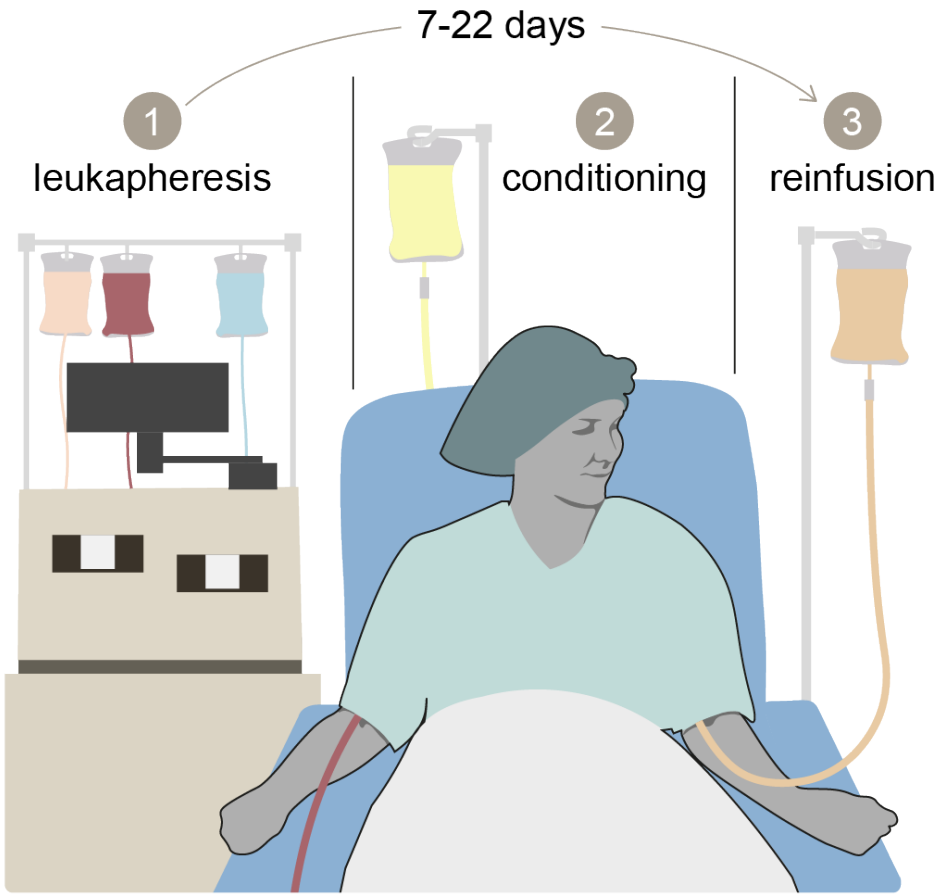
Carl June

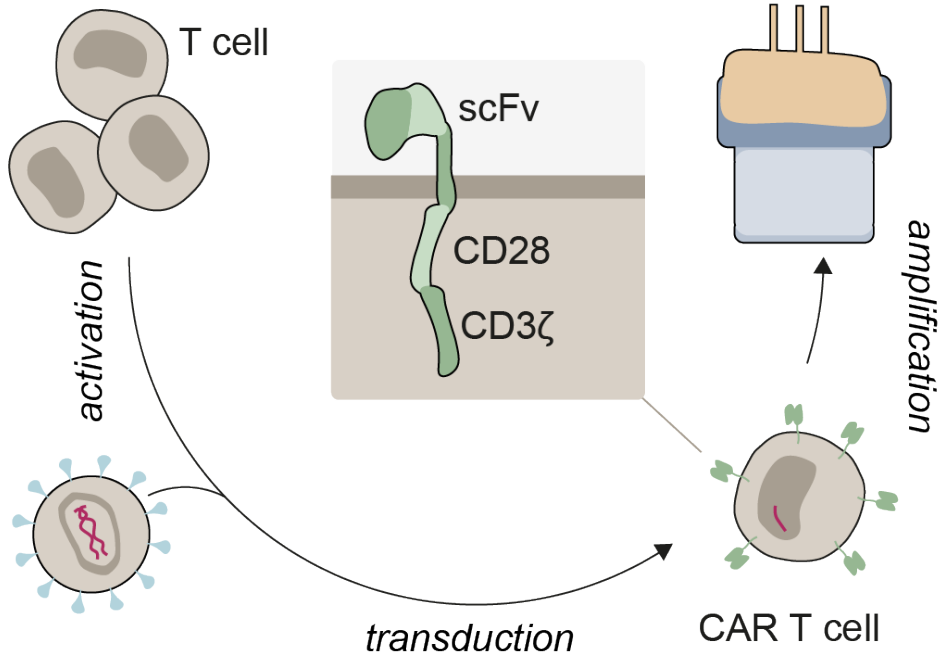
CAR mediated killing of tumor cells



De Marco et al. Int. J. Mol. Sci. (2023)

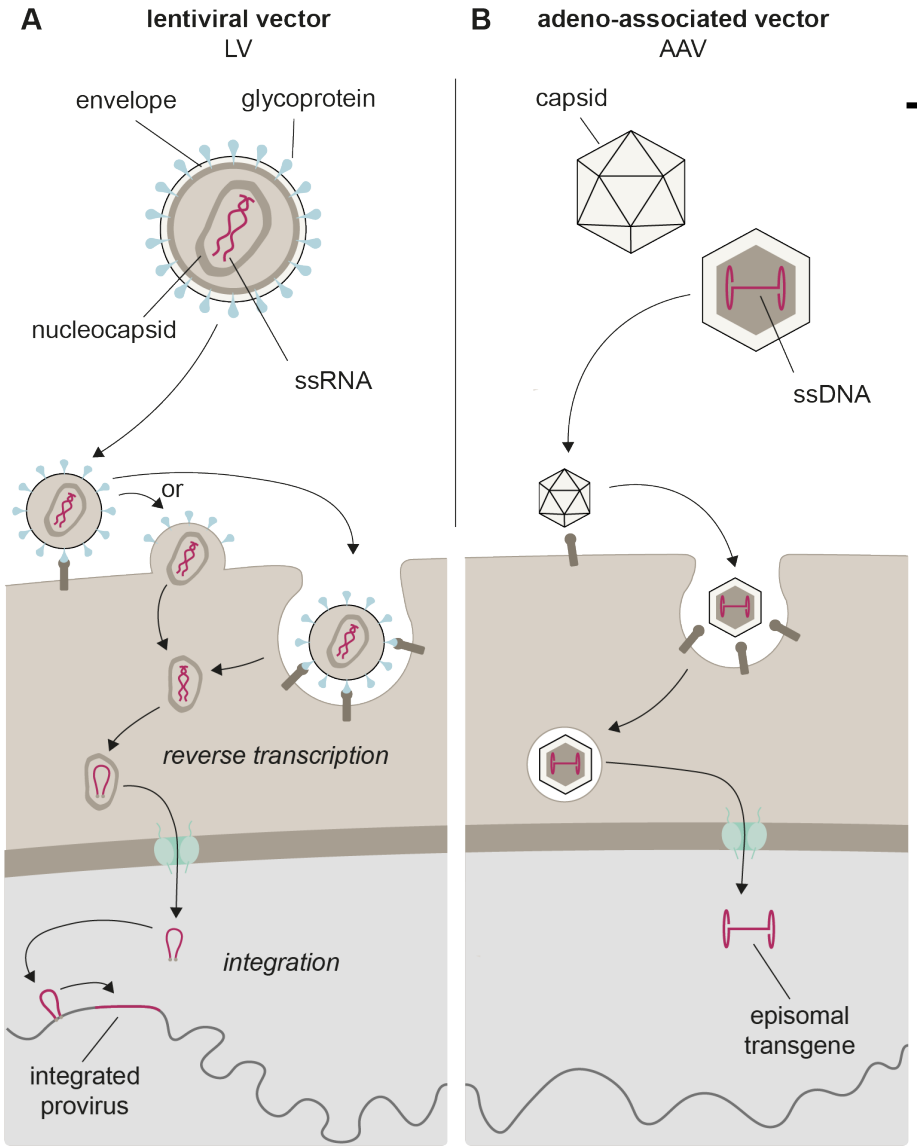
Conventional CAR therapy





modified from Michels, Ho & Buchholz (2022) Mol Ther

autologous difficult to manufacture expensive



Broad Tropism

- LV:
  - Vesicular stomatitis virus glycoprotein
    → **LDLR**
- AAV:
  - Proteoglycans serve as attachment factors
    - **Heparan sulfate** (AAV2)
    - **Scialic acid** (AAV5, -1, -6, -4)
    - **Galactose** (AAV9)

Michels, Ho & Buchholz (2022) Mol Ther

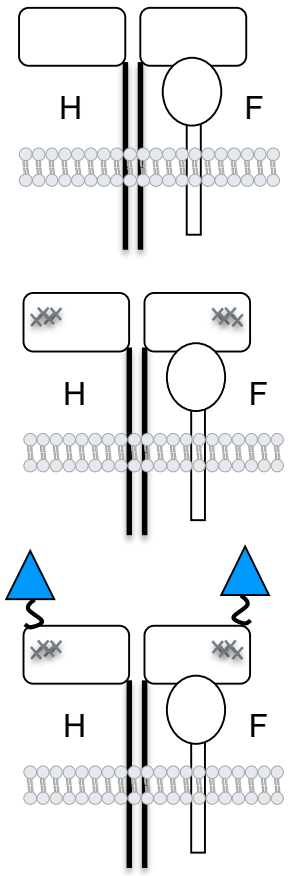
Engineering process for receptor-targeting

Unmodified envelope/particle

Mutate receptor binding sites

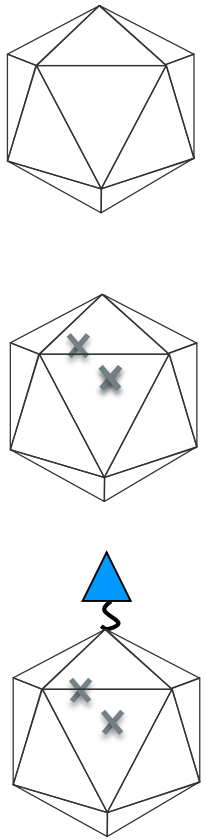
Add the targeting domain

Measles virus glycoproteins



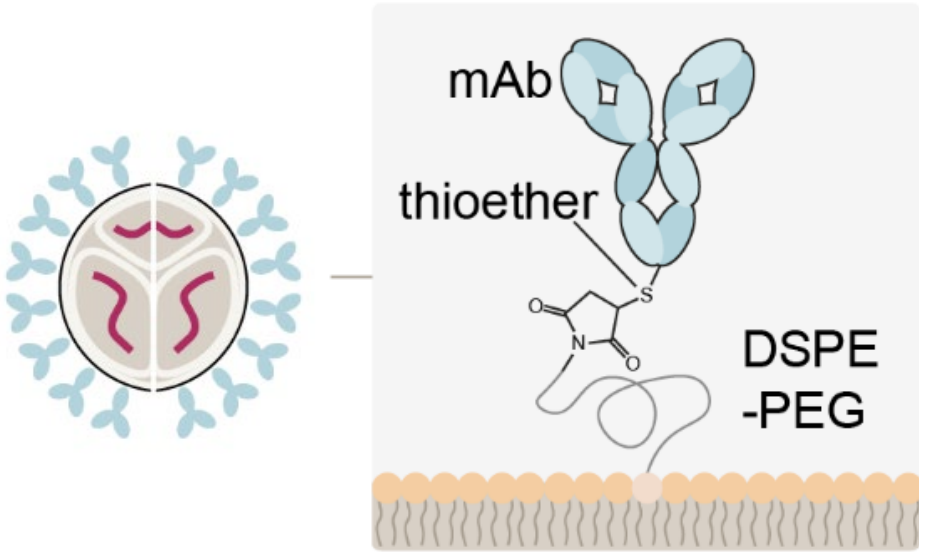
Funke et al., 2008

AAV particle



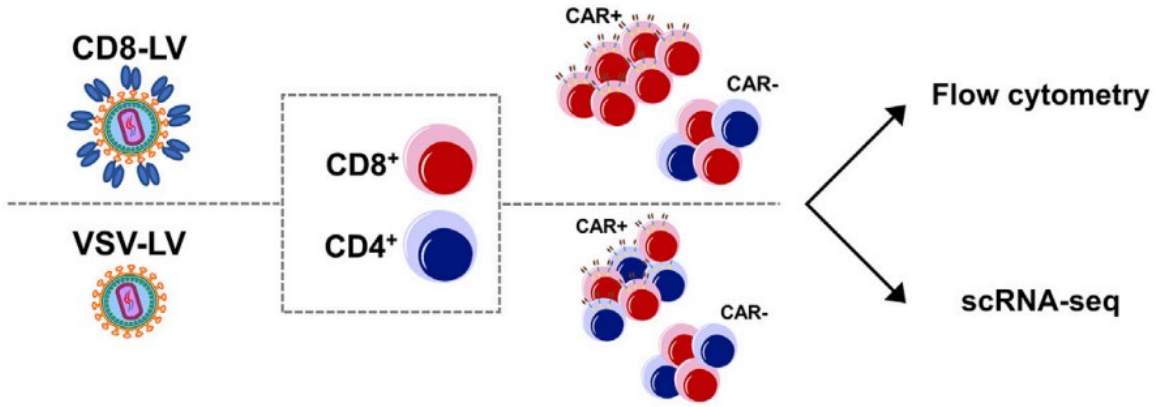
Münch et al., 2013

Lipid nanoparticle



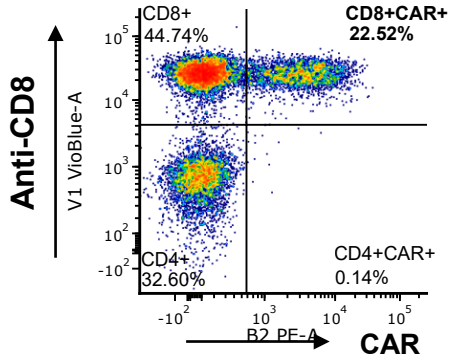
Michels et al., 2022

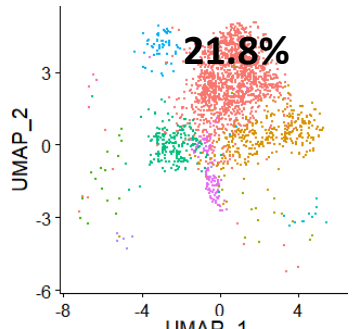
More than 99% target cell selectivity based on scRNA seq



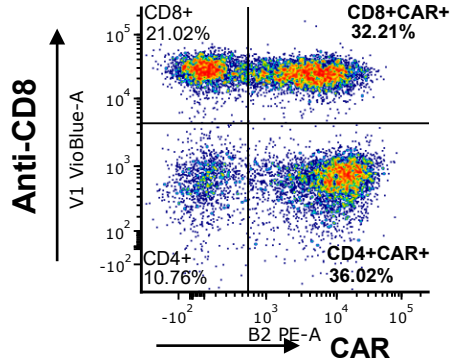
FACS scRNA

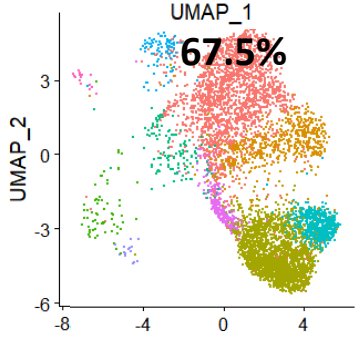
CD8-LV



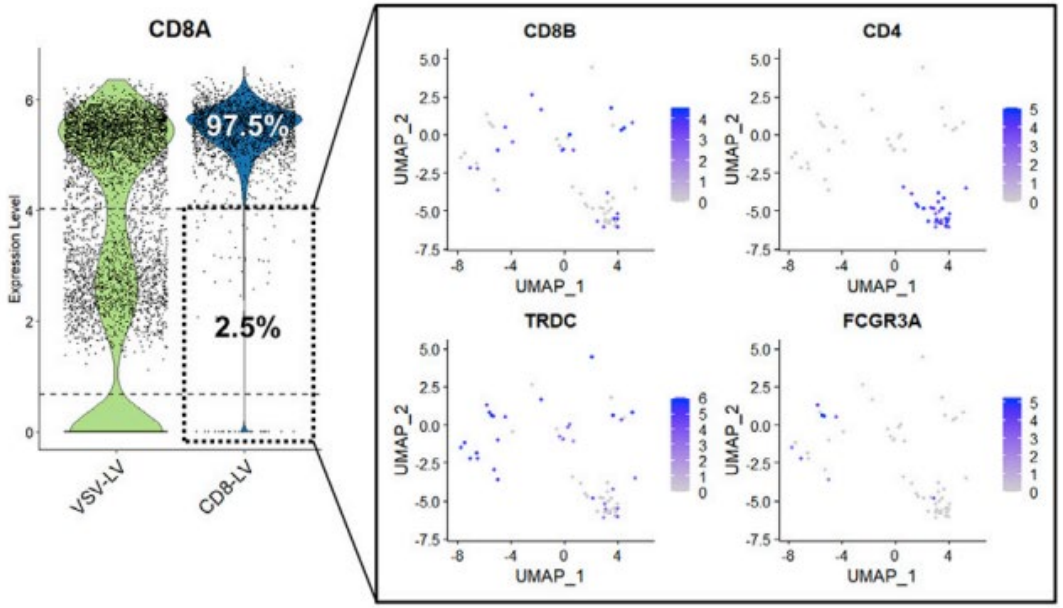


VSV-LV





CAR^{high} cells

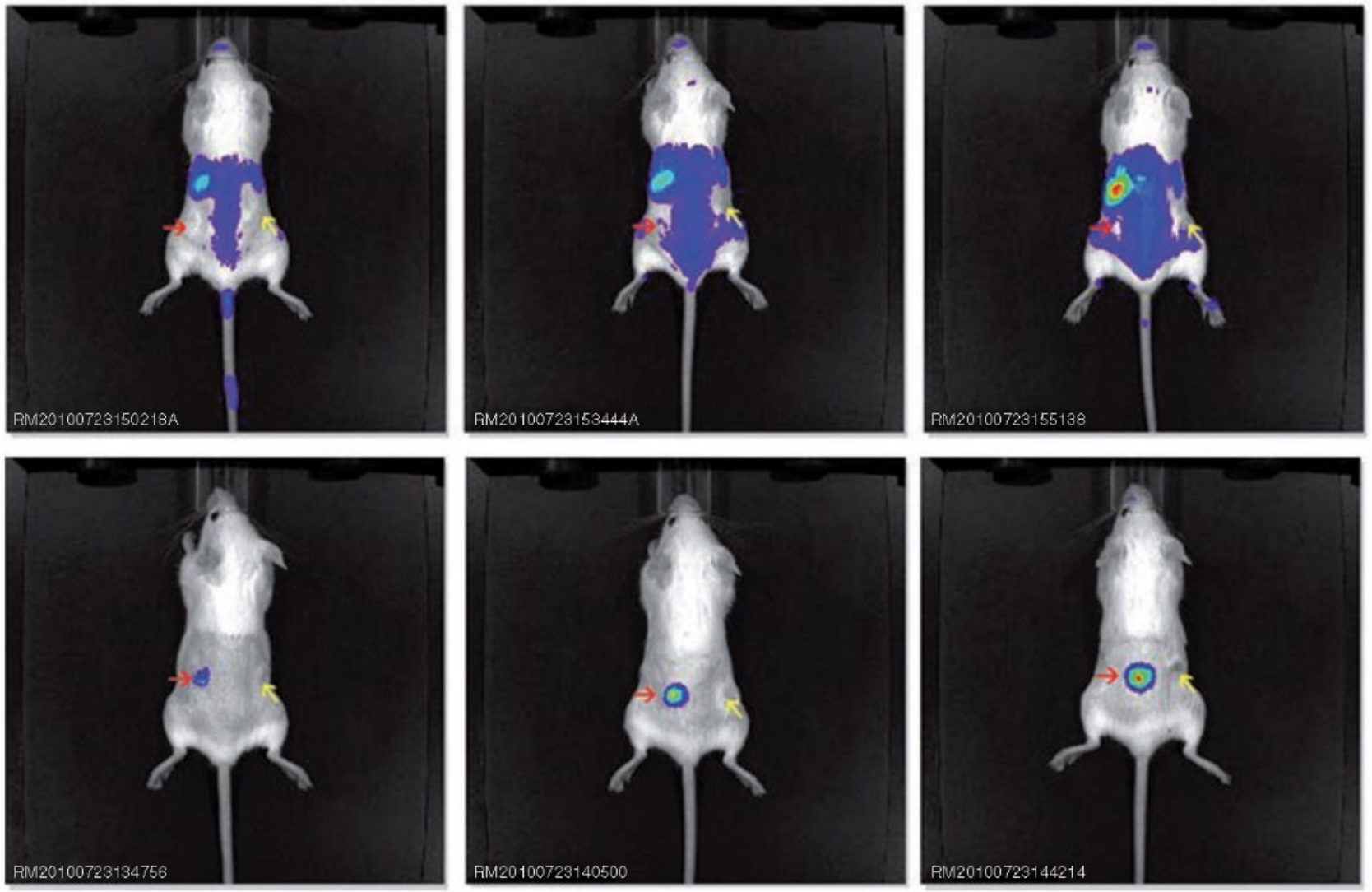


Charitidis et al. (2021)

Liver gene transfer can be prevented by receptor targeting

VSV-LV

Her2-LV



Münch et al., 2011

Proof of concept for *in vivo* generation of human CAR T cells

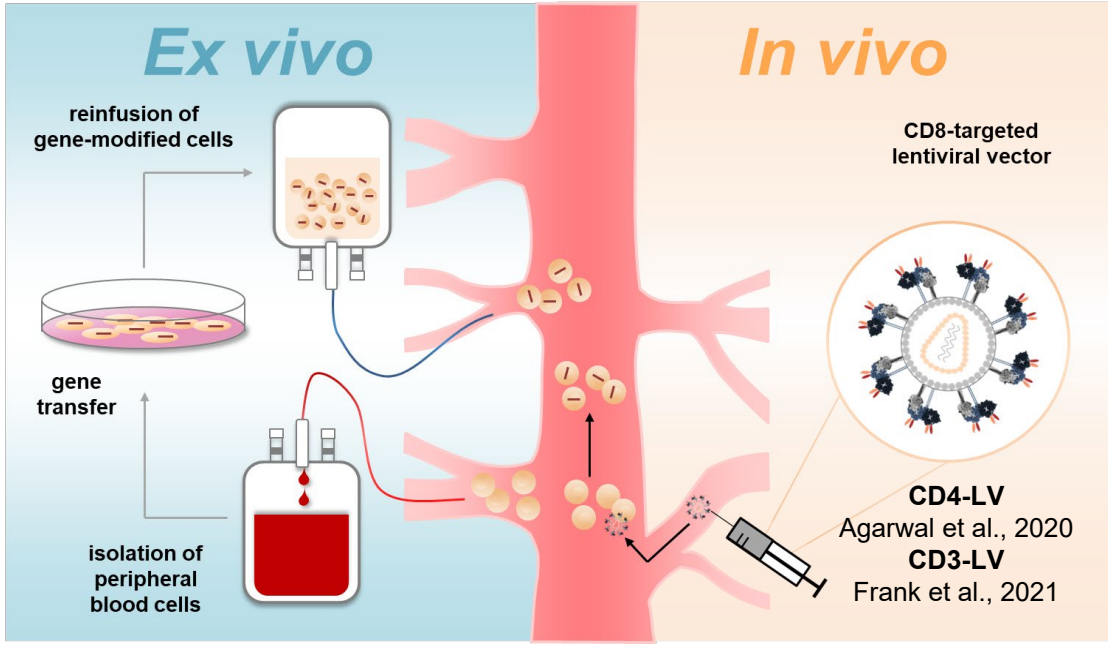
Report

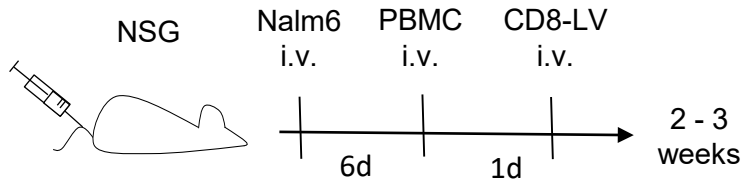
TRANSPARENT PROCESS OPEN ACCESS

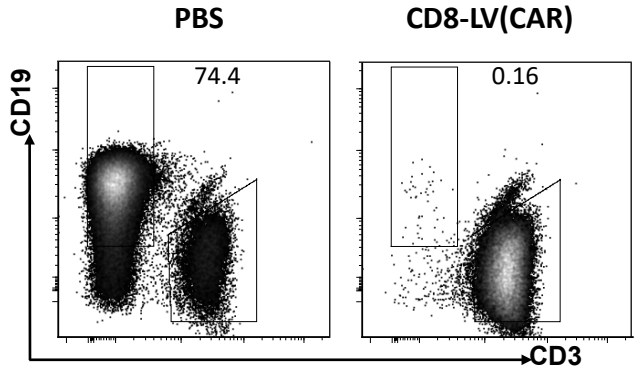
EMBO Molecular Medicine

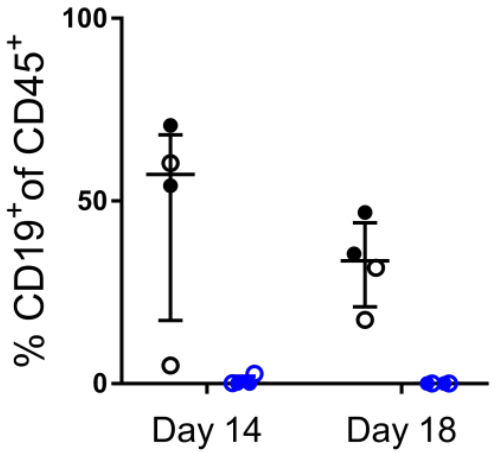
In vivo generation of human CD19-CAR T cells results in B-cell depletion and signs of cytokine release syndrome

Anett Pfeiffer[1,†], Frederic B Thalheimer[1,†], Sylvia Hartmann[2], Annika M Frank[1], Ruben R Bender[1], Simon Danisch[3], Caroline Costa[4], Winfried S Wels[5,6,7], Ute Modlich[8], Renata Stripecke[3], Els Verhoeyen[4,9] & Christian J Buchholz[1,7,10,*]



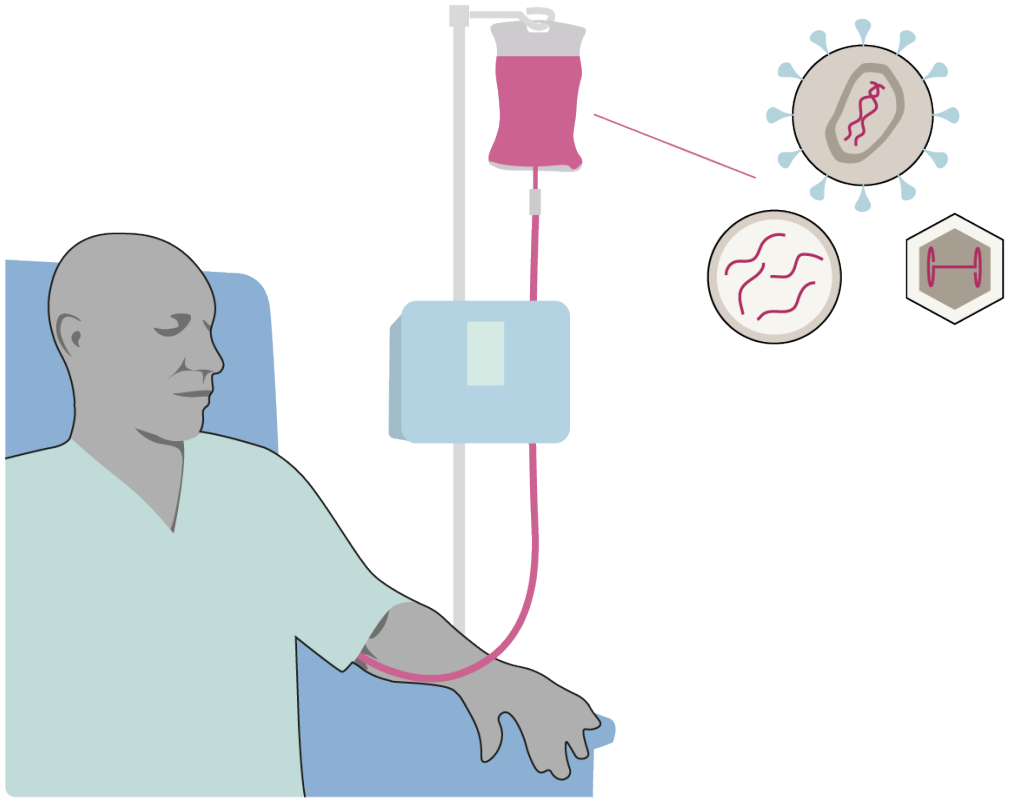






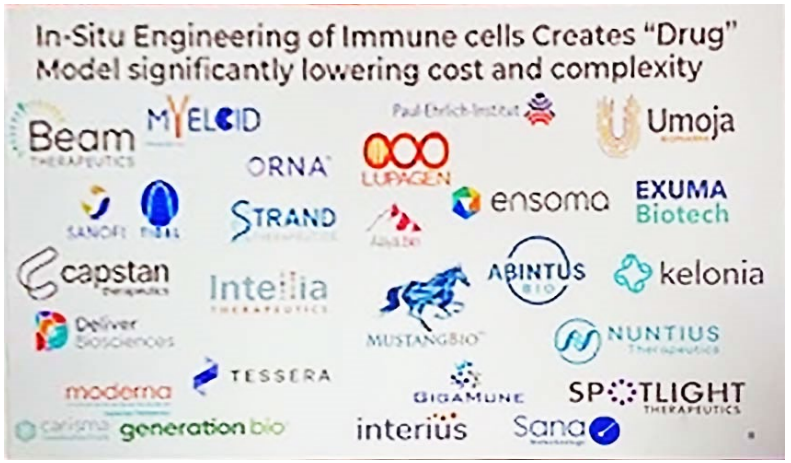
Pfeiffer et al., 2018/Agarwal et al, 2019

In vivo CAR therapy has arrived in biotech industry and the clinic



modified from Michels et al (2022) Mol Ther

off-the-shelf enables immediate treatment



Advanced Therapies Conference, London 2024

PRESS RELEASE

Interius BioTherapeutics Receives HREC Approval and CTN Clearance from the TGA to Commence a Phase 1 Clinical Trial for Its First-in-Class In Vivo CAR Therapeutic for B Cell Malignancies

July 09, 2024

PHILADELPHIA, July 9, 2024 /PRNewswire/ — Interius BioTherapeutics, a leading developer of in vivo cell-specific gene medicines, today announced that...

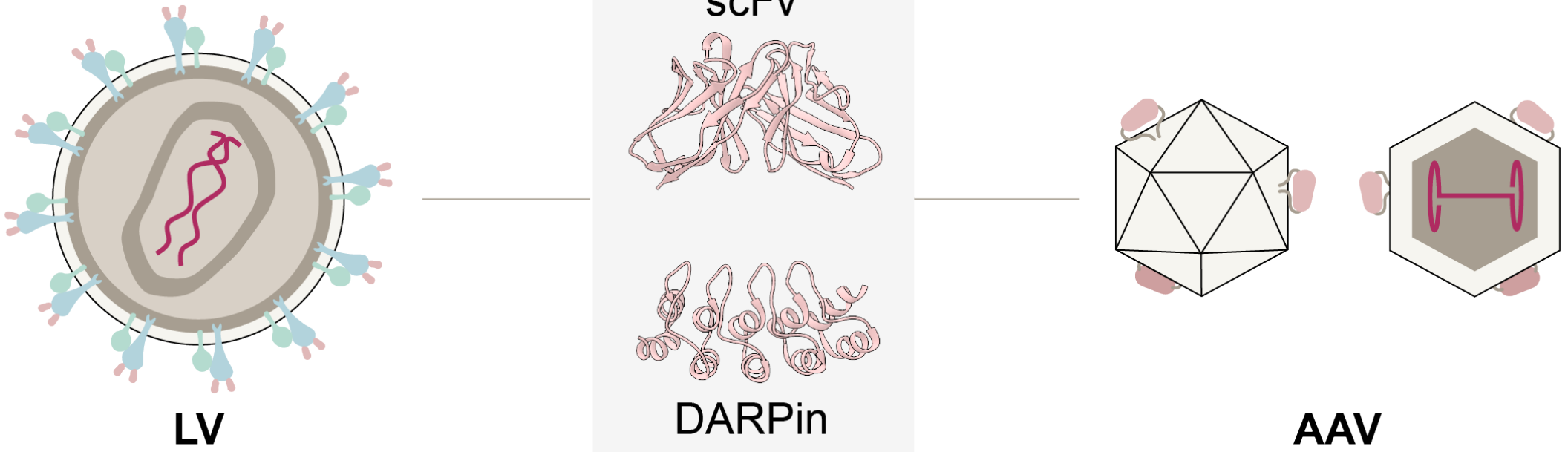
read more

Umoja Biopharma Announces FDA Clearance of IND Application for UB-VV111, a CD19 Directed in situ CAR T for Hematologic Malignancies

- *UB-VV111 is potentially the first in situ generated CD19 chimeric antigen receptor (CAR)-T cell therapy to be evaluated in humans in the hematology setting*
- *First patient expected to be dosed by end of 2024*

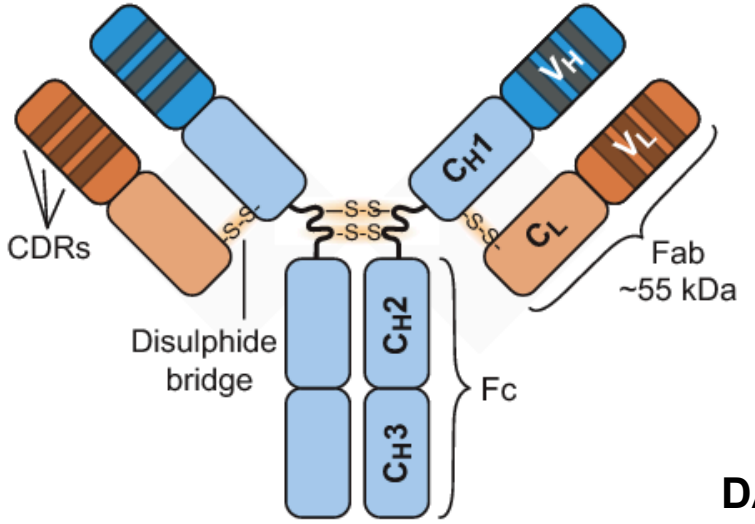
SEATTLE, WA, July 31, 2024 — Umoja Biopharma, Inc. (Umoja), a transformative immunotherapy company creating off-the-shelf treatments that aim to extend the reach and effectiveness of CAR-T cell therapies in oncology and autoimmunity, today announced the clearance of its Investigational New Drug (IND) application by the U.S. Food and Drug Administration (FDA) for UB-VV111, a gene therapy that generates CD19 CAR T-cells in situ, intended to treat hematologic malignancies. Umoja expects to initiate a Phase 1 study and dose the first patient in the trial by the end of 2024. UB-VV111 is the first asset from the VivoVecTM gene delivery platform to enter the clinic.

Targeting ligand is key for sucessful receptor-targeting



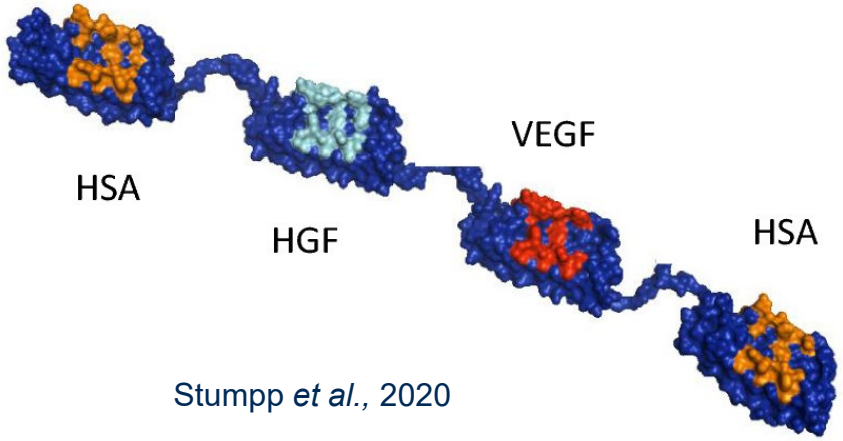
DARPins – a small and stable alternative to antibodies

Human IgG (~150 kDa)



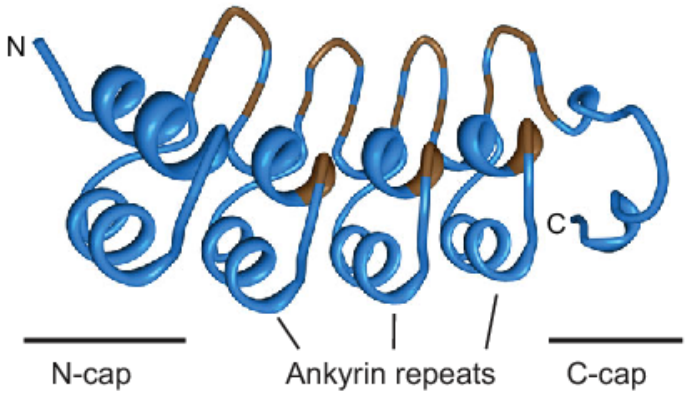
Harmansa & Affolter, 2018

scFv (~28 kDa)



Stumpp *et al.*, 2020

DARPin (~14-28 kDa)

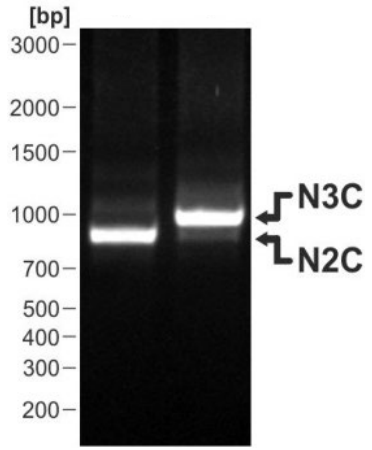


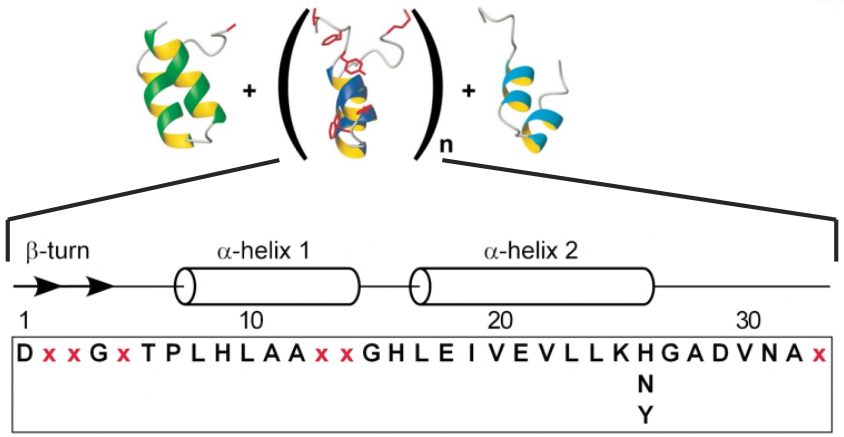
Designed **A**nkyrin **R**epeat **P**rote**ins**

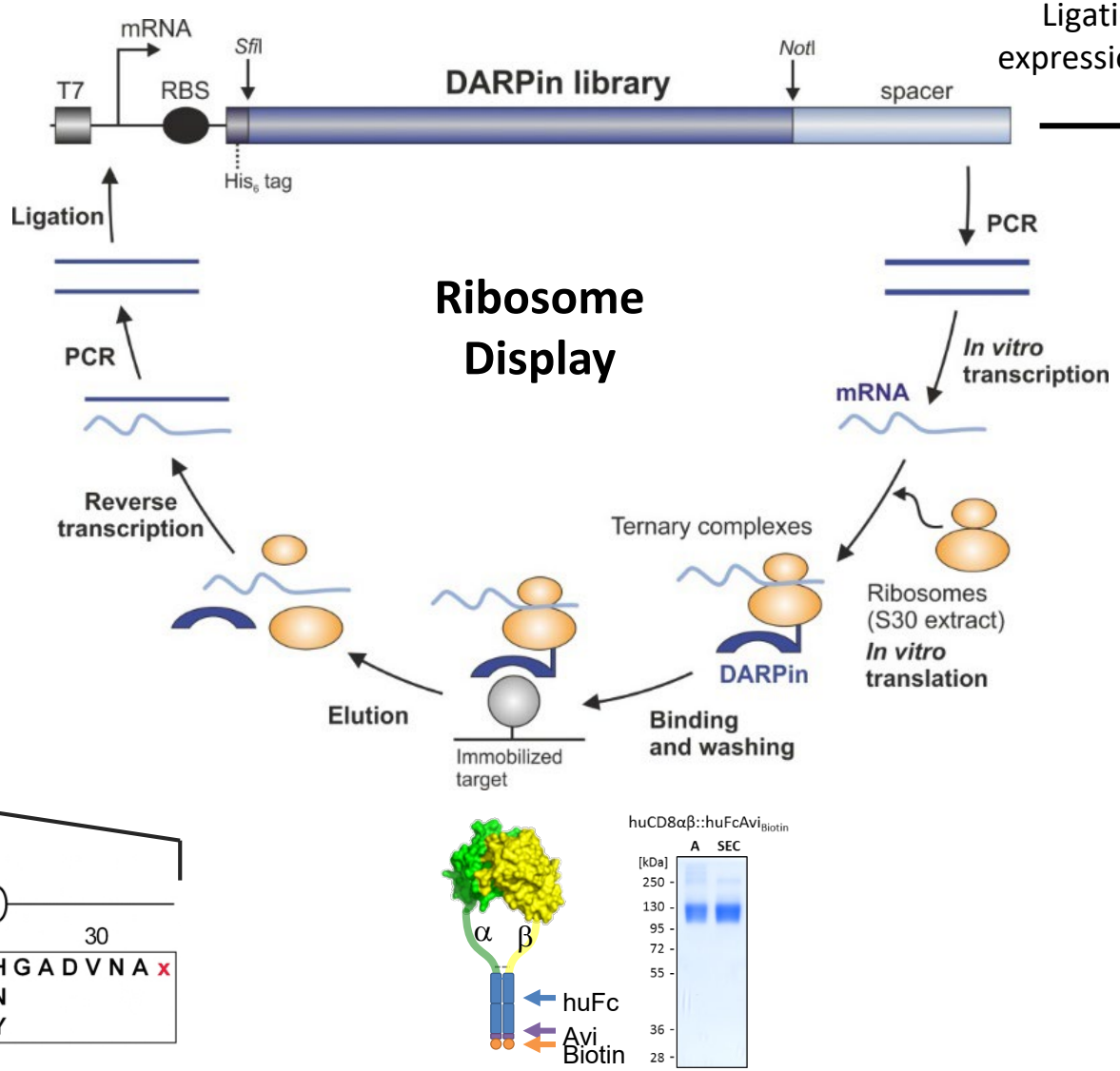
- High affinity binders (<5-100 pM)
- Increased thermodynamic stability (66-85°C)
- Small size (~14-21 kDa, 10% of an IgG)
- Expressed at high levels in E.coli (1-10 g/L)
- Absence of cysteines

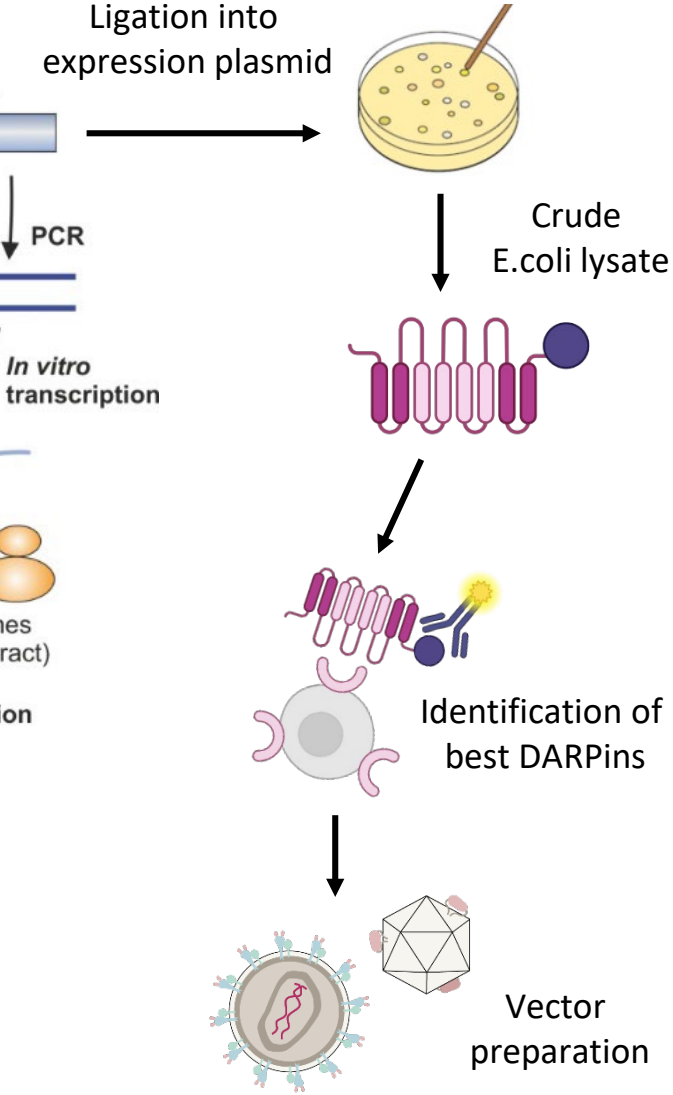
Screening procedure for target-receptor specific DARPins

Generated DARPin library covers <10^{13} variants



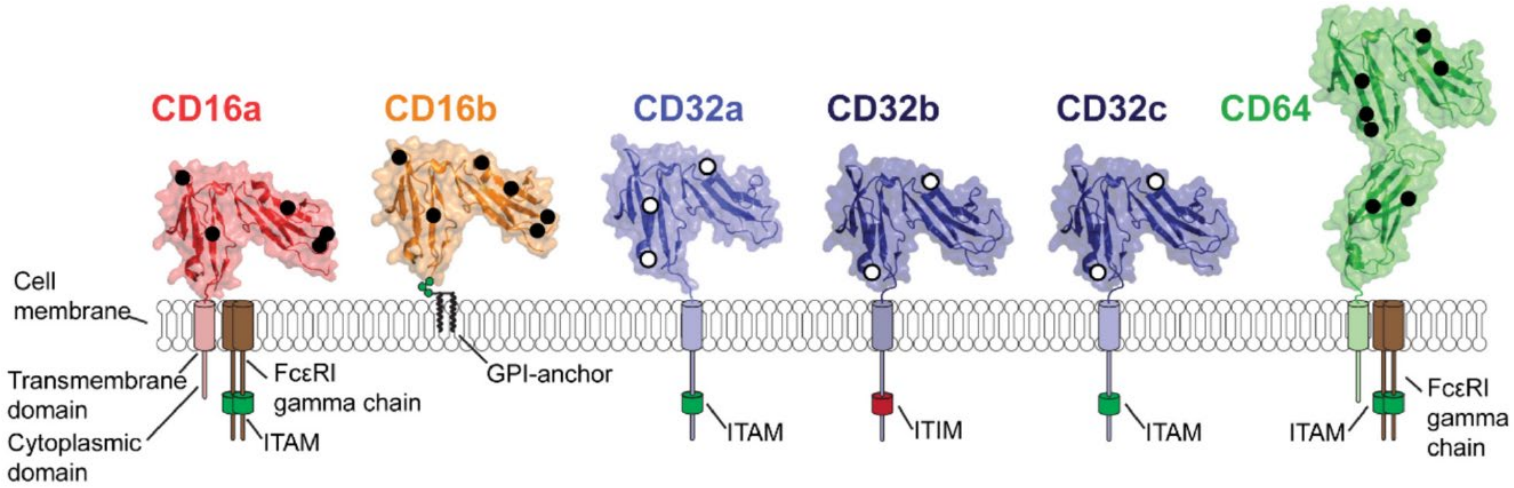




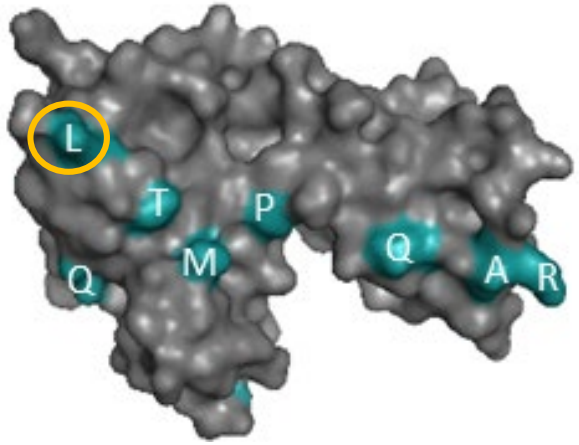


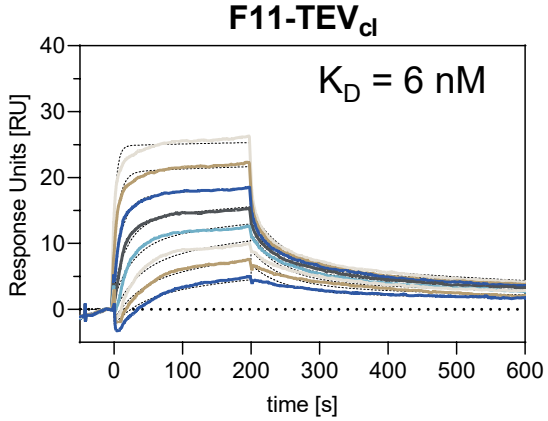
Hartmann et al., 2018

DARPin F11 binds CD32a (FcγRIIA) a target for HIV reservoir cells

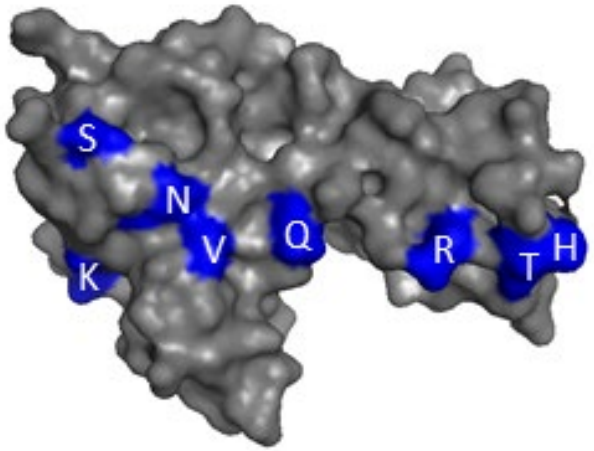


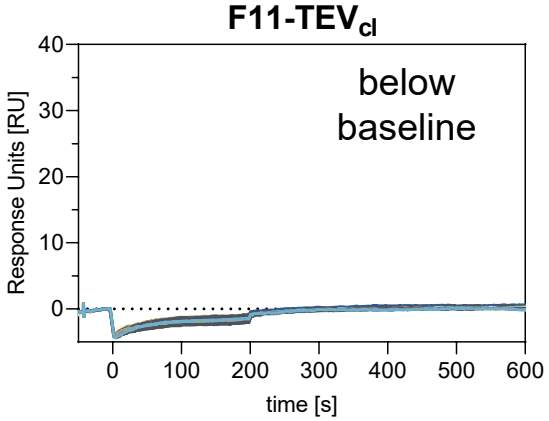
CD32a





CD32b





Riechert et al., 2023

Designing receptor-targeted AAVs

Münch et al, 2013 ⇨ Michels et al, 2021

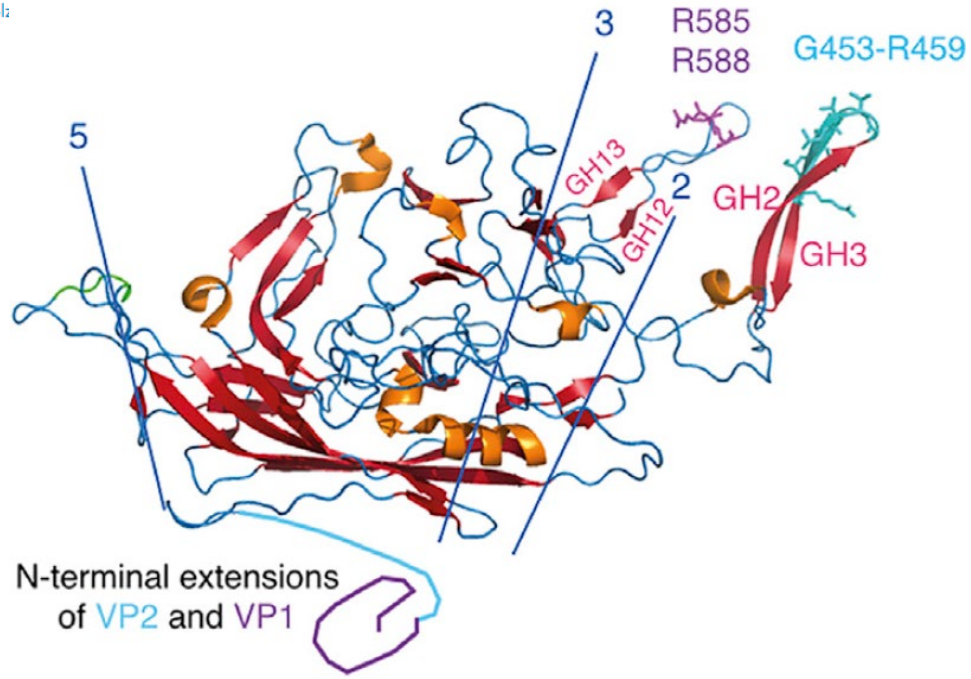
Molecular Therapy

Volume 21, Issue 1, January 2013, Pages 109-118

Original Article

Displaying High-affinity Ligands on Adeno-associated Viral Vectors Enables Tumor Cell-specific and Safe Gene Transfer

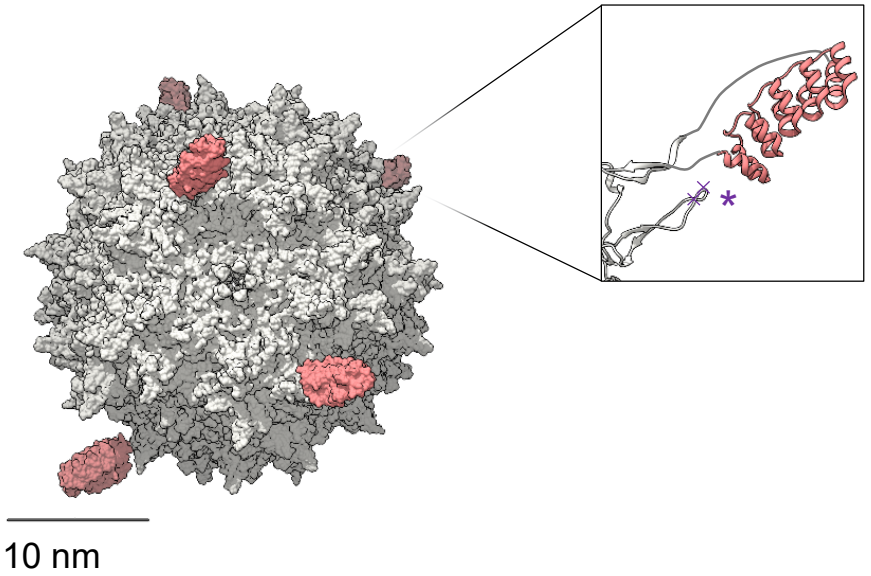
Robert C Münch [1,*], Hanna Janicki [2], Iris Völker [1], Anke Rasbach [1], Michael Hallek [2], Hildegard Büning [2], Christian J Buchholz [1]



Molecular Therapy
Methods & Clinical Development
Original Article

Lentiviral and adeno-associated vectors efficiently transduce mouse T lymphocytes when targeted to murine CD8

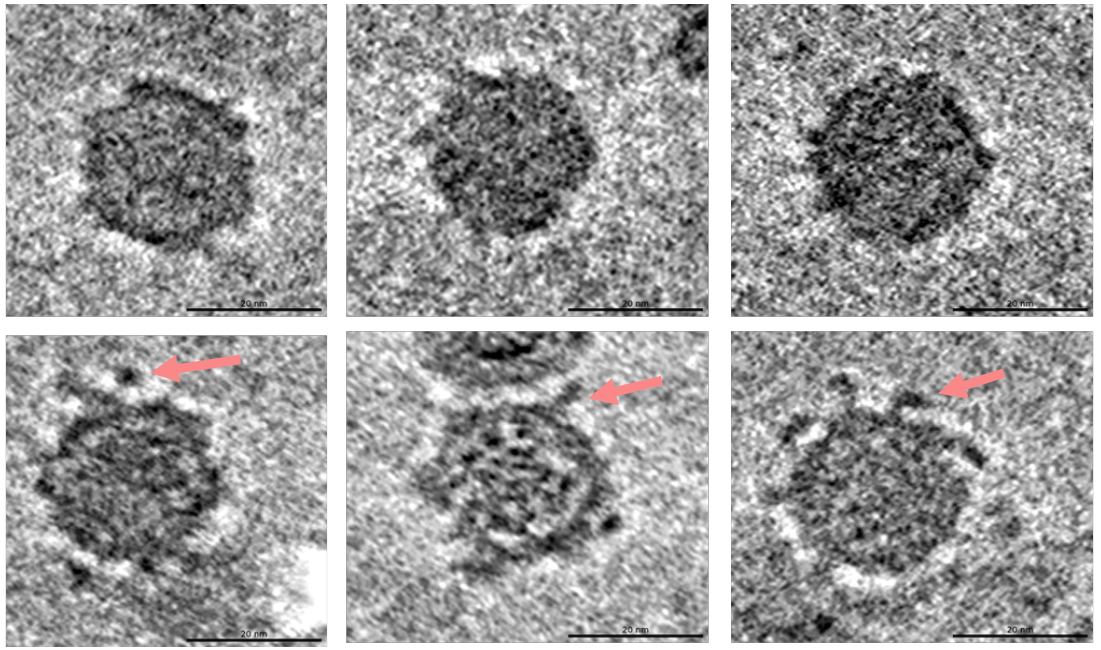
Alexander Michels,[1] Annika M. Frank,[2] Dorothee M. Günther,[1,3] Mehryad Mataei,[1] Kathleen Börner,[4] Dirk Grimm,[4,5,6] Jessica Hartmann,[2] and Christian J. Buchholz[1,2]



DARPins are visible on the surface of DART-AAVs by cryo-electron microscopy

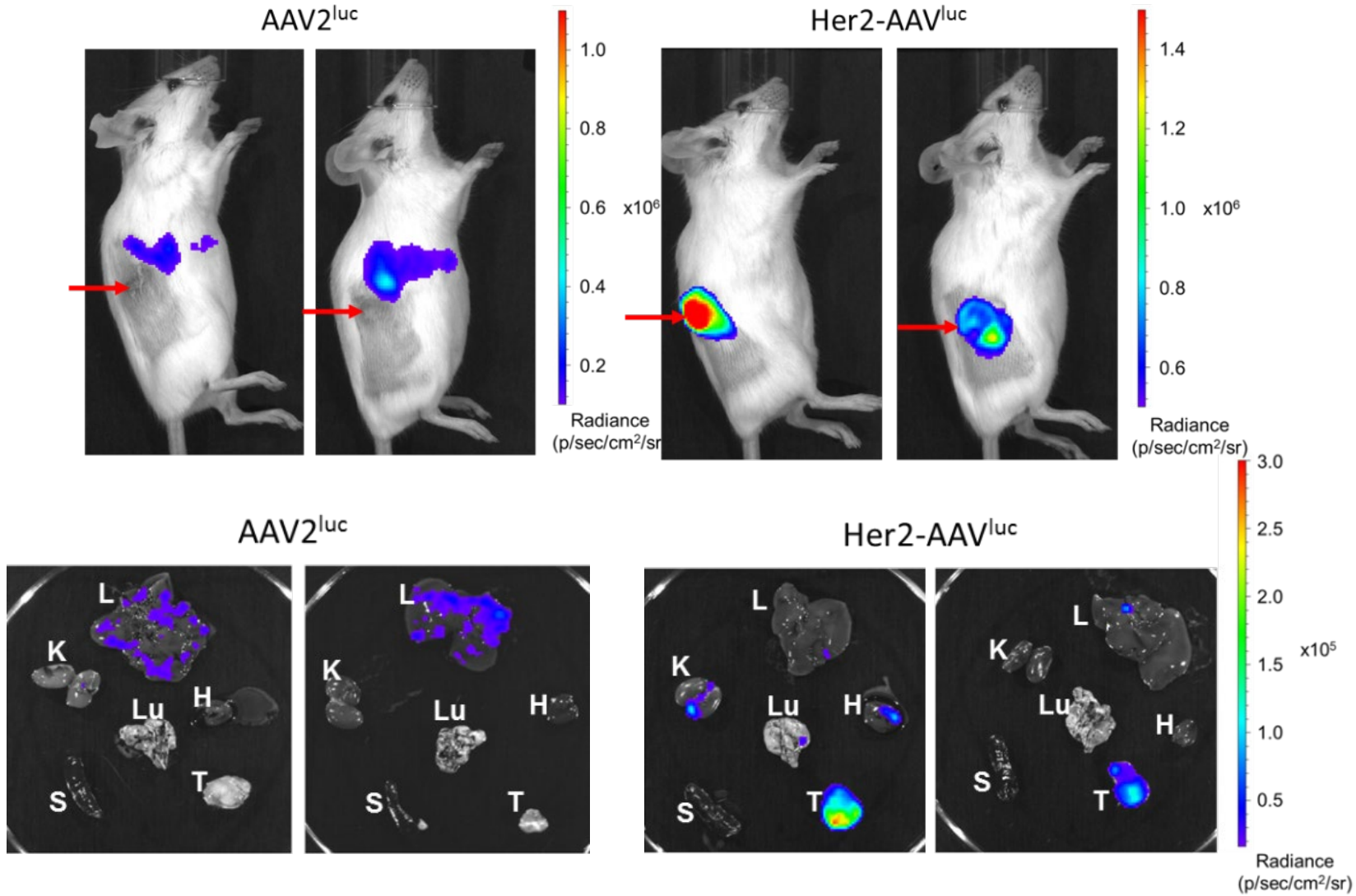
AAV2

8-AAV



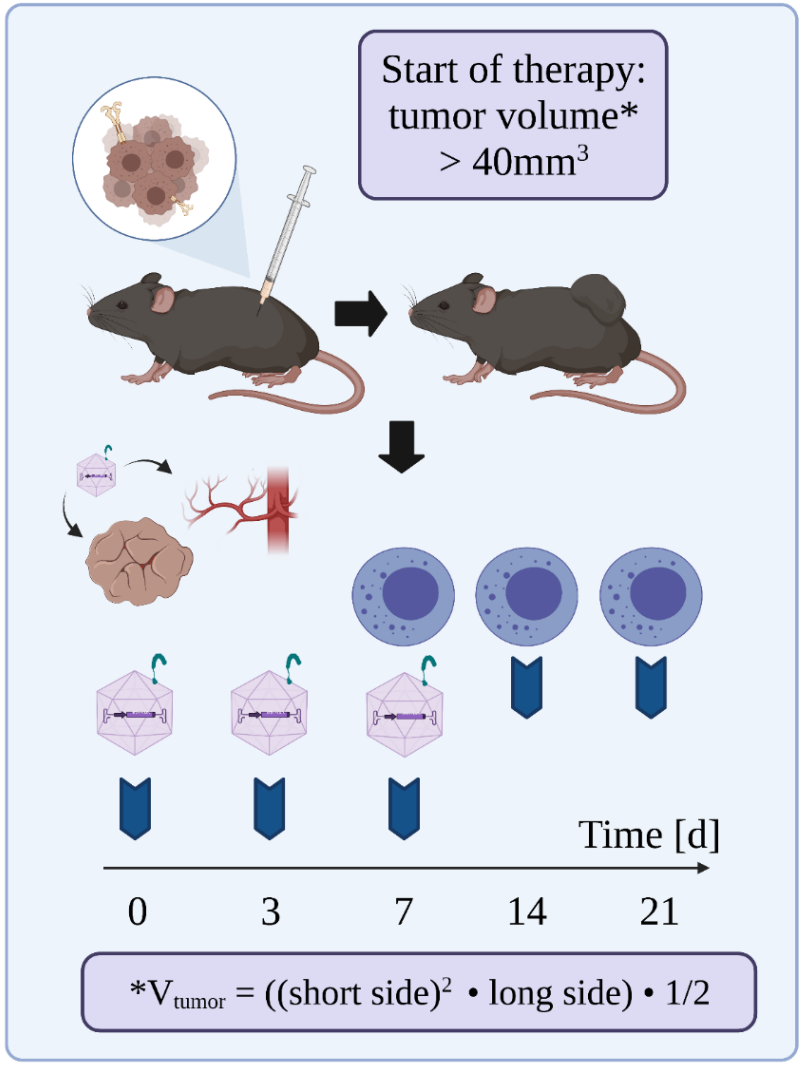
Demircan/Zinser et al, 2024

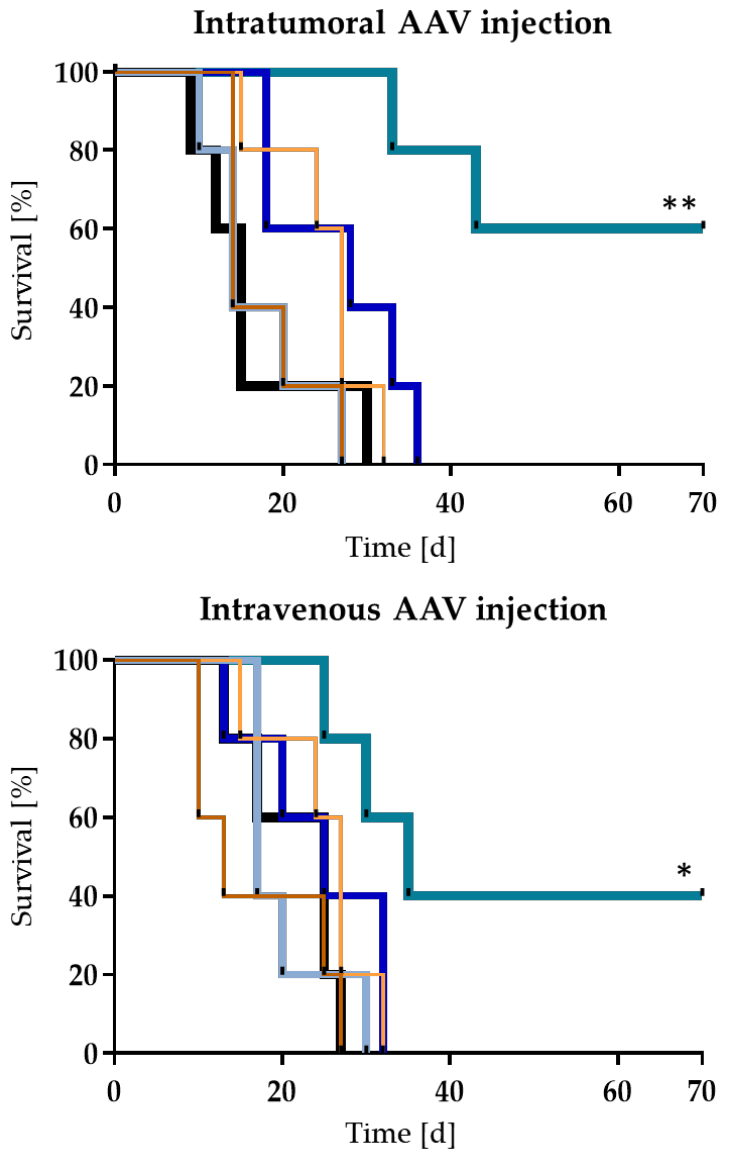
Her2-AAV delivers genes into tumors of immunocompetent mice



Reul/Frisch et al, 2019

Survival benefit in mice bearing subcutaneous GL261-HER2^{+} tumors





Strecker et al., 2022

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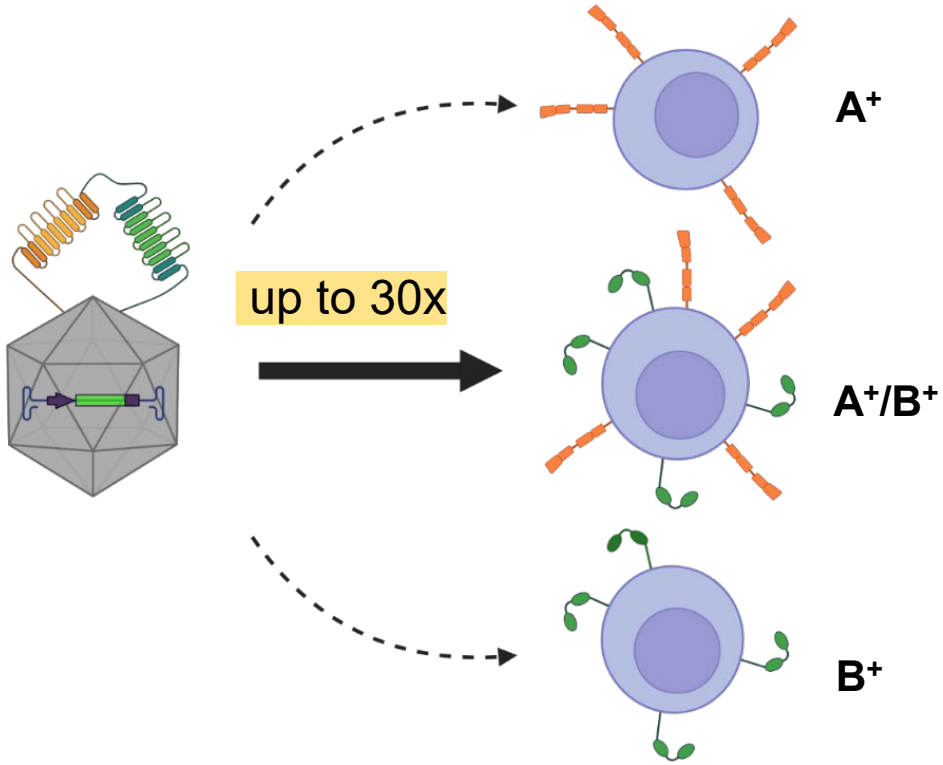
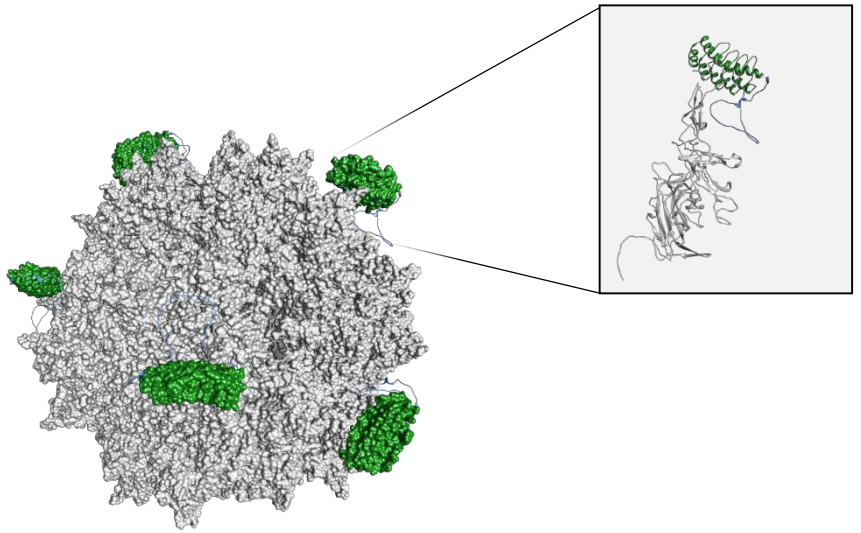
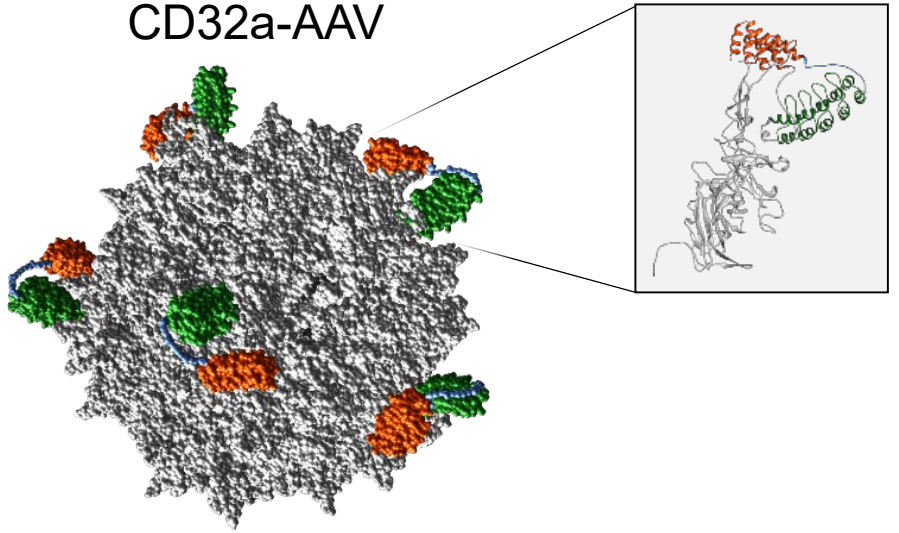
AAV vectors displaying bispecific DARPins enable dual-control targeted gene delivery

Samuel A. Theuerkauf [a], Elena Herrera-Carrillo [b], Fabian John [a,c], Luca J. Zinser [a], Mariano A. Molina [b], Vanessa Riechert [a], Frederic B. Thalheimer [a,c], Kathleen Börner [d,e,f], Dirk Grimm [e,f,g,h], Petr Chlanda [d,e,i], Ben Berkhout [b], Christian J. Buchholz [a,c,*]





CD32a-AAV

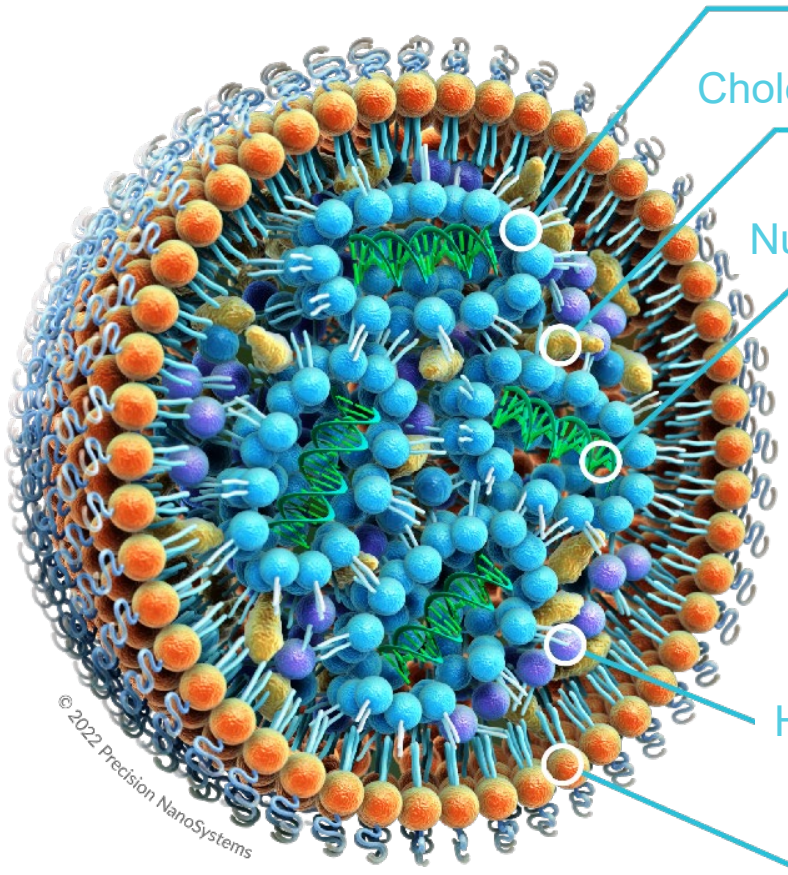


CD4/CD32a-AAV

Theuerkauf et al, 2023

RNA lipid Nanoparticles (RNA-LNPs)

Ionizable lipid e.g. MC3: payload encapsulation and endosomal escape



Cholesterol: stabilizer

Nucleic Acid e.g. siRNA or mRNA: payload

Helper-Lipid e.g. DSPC: stabilizer

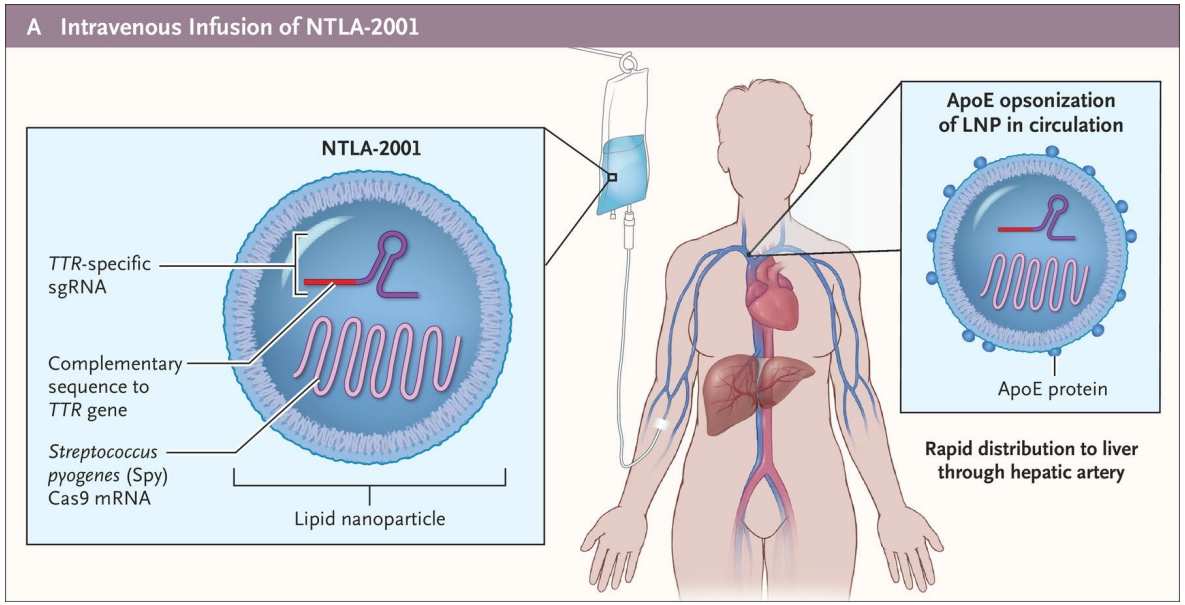
Stabilizer

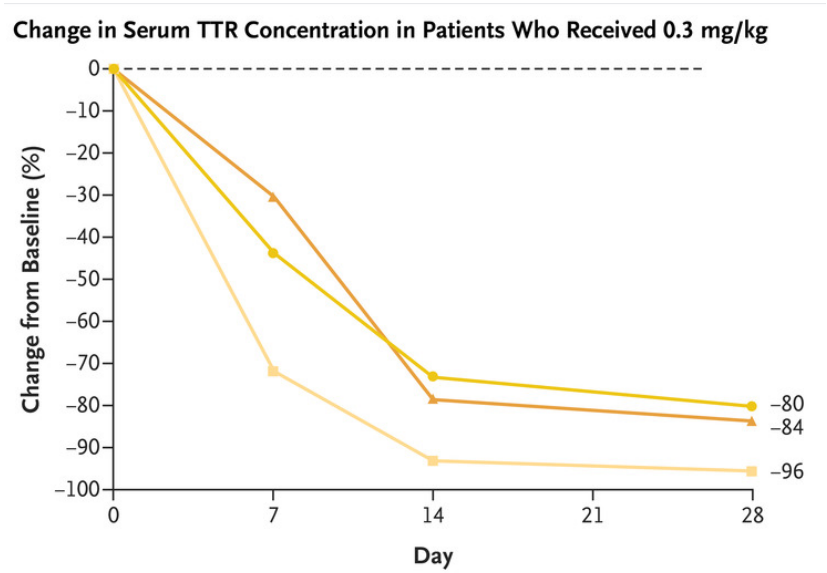
PEG-Lipid e.g. DMG-PEG: stabilizer, impedes LNP aggregation

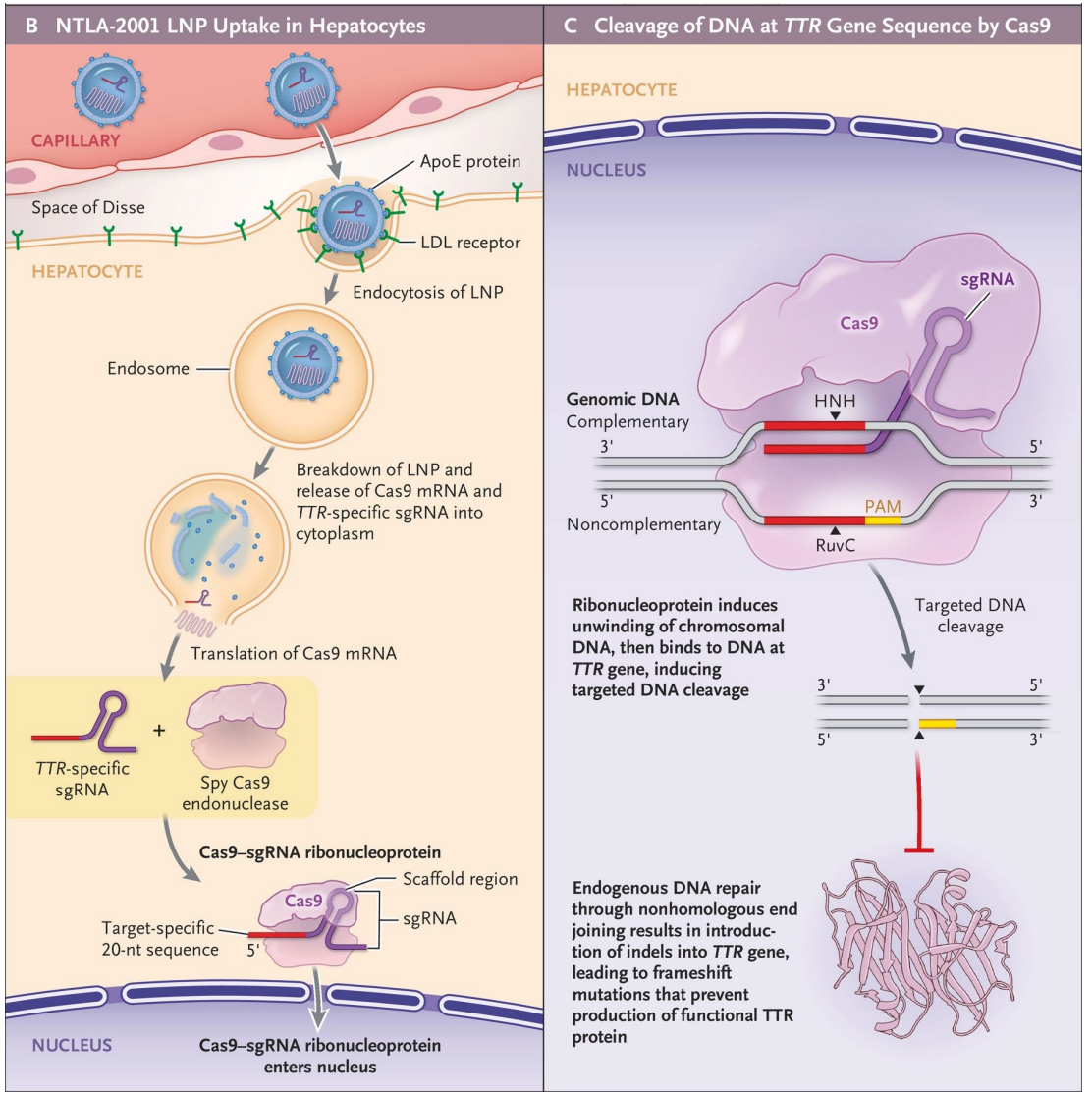
Onpattro® (2018)

siRNA to downmodulate expression of transthyretin in patients suffering from inherited transthyretin amyloidose

In Vivo Gene Editing for Transthyretin Amyloidosis



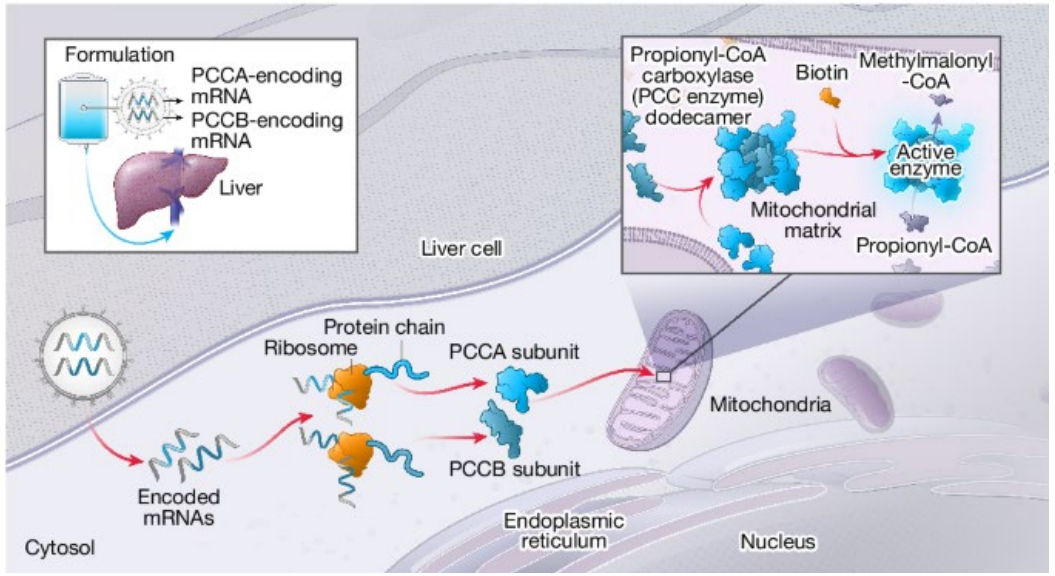


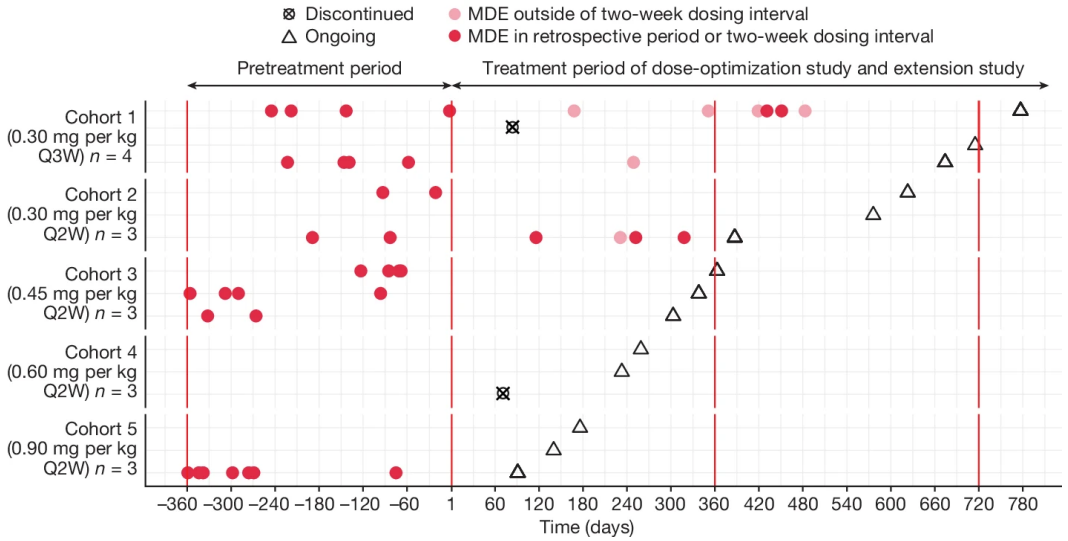


Gillmore et al 2021

RNA-LNPs for treatment of propionic acidemia

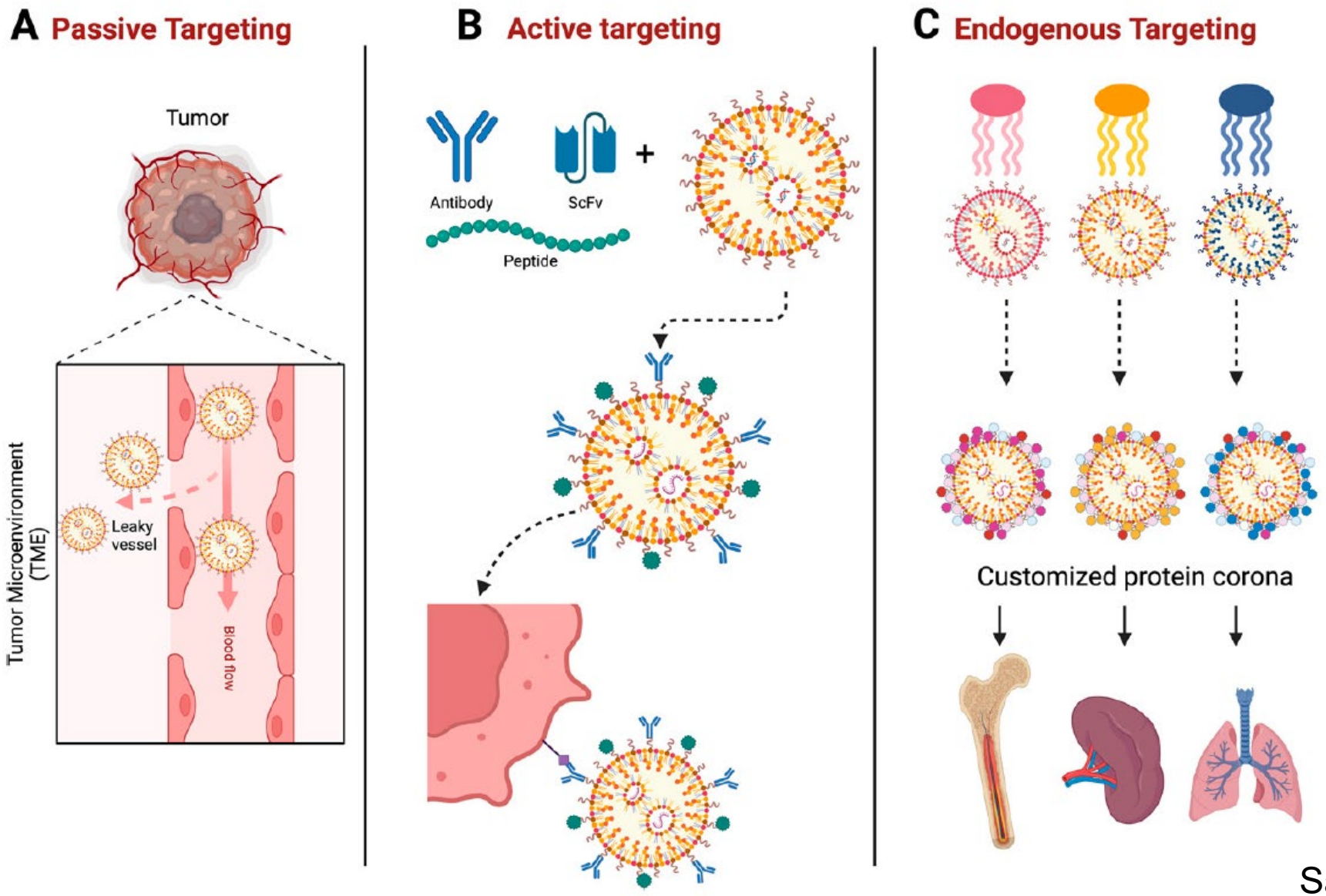
- Rare disease with defect αβ subunits of the propionyl-coenzyme A carboxylase
- Accumulation of life-threatening toxic metabolites
- Currently only liver transplantation is an option
- 0.30–0.90 mg per kg of mRNA-3927 (Moderna) intravenous infusion every two to three weeks, to ten doses over 20–30 weeks
- Adverse events:
  fever, vomitting, diarrhoea, (pancreatitis)
- Efficacy:
  Risk for metabolic decompensation reduced by 70%





Koeberl et al , Nature 2024

Tissue/cell targeting with RNA-LNPs



Saber et al, 2024

Take Home Message

- *In vivo* CAR delivery has the potential to bring a new therapeutic strategy into clinics.
  The first clinical trials based on this strategy are about to start and will tell about feasibility in patients.
  Highly specific T-cell targeted vectors are key.

- DARPins are ideal for receptor-targeting: they can be selected for any receptor of choice and
  even discriminate between closely related cell surface molecules

- The recent development of DART-AAVs expands the vector repertoire for targeted *in vivo* gene therapy.
  Bi-specific AAVs enable AND-gated receptor usage, which allows for the first time
  to target therapy relevant cells defined by two markers.

- DARPin-targeted RNA-LNPs are under development. Administration in mouse models will be
  crucial to assess their activities in comparison to viral vectors.

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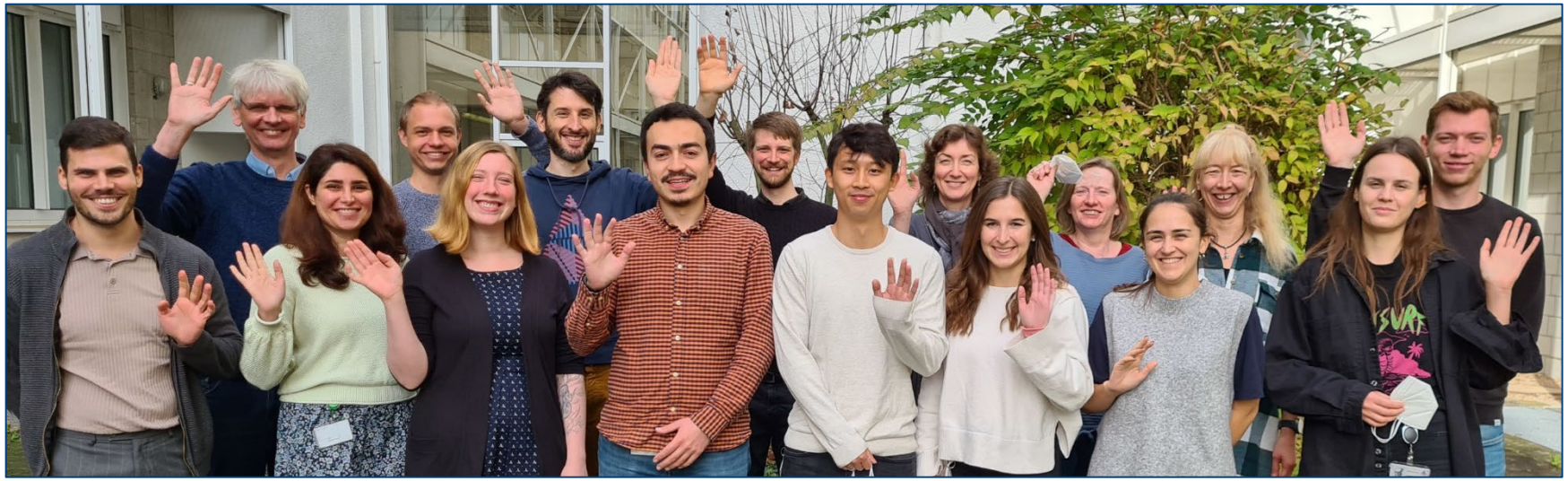

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Phospholipid Research Center

LOEWE
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