UNIVERSITÀ DEL PIEMONTE ORIENTALE

Dipartimento di Scienze della Salute

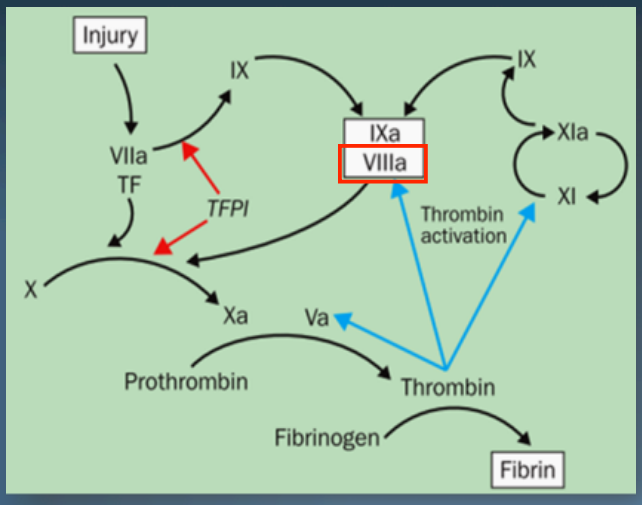
Terapia cellulare e genica per l'Emofilia

Torino, 25 Novembre 2017

Antonia Follenzi, MD, PhD

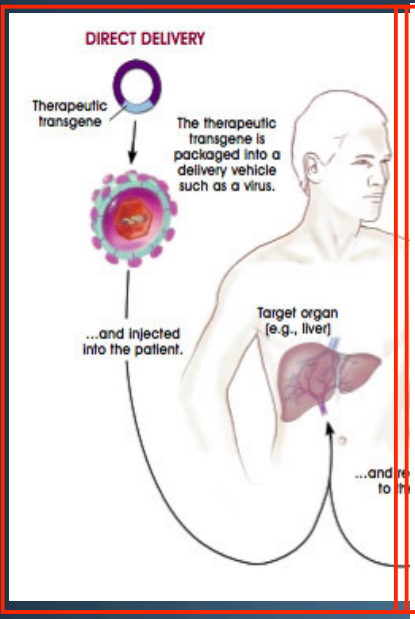
Hemophilia A

- Different degrees : mild (5-40% FVIII), moderate (1-5% FVIII) and severe (<1% FVIII)
- Treatment with plasma-derived or recombinant FVIII
- No definitive cure



Optimal target for cell and gene therapy approach

Hemophilia A
Gene and Cell Therapy



"Pure" Gene Therapy Approach

- Successful HB gene therapy by targeting FIX expression to hepatocytes using AAV
- FVIII can be too large for AAV but new clinicaL trials are coming
- Development of anti-FVIII neutralizing antibodies
- LV alternative approaches by targeting transgene expression to specific cells other than hepatocytes.

Combining gene and cell therapy could allow to develop an effective and safe treatment for HA

Endothelial Cell as Cellular Target

Transplanted endothelial cells repopulate the liver endothelium and correct the phenotype of hemophilia A mice

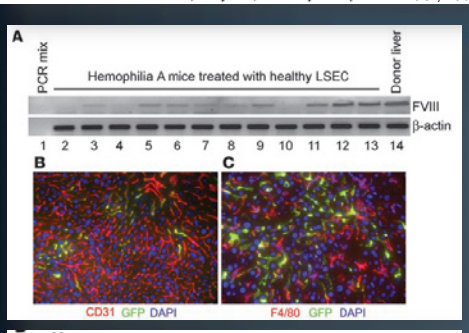
Antonia Follenzi,[1,2] Daniel Benten,[3] Phyllis Novikoff,[1] Louisa Faulkner,[4] Sanj Raut,[4] and Sanjeev Gupta[1,3,5]

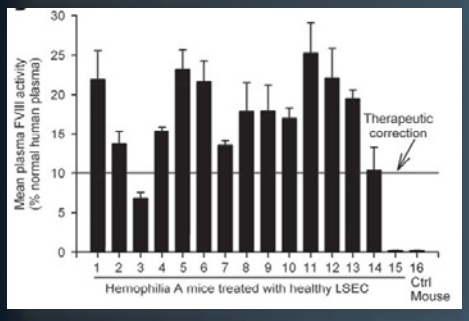
JCI, 2008

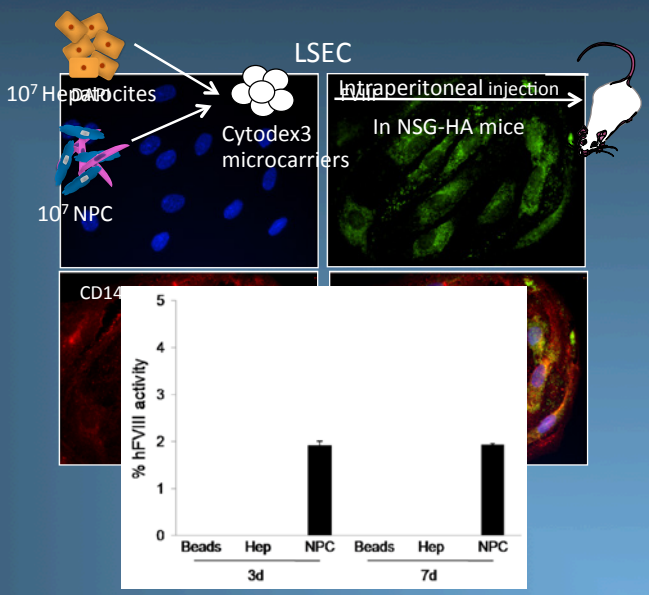
Extrahepatic sources of factor VIII potentially contribute to the coagulation cascade correcting the bleeding phenotype of mice with hemophilia A

Diego Zanolini,[1*] Simone Merlin,[1*] Maria Feola,[1] Gabriella Ranaldo,[1] Angela Amoruso,[1] Gianluca Gaidano,[2] Mauro Zaffaroni,[3] Alessandro Ferrero,[4] Sandra Brunelleschi,[1] Guido Valente,[2] Sanjeev Gupta,[5] Maria Prat,[1] and Antonia Follenzi[1]

Haematologica 2015

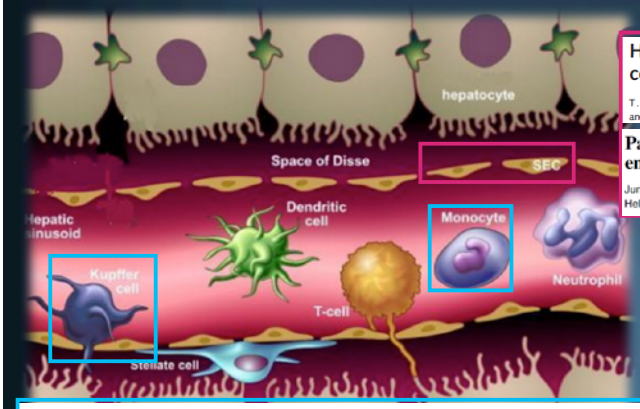






Target Cells for HA Gene Therapy

- Liver is the main FVIII source of the body



Human liver sinusoidal endothelial cells but not hepatocytes contain factor VIII
Journ Thromb Haemost, 2013
T. SHAHANI,* K. COVENS,† R. LAVEND'HOMME,† N. JAZOULI,‡ E. SOKAL,‡ K. PEERLINCK† and M. JACQUEMIN†

Patterns of expression of factor VIII and von Willebrand factor by endothelial cell subsets in vivo
Blood, 2016
Junliang Pan,[1-3] Thanh Theresa Dinh,[1,4] Anusha Rajaraman,[1,4] Mike Lee,[1,4] Alexander Scholz,[1,4] Cathrin J. Czupalla,[1,4] Helena Kiefel,[1,4] Li Zhu,[5] Lijun Xia,[6] John Morser,[2] Haiyan Jiang,[6] Laura Santambrogio,[7] and Eugene C. Butcher[1,2,4]

Liver sinusoids unique anatomy can facilitate direct or indirect priming of lymphocytes and contribute to some of the immunological properties of the organ (e.g., induction of antigen-specific tolerance)

Role of bone marrow transplantation for correcting hemophilia A in mice
Antonia Follenzi,[1,2] Sanj Raut,[3] Simone Merlin,[2] Rita Sarkar,[4] and Sanjeev Gupta[1,5]
Blood, 2012

Extrahepatic sources of factor VIII potentially contribute to the coagulation cascade correcting the bleeding phenotype of mice with hemophilia A
Haematologica, 2015
Diego Zanolini,[1*] Simone Merlin,[1*] Maria Feola,[1] Gabriella Ranaldo,[1] Angela Amoruso,[1] Gianluca Gaidano,[2] Mauro Zaffaroni,[1] Alessandro Ferrero,[1] Sandra Brunelleschi,[1] Guido Valente,[2] Sanjeev Gupta,[3] Maria Prat,[1] and Antonia Follenzi[1]

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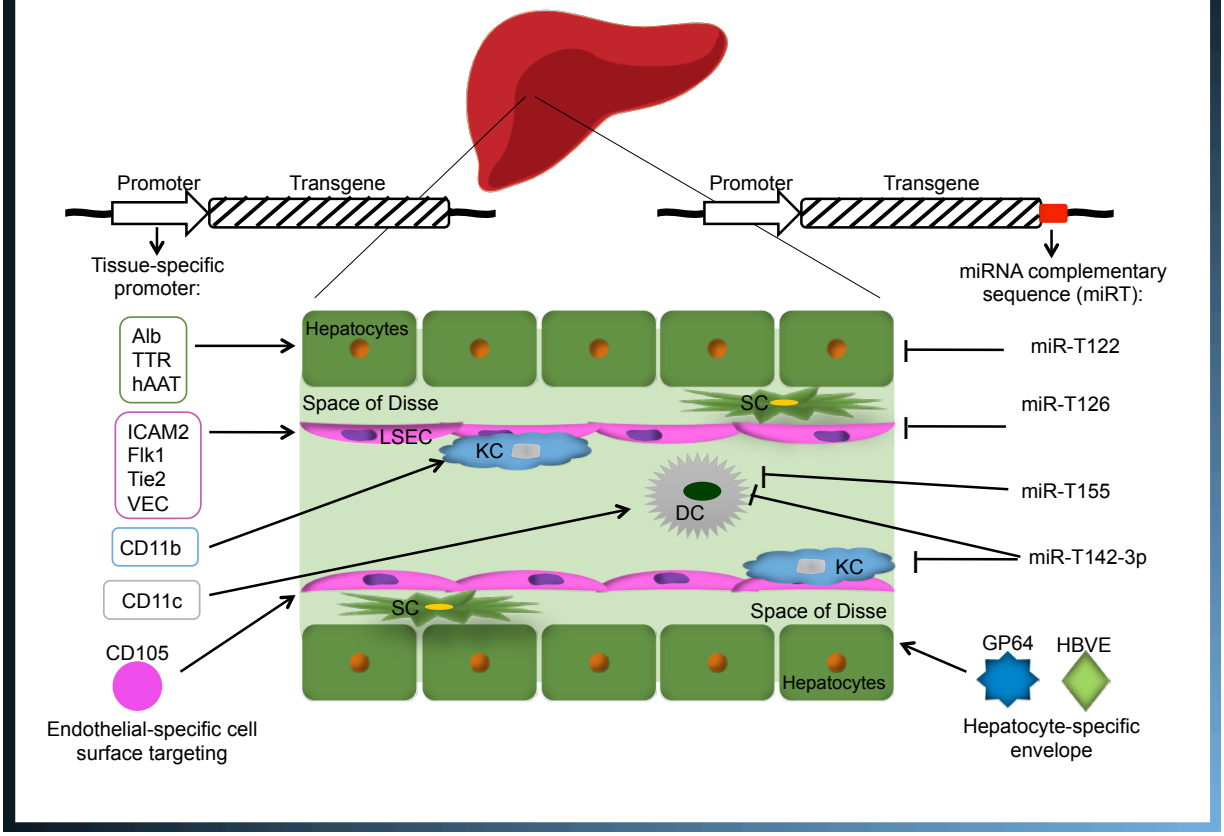
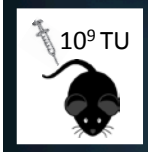
Tools to Optimize Cell-type Specific Transgene Expression



Aim

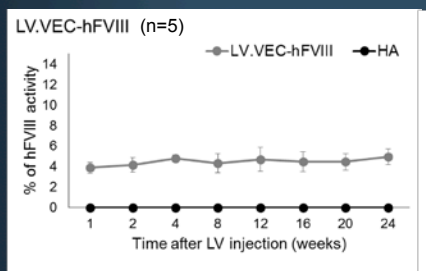
To identify the best combination of cell-specific promoter and miRT for transgene expression in liver sinusoidal endothelial cells (LSECs)

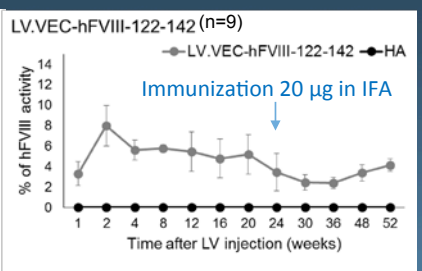
Targeting FVIII Expression in Endothelial Cells

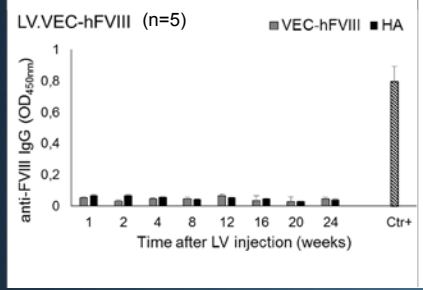


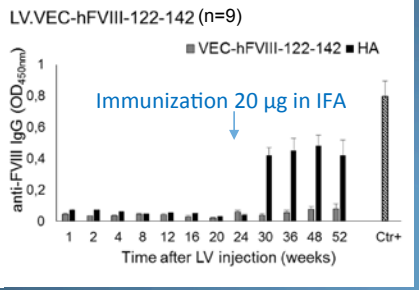
VEC → BDD hFVIII

VEC → BDD hFVIII (122 142)



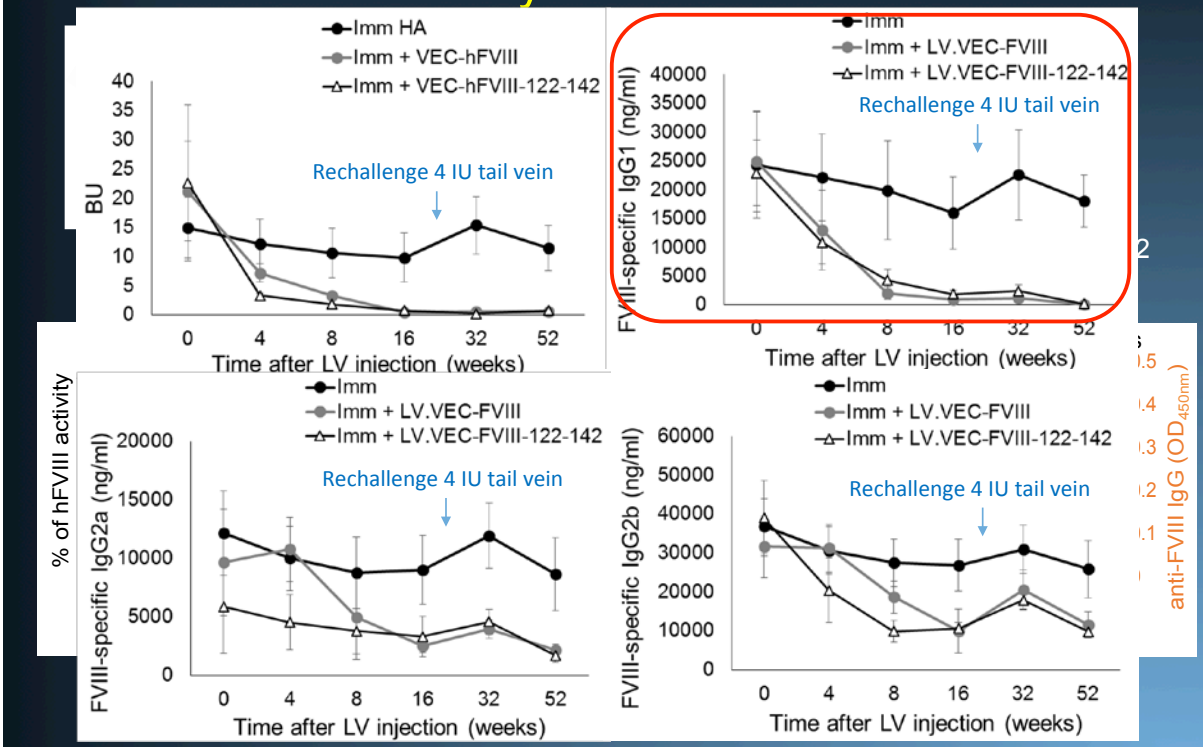




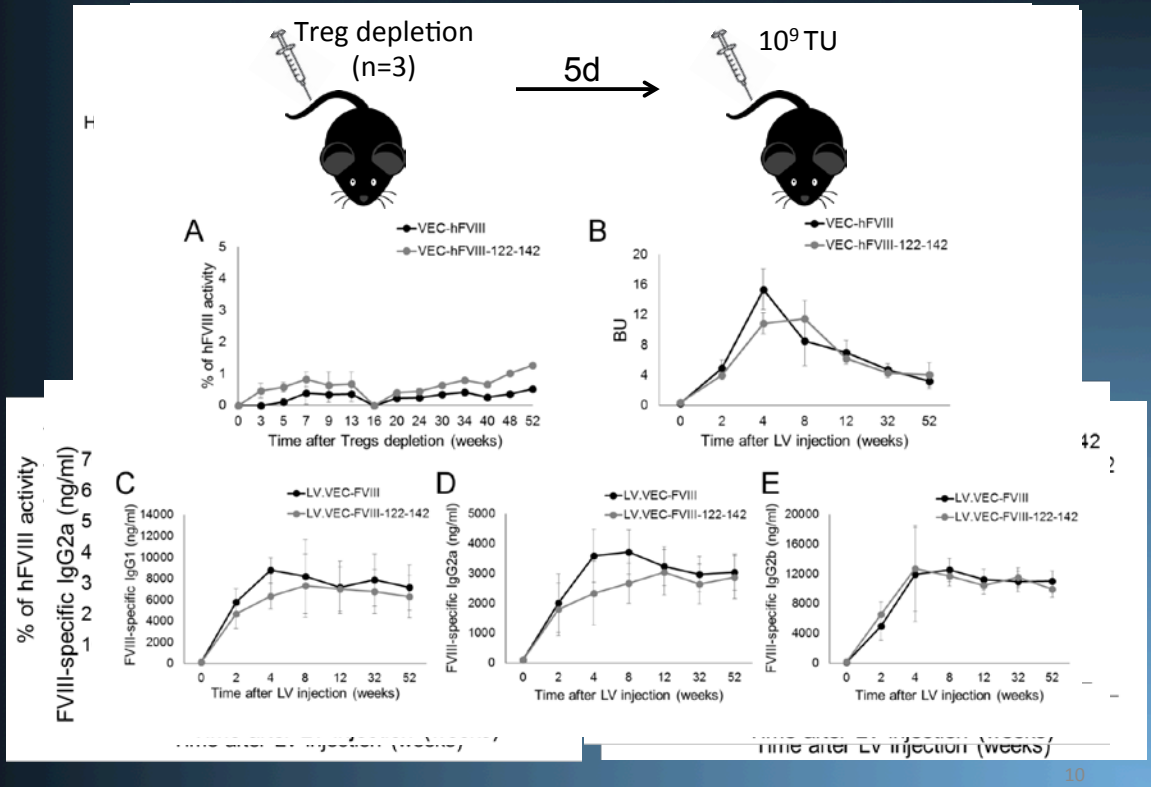


Plasma dilution 1:2000

hBDD-FVIII Activity of LV-VEC-FVIII 122-142 in Previously Immunized Mice



LSEC-induced Tregs Allow Tolerance to FVIII



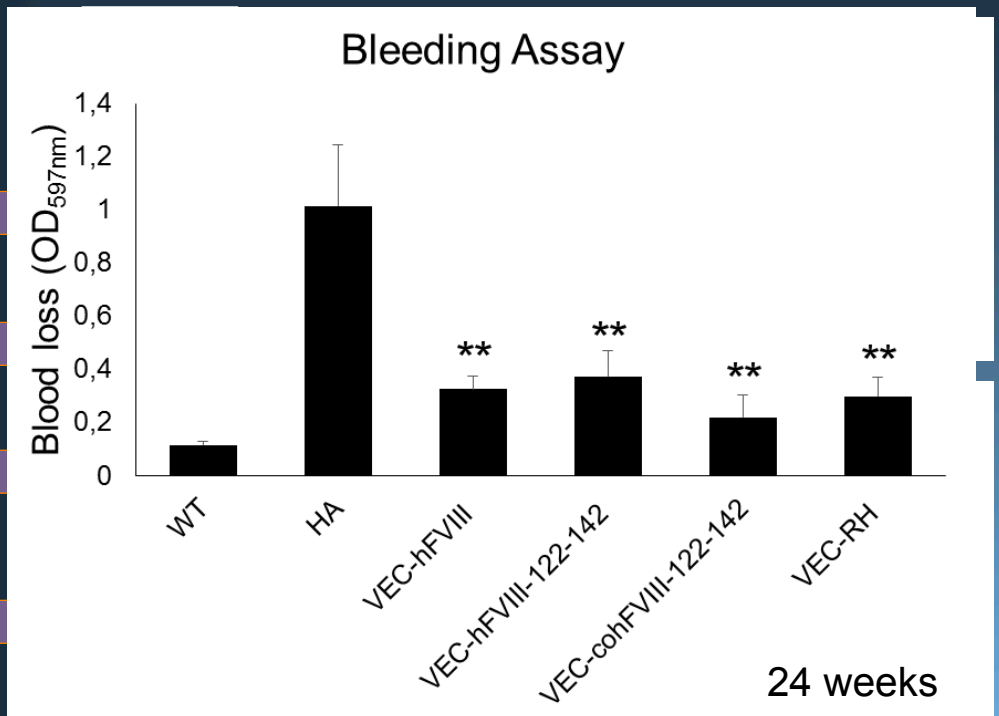
Engineered FVIII Forms

VEC

VEC

VEC

VEC



Conclusion I

The presence of endothelial or myeloid specific–promoter with specific miRTs in LV were able to restrict transgene expression in cell-types capable of efficient and long term FVIII-expression without anti-FVIII antibodies formation

Our data demonstrate a role for Tregs in establishing tolerance to FVIII during LV gene expression under the control of VEC promoter

Endothelial-specific expression of FVIII-RH and codon-optimized FVIII results in higher FVIII activity without antibody formation

FVIII expression by liver sinusoidal cells may provide cellular models to acheive antigen-specific tolerance in gene transfer approaches reaching phenotipic correction in several hemophilia A mouse strains

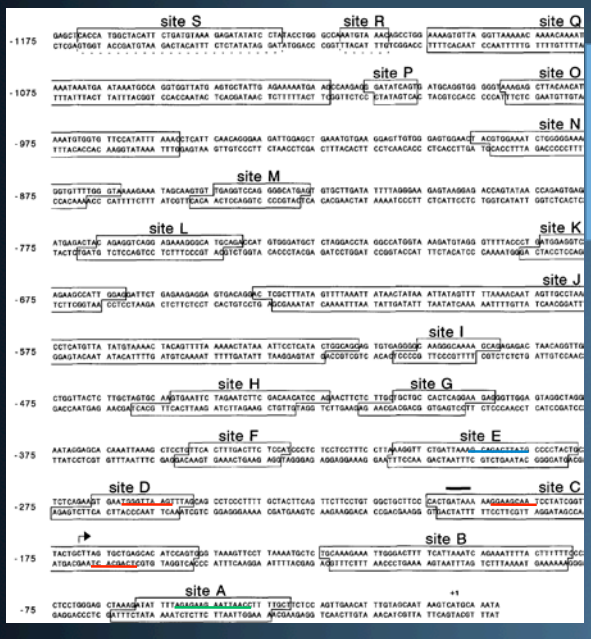
Merlin et al., Molecular Therapy 2017

FVIII promoter

cis*-Acting Elements and Transcription Factors Involved in the Promoter Activity of the Human Factor VIII Gene

(Received for publication, October 7, 1994, and in revised form, February 7, 1995)

Mauro S. Figueiredo‡ and George G. Brownlee



Role of Liver-Enriched Transcription factor HNF1 in transcriptional regilation of the FVIII gene

McGlynn, Mueller, Begbie, Notley, and Lillicrap

Mol Cell Biol, 16(5), 1936-1945 (1996)

Luciferase assay showed pF8 activity in hepatic cell lines

Study of TF that bind pF8 (in A-E regions) identified CEBP/α HNF3α and HNF1α by EMSA

Aims

- To characterize *in vitro* the activity of two FVIII promoter sequences

- To characterize *in vivo* and *ex vivo* the cell types in which FVIII promoter is active

- To investigate phenotypic correction of hemophilia A mice after gene therapy using a lentiviral vector carrying the FVIII under the control of FVIII promoter

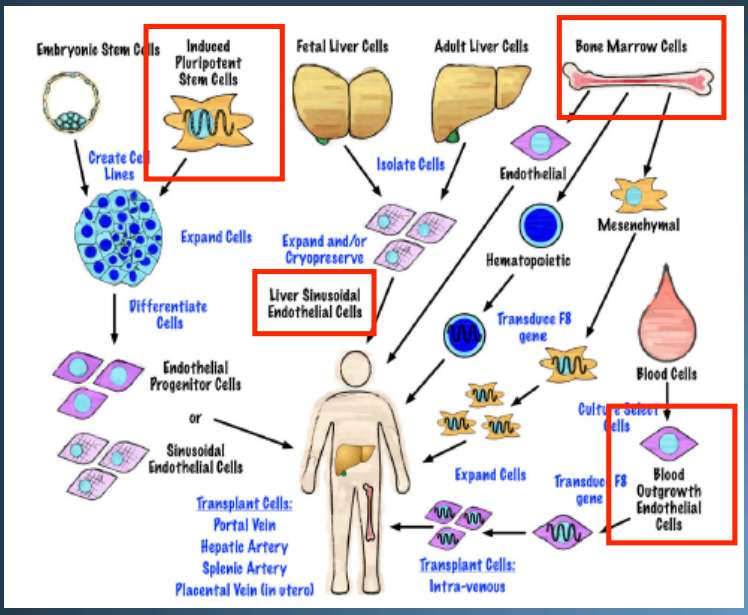
Transcriptional Factor	Expression and function
TFII-D (7 nt)	RNA Pol II
TBP (10 nt)	TATA binding protein
HNF3-alfa (8 nt)	Hepatocytes
HNF1-alfa (8 nt)	Hepatocytes
C/EBP-alfa (7 nt)	Hepatocytes, mieloyd differentiation
c-Ets-1 (7 nt)	Endothelial cells
c-Ets-2 (9 nt)	Endothelial cells
PEA 3 (9 nt)	c-Ets family
STAT4 (6 nt)	Mieloyd lineage
GATA-1 (6 nt)	Mieloyd lineage
NF-Y (8 nt)	Increasing during monocytes-macrophages differentiation
IRF-2 (6 nt)	Monocytes
STAT1 (10 nt)	Hematopoietic cells
TCF-4E (10 nt)	B cells
Pax5 (7 nt)	B cells
NF-AT1 (10 nt)	T cells
Fox P3 (7 nt)	T regulatory cells
LEF-1 (8 nt)	Pre B pre T cells

http://alggen.lsi.upc.edu/recerca/menu_recerca.html

Dissimilarity margin less than 5%

In silico Analysis of TF Binding the F8 Promoter Sequences

Hemophilia A
Cells Sources for HA Treatment



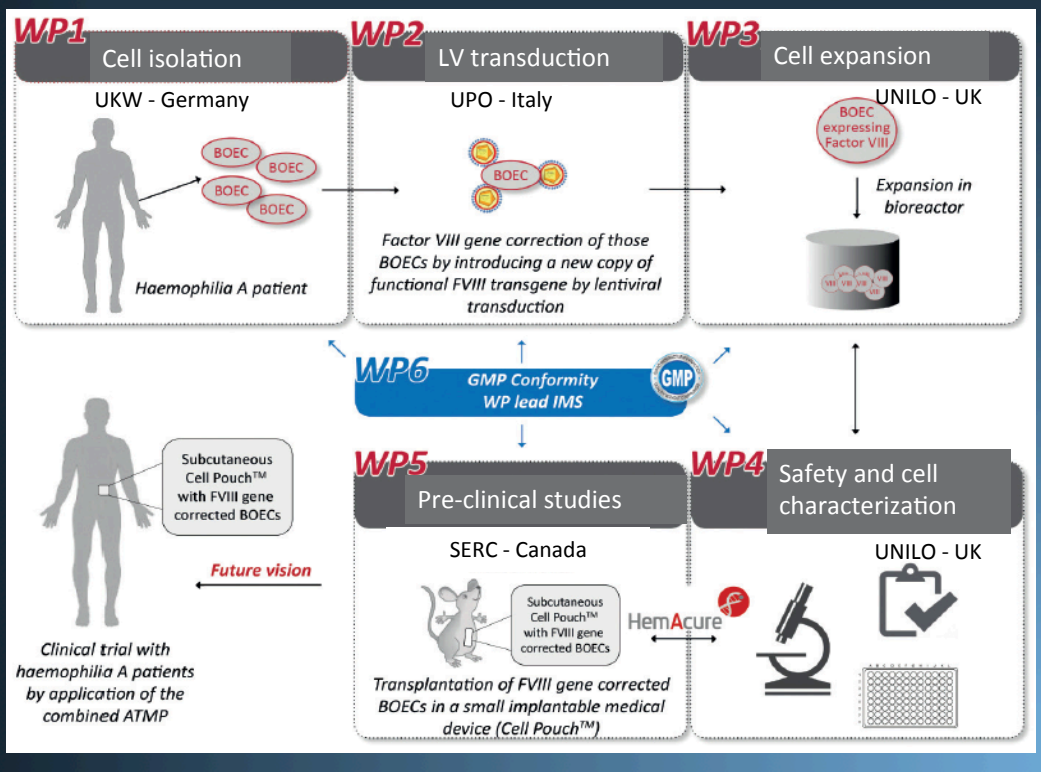
Fomin et al., 2014

INTRODUCTION to BOECs

- Blood outgrowth endothelial cells (BOECs) belong to the family of endothelial progenitors and they are generated from circulating endothelial progenitors found in adult peripheral blood
- BOECs are self-renewing, clonogenic, able to form capillary-like structures and integrate into functional blood vessels both *in vitro* and *in vivo*
- They are a valuable source of cells to understand endothelial cell biology, to perform disease modeling and they can be a substrate for the generation of induced pluripotent stem cells (iPSCs)
- BOECs might represent a good target for gene delivery by lentiviral vector (LV) to cure HA

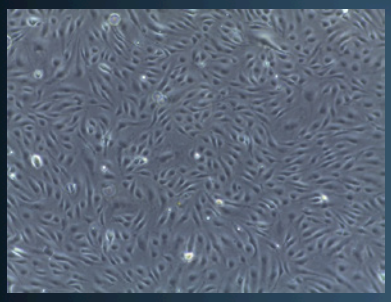
AIM

Development of tools and technologies for a novel ex vivo cell-based therapy to treat HA

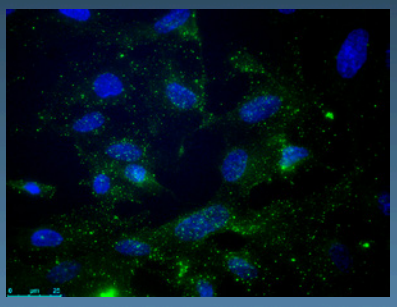


IN VIVO

Non-transduced or transduced BOECs (10^7) were transplanted intraperitoneally in NSG-HA mice in association with microcarrier beads



VEC — BDD hFVIII



NSG HA

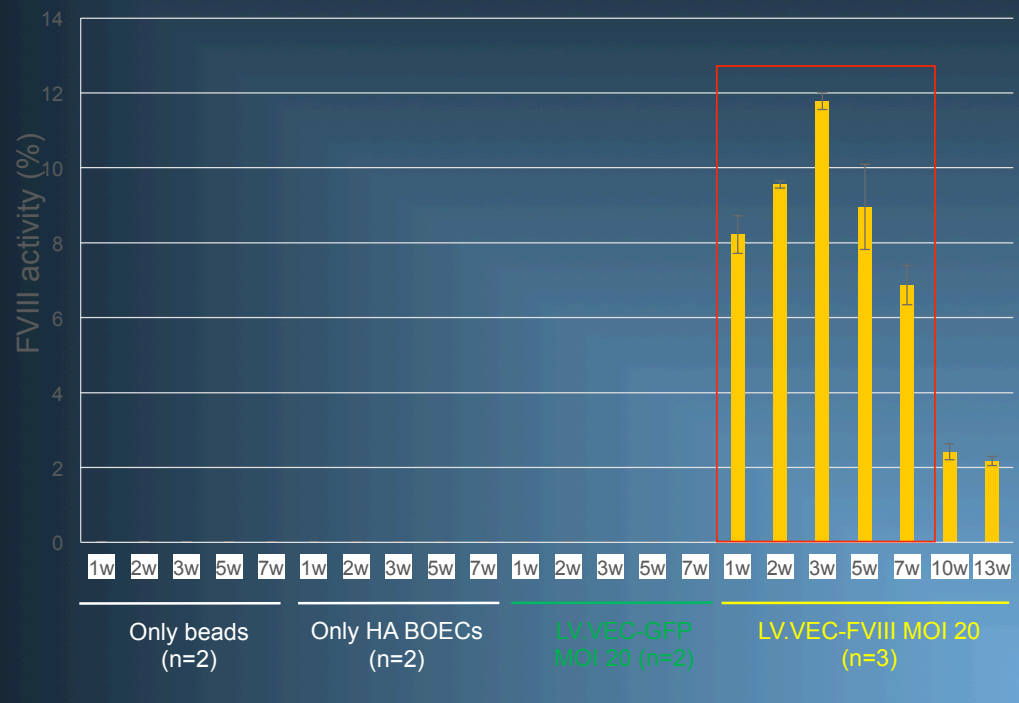
HA BOECs:

Only beads, n=2
Only HA BOECs, n=2
LV.VEC-GFP MOI 20, n=2
LV.VEC-FVIII MOI 20, n=3

→ Weekly aPTT assay
End point: bleeding assay

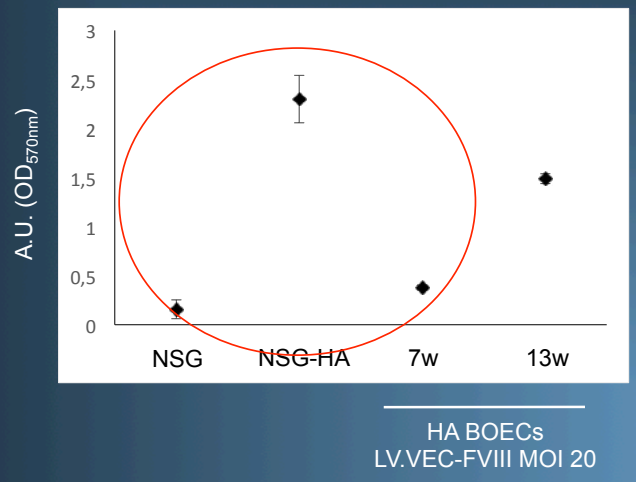
HA BOECs secreted FVIII in vivo up to 7 weeks after transplantation

aPTT assay on plasma obtained from HA BOECs-injected mice



NSG-HA mice ip injected with FVIII-corrected HA BOECs showed a blood loss similar to NSG control mice

Bleeding assay performed on NSG-HA mice ip injected with LV.VEC-FVIII BOECs



CONCLUSIONS AND FUTURE PLAN

- BOECs were isolated and expanded by both healthy and hemophilic donors
- BOECs were efficiently transduced by LV carrying FVIII under the VEC promoter
- LV.VEC-FVIII transduced BOECs survived and secreted FVIIII up to 10 weeks in NSG-HA mice

NEXT:

- Long term evaluation of BOEC tumorigenesis
- Transplantation of FVIII-corrected BOECs in a small implantable device (Cell Pouch™)

Thanks to…

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