Can Red Cells Cure High Cholesterol and Type I Diabetes? Modified Red Blood Cells as Novel Biotherapeutics

Professor Harvey Lodish
Whitehead Institute for Biomedical Research
Departments of Biology and Biological Engineering,
MIT

Supported by NIH, DARPA and Flagship Pioneering

Although I have helped start several successful biotechnology companies, at heart I am a cell and developmental biologist focused on understanding basic life processes

- 1979 Damon Biotech †
- 1979 BioInformation Associates
- 1981 Genzyme
  Sold to Sanofi for $20.2 billion
- 1983 Arris (now Axys) Pharmaceuticals
- 1993 Millennium Pharmaceuticals
  Sold to Takeda for $9 billion
- 2005 Allozyne †
- 2014 Rubius
- 2017 Tevard

From 2007 to 2016 I was the Founding Chair of the Scientific Advisory Board of the Massachusetts Life Sciences Center, the group charged with oversight of the state's 10- year $1 billion investment in the life sciences.

Since 2007 I have served on the Board of Trustees of Boston Children's Hospital and have been the Chair of the Board Research Committee

BCBS Island Run powered by Boston.com

Biotech startup Rubius raises $120m to develop red blood cell technology

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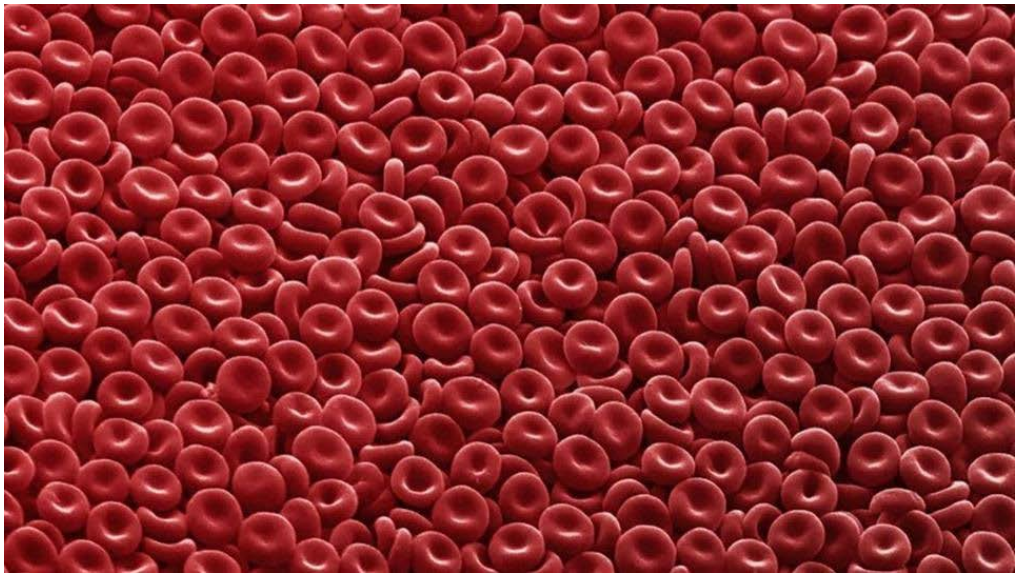


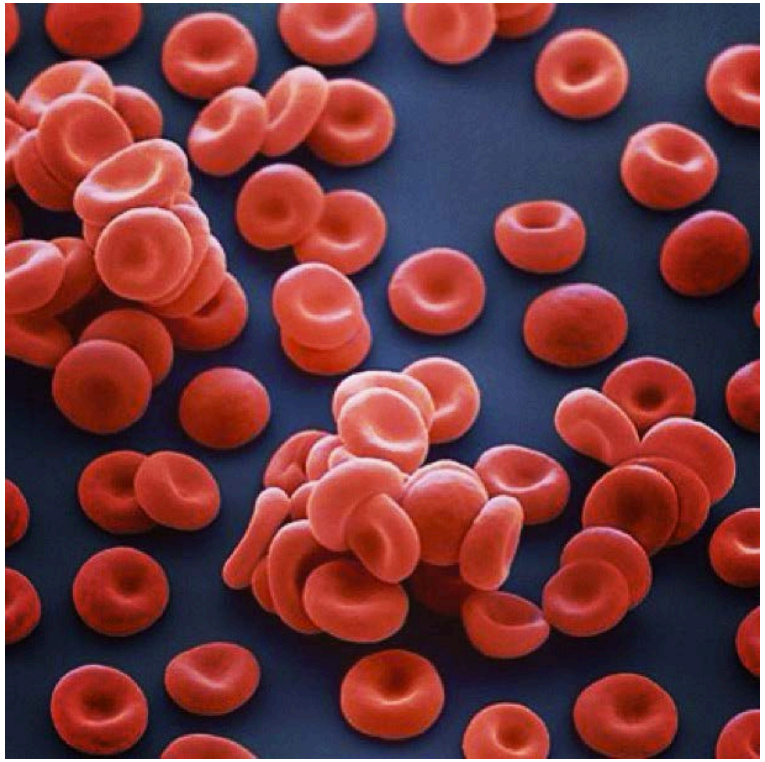
ANNIE CAVANAGH/WELLCOME IMAGES

Red blood cells.

By Robert Weisman | GLOBE STAFF JUNE 21, 2017

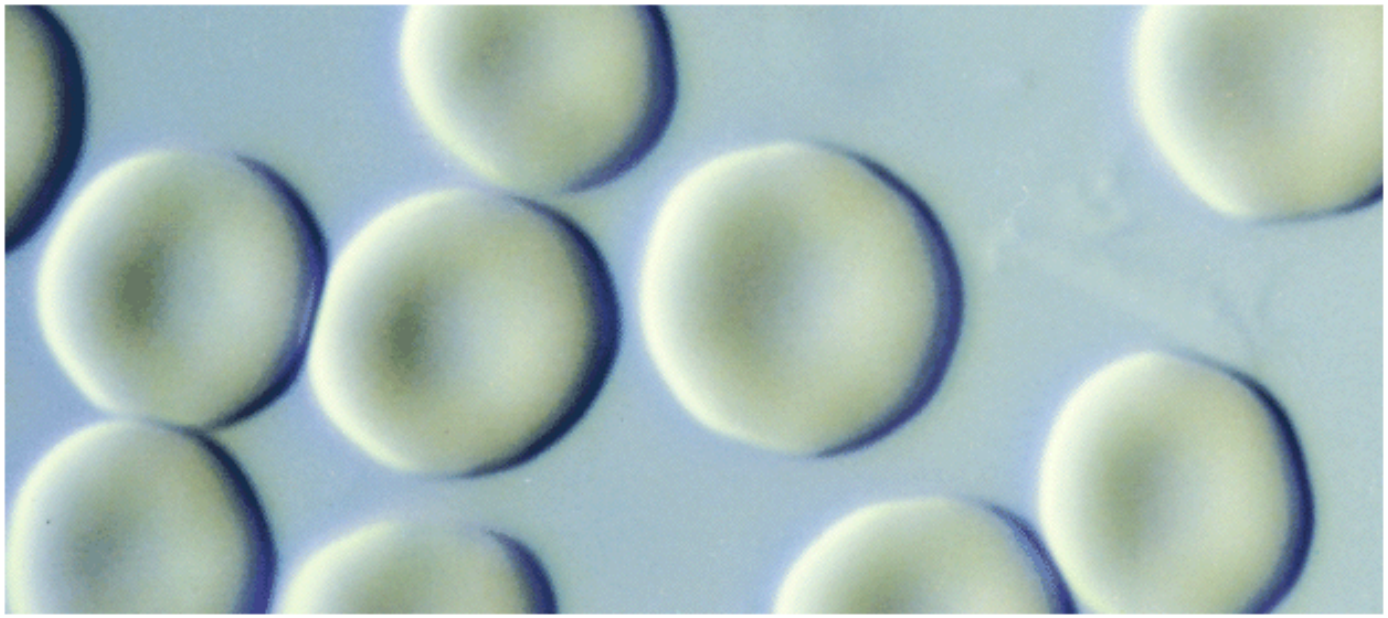
Rubius Therapeutics Inc. of Cambridge is set to announce Wednesday that it has raised $120 million — one of the largest biotech financing rounds this year — to develop a novel drug-making technology.

Red Blood Cells (Erythrocytes):



- The most common type of blood cell - ~45 – 50% of blood volume; one quarter of all human cells
- Transports oxygen from the lungs to body tissues and waste carbon dioxide back to the lungs
- Adult humans produce ~ 2.4 million red cells per second
- Red cells circulate for 100 - 120 days before being degraded.

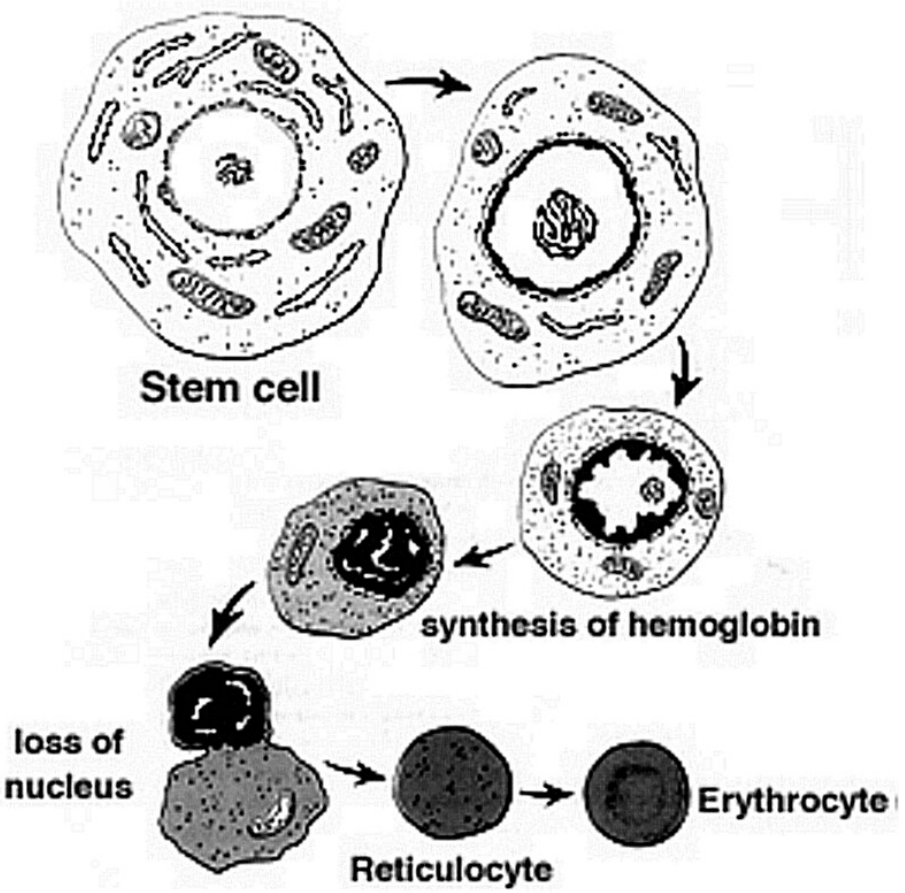
Red cells are attractive microparticles for introducing therapeutics & diagnostics into the human body



- Blood transfusion is a widely used therapeutic
- 7 μm diameter flexible biconcave discs
- Long lifespan: 120 days in blood stream
- Large cell surface area and excellent biocompatibility
- Cytosolic and membrane proteins and metabolism well- characterized
- Genes encoding foreign or chimeric proteins can be ectopically expressed at will in cultured erythroid progenitor cells
- Lose nucleus and mitochondria: no remnants of introduced DNAs

Production of human red blood cells expressing a foreign protein

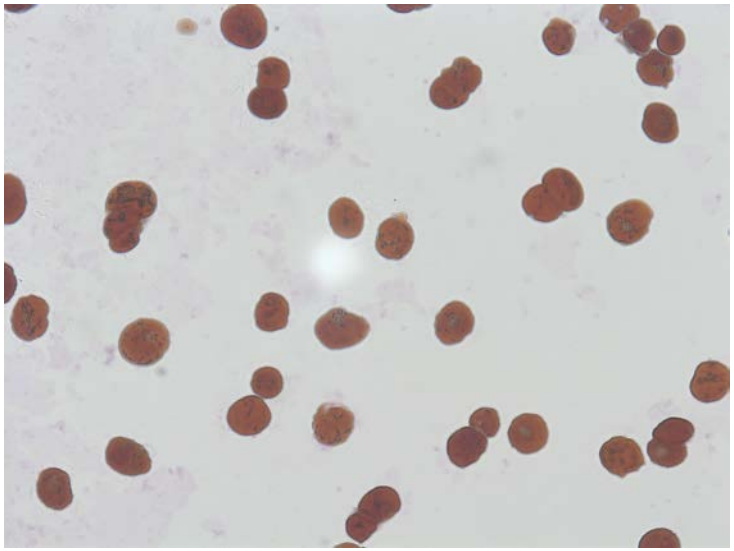
- Use recombinant DNA to introduce foreign genes into red blood cells produced in culture from bone marrow- derived CD34+ stem/ progenitor cells
- Use sortase enzymes to covalently link ~3000 - 8,000 molecules of any desired peptide or proteins to the surface of normal mouse and human red cells
- In the future, produce human red cells in culture from immortal lines of ES or iPS cells

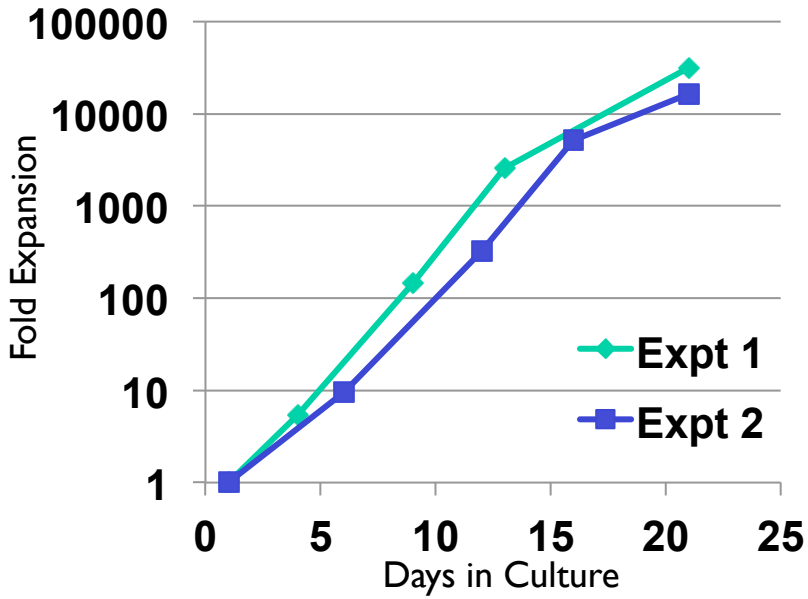


Covalently linking unique functional modalities to mouse or human red cells produced in cell culture:

- Single- chain antibodies that neutralize foreign pathogens and toxins
- Proteins to treat enzyme deficiencies.
- Receptors that bind and remove pathological macromolecules (Low Density Lipoproteins, immune complexes)
- Inducing tolerance rather than an immune response to foreign peptides and proteins – treatment of autoimmune diseases

We developed a 21- day culture system for human peripheral blood CD34+ stem/progenitor cells that generates normal enucleated red blood cells





LETTER

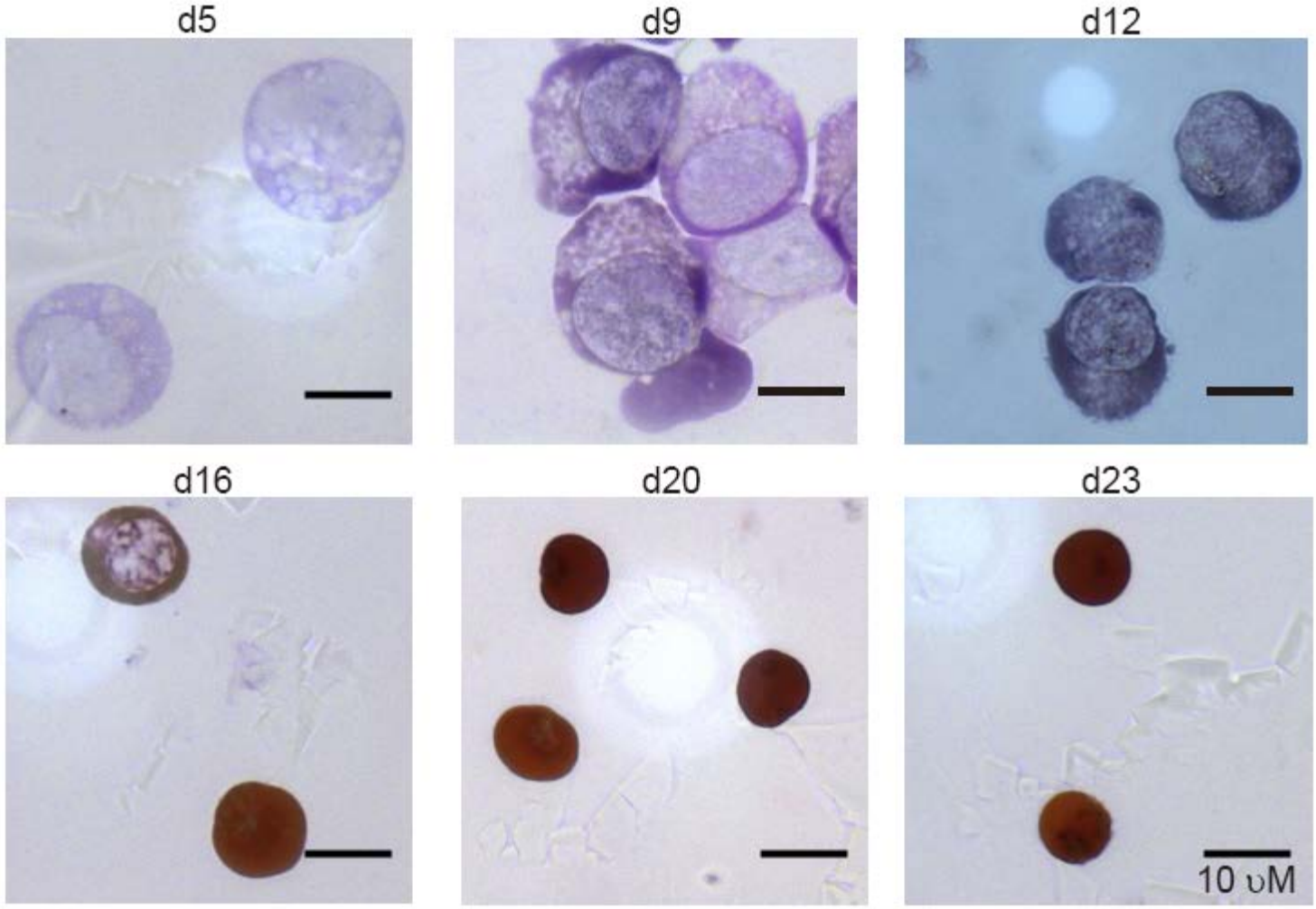
doi:10.1038/nature14326

PPAR-α and glucocorticoid receptor synergize to promote erythroid progenitor self-renewal

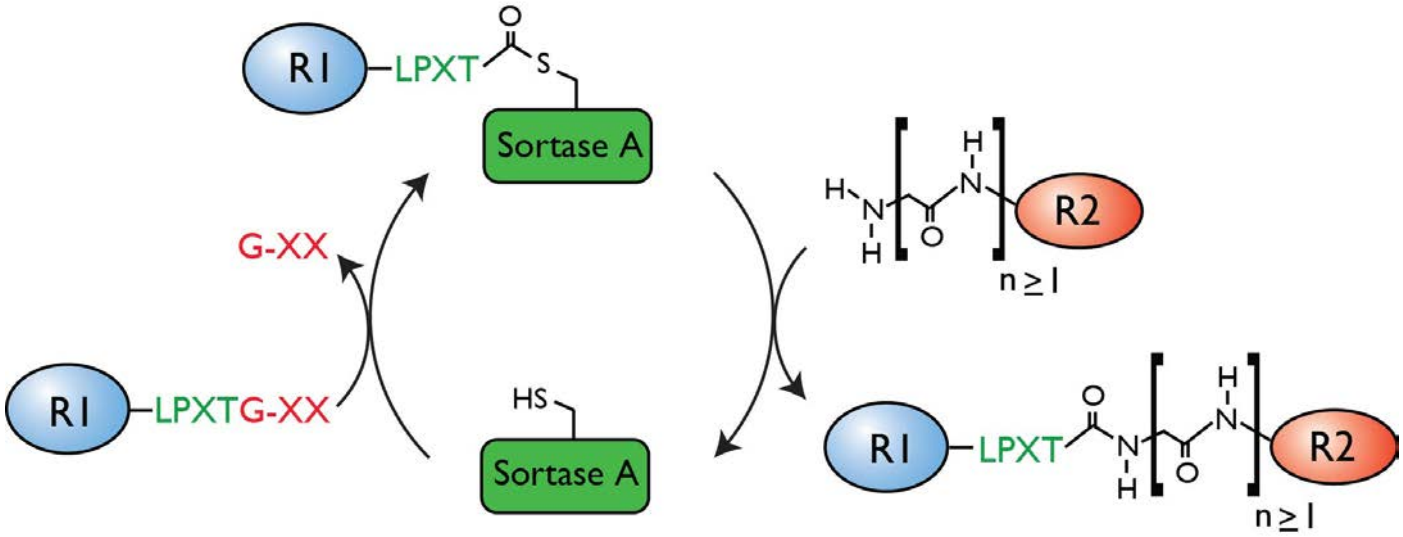
Hsiang-Ying Lee[1]*, Xiaofei Gao[1]*, M. Inmaculada Barrasa[1], Hu Li[2], Russell R. Elmes[1], Luanne L. Peters[3] & Harvey F. Lodish[1,4]

Nature 522, 474–477 (2015).

Normal morphology, size, and hemoglobin composition of human RBCs made in culture

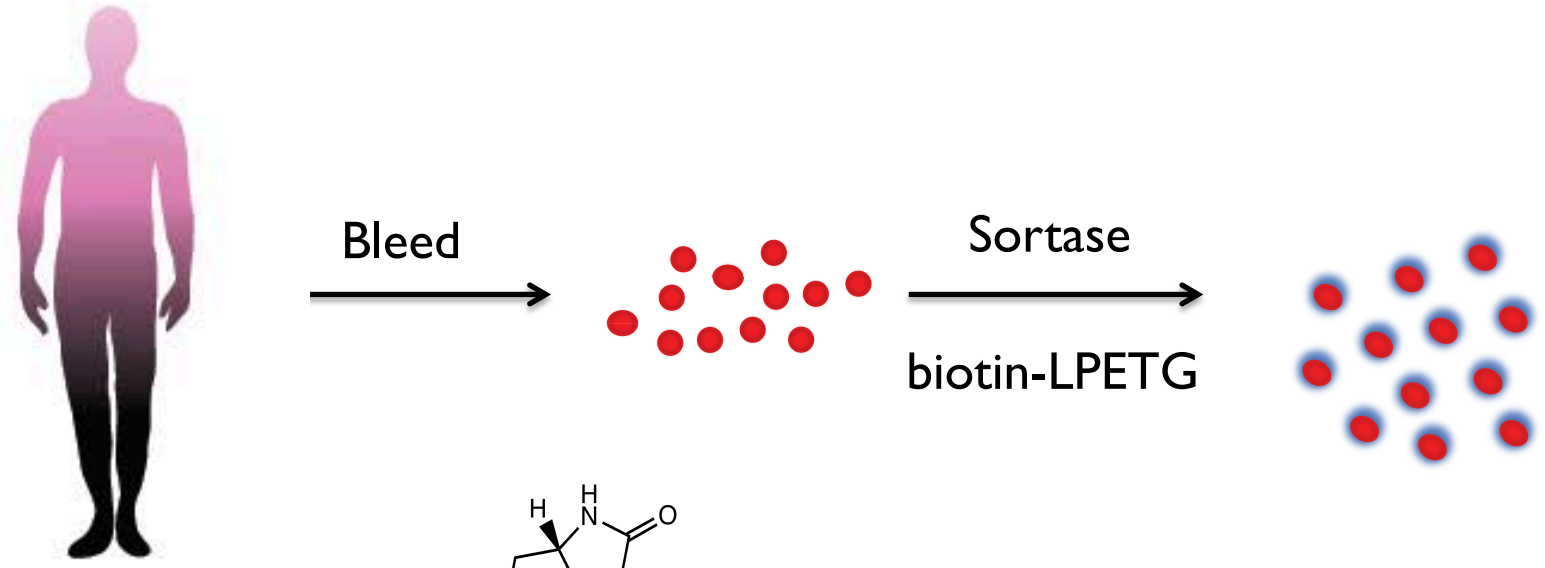


Sortase A is a bacterial transpeptidase that covalently links a "donor" molecule (R1) containing a C- terminal LPETGG sequence to an "acceptor" (R2) that contains amino-terminal glycine residues

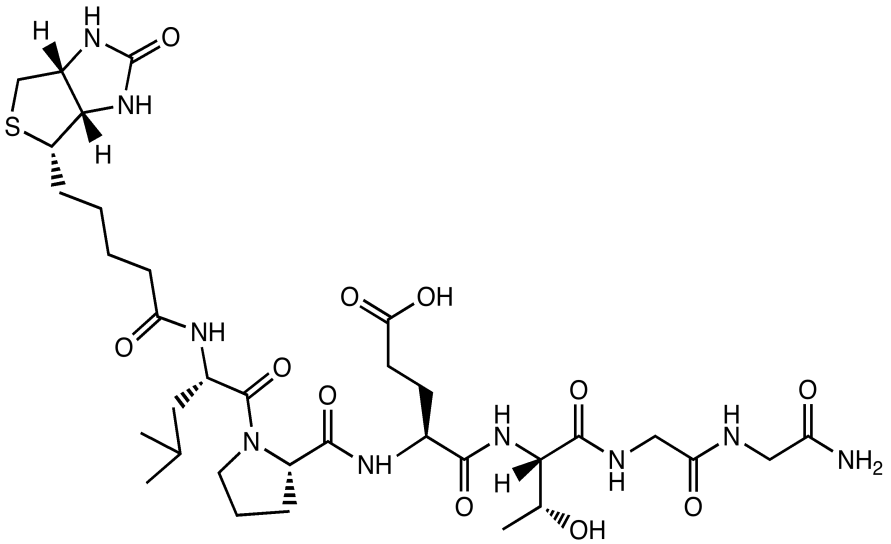


R1 and R2 can be proteins, peptides, small molecules, lipids, carbohydrates

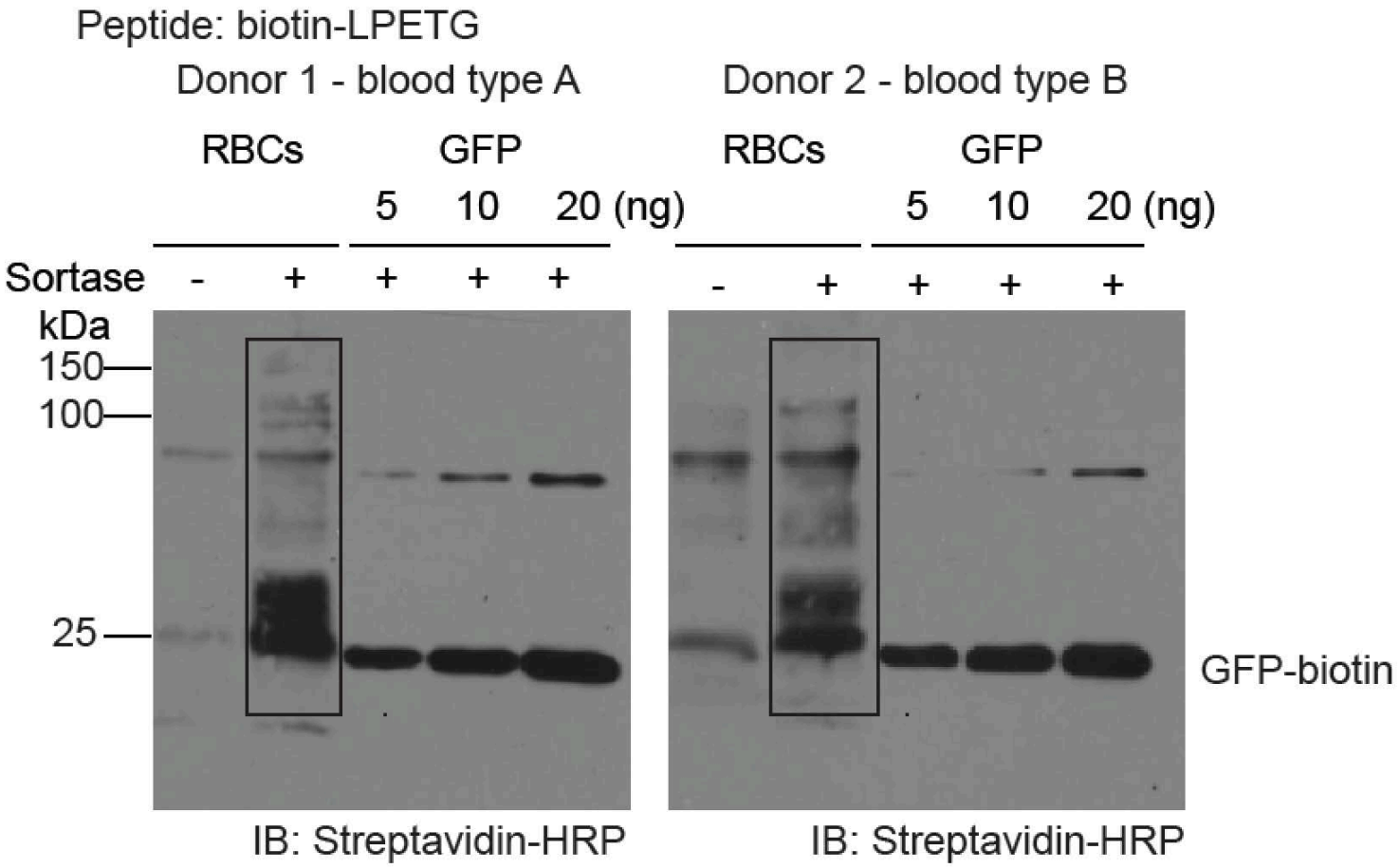
Engineering Normal Human Red Blood Cells



biotin-LPETG



Sortagging native human RBCs: Covalent linkage of biotin-LPETG to glycine residues at the extracellular N- termini of (as yet unknown) red cell membrane proteins



Quantification: ~3,000 sortagged biotin-containing peptides/cell

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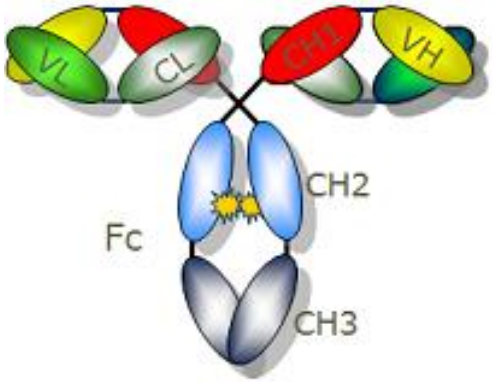
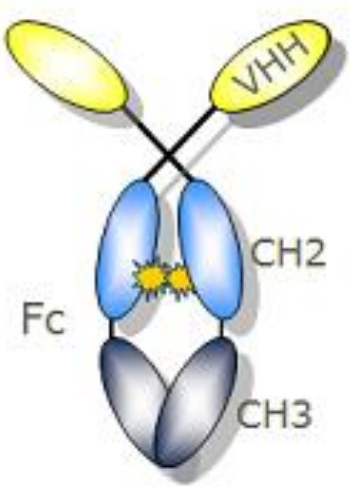
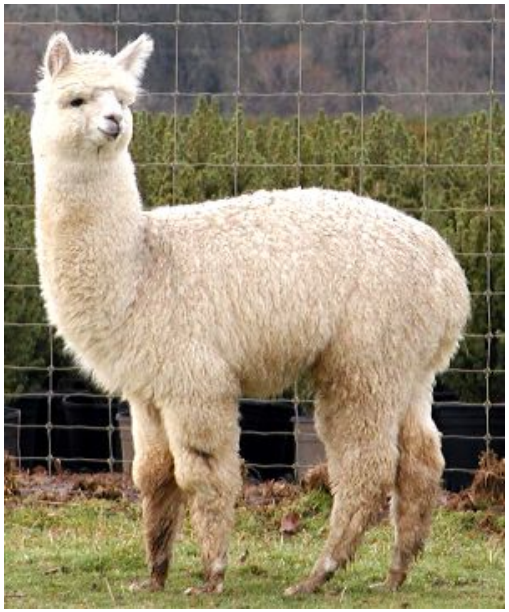
Alpaca heavy- chain only single-domain antibodies, called VHHs



Conventional antibody (Ab)

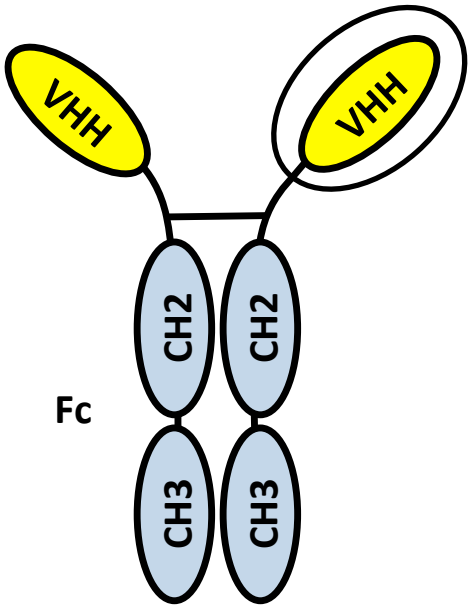


Heavy-chain only antibody (HcAb)



http://www.structuralbiology.be/chaperones

VH domains from alpaca heavy - chain only antibodies (VHHs)



Camelid heavy chain only Ab

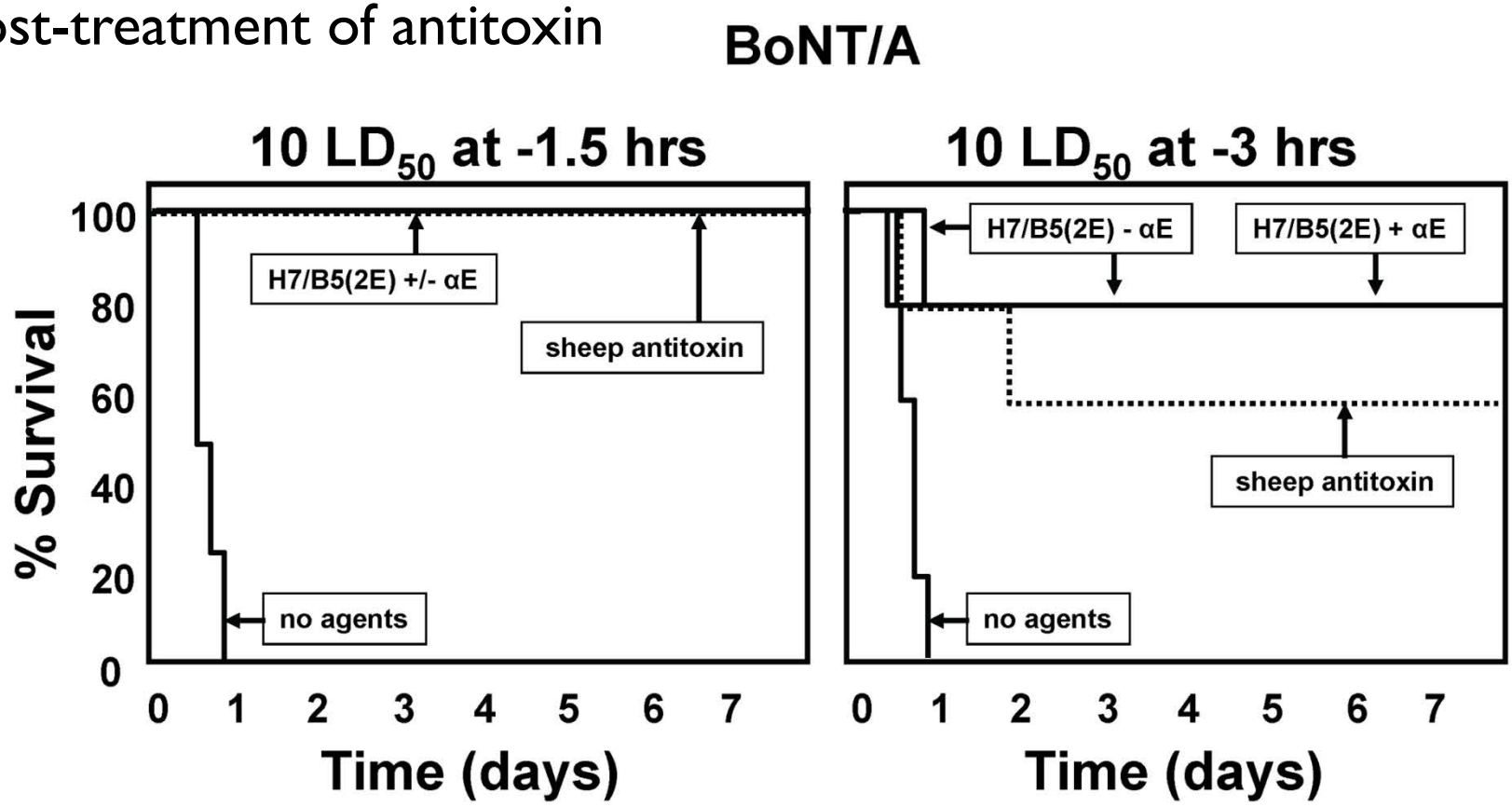
Favorable features of VHHs

- Small proteins (14 KDa)
- Easy to clone coding DNA (single domain)
- Functionally expressed at high levels in bacteria and mammalian cells
- More stable to extreme pHs and temperatures
- Commonly bind conformational epitopes
- Amenable to crystallization when bound to target
- Easy to 'multimerize' for improved properties

Botulinum toxin A (BoTox) causes nerve paralysis and death.

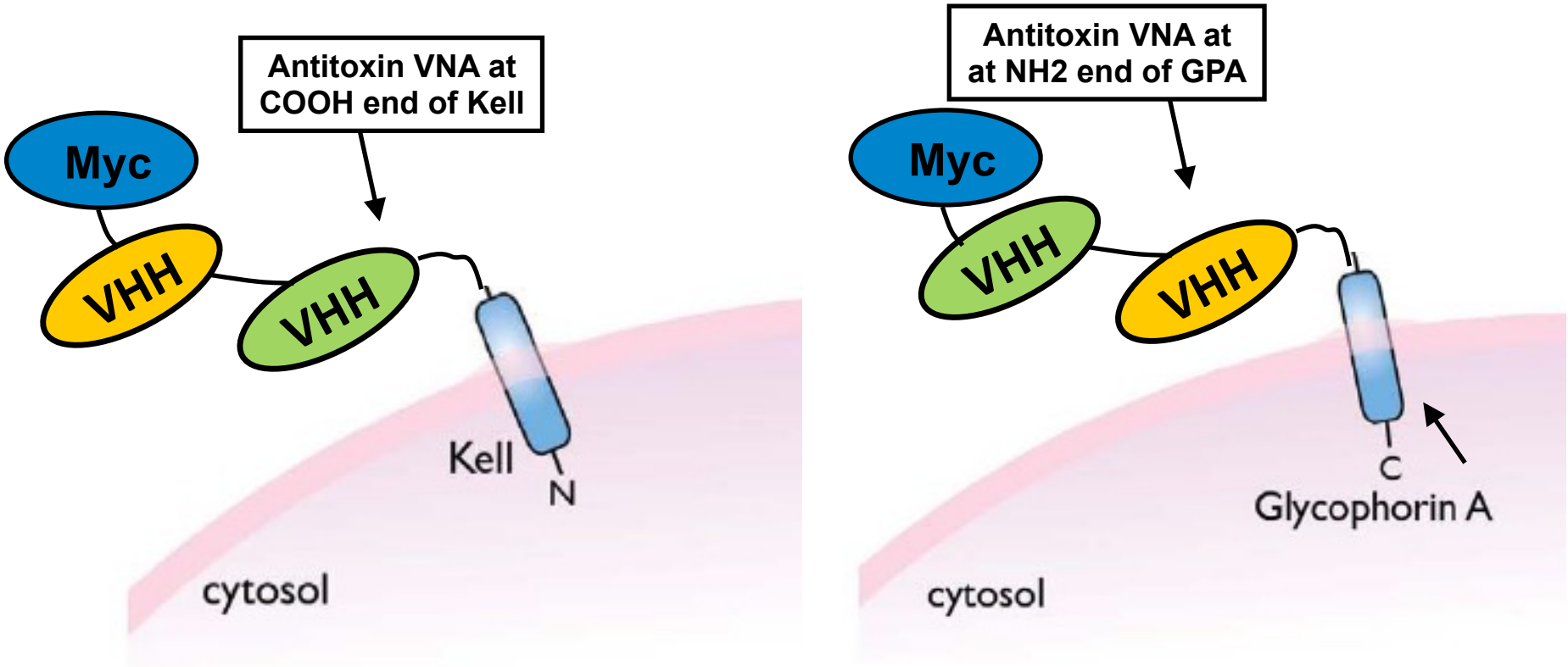
A chimera of two different anti- Botulinum toxin VHHs is highly effective as an antitoxin *in vivo* but only for a few hours

Post-treatment of antitoxin

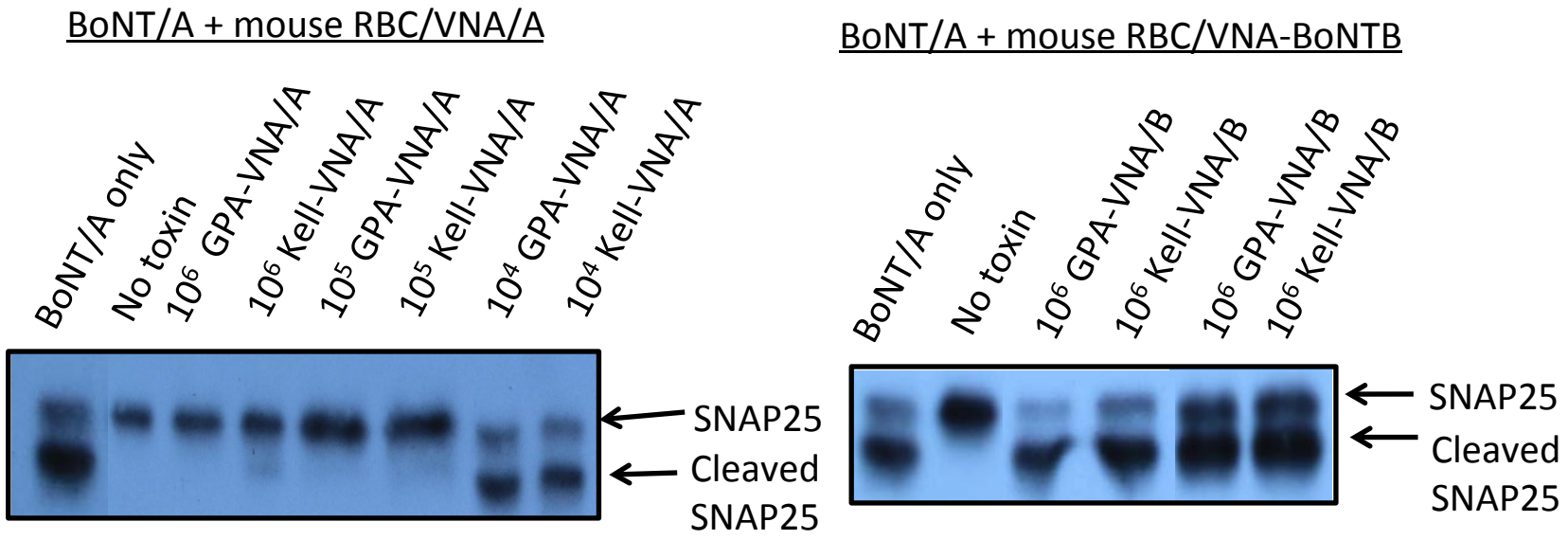


PLoS One. 2012;7(1)

Camelid single-domain antibodies, VHHs, can be linked by gene fusion to red cell membrane proteins and become localized to the surface of red blood cells made in culture

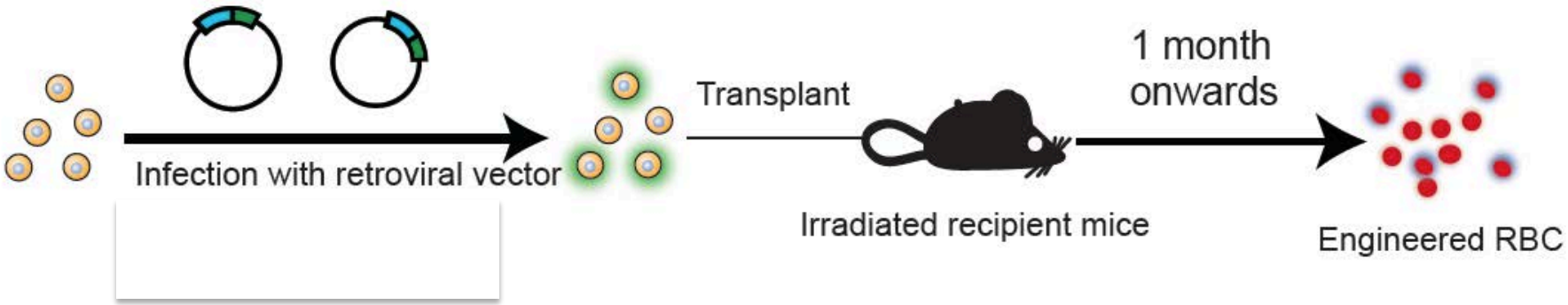


As demonstrated by coculture with nerve cells, BoNT/A is neutralized by *in vitro*- produced mouse red cells bearing VNA's specific for BoNT/A but not BoNT/B



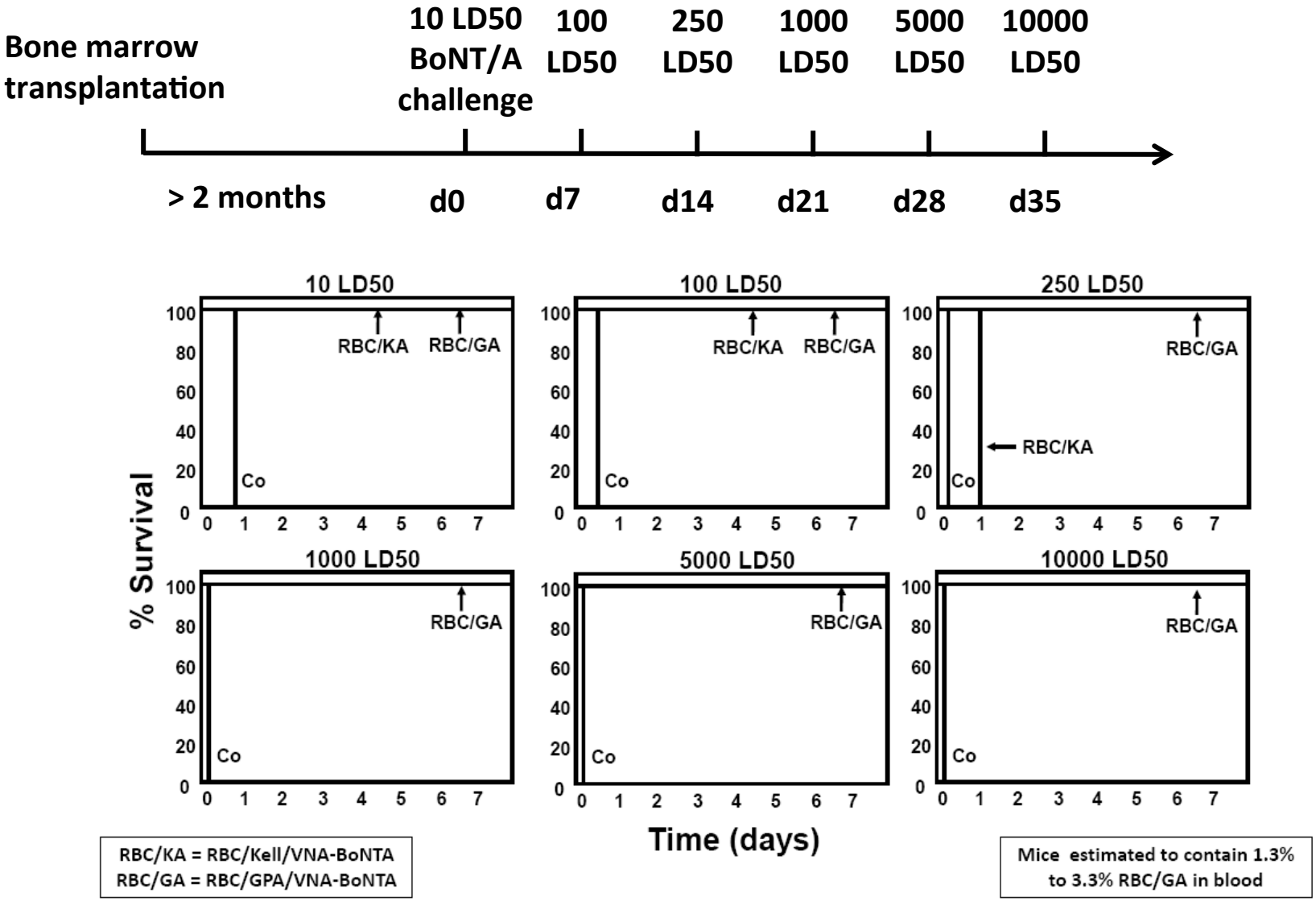
SNAP25 is a protein in nerve synapses that is essential for synaptic transmission. Treatment with BoNT/A causes cleavage of SNAP25, killing the nerve cell

Bone marrow stem cell transplantation provides a rapid way of generating normal mouse red blood cells engineered to express an antitoxin VHH on their surface



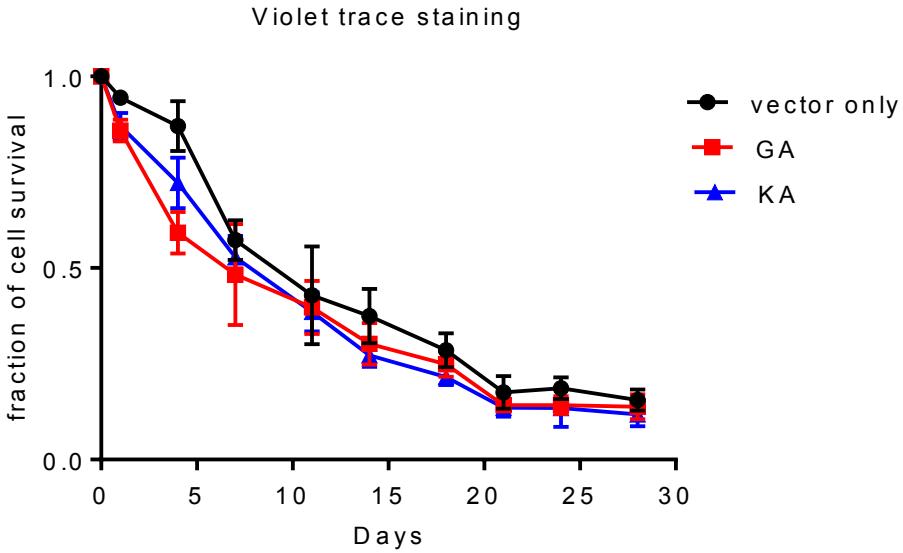
The retroviral vector encodes green fluorescent protein as well as a chimera of Kell or glycophorin A with the desired VHH antibody.

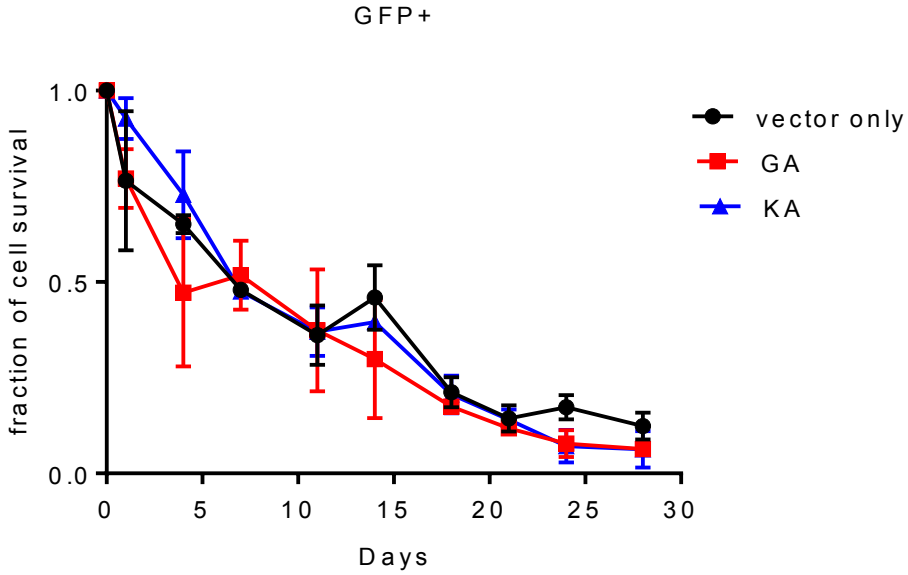
Bone marrow transplanted mice in which 1 – 3% of their red cells express on their surface a Glycophorin A-VNA chimera specific for Botulinum Toxin A (RBC/GA) are protected from a 10,000 LD50 BoNT/A challenge.



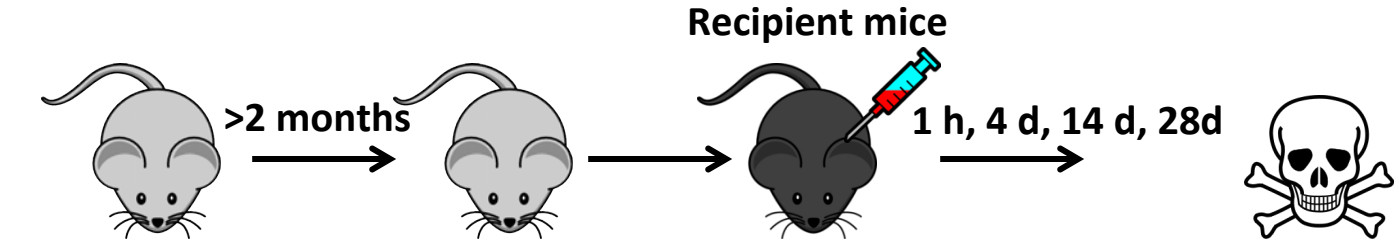
5 mice each group

Following transfusion RBC/VNAs circulate for at least 30 days - a normal lifetime in the circulation.





Mice transfused with RBC/GAs are protected from a 10 LD50 BoNT/A challenge even 28 days post-transfusion



Challenge time after transfusion	1 h	4 d	7 d	14 d	28d
Ctrl survival (%)	0	0	0	0	0
RBC/GA Survival (%)	100	100	100	100	100

200 µl WT or RBC/GA blood transfused;
3 mice each group; repeated twice

Generation of alpaca nanobodies against Ebola and Zika viruses

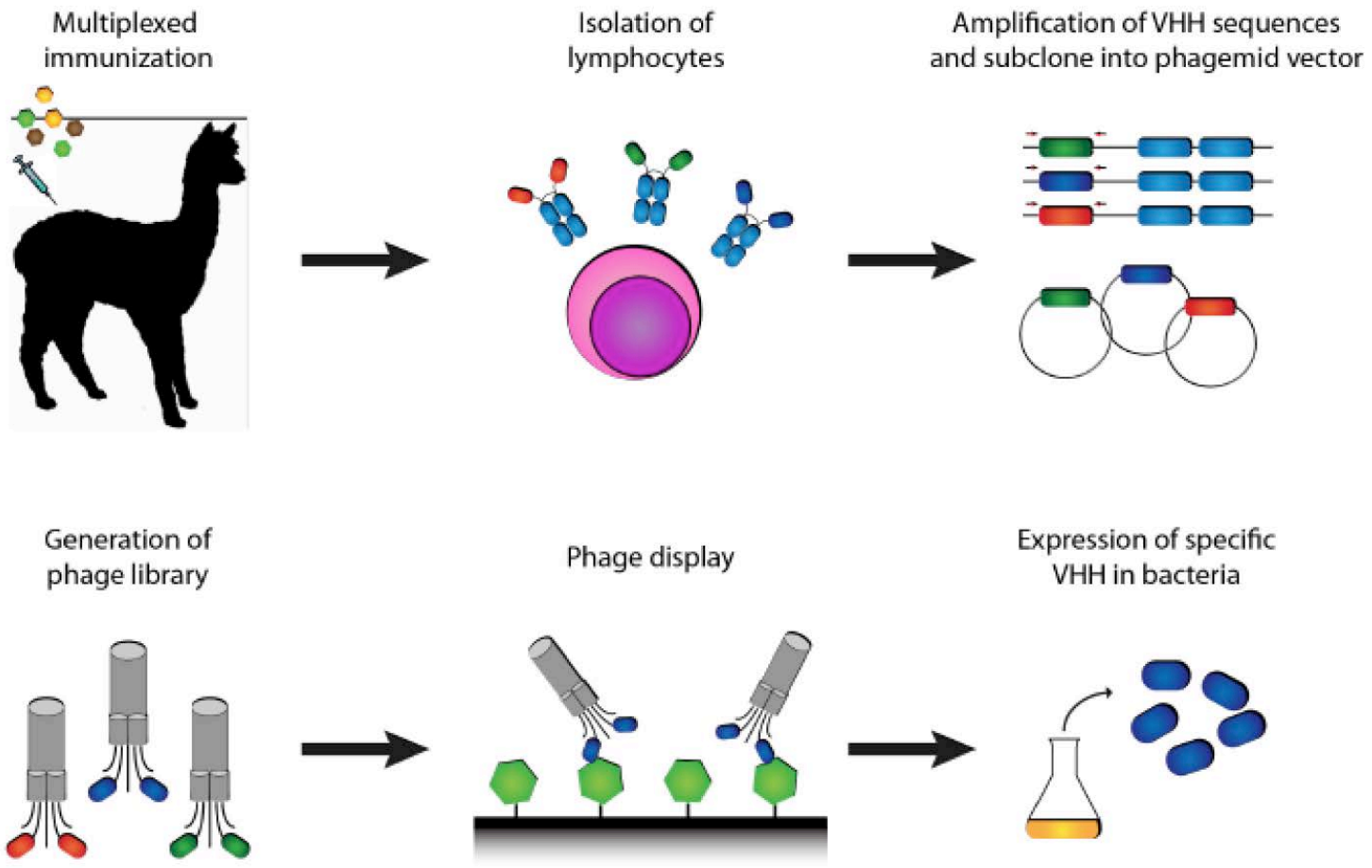


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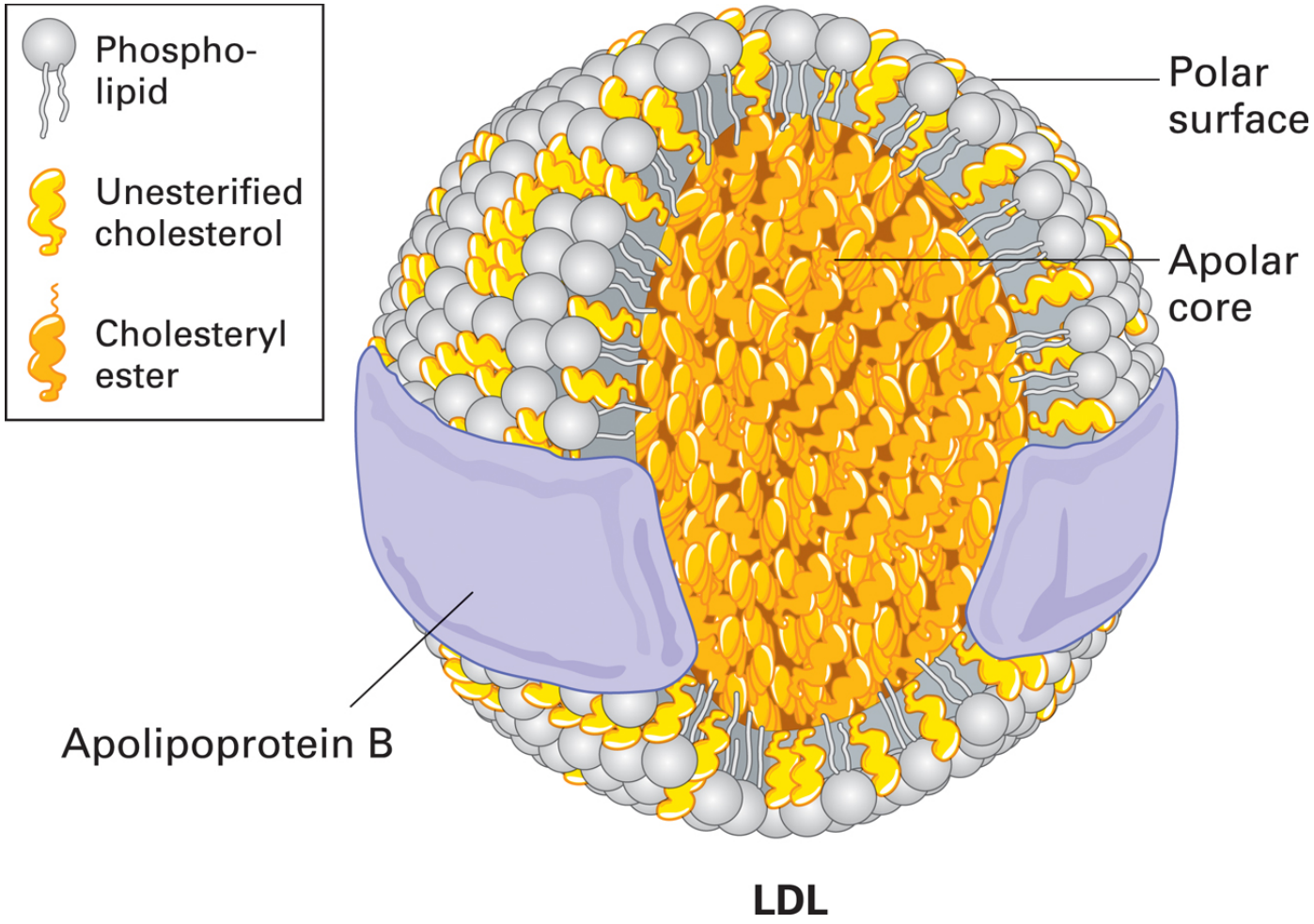
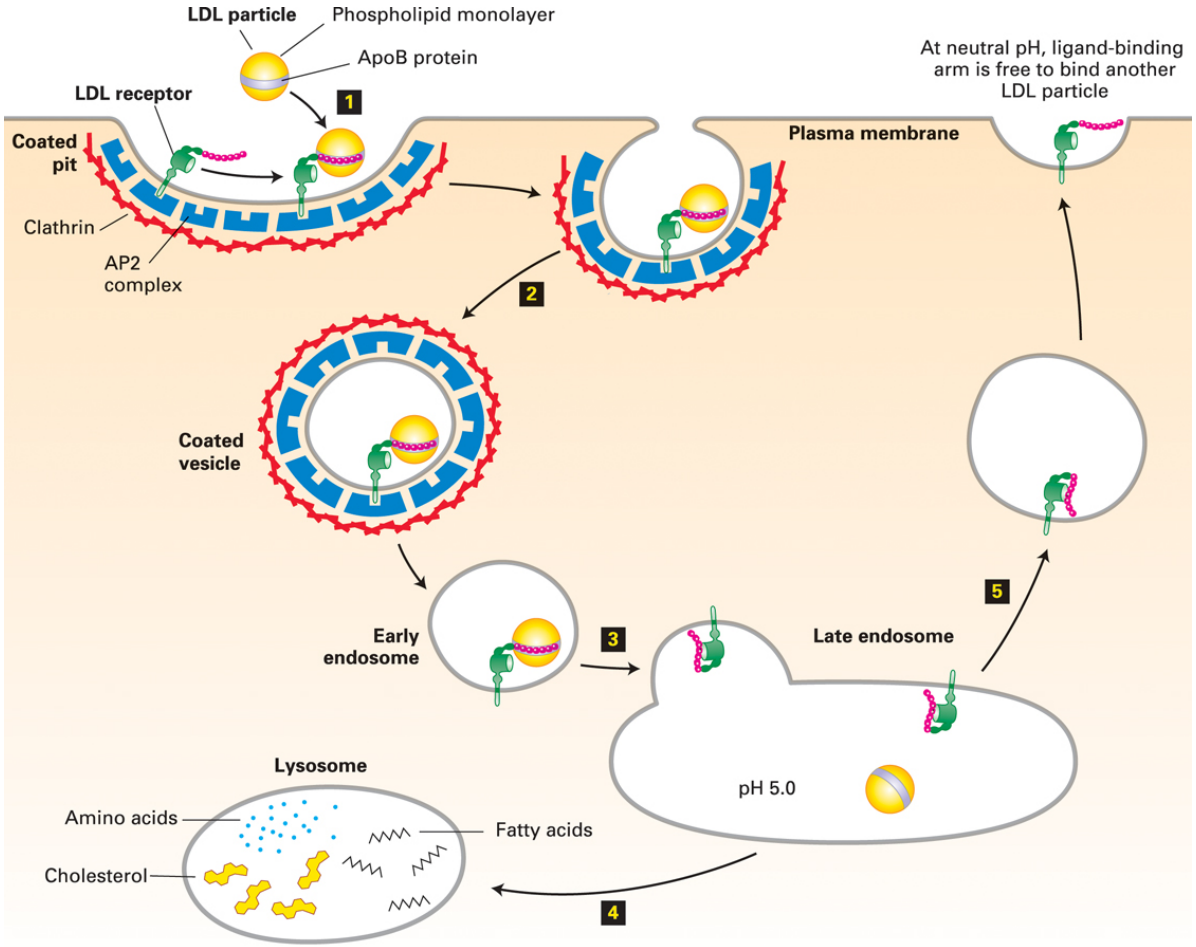
Figure 14.27 **Model of low-density lipoprotein (LDL).**



High levels of LDL ("bad cholesterol") in the blood are correlated with increased risk of cardiovascular disease and death.

Lodish et. al., Molecular Cell Biology 8th edition 2016

Figure 14.29 Endocytic pathway for internalizing low-density lipoprotein (LDL).



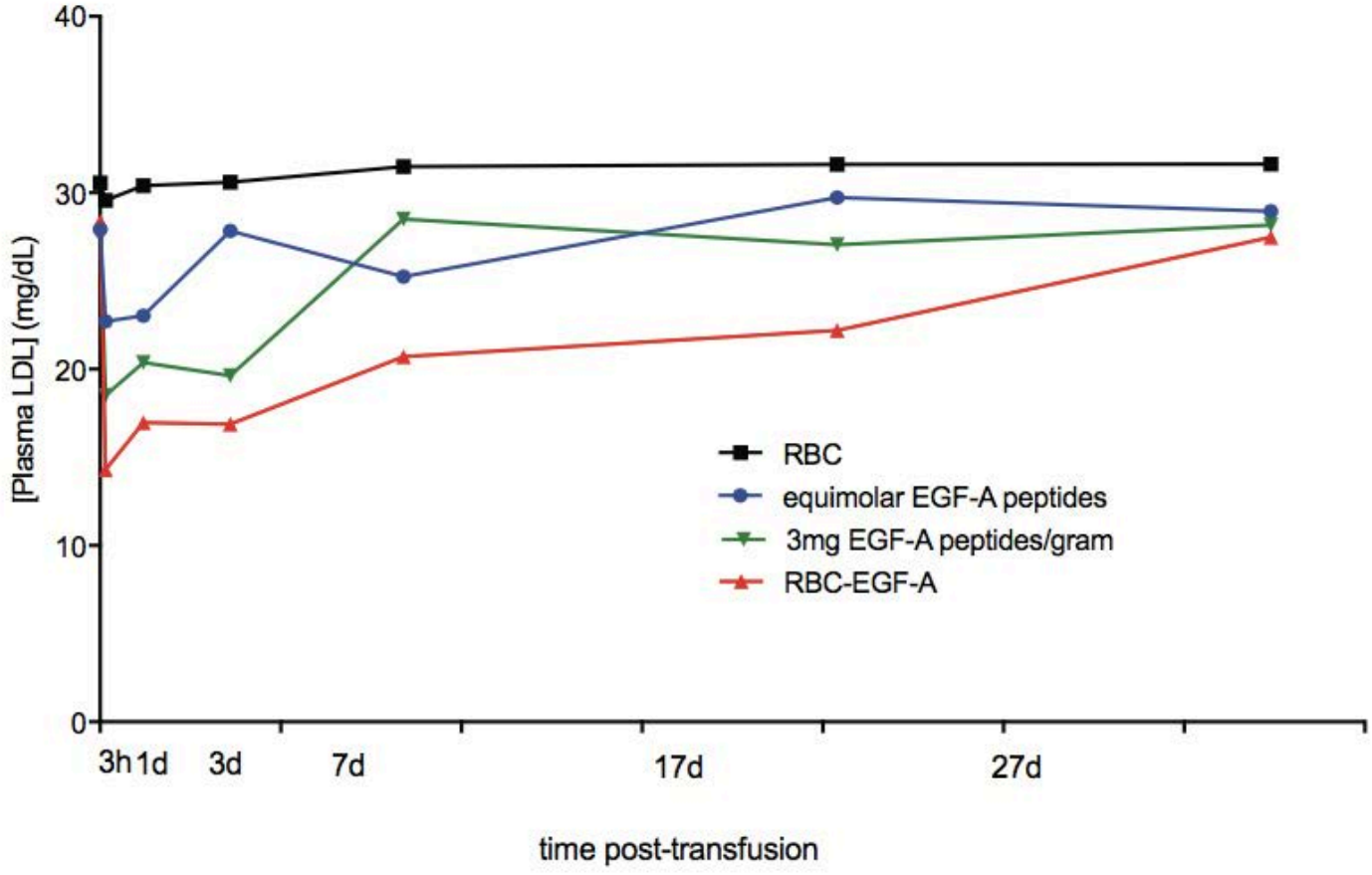
Endocytosis of LDL by LDL receptors in the liver is the major pathway for removing LDLs from the circulation.

Lodish et. al., Molecular Cell Biology 8th edition 2016

PCSK9
(Proprotein Convertase Subtilisin/Kexin type 9)

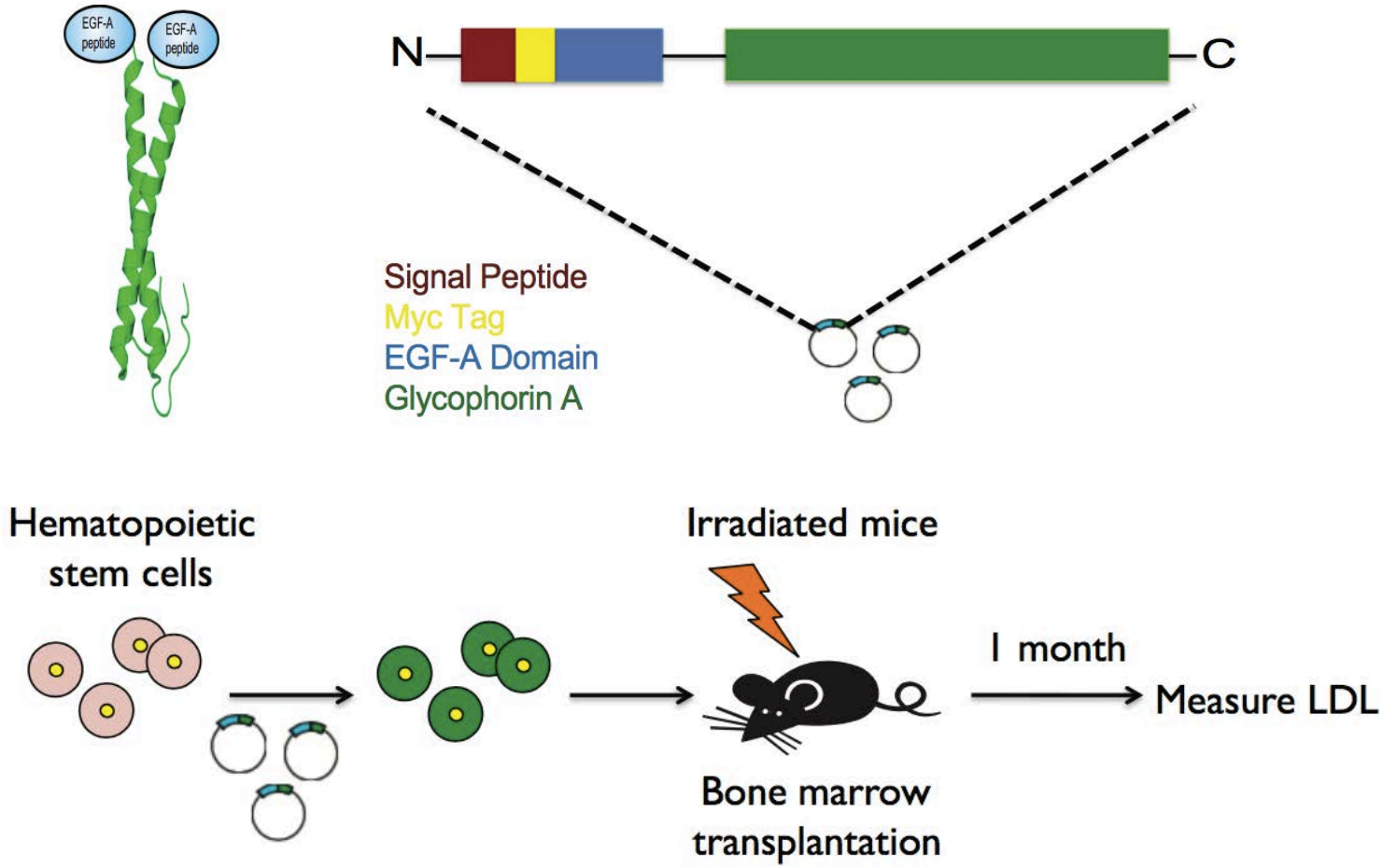
- PCSK9 binds to the EGF-A domain of the low-density lipoprotein receptor (LDL receptor), leading to accelerated degradation of the LDL receptor, reduced levels of the LDL receptor on the cell surface, and to increased LDL levels in the circulation.
- Individuals homo- or heterozygous for PCSK9 loss- of- function mutations have elevated levels of cell surface LDL receptors, very low serum cholesterol, and low risk of cardiovascular disease. Otherwise they are normal.
- PCSK9 inhibition can be accomplished by monoclonal antibodies targeting PCSK9, e.g. alirocumab and evolocumab, decreasing [LDL] up to 70%

Long- term lowering of plasma LDL cholesterol following transfusion of mouse red cells to which the ~4500 molecules of the 40 amino acid EGF-A peptide derived from the LDL receptor have been covalently attached using sortase

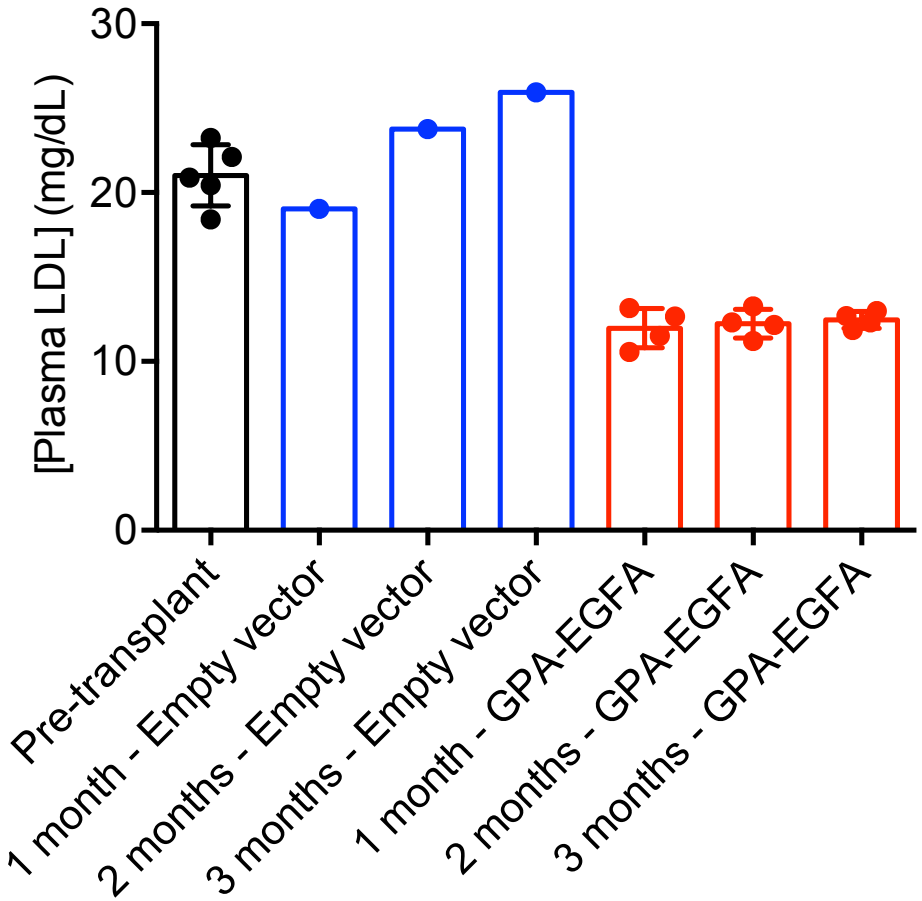


200 µl of mouse blood (= ~90 µl red cells) were reacted with sortase and a synthetic LDL-R EGFA domain extended with LPETGG; about 4,500 molecules were attached per red cell. The blood was then transfused into recipient mice, equivalent to ~ 10^{12} EGFA peptides.

Chimera of EGF-A domain and Glycophorin A



Persistent *in vivo* lowering of plasma LDL in transplanted mice



Rhogerry "Gerry" Deshycka won the top MIT prizes for undergraduate research in Biology and Biological Engineering

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Rubius™
Therapeutics
A Flagship VentureLabs Company

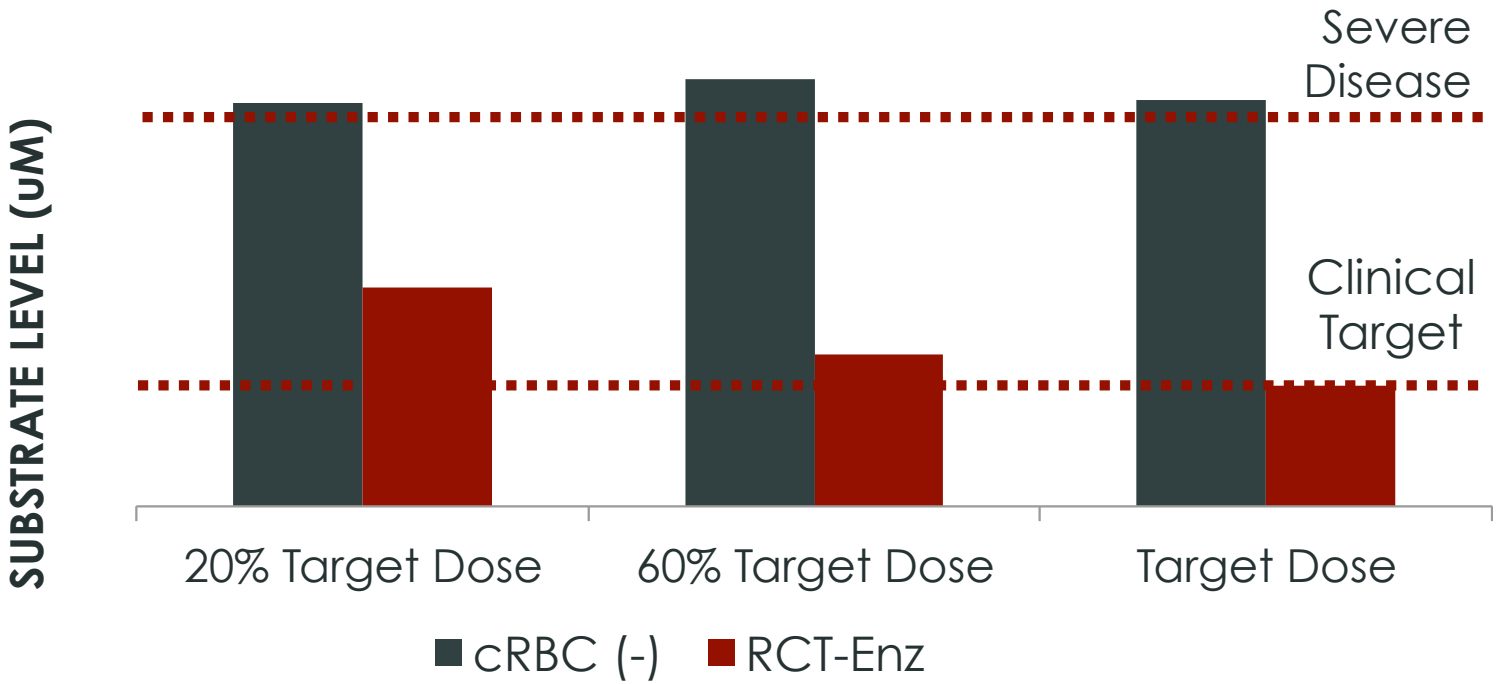
Phenylketonuria Is An Autosomal Recessive Enzyme Deficiency Which Can Lead To Irreversible Brain Damage

- Phenylketonuria (PKU) is a deficiency of phenylalanine hydroxylase (PAH), an enzyme that breaks down phenylalanine
- PKU is diagnosed at birth by a routine blood test (toe prick)
- PKU if not properly managed results in nerve damage and intellectual disability
- Goal of PKU therapies: Achieve blood Phe levels less than 360μM
- Mainstay of therapy is dietary restriction of Phe through medical foods (cost: $60,000 - $100,000/year)
- BH4 (Kuvan), a pterin cofactor that is required for PAH activity, is the only approved PKU therapy
  - 10% - 20% of patients are on Kuvan therapy due to cost, pill burden and low response rates
  - Moderate/severe PKU patents do not respond to Kuvan and remain on a restricted diet

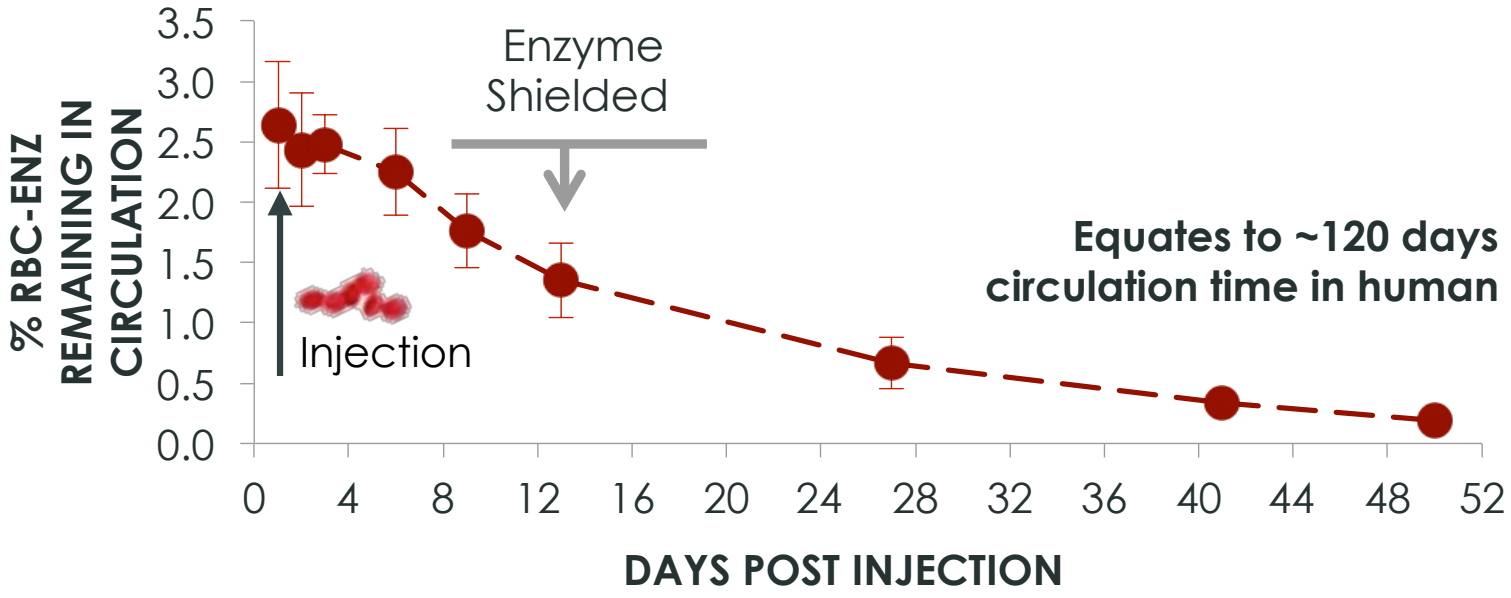
Rubius' solution

- Use recombinant DNA technology to introduce into blood stem cells the gene for a bacterial enzyme that degrades phenylalanine.
- Culture these blood stem cells under conditions where they divide and differentiate into otherwise normal red blood cells that contain the phenylalanine- degrading enzyme.
- Transfuse these red cells into a PKU patient, lowering the level of phenylalanine in the circulation.

Human RBC- ENZ metabolizes excess phenylalanine in human serum



Mouse RBC- ENZs have a normal lifetime in transfused mice



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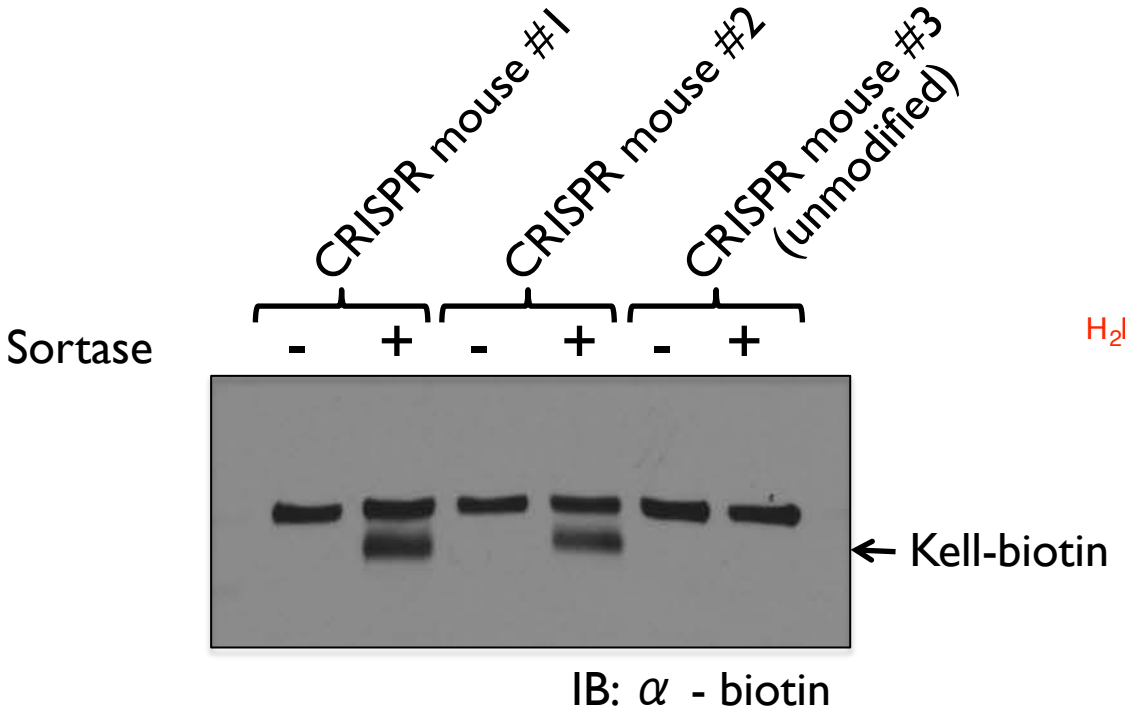
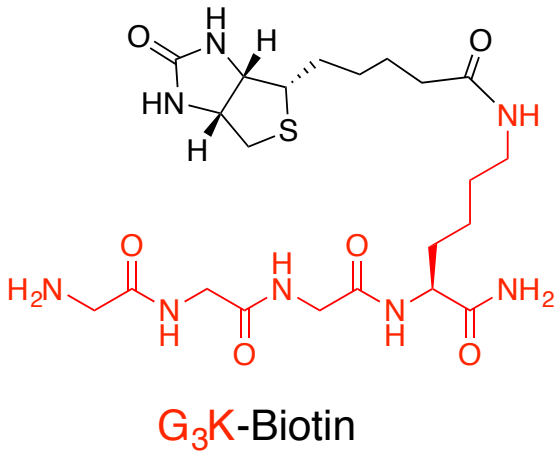
Generation of Sortaggable Mature RBCs: CRISPR mice expressing endogenous Kell-LPETG

Endogenous mouse Kell C-terminus: … R C K L W *

LPETG insertion to endogenous Kell C-terminus: … R C K L G G S G G S **L P E T G** G W *

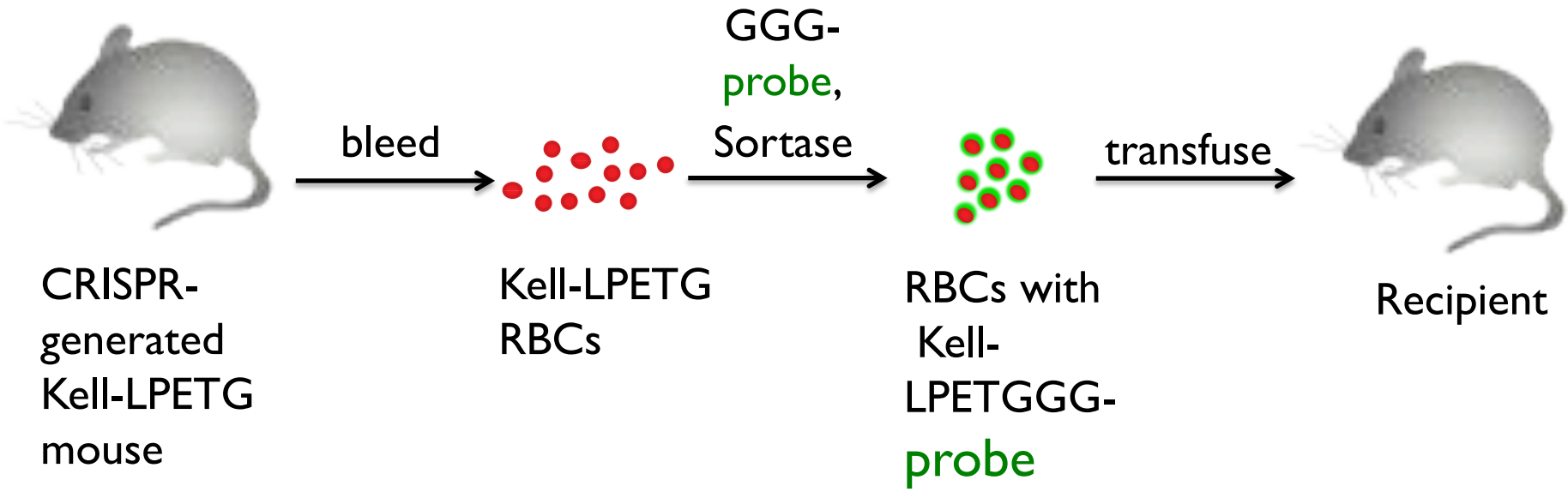




~ 9,000 Kell LPETGG molecules / red blood cell

Lenka Kundrat
Takeshi Maruyama
Stephanie Dougan

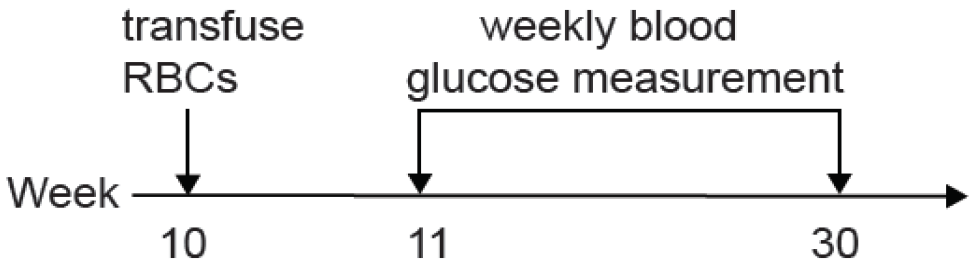
Overall experimental scheme

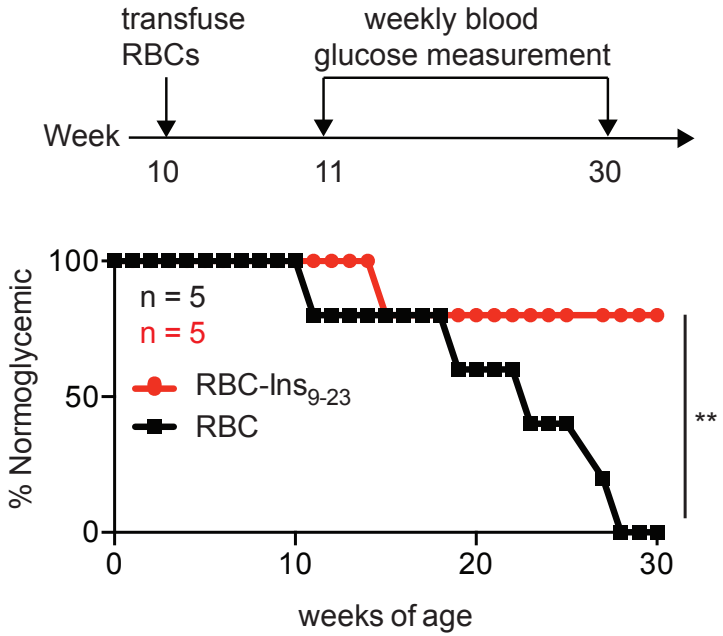


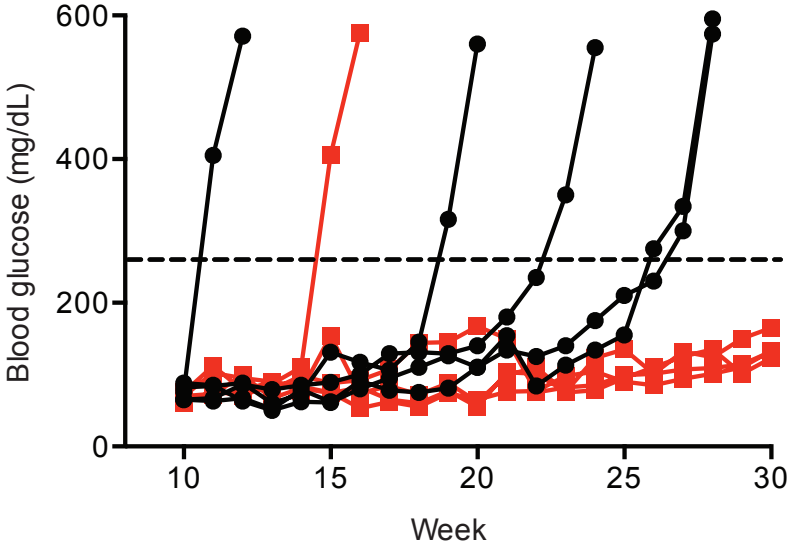
Type 1 Diabetes in NOD mice

- Polygenic model for type 1 diabetes
- Antigens: Insulin, GAD65, HSP-60, etc
- Characterized by insulitis and leukocytic infiltration of the pancreatic islets.
- Decrease in pancreatic insulin content occurs in females at about 12 weeks of age and several weeks later in males
- Plasma glucose levels increase to greater than 250 mg/dl (normal is ~80 mg/dl)

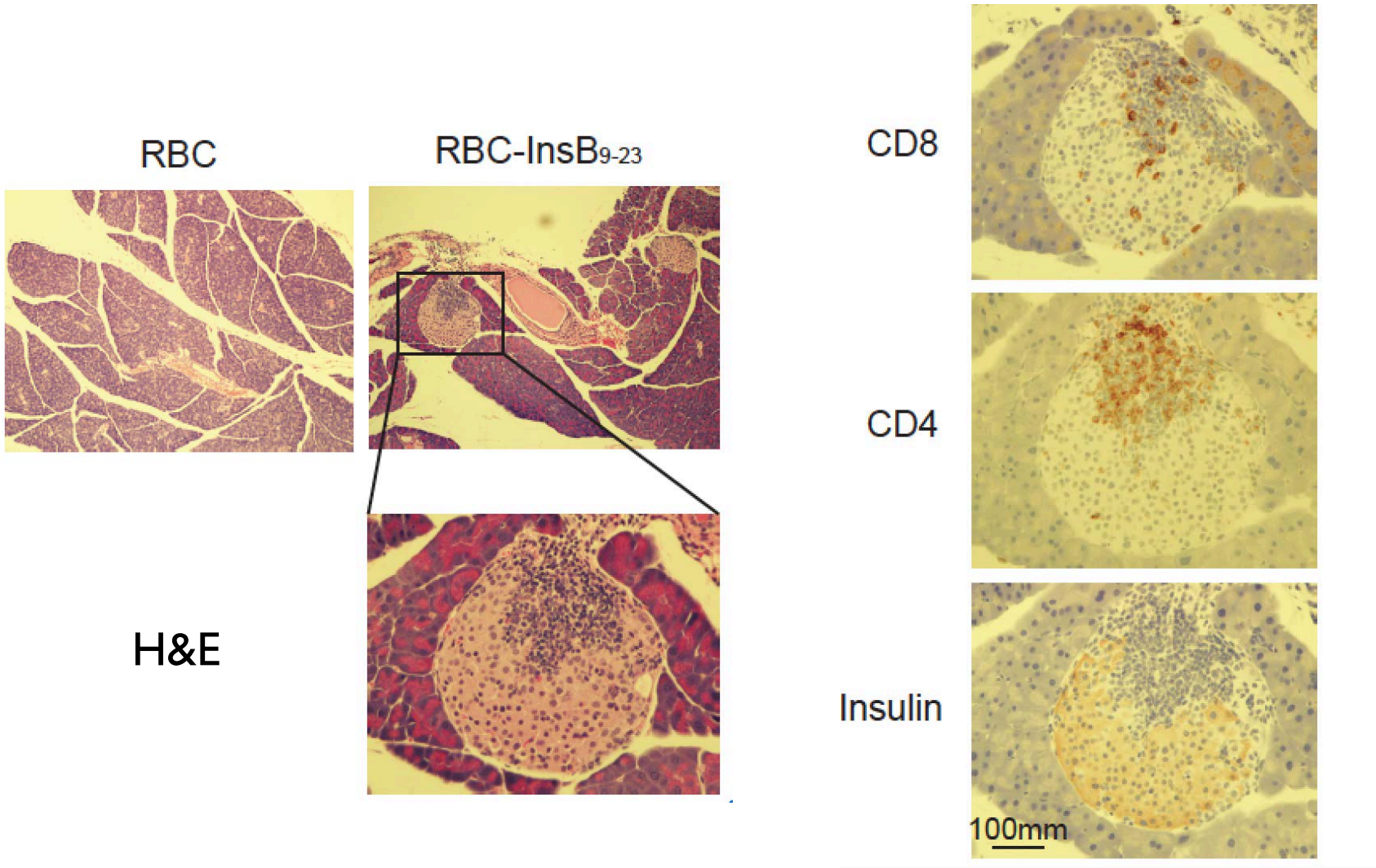
RBCs bearing $InsB_{9-23}$ peptide block progression of Type I Diabetes in NOD mice.



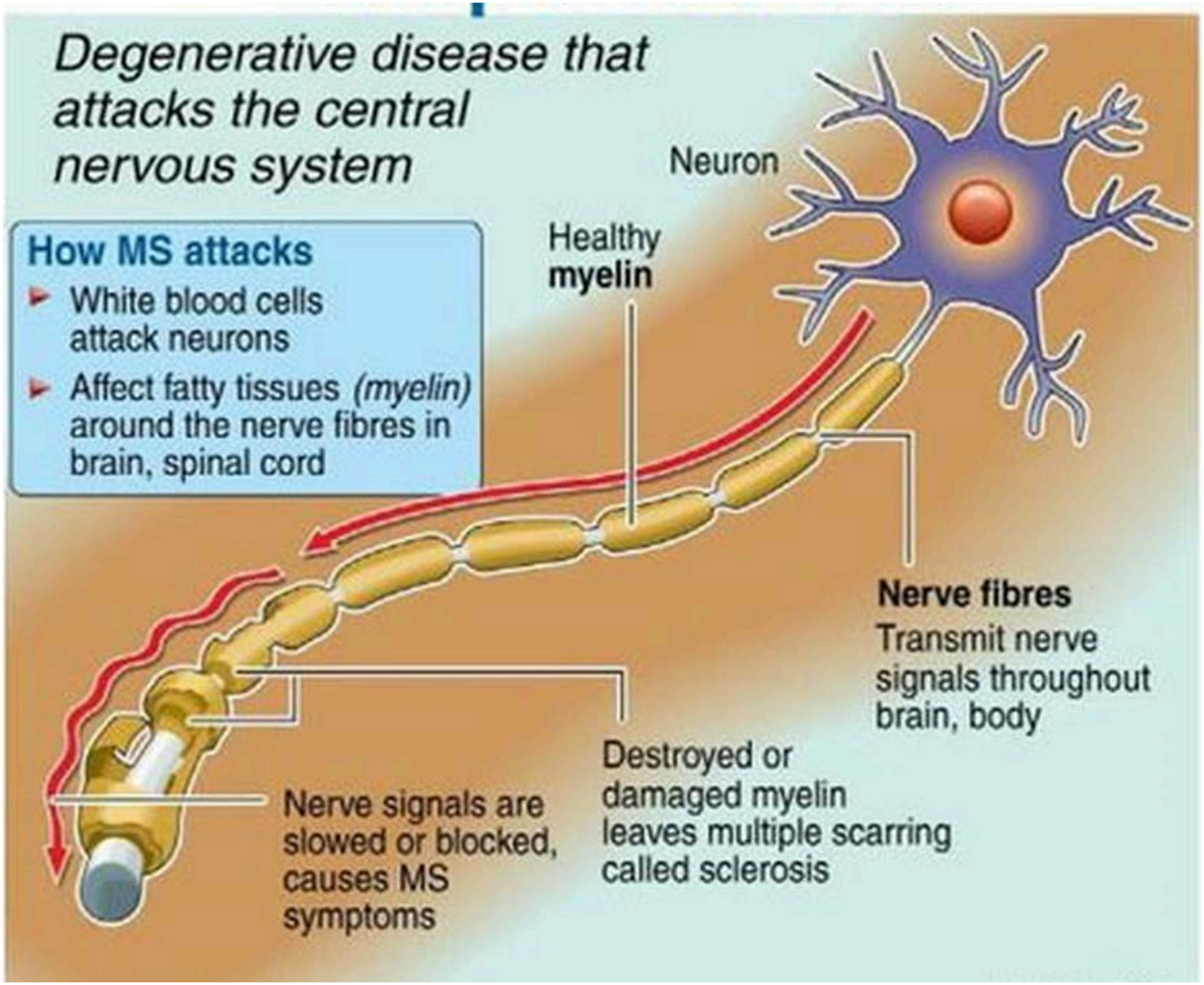




RBCs bearing $InsB_{9-23}$ peptides prevent destruction of insulin- containing islets in NOD mice.



Multiple Sclerosis



Novalia Pishesha

EAE (Experimental Autoimmune Encephalomyelitis) – a mouse model of multiple sclerosis

Days

challenge with:
adjuvant/MOG_{35-55}
pertussis toxin

0

~10
onwards

MOG = Myelin
Oligodendrocyte
Glycoprotein

Clinical scores:
1 - limp tail
2 - partial hind leg paralysis
3 - complete hind leg paralysis
4 - complete hind and partial front leg paralysis
5 - moribund

EAE (Experimental Autoimmune Encephalomyelitis) – a mouse model of multiple sclerosis

RBC-MOG$_{35-55}$ Transfusion → Days -7

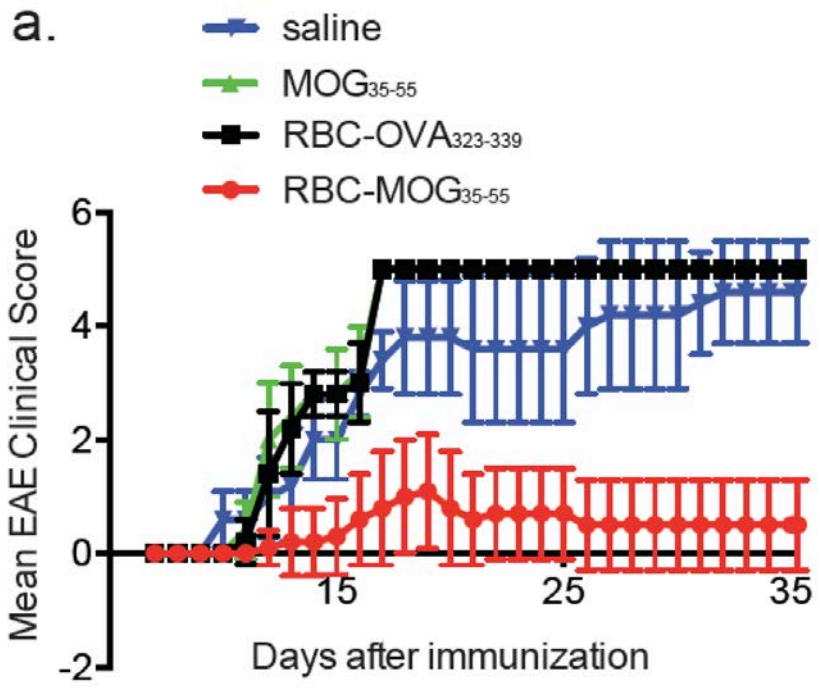
challenge with: adjuvant/MOG$_{35-55}$ pertussis toxin → 0

MOG = Myelin Oligodendrocyte Glycoprotein

~10 onwards

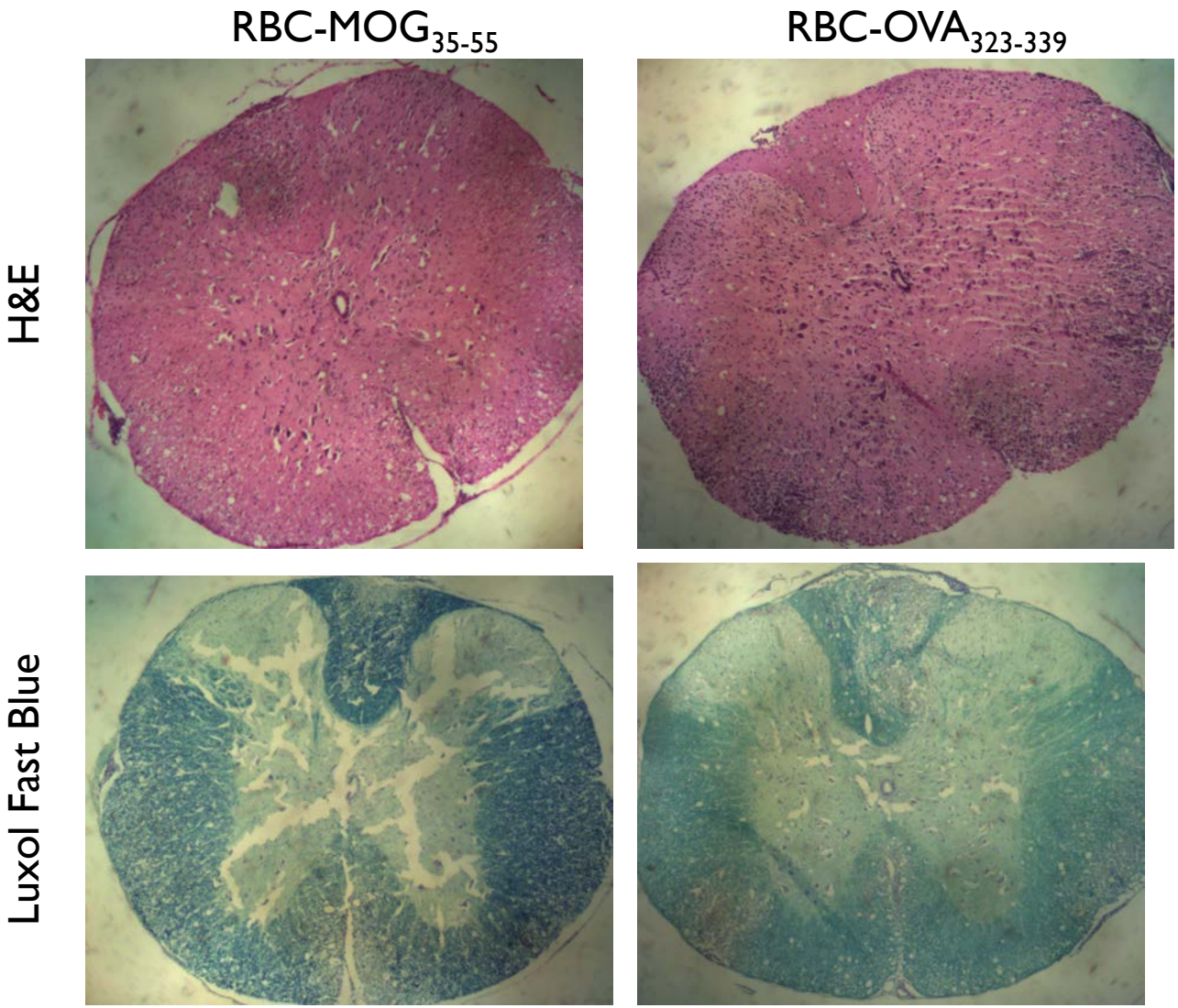
Clinical scores:
1 - limp tail
2 - partial hind leg paralysis
3 - complete hind leg paralysis
4 - complete hind and partial front leg paralysis
5 - moribund

RBCs carrying the MOG$_{35-55}$ peptide protect against EAE- delays or halt disease progression.



n = 10

Representative spinal cord histology shows that inflammation is suppressed by treatment with red cells bearing MOG_{35-55} peptide



Even after disease onset EAE can be reversed by transfusion of RBC-MOG_{35-55}

C57BL/6 mouse

challenge with:
s.c. - Complete Freund's Adjuvant/MOG_{35-55}
i.p. – pertussis toxin

i.v.

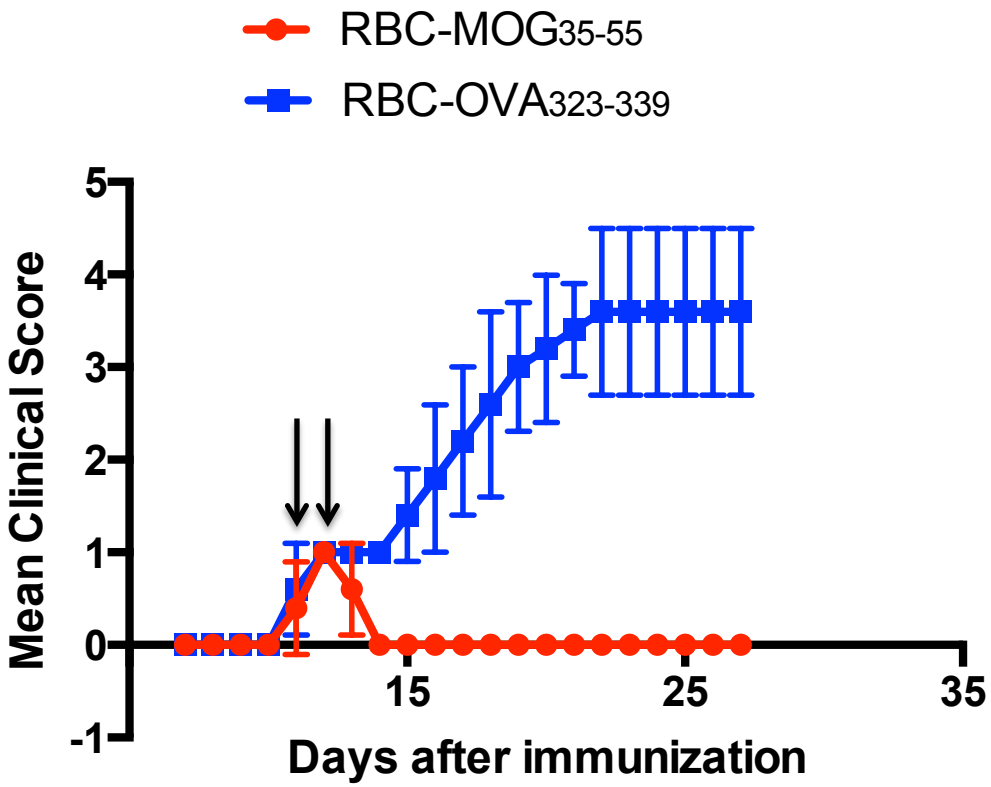
Check clinical score

Transfusion:
RBC-MOG_{35-55}
RBC-$OVA_{323-339}$

Days: 0 … 10 … 1 day after clinical score = 1

MOG = Myelin Oligodendrocyte Glycoprotein

Even after disease onset EAE can be reversed by transfusion of RBC-MOG$_{35-55}$



↓ = day 11 or 12, when transfusion is carried out

n = 5

Covalently linking unique functional modalities to mouse or human red cells produced in cell culture:

- Single- chain antibodies that neutralize foreign pathogens and toxins
- Receptors that bind and remove pathological macromolecules (Low Density Lipoproteins, immune complexes)
- Proteins to treat enzyme deficiencies.
- Proteins that kill tumor cells
- Inducing tolerance rather than an immune response to foreign peptides and proteins – treatment of autoimmune diseases