Therapeutic potential of fetal liver cell transplantation in hemophilia A mice

Simone Merlin,[1+] Saicharan Akula,[1+] Alessia Cottonaro,[1] Tamara Garcia-Leal,[2] Luis Javier Serrano,[3] Ester Borroni,[1] Vakhtang Kalandadze,[1] Rocio Galiano,[2] Chiara Borsotti,[1] Antonio Liras,[3] María José Sanchez[2+] and Antonia Follenzi[1]

[1]University of Piemonte Orientale, Department of Health Sciences, School of Medicine, Novara, Italy; [2]Centro Andaluz de Biologia del Desarrollo (CABD), University Pablo de Olavide, CSIC, Seville, Spain and [3]Department of Genetics, Physiology and Microbiology, School of Biology, Complutense University of Madrid, Madrid, Spain

[+]SM, SA and MJS contributed equally.

%GFP vs %mFVIII

$R^2 = 0.9626$
$\rho = 0.9811$
$p < 0.0001$
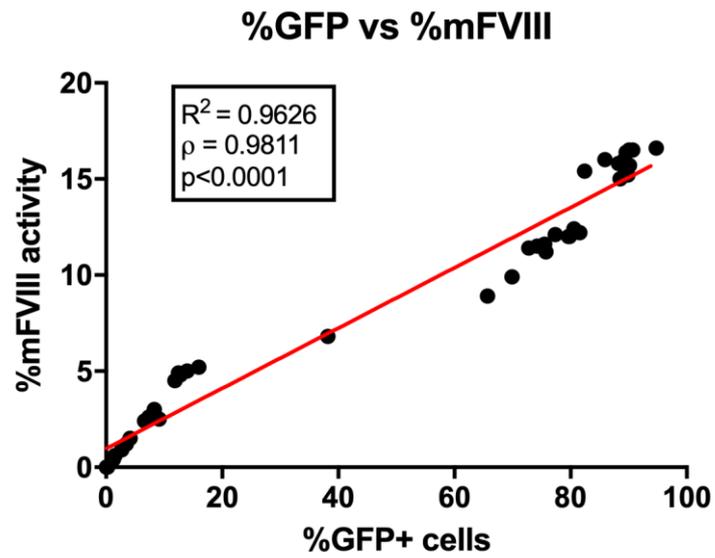
**Supplementary Figure 1. GFP+ cells engraftment and mFVIII production correlation in transplanted mice.** Pearson's correlation showing mFVIII levels correlating with percentage of engrafted GFP+ cells in a significant manner ($p < 0.0001$) at 6 months post-transplant in HA mice with or without busulfan preconditioning.
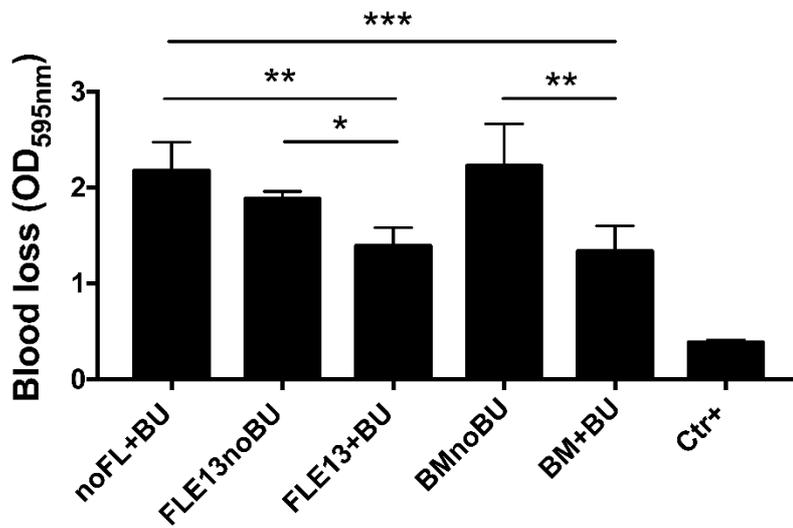
Supplementary Figure 2. Bleeding assay in newborn HA following transplantation. Tail clip assay performed 6 months after transplantation showed correction in recipient mice pre-treated with BU and a lower correction only in not pre-treated mice receiving FLE13 cells. n=8-10 each group. Graphs are showing mean values ± SD. Ctr+ = GFP+ mice (n=3). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
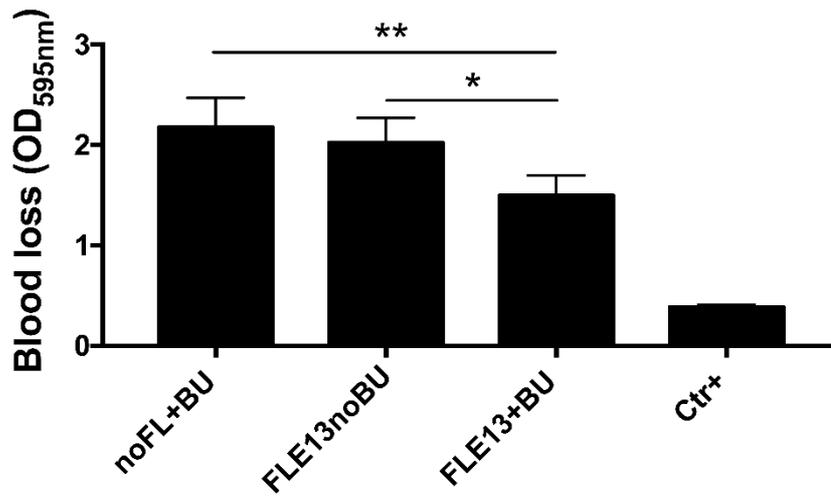
Supplementary Figure 3. Bleeding assay in adult HA following transplantation. Tail clip assay performed 6 months after transplantation showed correction only in recipient mice pre-treated with BU (n=5), while mice receiving cells without BU pre-treatment did not show correction (n=4). Graphs are showing mean values ± SD. Ctr+ = GFP+ mice (n=3). * $p<0.05$; ** $p<0.01$.

Supplementary table 1. List of antibodies used for immunofluorescence and flow cytometry

Primary antibodies			
Antibody	Clone	Brand	Dilution
Rat anti-mouse CD45	IBL-5/25	Immunotools	1:200
Rat anti-mouse CD31	MEC 13.3	BD Biosciences	1:100
Rat anti-mouse F4/80	Cl:A3-1	AbD serotec	1:400
Rat anti-mouse Lyve-1	ALY7	eBiosciences	1:200
Rabbit anti-GFP	polyclonal	Molecular Probes	1:500
Secondary antibodies			
Antibody	Clone	Brand	Dilution
Goat anti-rat IgG	polyclonal	Molecular Probes	1:500
Goat anti-rabbit IgG	polyclonal	Molecular Probes	1:500
Flow cytometry antibodies			
Antibody	Clone	Brand	Dilution
Rat anti-mouse CD45	30-F11	eBiosciences	1:100
Rat anti-mouse CD31	390	eBiosciences	1:100
Rat anti-mouse CD309	Avas12a1	eBiosciences	1:50
Rat anti-mouse CD146	P1H12	eBiosciences	1:50

Supplementary table 2. Engraftment (% GFP+ cells) and correction (% mFVIII activity) levels in plasma of newborn mice according to BU dosage and cell transplantation.

Group	Treatment	6 weeks		4 months		6 months	
		% GFP	% FVIII	% GFP	% FVIII	% GFP	% FVIII
no FL	no BU (n = 7)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	BU (n = 10)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
FLE13	no BU (n = 12)	6.3 ± 1.7	2.7 ± 1.9	7.2 ± 2.2	2.4 ± 1.2	7.5 ± 4.5	2.3 ± 1.7
	BU 1x (n = 7)	30.9 ± 14.4	4.6 ± 1.2	38.9 ± 16.7	6.7 ± 2.7	30.4 ± 16.9	7.7 ± 3.7
	BU 2x (n = 12)	79.5 ± 4.5	12.8 ± 2.6	75.2 ± 8.8	12.5 ± 2.7	90.2 ± 2.6	15.7 ± 0.4

List of abbreviations

Abbreviation	Definition
HA	Hemophilia A
FVIII	Factor VIII
LSEC	Liver sinusoidal endothelial cells
HSC	Hematopoietic stem cells
BU	Busulfan
FL	Fetal liver
BM	Bone marrow
AAV	Adeno associated virus
MCT	Monocrotaline
GFP	Green fluorescent protein
aPTT	Activated partial thromboplastin time
NPC	Non parenchymal cells
qRT-PCR	Quantitative real-time polymerase chain reaction
PB	Peripheral blood
AGM	Aorta-gonads-mesonephros region
CB	Cord blood
ECFCs	Endothelial colony forming cells
iPSCs	Induced pluripotent stem cells