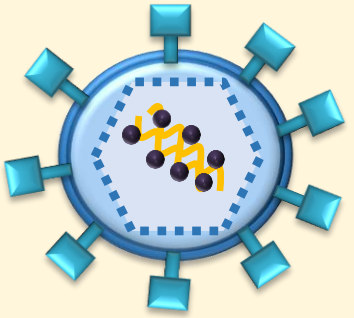
Targeted Gene Therapy Vectors for Enhanced B-Cell production of Antibodies and Factor IX protein

Els Verhoeyen

1) International center for research in infectiology CIRI – EVIR, Lyon
2) C3M, Equipe 3: Cancer metabolism and immunity– NICE

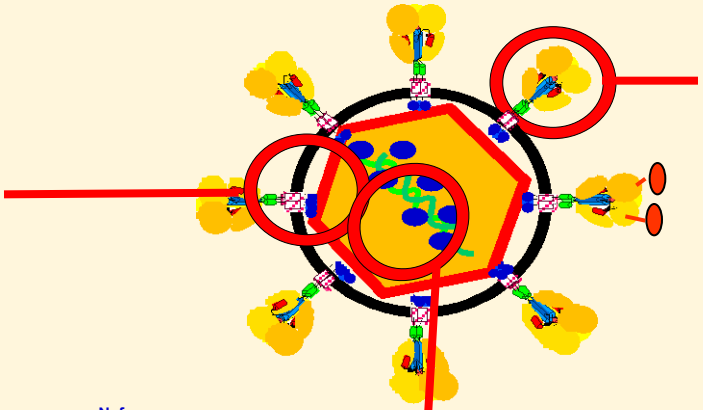


SFTH meeting , Lyon, 1 october, 2025

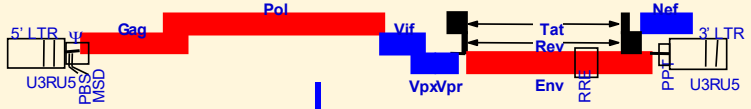
SFTH
SOCIÉTÉ FRANÇAISE
DE THROMBOSE
ET D'HÉMOSTASE

LENTIVIRAL VECTORS

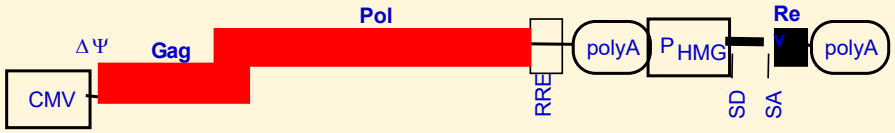
Modification
of viral "core"



Viral surface
modifications



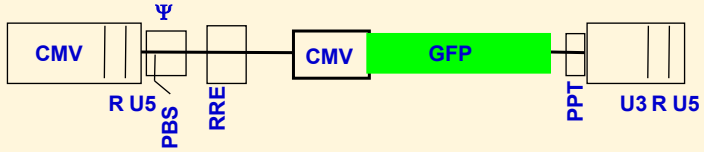
Modification
of viral genome



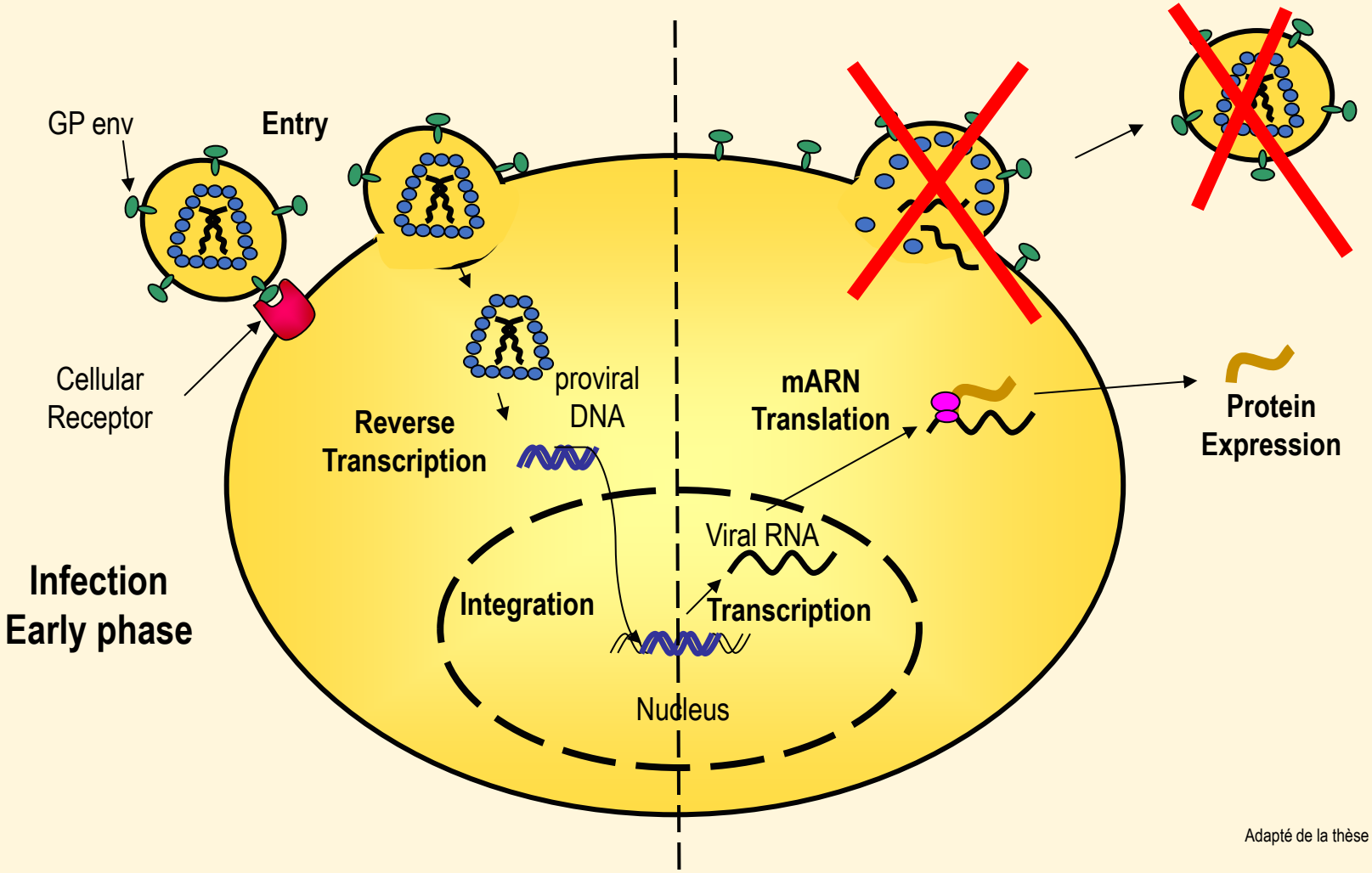
Viral glycoprotein

Viral GP

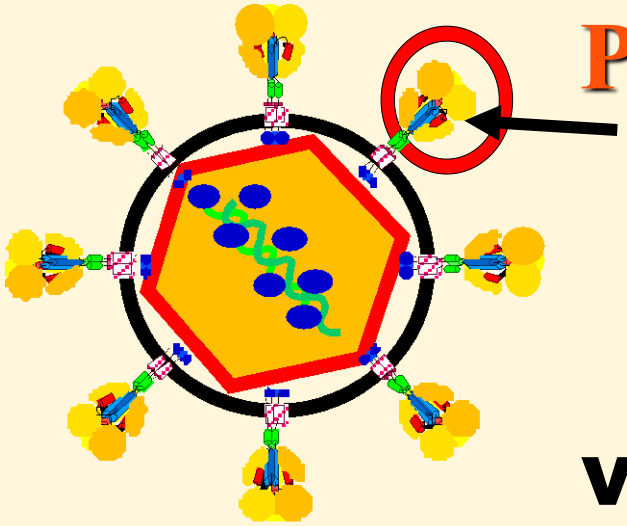
Transfer vector



The viral cycle of a lentiviral vector



Adapté de la thèse de Lise Rivière

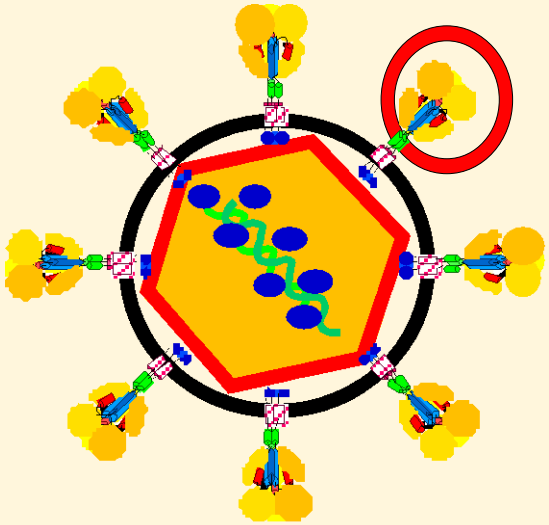


Pseudotyping of LVs

VSV-G-LVs present limitations

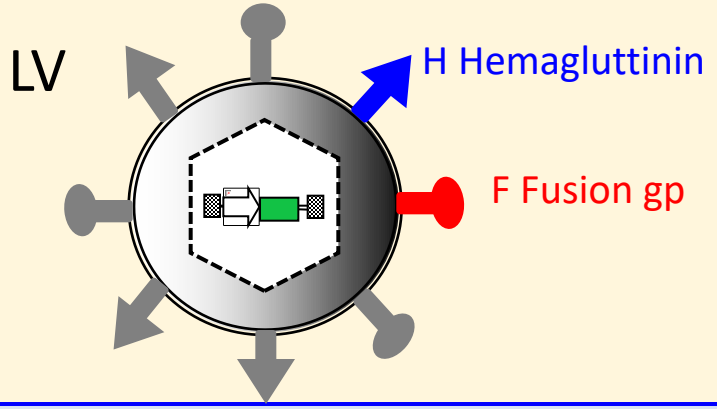
- Cytotoxic → no stable producer cell line
- Sensitive to human complement → no in vivo use
- High vector dose
- **Resting hematopoietic cells such as T cells or HSCs: need cytokine stimulation for efficient transduction**
- **human B cells are refractory**

Pseudotyping of LVs



URGENT NEED FOR NEW ALTERNATIVE LENTIVIRAL VECTOR PSEUDOTYPES

B cells??



B-cell gene therapy:

✓Induction of tolerance to treat autoimmune diseases (B cells = potent APCs)

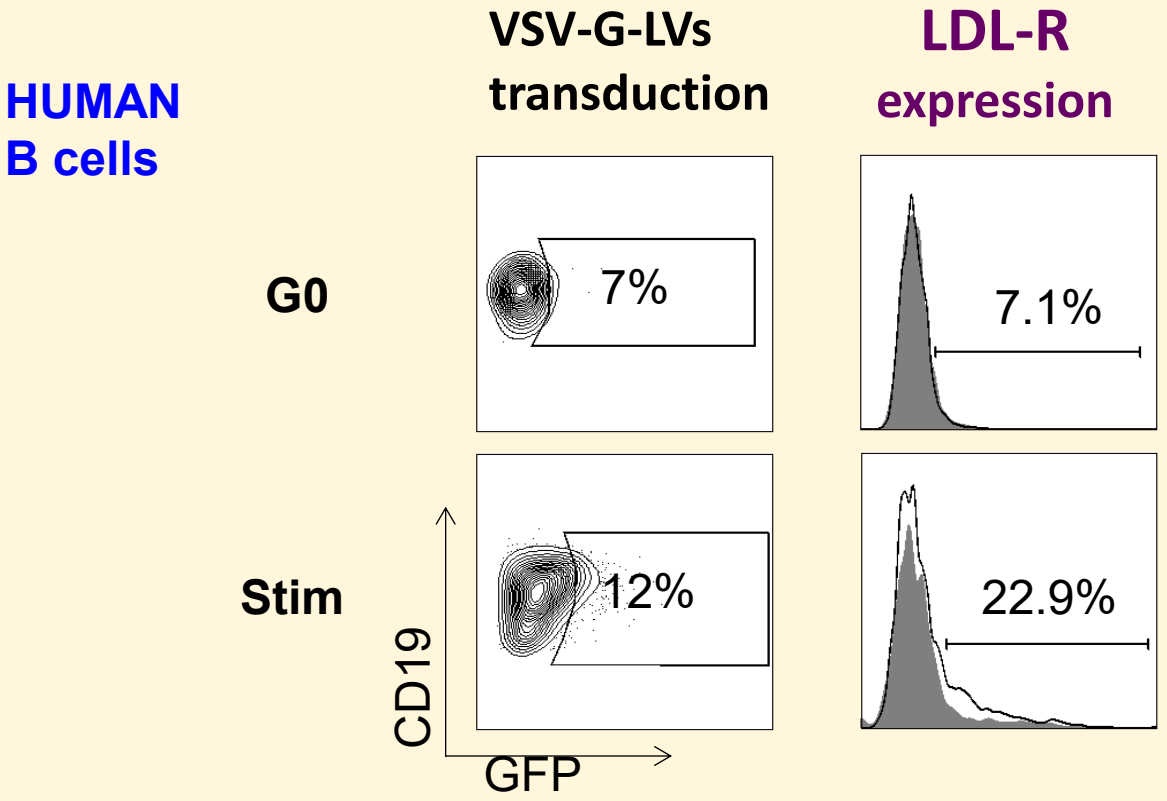
✓Activation of the immune system by increase of immunogenicity of cancer B cells

✓Immunotherapy = B cells are potent antibody producing cells (HCV, HIV)

OBSTACLE

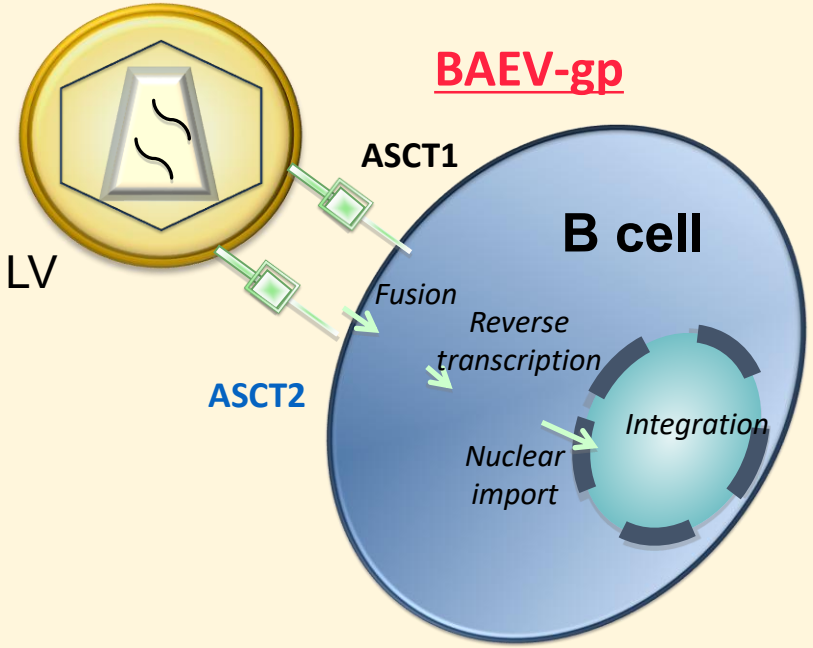
Classical VSV-G-LVs do not allow efficient transduction of human B cells

VSV receptor = low density lipid receptor **LDL-R** (Finkelstein et al., PNAS 2013)



Lévy et al. Blood 2010
Amirache et al. Blood, 2014

Comparing measles virus and baboon gp LV pseudotypes for B cell transduction



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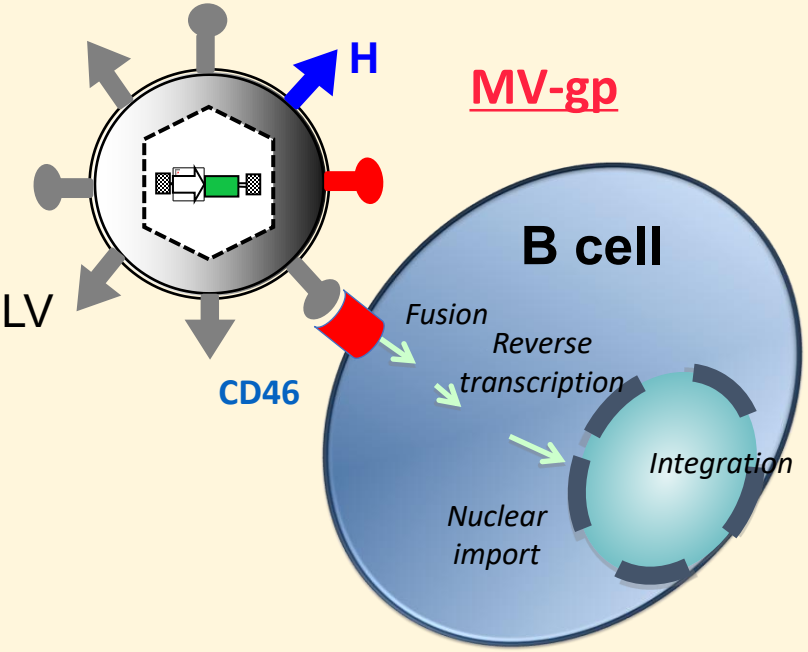
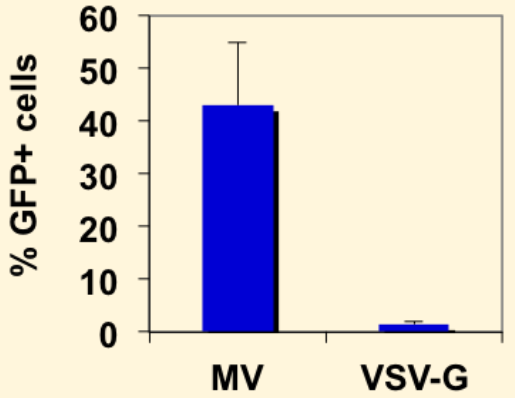
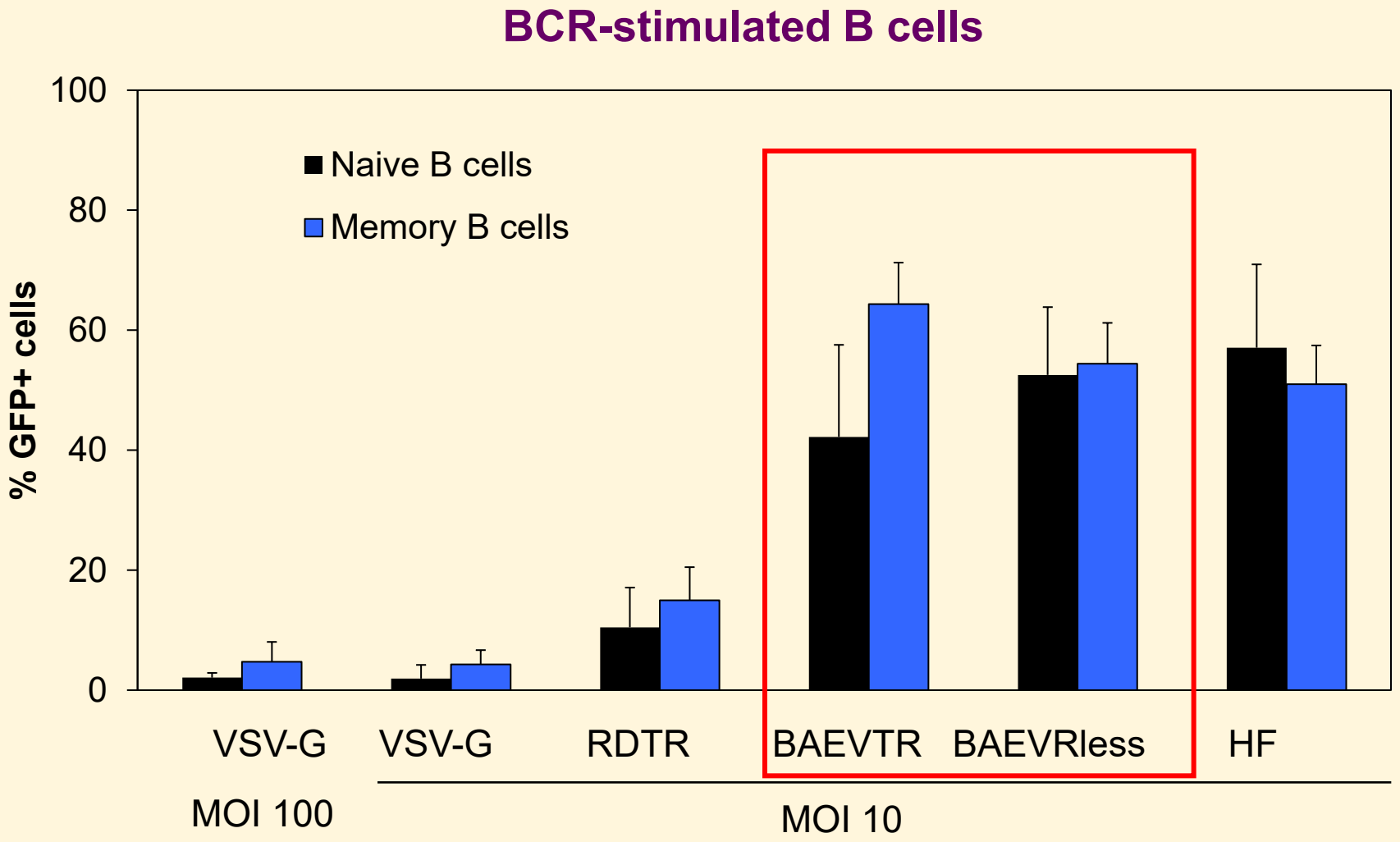




Frecha, Blood 2009,
Lévy Blood 2010; Mol ther. 2012;
Schoenhals et al, Leukemia 2012

BAEV-LVs allow high-level transduction of human memory and naive B cells



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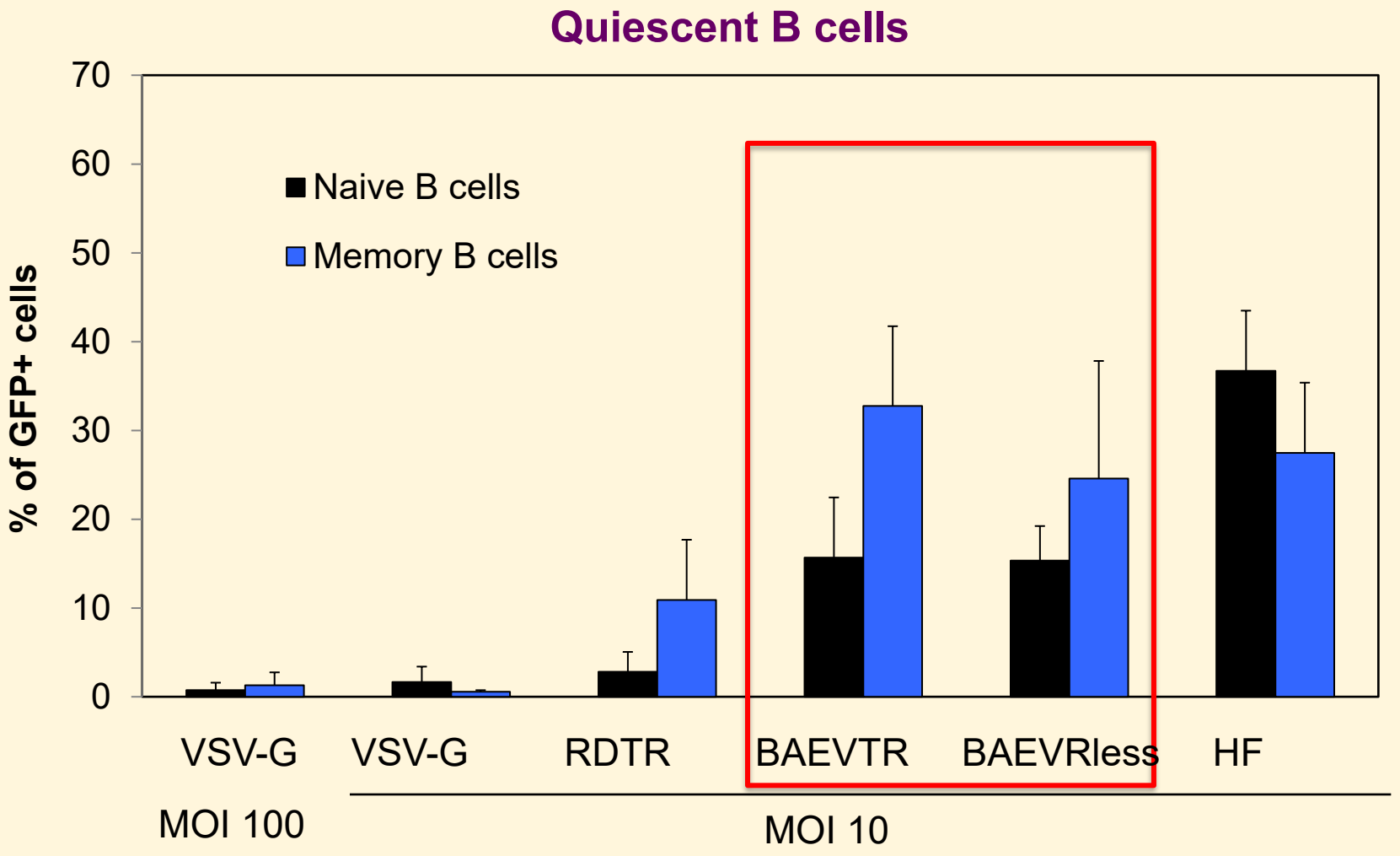
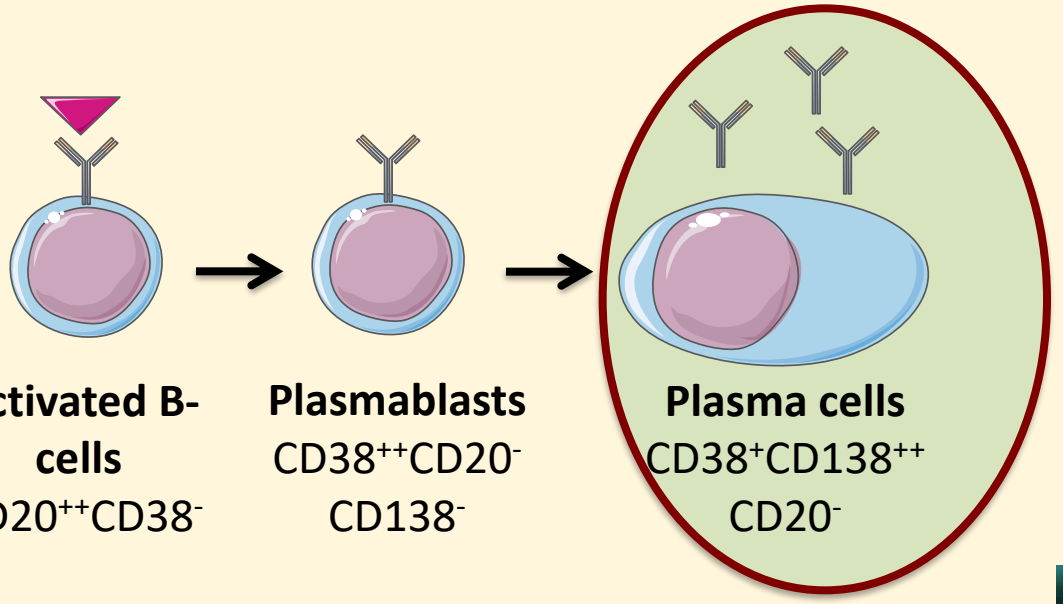


B-cells as attractive targets for gene therapy

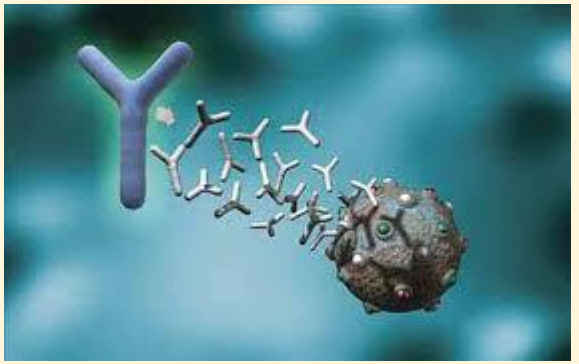


Activated B-cells
$CD20^{++}CD38^{-}$

Plasmablasts
$CD38^{++}CD20^{-}$
$CD138^{-}$

Plasma cells
$CD38^{+}CD138^{++}$
$CD20^{-}$

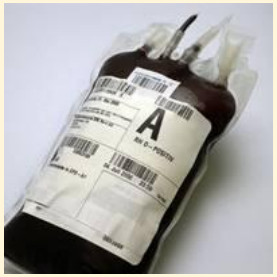
Differentiation into plasma cells : antibody producer cells



- ✓ Induction of tolerance (B cells = potent APCs)
  - ➢ Autoimmune disease: Diabetic

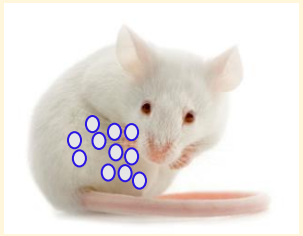
- ✓ **Immunotherapy = B cells = Potent protein-secreting cells that can persist life long**

Adoptive transfer of TRANSDUCED human B-cells into NOD/SCID/γC$^{-/-}$ (NSG) mice



Isolation of B cells and CD4 T cells from the donor

28j



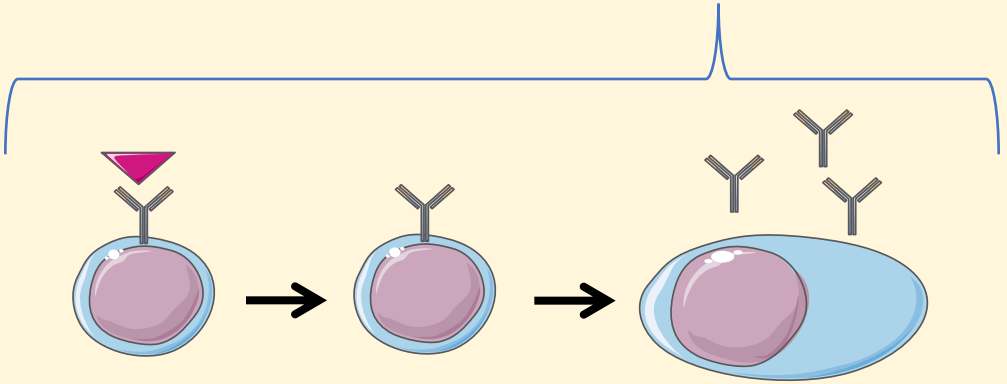
sacrifice

Splenocyte isolation

hB cells

+

hCD4⁺ T cells

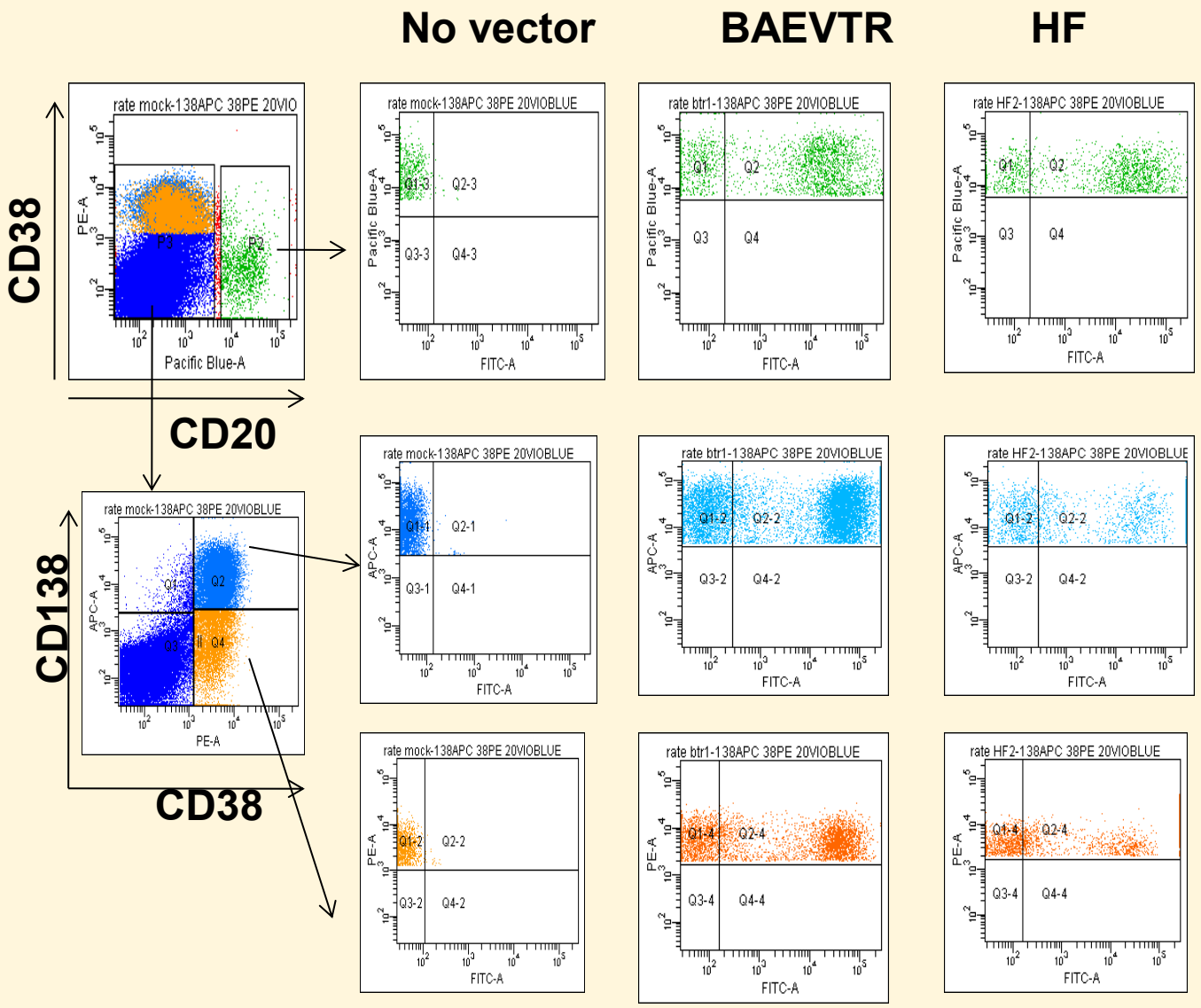


Activated B-cells
CD20^{++}CD38^{-}

Plasmablasts
CD38^{++}CD20^{-}
CD138^{-}

Plasma cells
CD38^{+}CD138^{++}
CD20^{-}

BAEV-LVs allow efficient transduction of human plasmablast and plasma B cells



Activated B-cells

20++ 38-

Plasma B cells

38+ 138++ 20-

Plasmablast

38++20-CD138-

Adaptive transfer of human TRANSDUCED B cells into NOD/SCID $\gamma^{-/-}$ (NSG) mice

d0

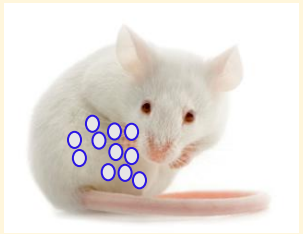
Isolation B cells and CD4 T cells from the donor

d1

d7

d14

28j



d25

sacrifice

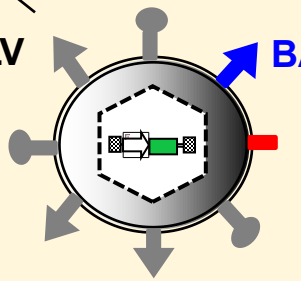
-Spleen
-BM
-Lymph nodes
-Blood

hB cells
+
hCD4⁺ T cells

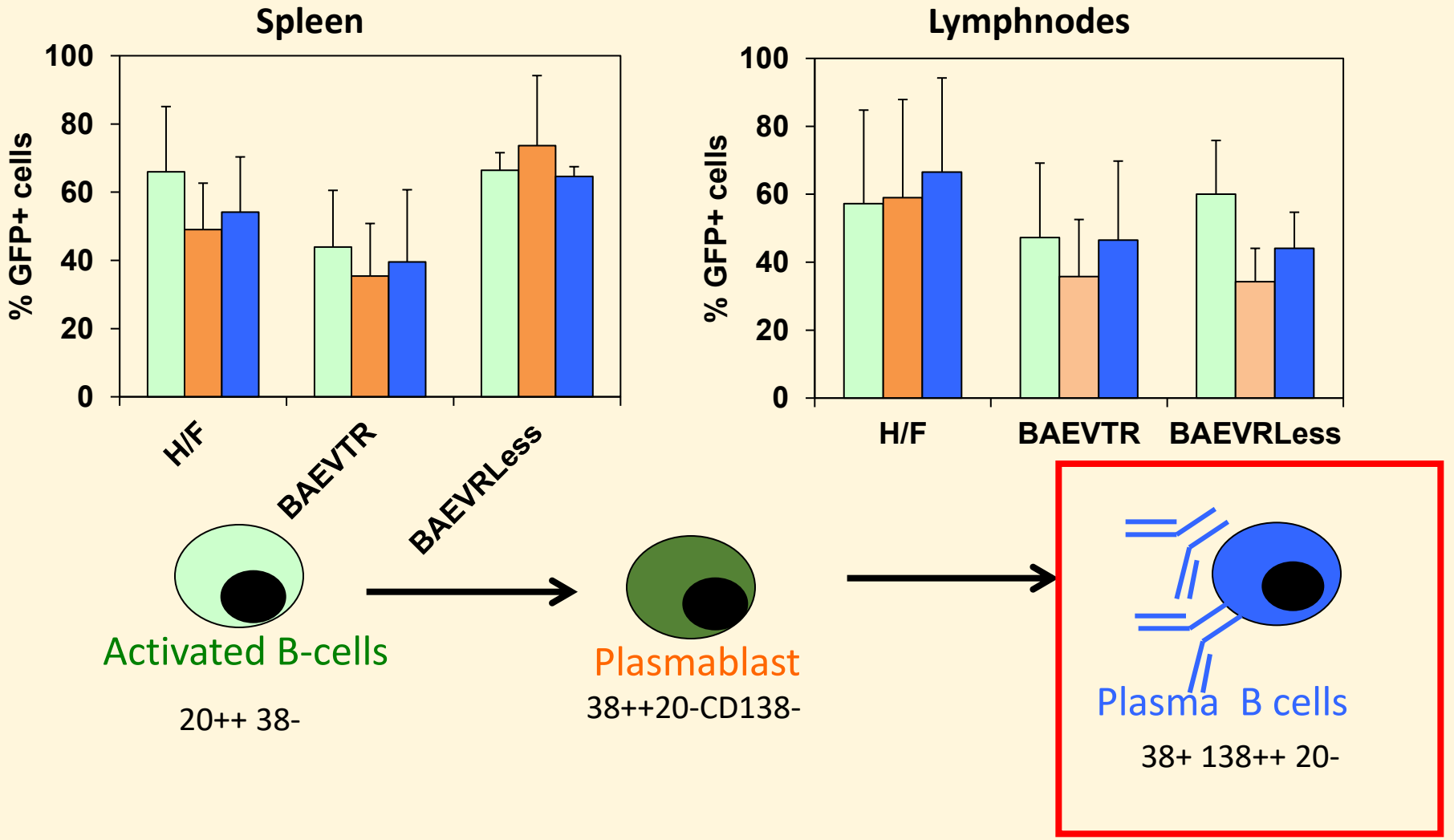
TRANSDUCTION

LV

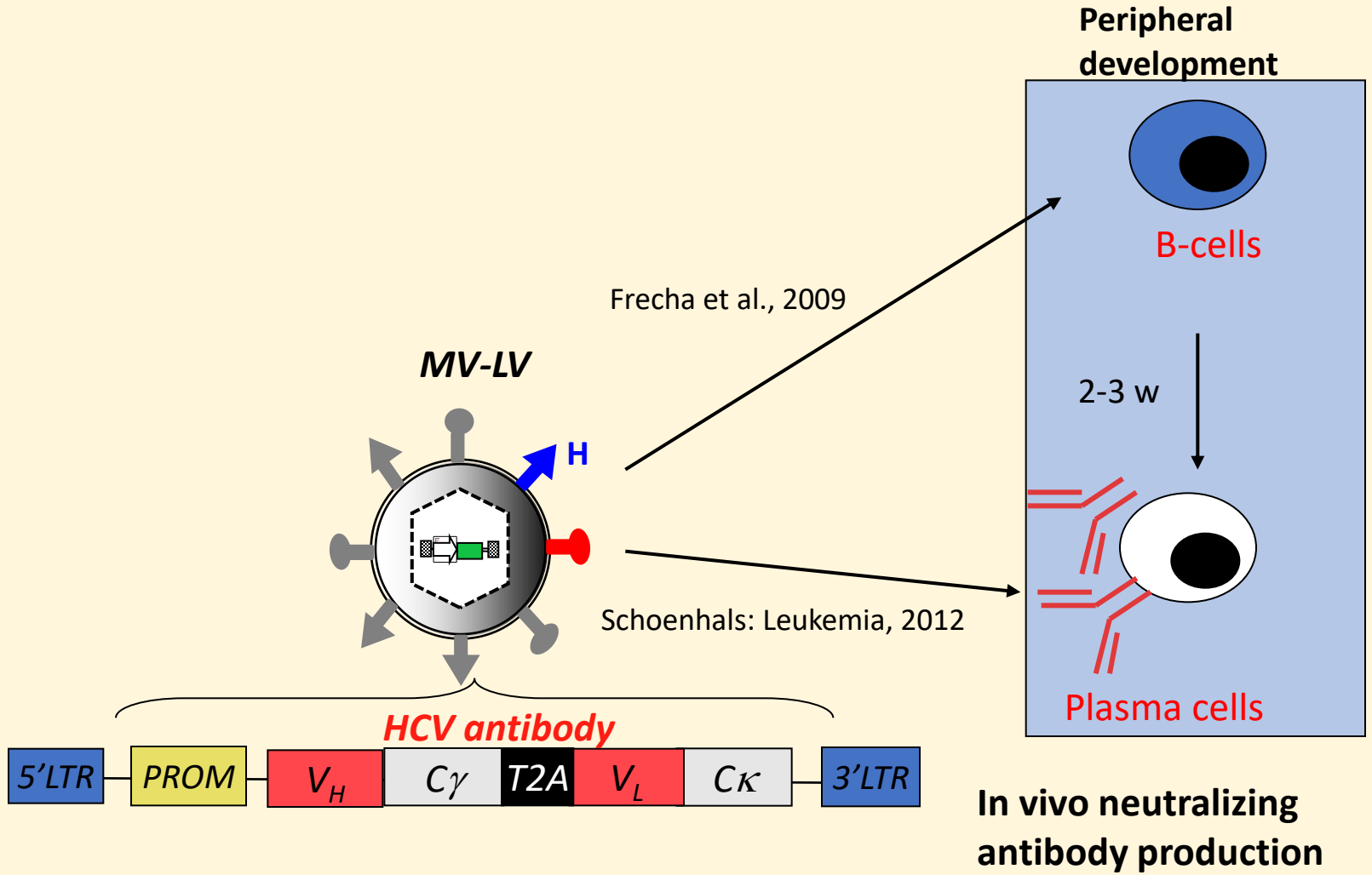
BAEV



High-level transduction in plasmocytes persists upon adaptive transfer of hB cells into NSG mice

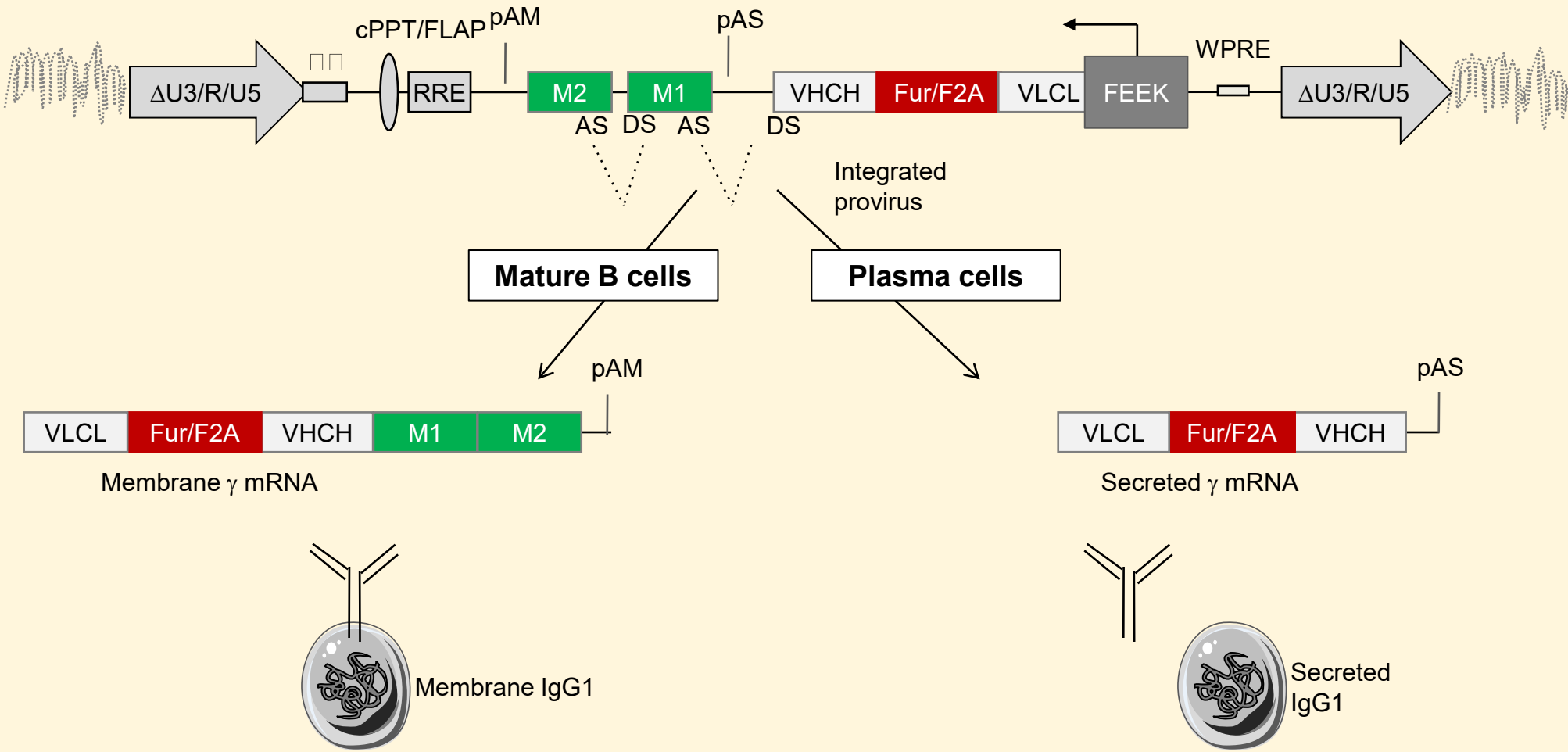


B-cells producing Hepatitis C virus neutralising antibodies *in vivo*

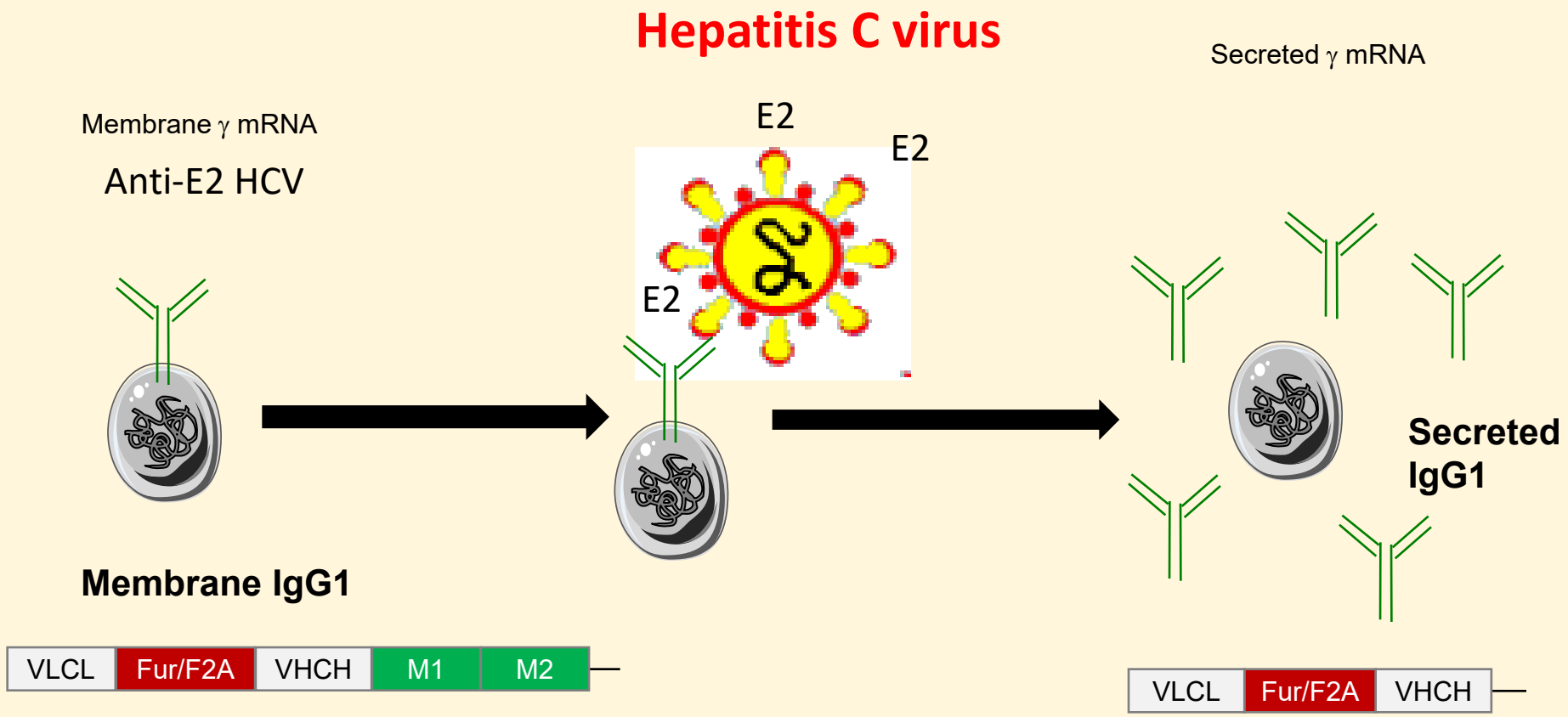


In vivo neutralizing antibody production

B cells producing HCV neutralising antibodies *in vivo*

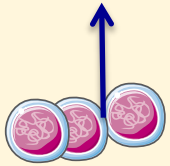


B cells producing HCV neutralising antibodies *in vivo*

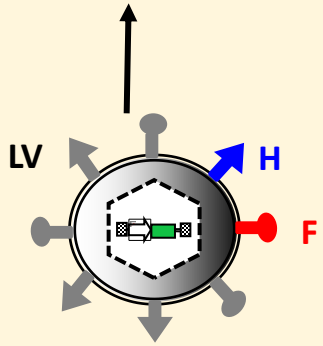


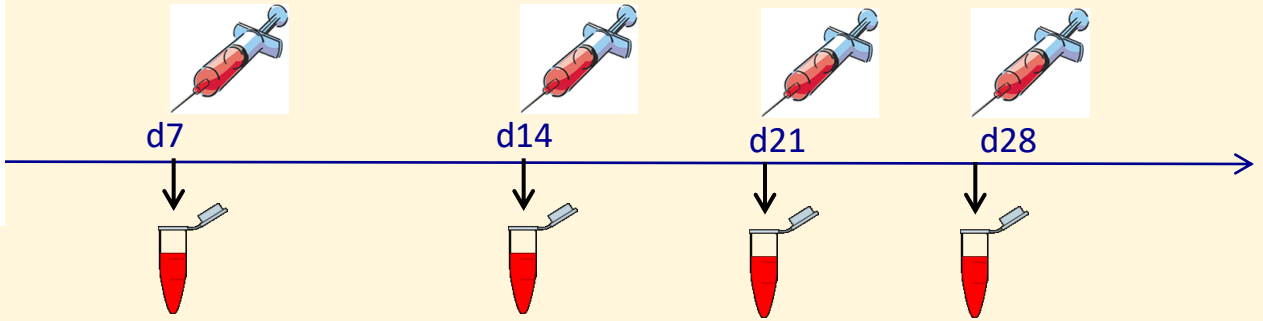
B-cells producing HCV neutralising antibodies *in vivo* in NOD/SCID γ-/- (NSG) mice

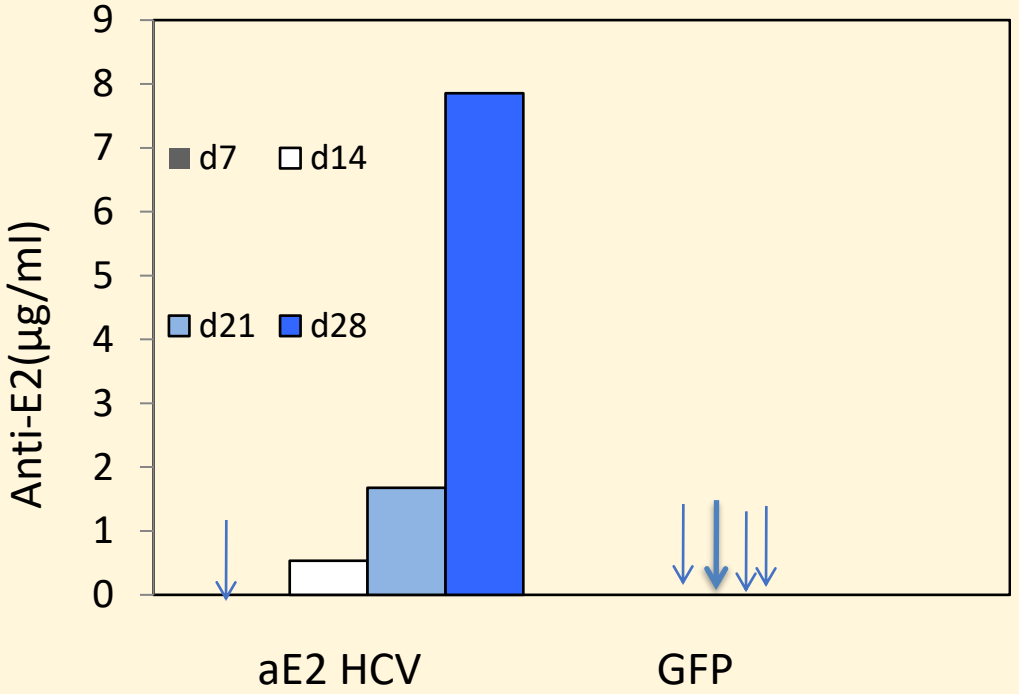
d1



3 10^6 B cells



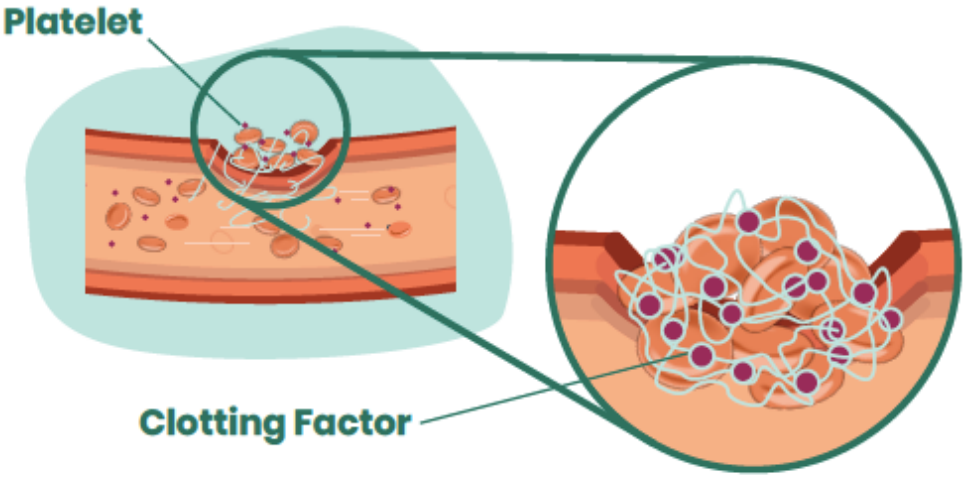




Hemophilia and Gene Therapy

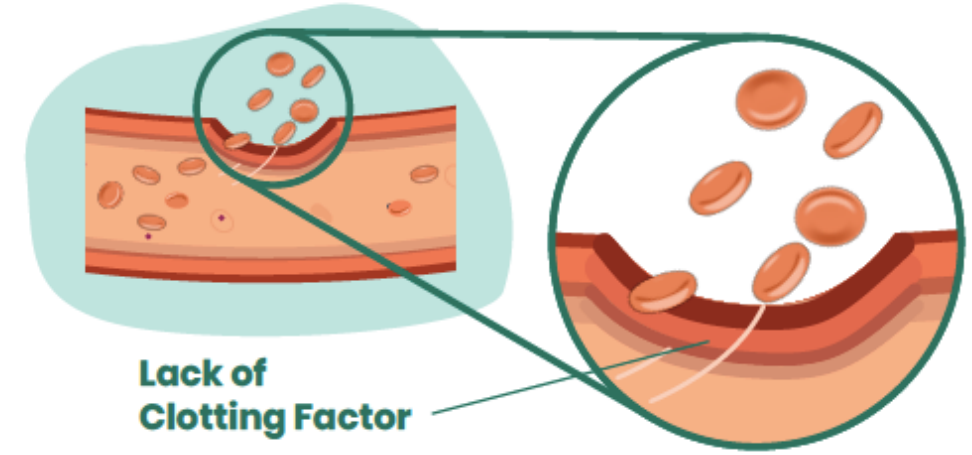
Blood Clots

Blood clots form in response to a cut or other injury to a blood vessel. Platelets in the bloodstream stick to the cut edges of the ruptured vessels. Once the platelet plug is formed, proteins called clotting factors create a net-like mesh called fibrin that reinforces the plug and seals the wound.

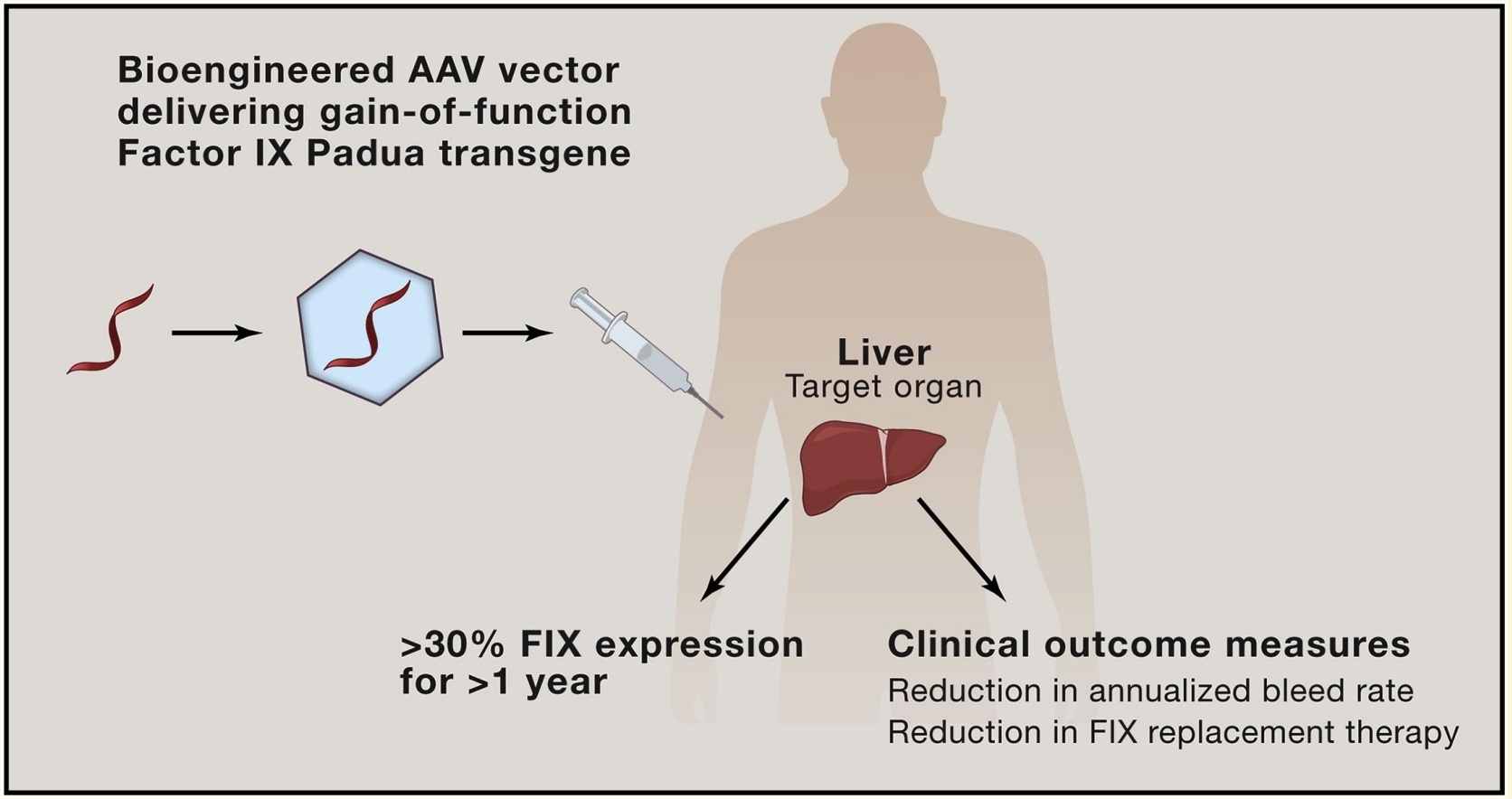


Effect of Hemophilia

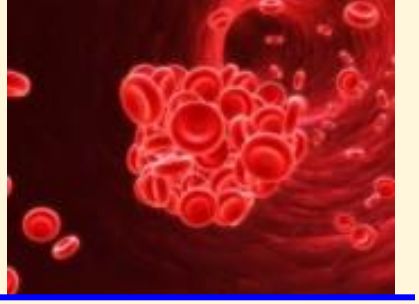
Hemophilia is a genetic disease that occurs when the genes that are vital to creating clotting factors are faulty. This results in prolonged external or internal bleeding in the body. The most common types of the disease—hemophilia A and hemophilia B—are caused by deficiencies of clotting factors VIII and IX.



Une approche = sécréter le FIX par le foie du patient Utilisant un vecteur AAV

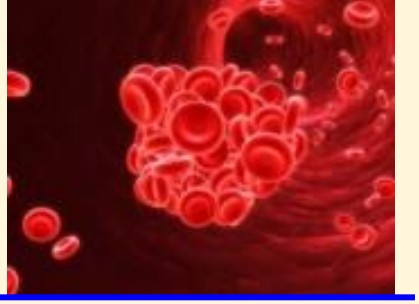


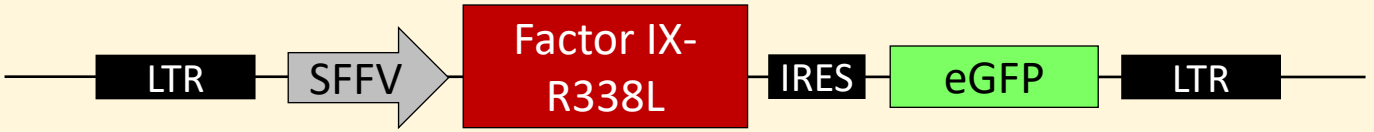
Gene therapy to treat hemophilia B

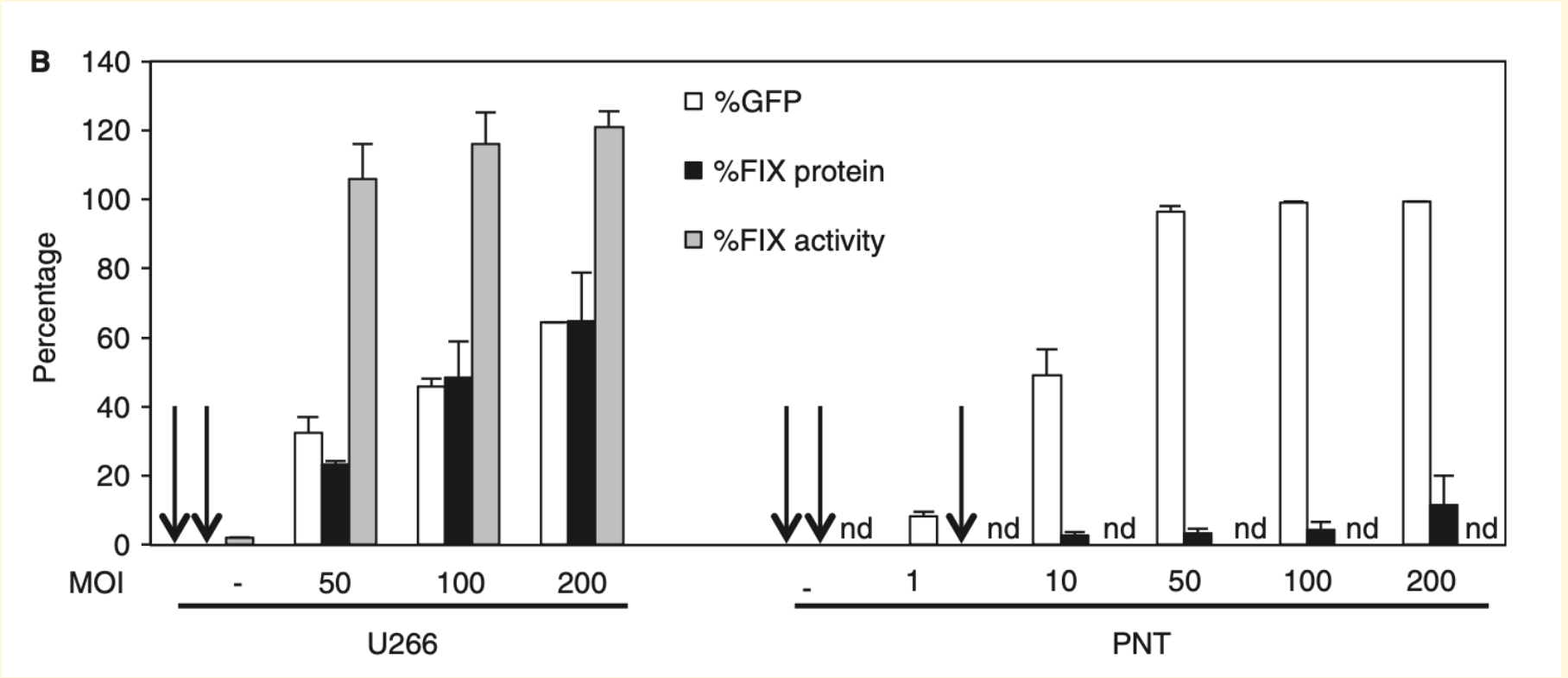


- Monogenic disease
- Long lasting delivery of transgene
- Induction of tolerance ?
- Therapeutic level with only a low plasma levels (5%) suffisant to reduce the risk of mortality and morbidity

Gene therapy to treat hemophilia B



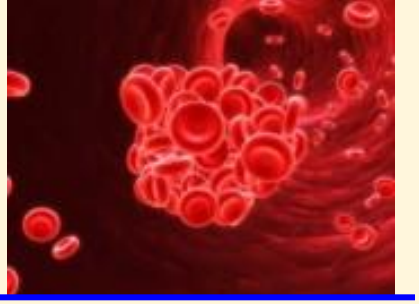


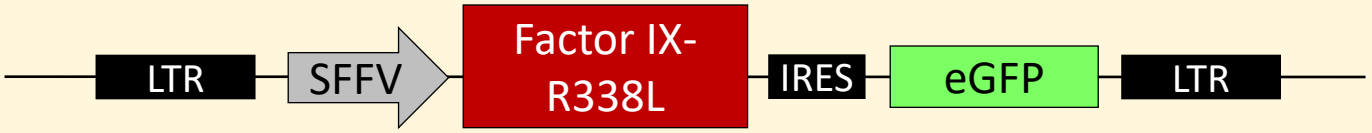


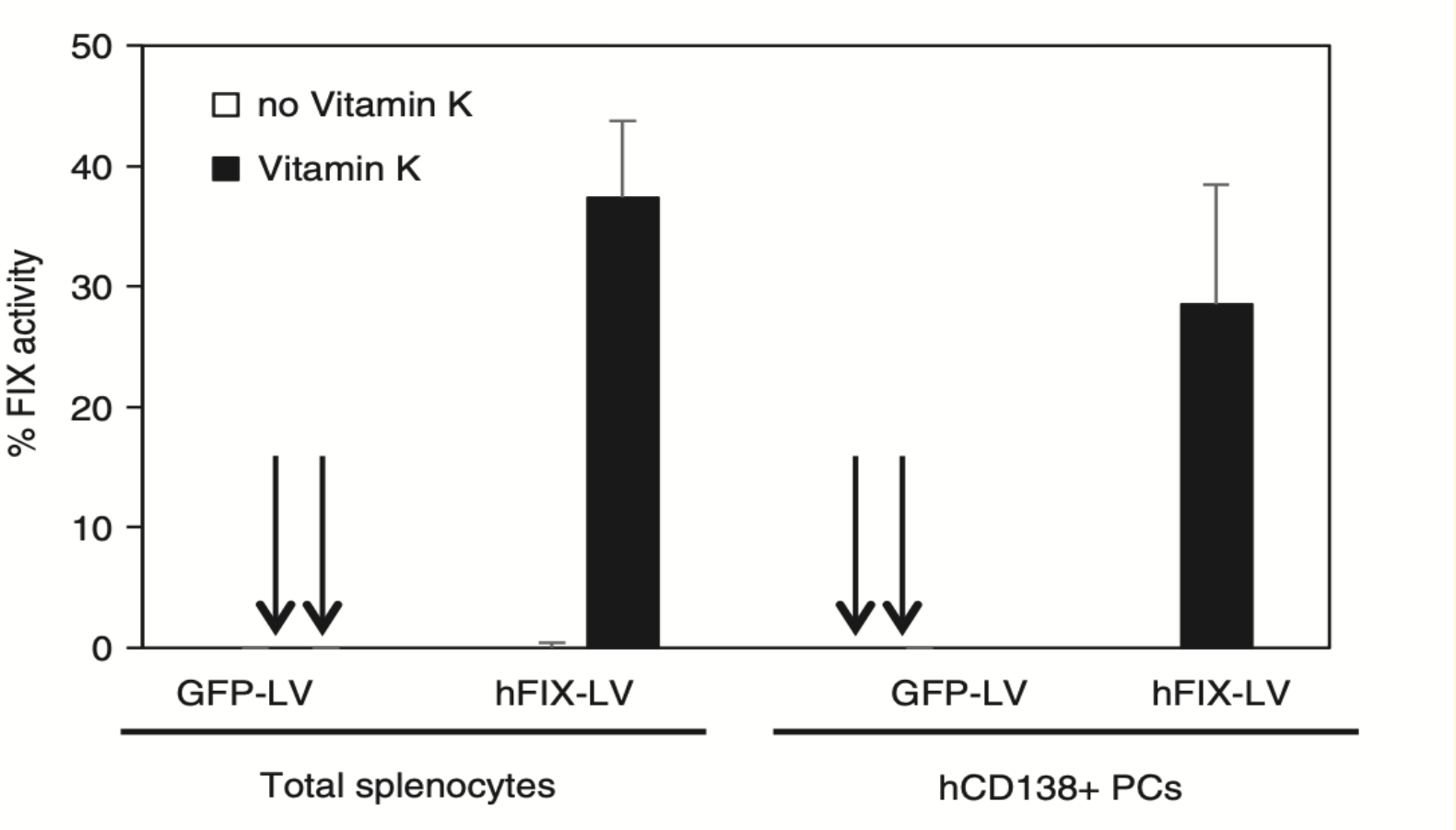
Plasma B cell line

Non plasma B cell line

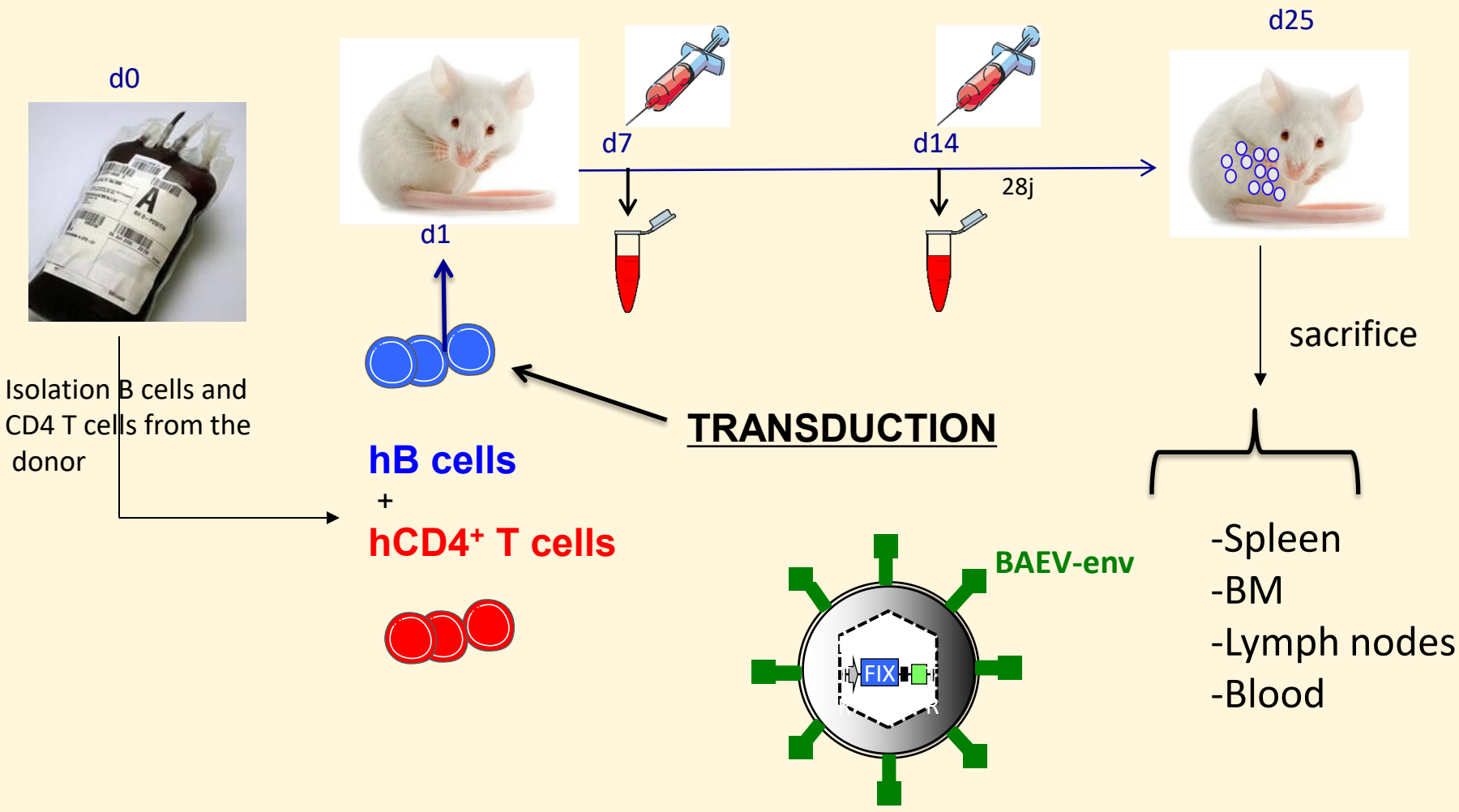
Gene therapy to treat hemophilia B



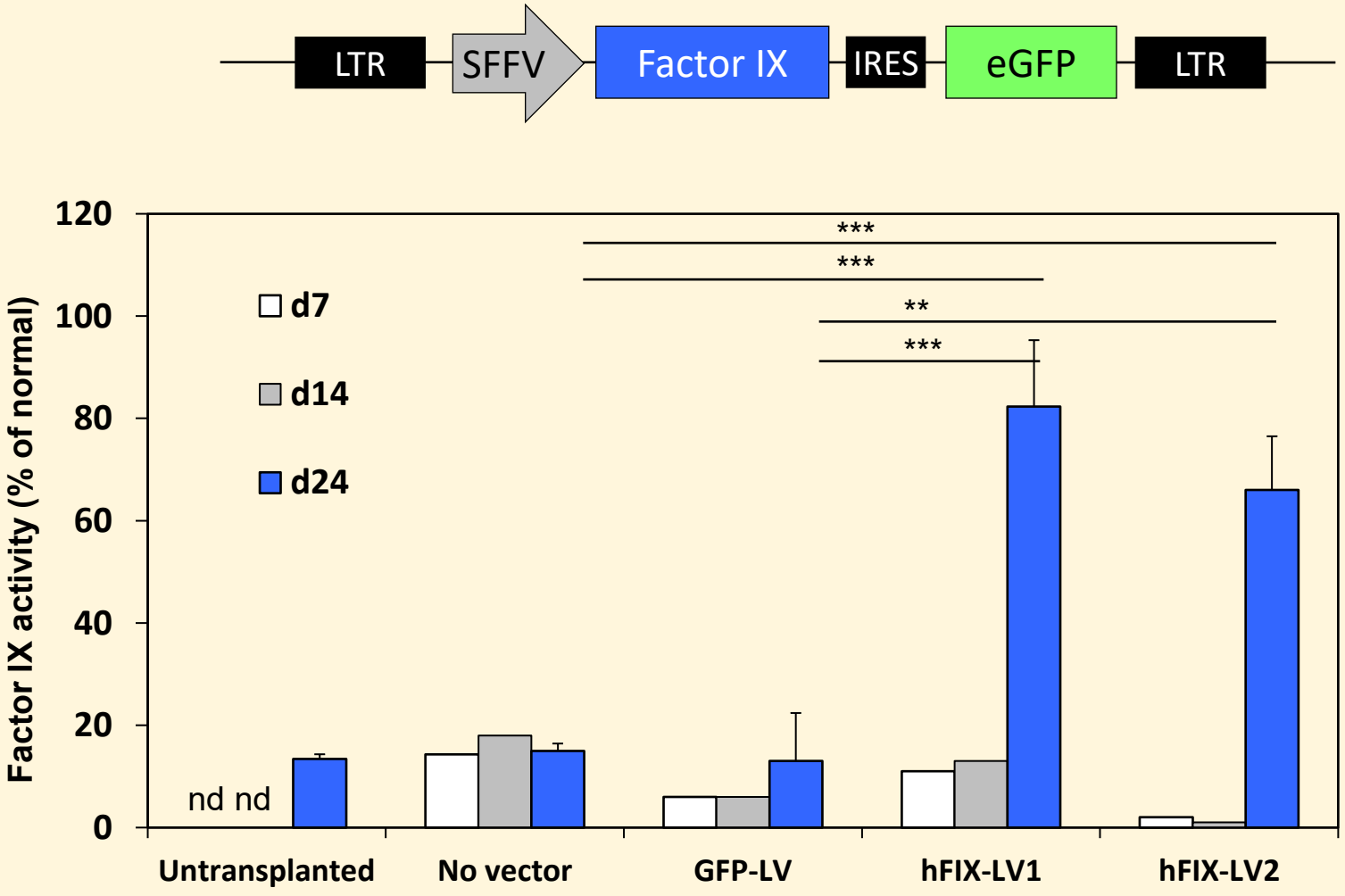




Adoptive transfer of TRANSDUCED human B-cells into NSG mice

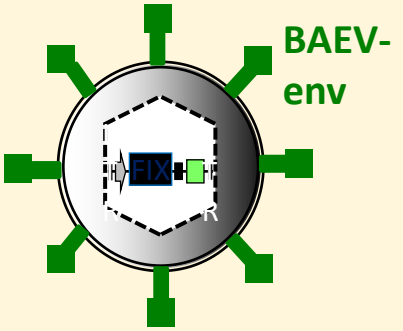


Adaptive transfer in NSG mice of BAEV-FIX LV transduced hB cells allows FIX secretion in vivo



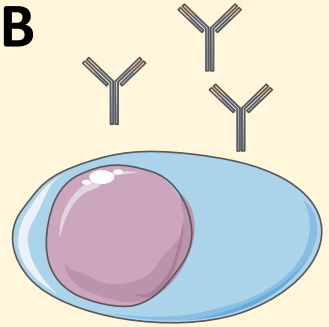
Lévy et al. 2016 Journal of Thromb. and haemostasis

Conclusions



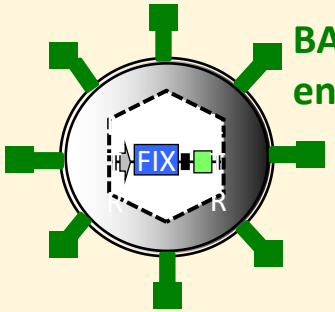
- ✓ Baboon enveloped lentiviral vectors are excellent tools for gene delivery into B cells

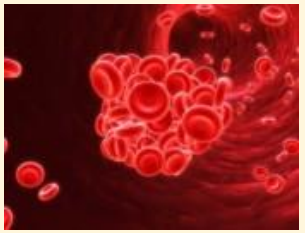
- ✓ Baboon enveloped lentiviral vectors are excellent tools for B cell gene therapy in order to express antibodies in vivo
  - infections
  - immune check point inhibitors



Plasma cells

- ✓ Baboon enveloped lentiviral vectors are excellent tools for B cell gene therapy to express Factor IX to treat hemophelia





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-FL Cosset

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Boro Dropolic
Rimas
Ian Johnston

And many many more……

Lyon 1

cnrs

Inserm
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PBES

E-RARE
FP 7

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a Miltenyi Biotec Company

anRS
Agence nationale de recherches sur le sida et les hépatites virales

Agence Nationale de la Recherche GIP
ANR

ENS DE LYON

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Plateforme imagerie
Equipe Animalerie
Equipe Gestion
Equipe Laverie

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