CFM
CONSELHO FEDERAL DE MEDICINA

HORIZONTES TERAPEUTICOS

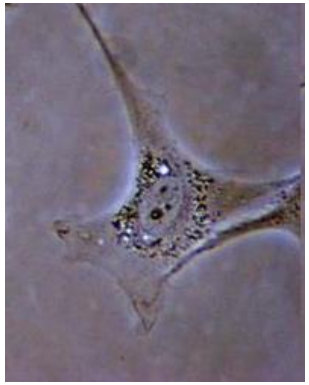
Profa. Dra. Ursula Matte

Depto. Genética/UFRGS

Centro de Terapia Gênica/HCPA

Ensaios pré-clínicos

- Testes *in vitro*
  - Cultura de células
- Testes *in vivo*
  - Modelos animais



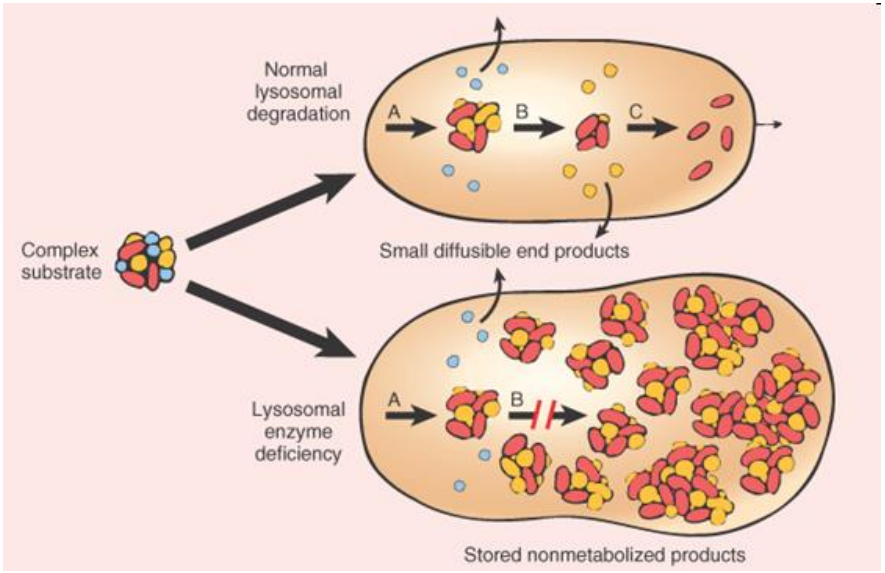
Mucopolissacaridoses (MPS)

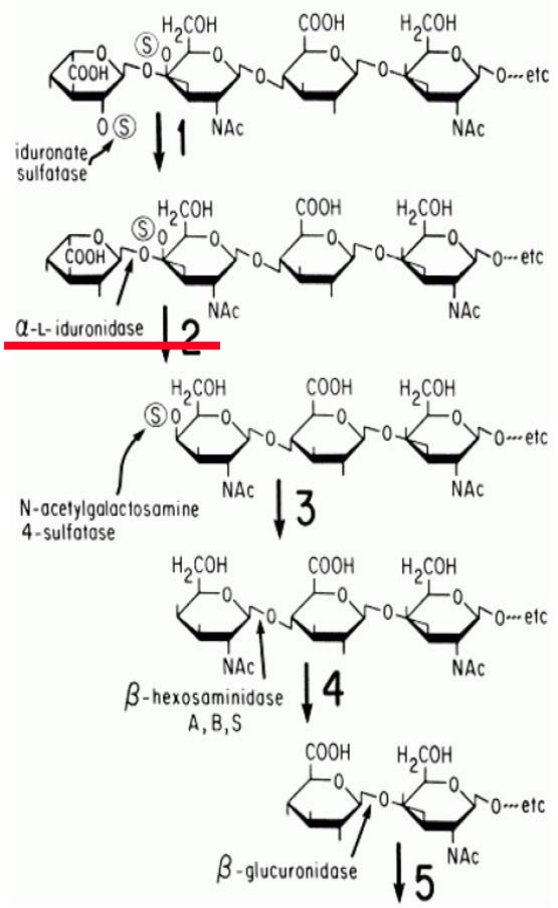
Terapia Gênica
CP-HCPA

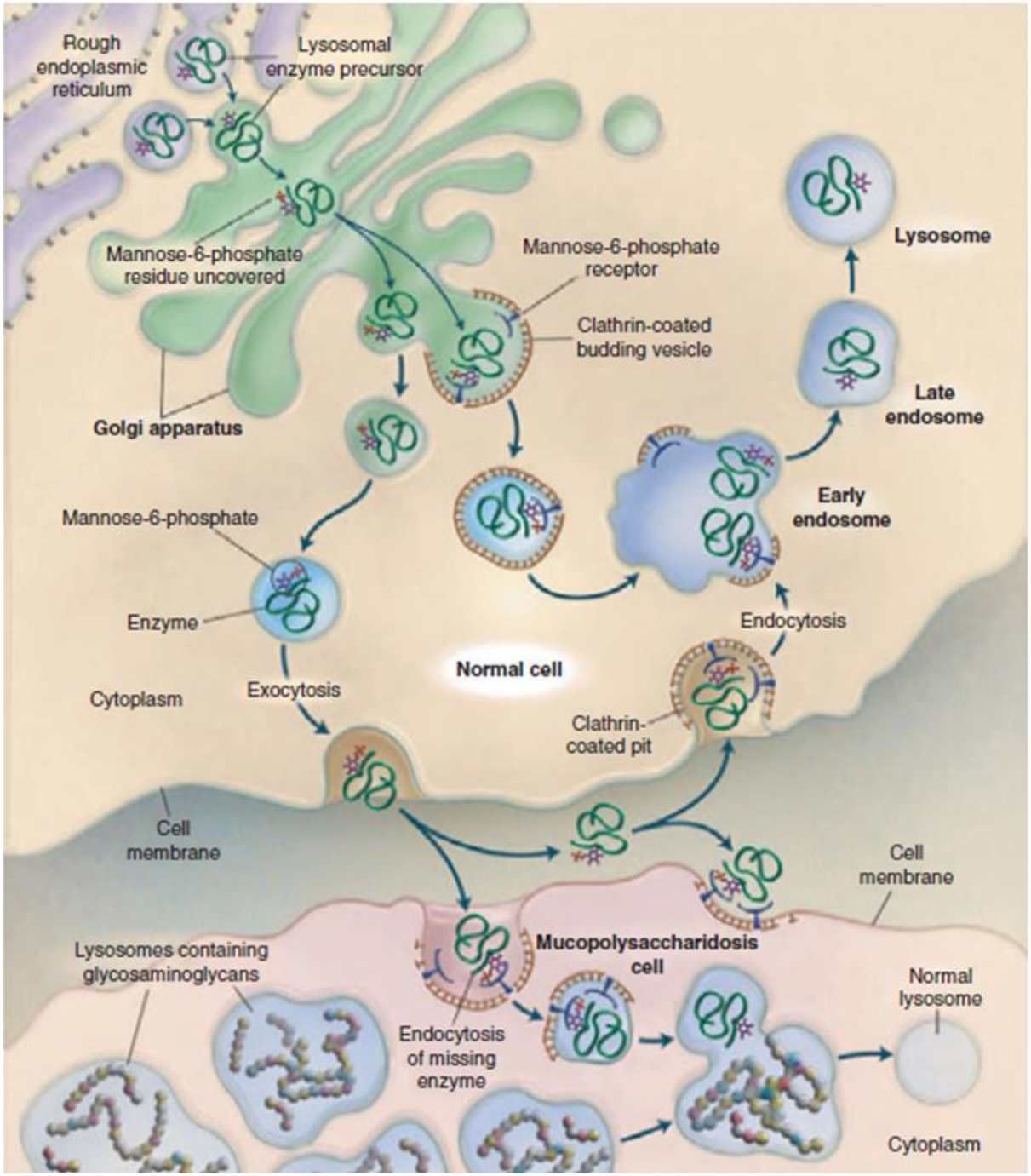
Erros inatos do metabolismo

Deficiência de enzimas lisossômicas

Acumulam substrato – glicosaminoglicanos (GAGs)







Terapia Gênica
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Enzima exógena pode ser captada e usada pelas células deficientes.

TCTH

TRE

Células encapsuladas

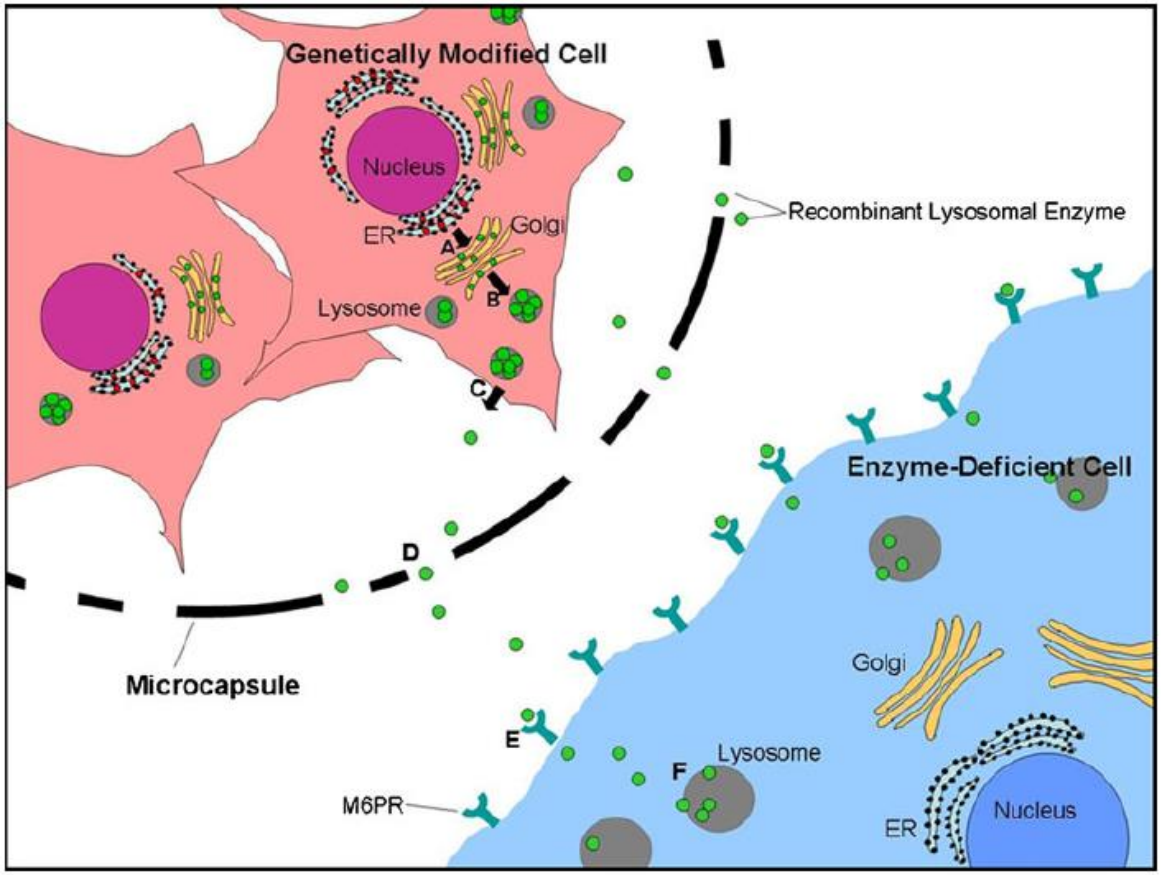
Terapia Gênica
CP-HCPA

J Inherit Metab Dis
DOI 10.1007/s10545-011-9350-4

REVIEW

Cell microencapsulation: a potential tool for the treatment of neuronopathic lysosomal storage diseases

Ursula Matte · Valeska Lizzi Lagranha · Talita Giacomet de Carvalho · Fabiana Quoos Mayer · Roberto Giugliani



Original Paper

Cells Tissues Organs

Cells Tissues Organs
DOI: 10.1159/000327532

Accepted after revision: March 15, 2011
Published online: July 19, 2011

Terapia Gênica
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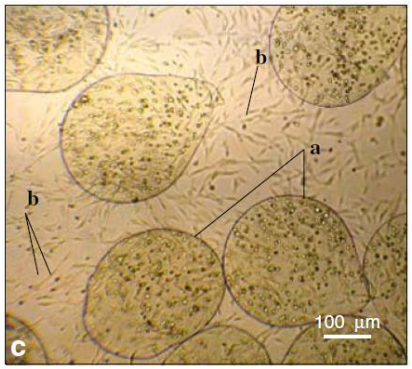
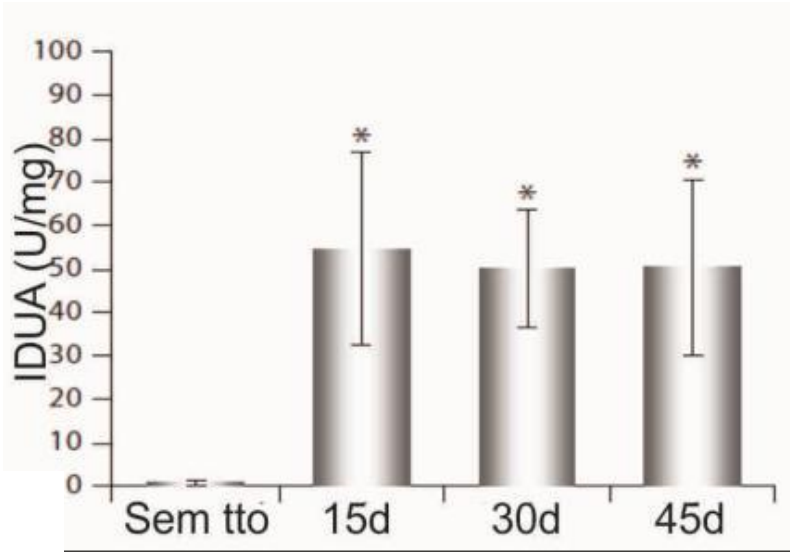
Recombinant Encapsulated Cells Overexpressing Alpha-L-Iduronidase Correct Enzyme Deficiency in Human Mucopolysaccharidosis Type I Cells

Guilherme Baldo[a, c] Fabiana Quoos Mayer[a, d] Maira Burin[b]
Joaquín Carrillo-Farga[e] Ursula Matte[a, d] Roberto Giugliani[a, c, d]

[a]Gene Therapy Center and [b]Medical Genetics Service, Hospital de Clinicas de Porto Alegre, and Post Graduation Programs in [c]Biochemistry and [d]Genetics and Molecular Biology, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil; [e]Institute of Hemopathology, México City, México





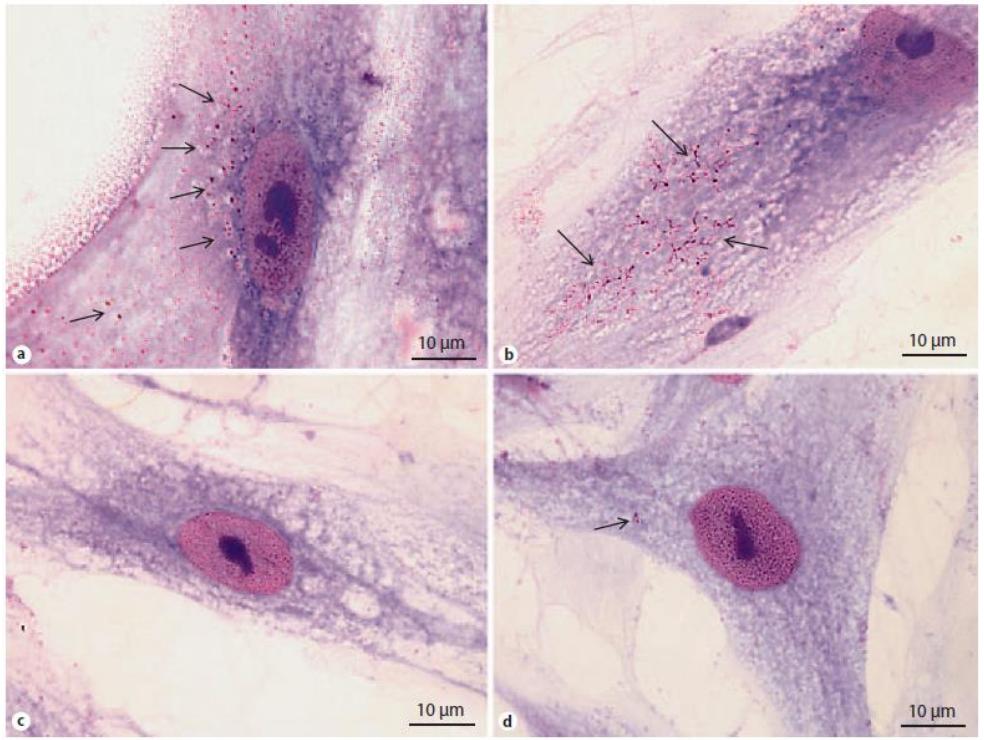


Fig. 3. GAG storage in MPS I fibroblasts before (**a**, **b**) and after 45 days of coculture with the capsules (**c**, **d**) using Wright staining. GAGs are shown as abundant magenta (metachromatic) dots within the MPS cells, almost undetectable in the treated fibroblasts (arrows).

Cytotherapy, 2012; Early Online: 1–8

Terapia Gênica
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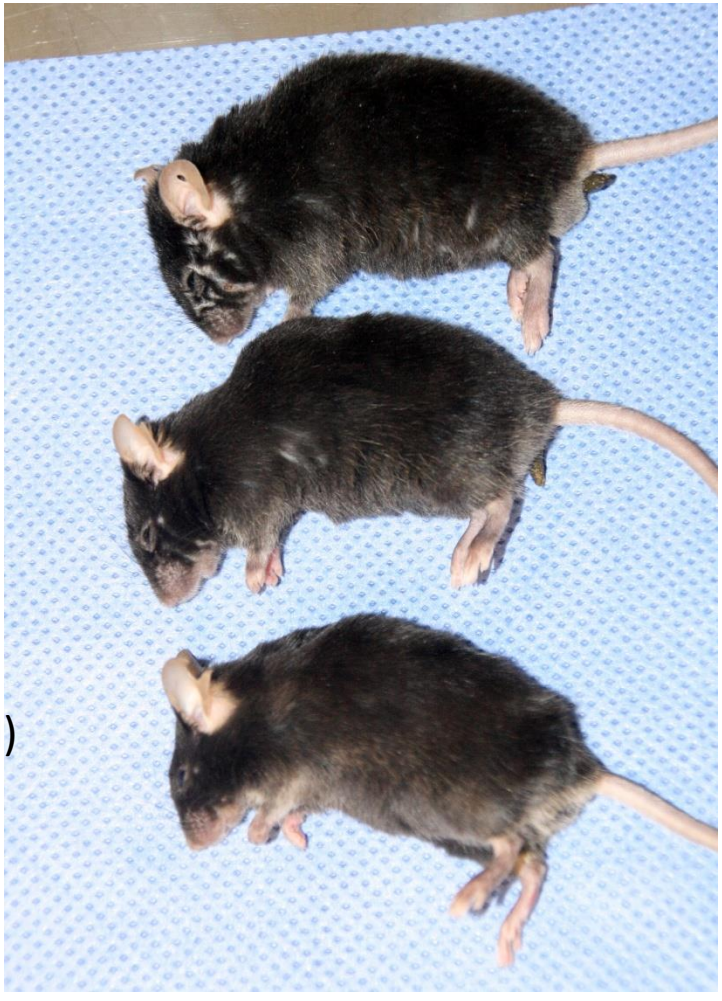
Intraperitoneal implant of recombinant encapsulated cells overexpressing alpha-L-iduronidase partially corrects visceral pathology in mucopolysaccharidosis type I mice

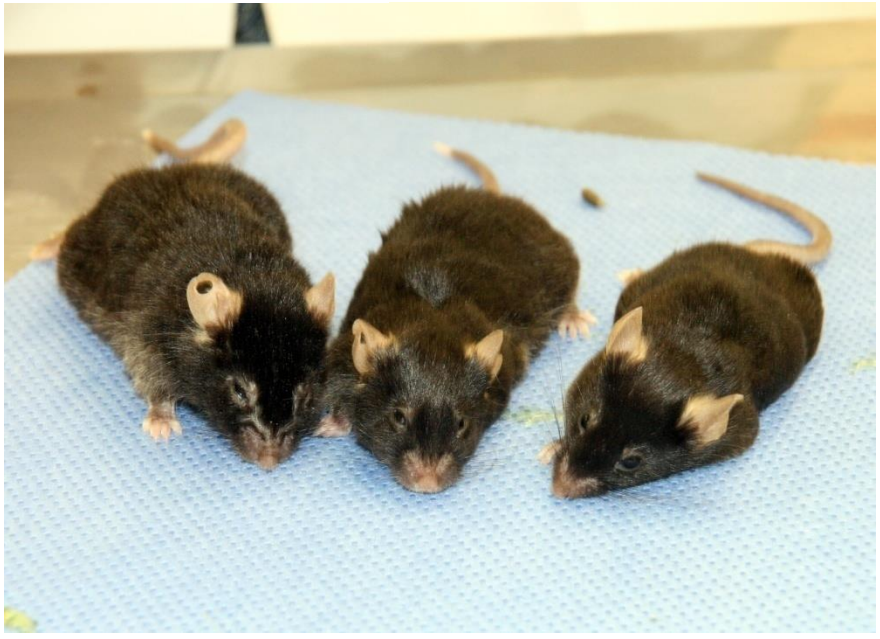
GUILHERME BALDO[1,2], FABIANA QUOOS MAYER[1,3], BARBARA MARTINELLI[1], FABIOLA SCHONS MEYER[4], MAIRA BURIN[5], LUISE MEURER[6], ANGELA MARIA VICENTE TAVARES[7], ROBERTO GIUGLIANI[1,2,3,5] & URSULA MATTE[1,3]

MPS

Capsules

WT(normal)

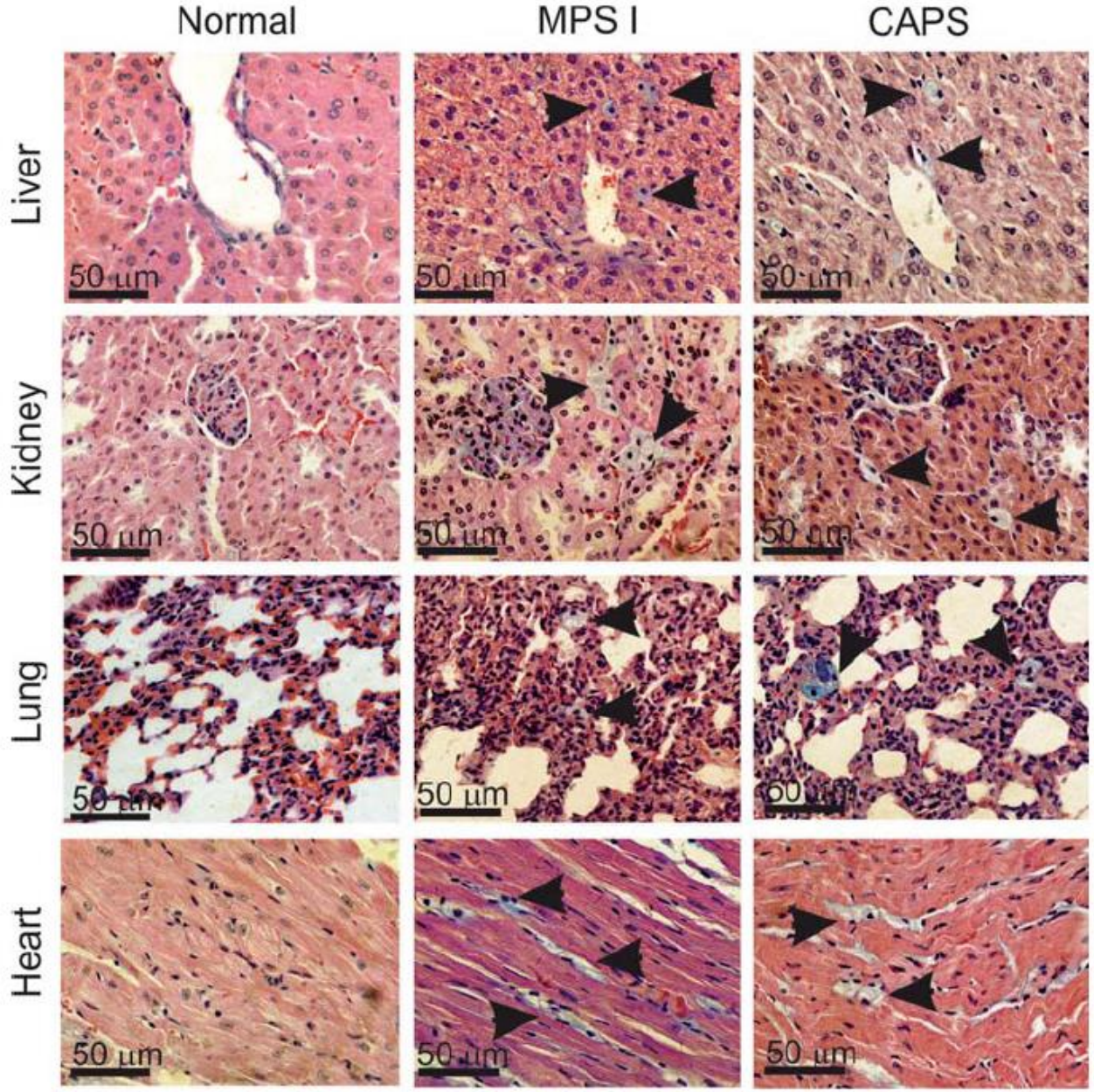




MPS Capsules WT

Capsules implanted i.p. at 30, 90, 150 days.

Euthanasia at 180 days.



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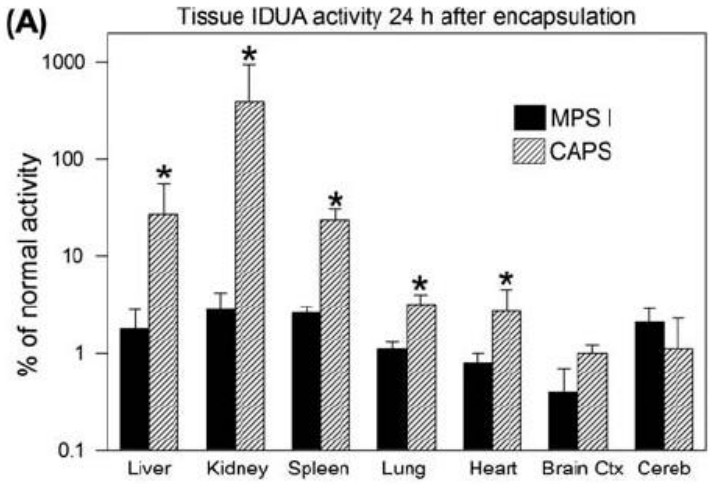
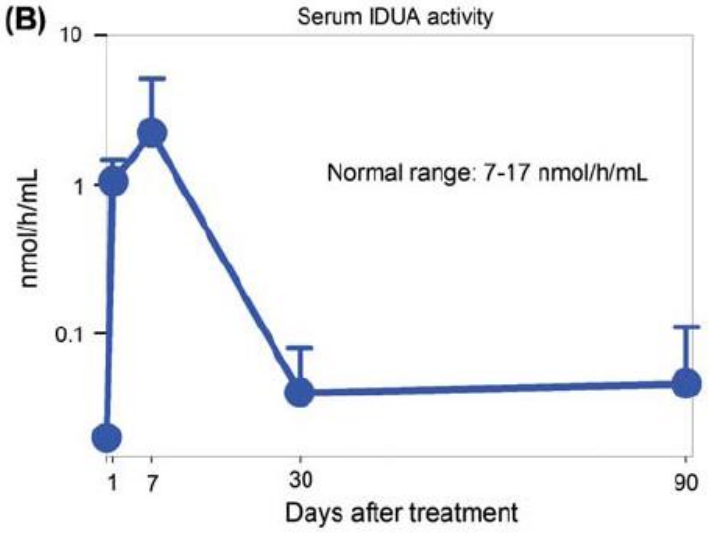
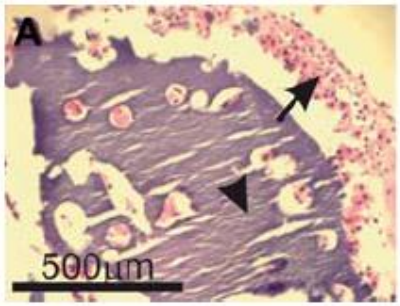
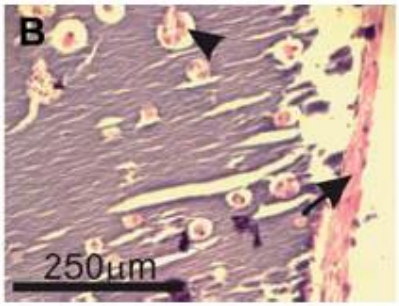
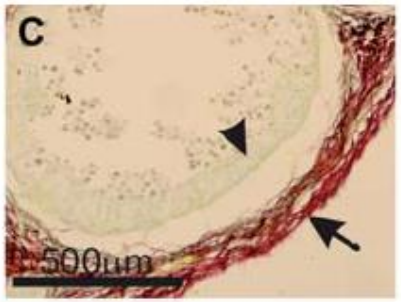
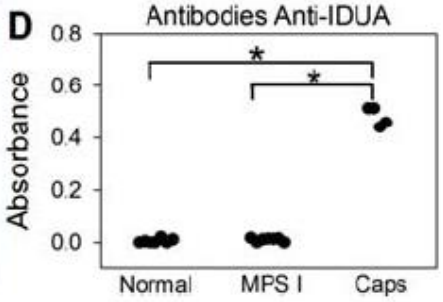

(A)
Tissue IDUA activity 24 h after encapsulation
% of normal activity
1000
100
10
1
0.1
MPS I
CAPS
*
Liver
Kidney
Spleen
Lung
Heart
Brain Ctx
Cereb


(B)
Serum IDUA activity
nmol/h/mL
10
1
0.1
Normal range: 7-17 nmol/h/mL
1
7
30
90
Days after treatment


A
500µm


B
250µm


C
500µm


D
Antibodies Anti-IDUA
Absorbance
0.8
0.6
0.4
0.2
0.0
*
*
Normal
MPS I
Caps

Alterações propostas

Terapia Gênica
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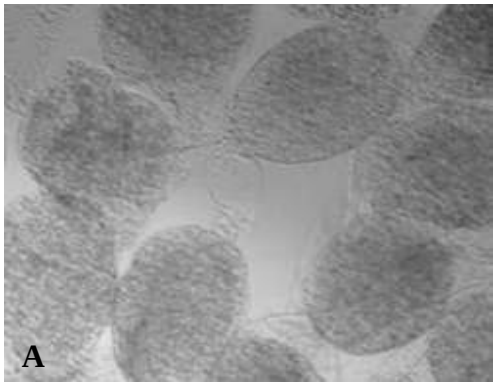
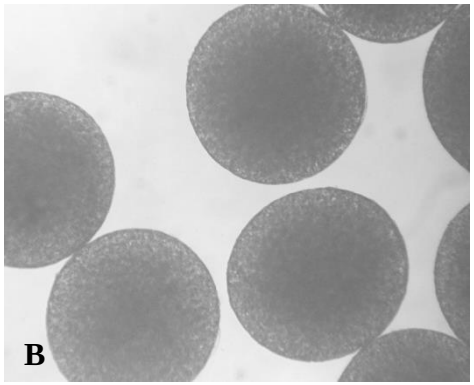
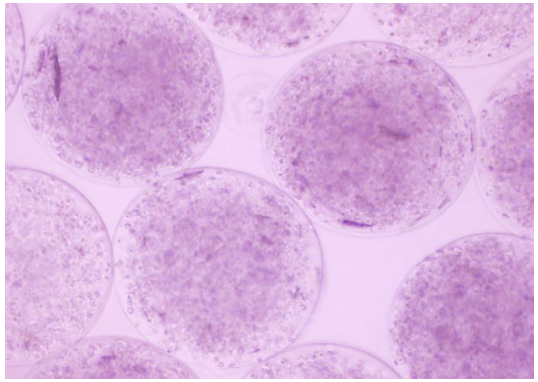
Cápsulas APA usando o '*Ultra Pure Low Vicosity Guluronic'* (UP LVG) da FMC Nova Matrix™.





Modificação do biomaterial

Cristais de Dexametasona



Uso combinado de anti-inflamatório

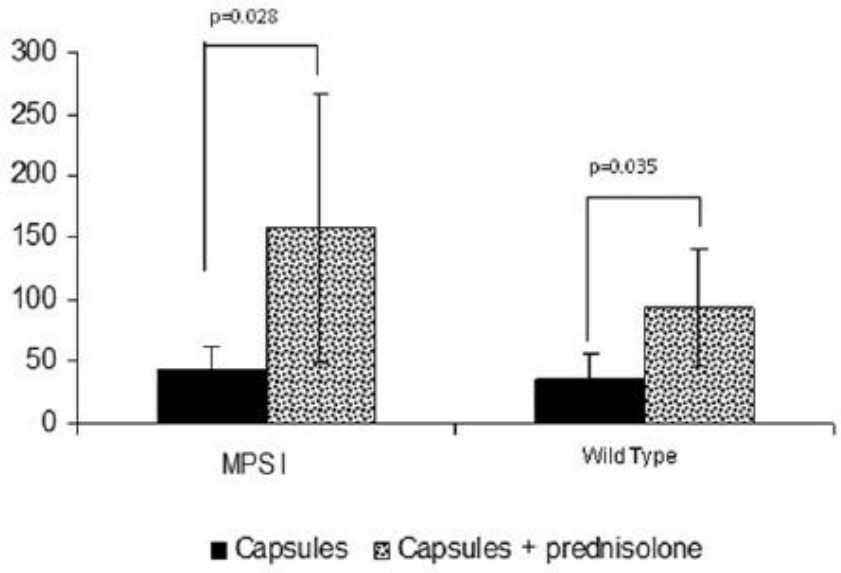
Journal of Microencapsulation, 2013; 30(4): 383–389

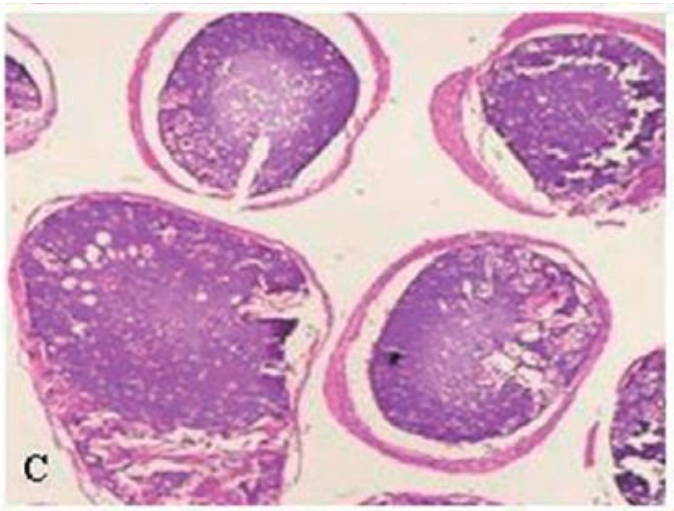
ISSN 0265-2048 print/ISSN 1464-5246 online
DOI: 10.3109/02652048.2012.746745

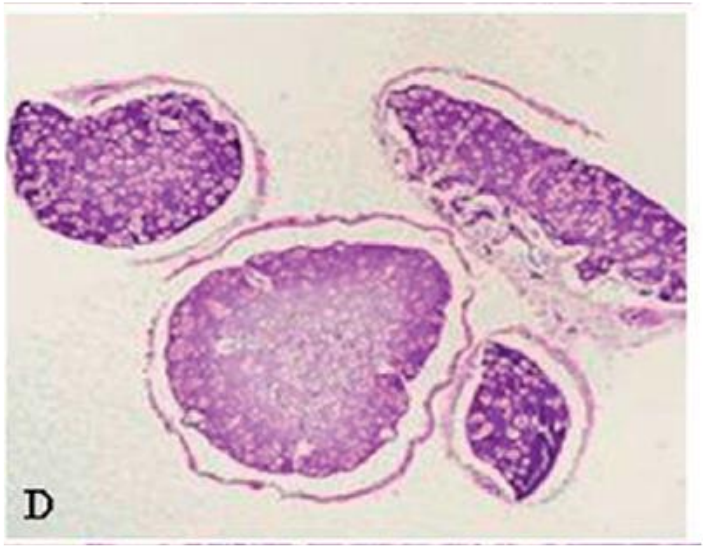
Terapia Gênica
CP-HCPA

Treatment of MPS I mice with microencapsulated cells overexpressing IDUA: effect of the prednisolone administration

Valeska Lizzi Lagranha[1,2], Talita Giacomet de Carvalho[1,2], Roberto Giugliani[1,2,3] and Ursula Matte[1,2]







Terapia Gênica
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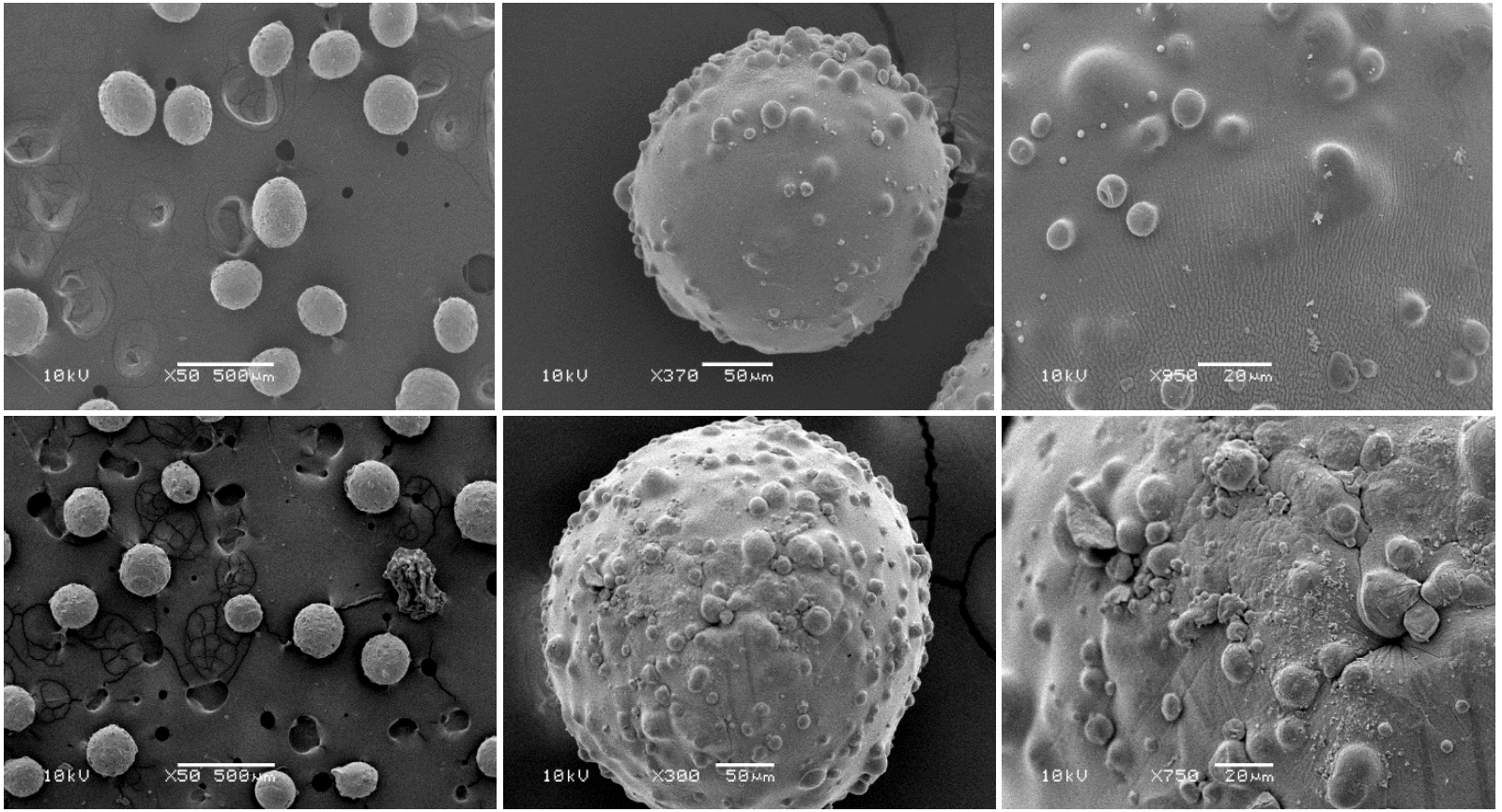
Controle do crescimento celular dentro das cápsulas

1 week

3 weeks



Órgãos de difícil acesso

TECNOLOGIA | BIOTECNOLOGIA

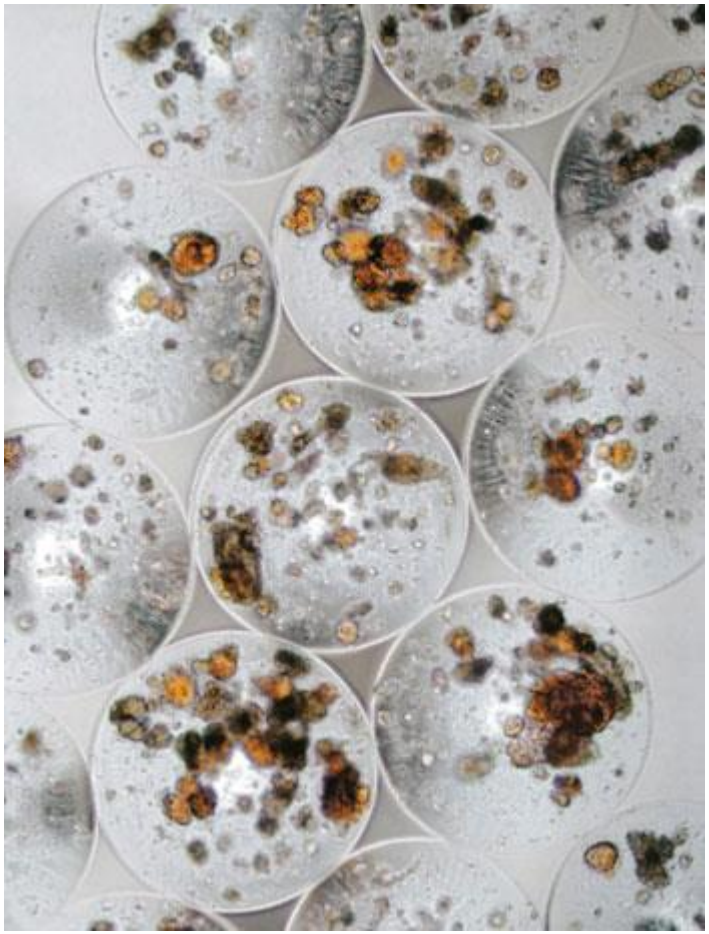
A proteção das esferas

Uso de microcápsulas para revestir ilhotas do pâncreas pode impulsionar tratamento do diabetes

Marcos Pivetta

Edição Impressa 182 - Abril de 2011

Terapia Gênica
CP-HCPA



NUCEL/CELLPROTECT®

Núcleo de Terapia Celular e Molecular

Profa. Mari Cleide Sogayar

Injeção intra-articular
Ensaios de segurança em ovelhas

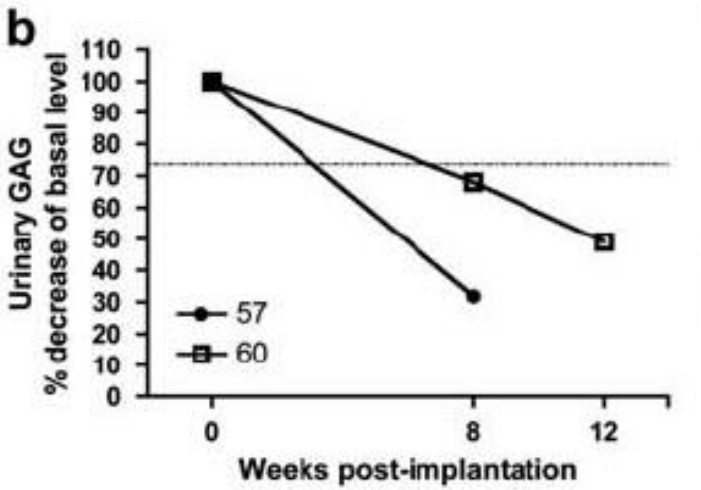
Gene Therapy (2011), 1–10

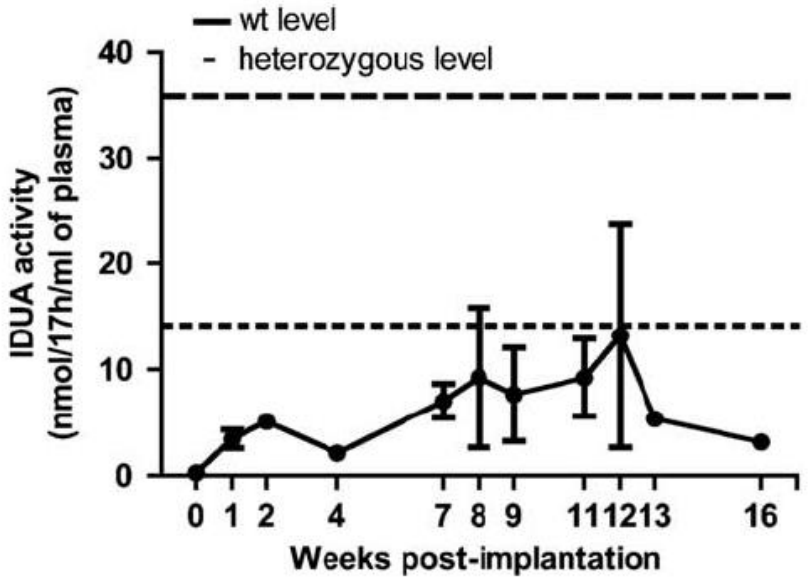
www.nature.com/gt

ORIGINAL ARTICLE

Encapsulated engineered myoblasts can cure Hurler syndrome: preclinical experiments in the mouse model

E Piller Puicher[1,4], R Tomanin[1,4], M Salvalaio[1], A Friso[1], G Hortelano[2], O Marin[3] and M Scarpa[1]





Edição Genômica

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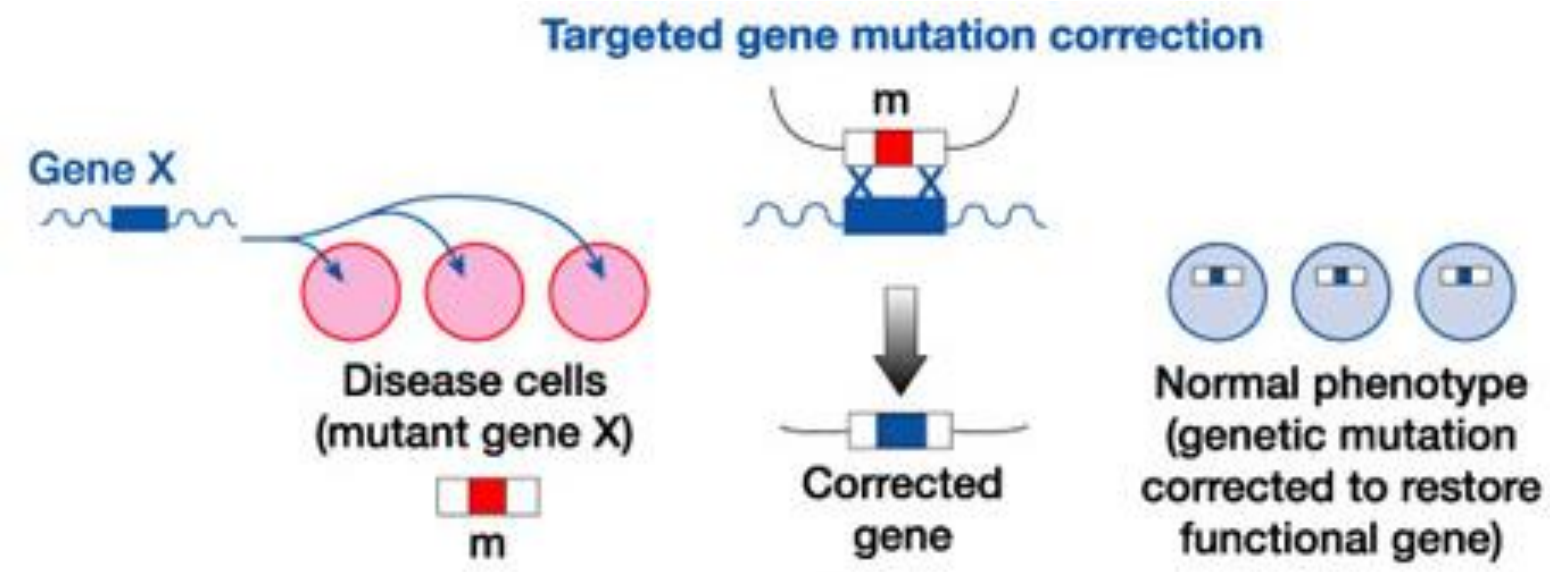

Correção de uma mutação específica utilizando um sistema chamado CRISPR-Cas9 (derivado de bactérias).

Terapia Gênica
CP-HCPA

Multiplex Genome Engineering Using CRISPR/Cas Systems

Le Cong,[1,2]* F. Ann Ran,[1,4]* David Cox,[1,3] Shuailiang Lin,[1,5] Robert Barretto,[6] Naomi Habib,[1] Patrick D. Hsu,[1,4] Xuebing Wu,[7] Wenyan Jiang,[8] Luciano A. Marraffini,[8] Feng Zhang[1]†

Functional elucidation of causal genetic variants and elements requires precise genome editing technologies. The type II prokaryotic CRISPR (clustered regularly interspaced short palindromic repeats)/Cas adaptive immune system has been shown to facilitate RNA-guided site-specific DNA cleavage. We engineered two different type II CRISPR/Cas systems and demonstrate that Cas9 nucleases can be directed by short RNAs to induce precise cleavage at endogenous genomic loci in human and mouse cells. Cas9 can also be converted into a nicking enzyme to facilitate homology-directed repair with minimal mutagenic activity. Lastly, multiple guide sequences can be encoded into a single CRISPR array to enable simultaneous editing of several sites within the mammalian genome, demonstrating easy programmability and wide applicability of the RNA-guided nuclease technology.

RNA-Guided Human Genome Engineering via Cas9

Prashant Mali,[1]* Luhan Yang,[1,3]* Kevin M. Esvelt,[2] John Aach,[1] Marc Guell,[1] James E. DiCarlo,[4] Julie E. Norville,[1] George M. Church[1,2]†

Bacteria and archaea have evolved adaptive immune defenses, termed clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems, that use short RNA to direct degradation of foreign nucleic acids. Here, we engineer the type II bacterial CRISPR system to function with custom guide RNA (gRNA) in human cells. For the endogenous AAVS1 locus, we obtained targeting rates of 10 to 25% in 293T cells, 13 to 8% in K562 cells, and 2 to 4% in induced pluripotent stem cells. We show that this process relies on CRISPR components; is sequence-specific; and, upon simultaneous introduction of multiple gRNAs, can effect multiplex editing of target loci. We also compute a genome-wide resource of ~190 K unique gRNAs targeting ~40.5% of human exons. Our results establish an RNA-guided editing tool for facile, robust, and multiplexable human genome engineering.

Science, Fev 2013

Clustered Regurlarly Interespaced Short Palindromic Repeat (CRISPR) associated protein 9 (CRISPR-Cas9)

Terapia Gênica
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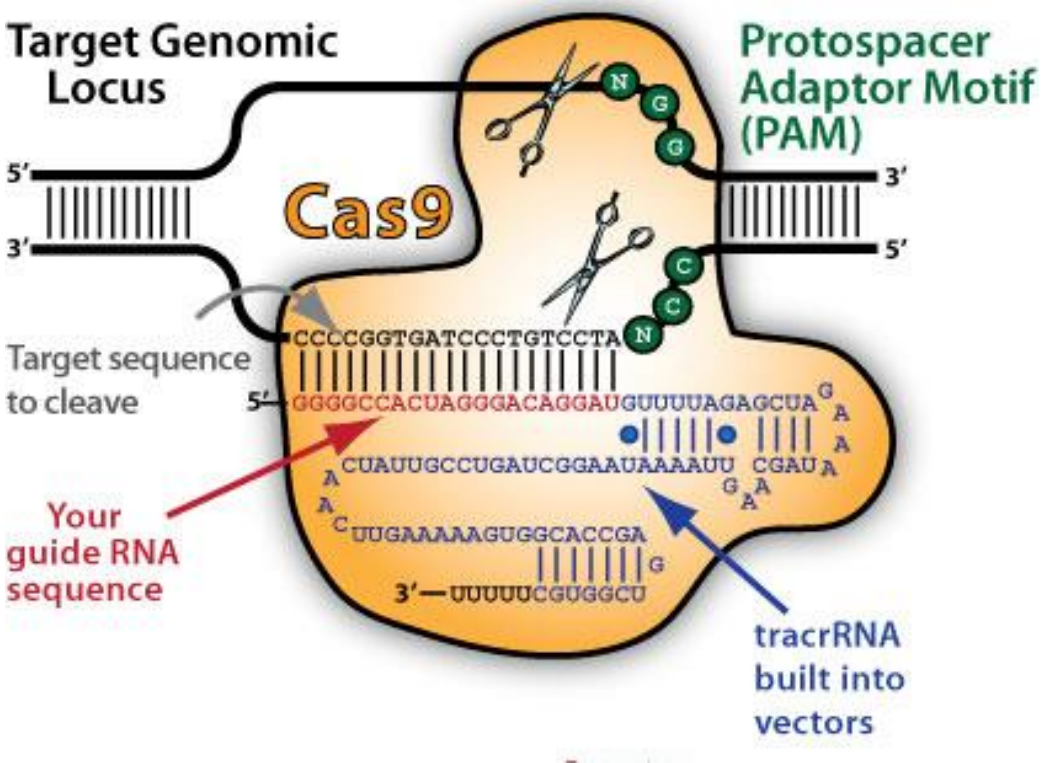
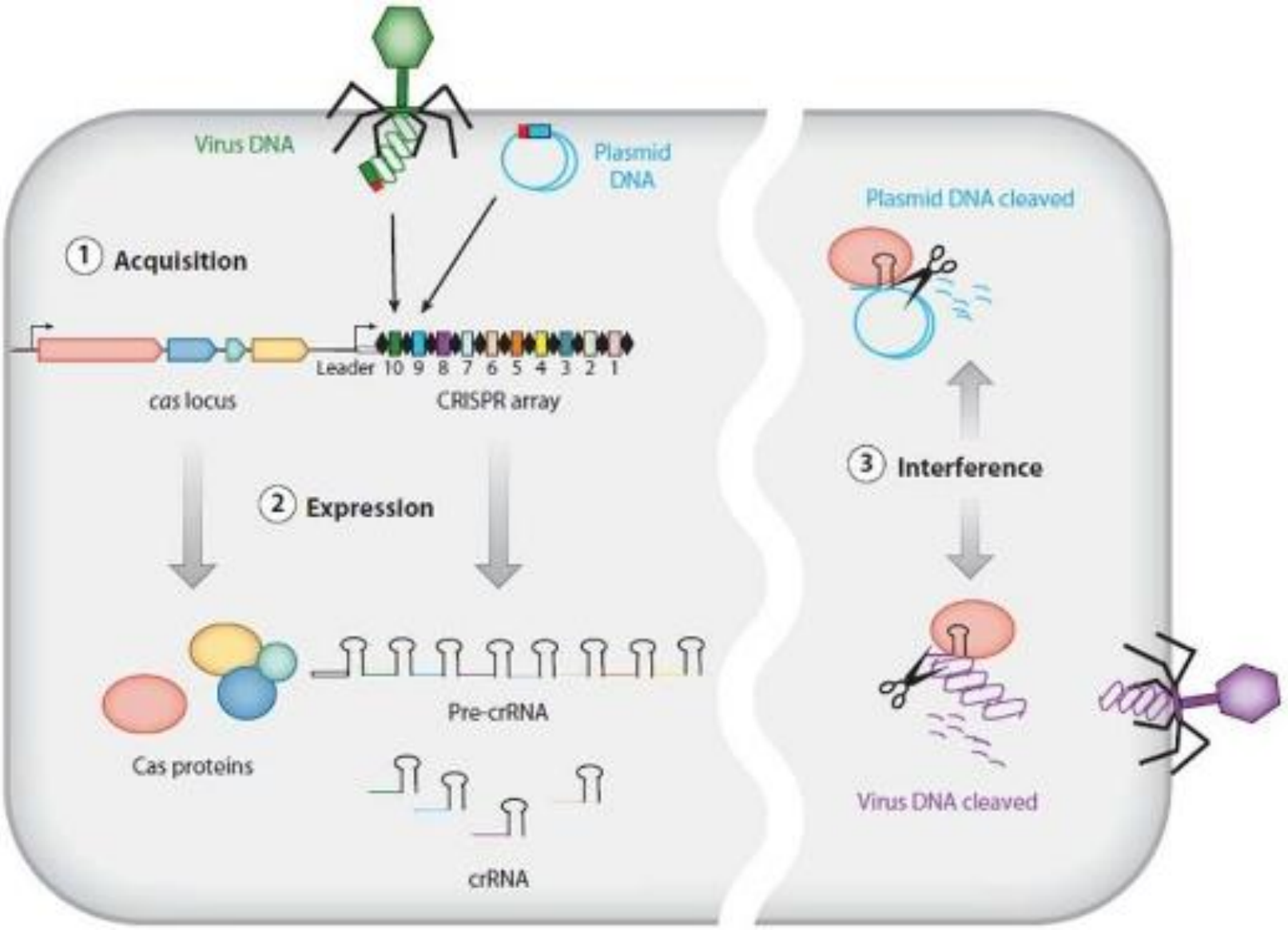
O sistema usa uma nuclease (Cas9) que se complexa com pequenos RNAs guias (gRNAs) para clivar o DNA de maneira específica *upstream* à sequencia PAM em qualquer localização genômica.





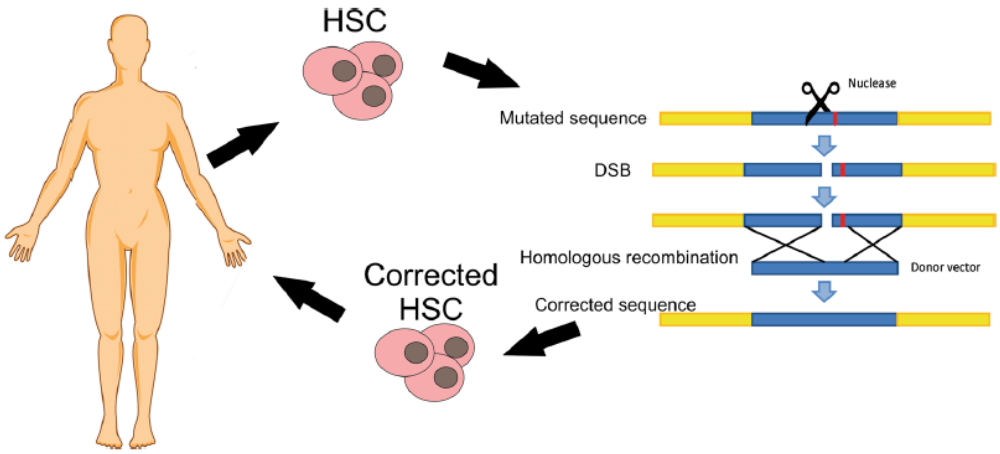
Curr Stem Cell Rep (2015) 1:9–15
DOI 10.1007/s40778-014-0007-8

Genome Editing: Potential Treatment for Lysosomal Storage Diseases

Talita Giacomet de Carvalho · Ursula da Silveira Matte · Roberto Giugliani · Guilherme Baldo

Table 1 List of main pre-clinical studies using nuclease-mediated genome editing to correct mutations in genetic diseases

Genetic disease	Gene	Model organism	Strategy	Reference
X-linked severe combined immunodeficiency	IL2RG	Human hematopoietic stem cells	ZFN	[5•]
α-1-antitrypsin deficiency	A1AT	iPSCs derived from patient	ZFN	[6]
Parkinson's disease	LRRK2	iPSCs derived from patient	ZFN	[7]
Sickle cell disease	HBB	iPSCs derived from patient	TALEN	[8]
X-linked severe combined immunodeficiency	IL2RG	Jurkat cells	TALEN	[9]
Retinal disease	CRB1	Fertilized oocytes from a mouse model	TALEN	[10]
β-thalassemia	HBB	iPSCs derived from patient	CRISPR-Cas9	[11]
Hereditary tyrosinemia	FAH	mouse model	CRISPR-Cas9	[12]
Duchenne muscular dystrophy	DMD	Germ line of mdx mice	CRISPR-Cas9	[13]



LSD	Common mutations	Combined mutant alleles frequency in the population (approximate) (%)	Reference
Aspartylglucosaminuria	p.C163S; c.488G>C	98	[26]
Gaucher disease	p.L444P; c.1448T>C p.N370S; c.1226A>G	67	[27]
Krabbe disease	c.1161+6532_polyA+9kbdel c.1586C>T, c.1700C>T, c.1472delA	60	[28]
Lysosomal acid lipase deficiency	p.delS275_Q298; c.894G>A	50–60	[29]
α-mannosidosis	p.R750W; c.2248C>T	27	[30]
Metachromatic leukodystrophy	c.459+1G>A	25	[31]
Mucopolysaccharidosis type I	p.W402X; c.1293G>A p.Q70X; c.296C>T	70	[32]

Terapia Gênica
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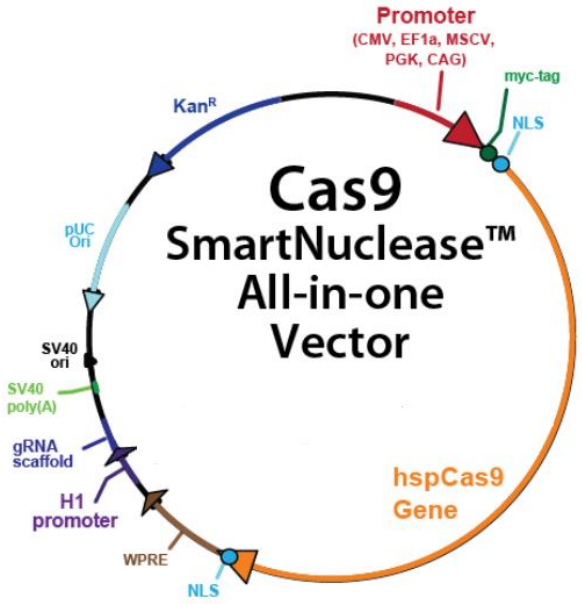
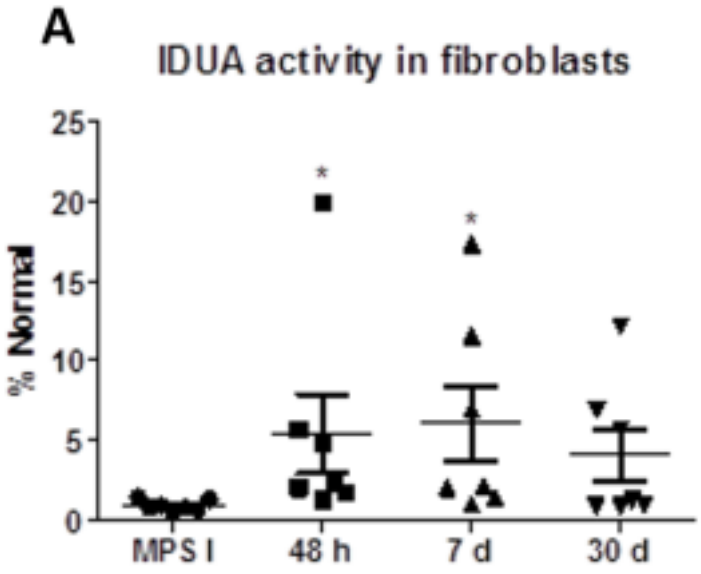
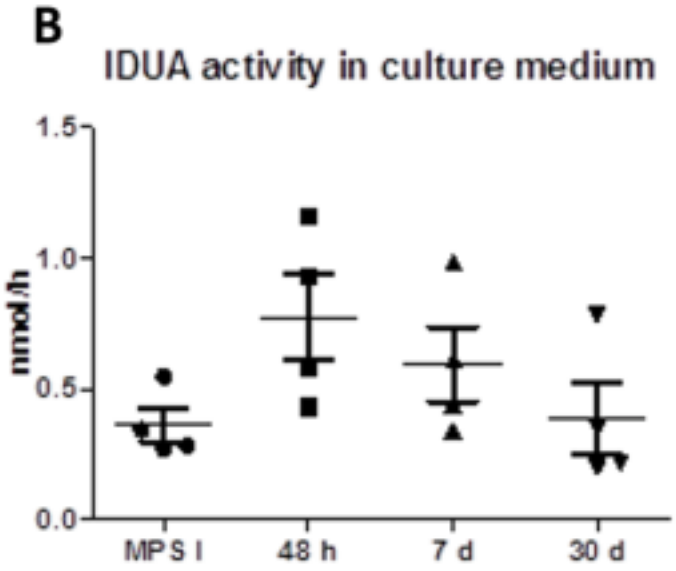
Fibroblastos de pacientes homozigotos para mutação p.W402X

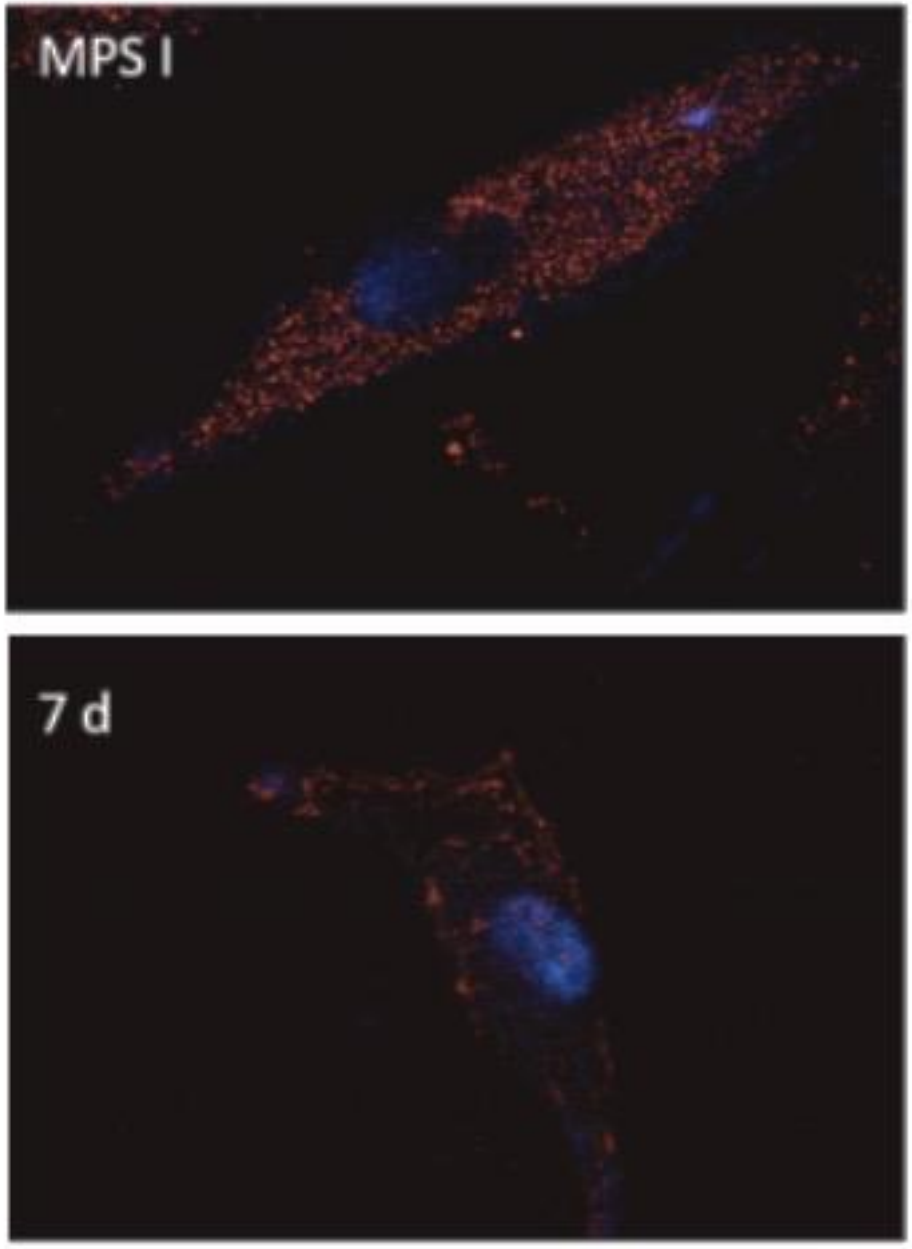
- 1293 TGG → TAG (Stop codon)
- Mutação mais comum na MPS I
- Fenótipo grave (sem atividade enzimática)

GCGGCTGGGCAACGACCCCACGCGGCGACGGCCCCCCCCCGCCC
CGCAGATGAGGAGCAGCTCT**G**GGCCGAAGTGTCGCA**A**GCCGGGAC
CGTCCTGGACAGCAACCACACGGTGGGCGTCCTGGCCAGCGCCCA


Promoter
(CMV, EF1a, MSCV,
PGK, CAG)
myc-tag
NLS
Kan^R
pUC
Ori
Cas9
SmartNuclease™
All-in-one
Vector
SV40
ori
SV40
poly(A)
gRNA
scaffold
H1
promoter
WPRE
NLS
hspCas9
Gene


A
IDUA activity in fibroblasts
% Normal
25
20
15
10
5
0
*
*
MPS I
48 h
7 d
30 d


B
IDUA activity in culture medium
nmol/h
1.5
1.0
0.5
0.0
MPS I
48 h
7 d
30 d



Aparente redução no número de lisossomos 7 dias após o tratamento.

Terapia Gênica
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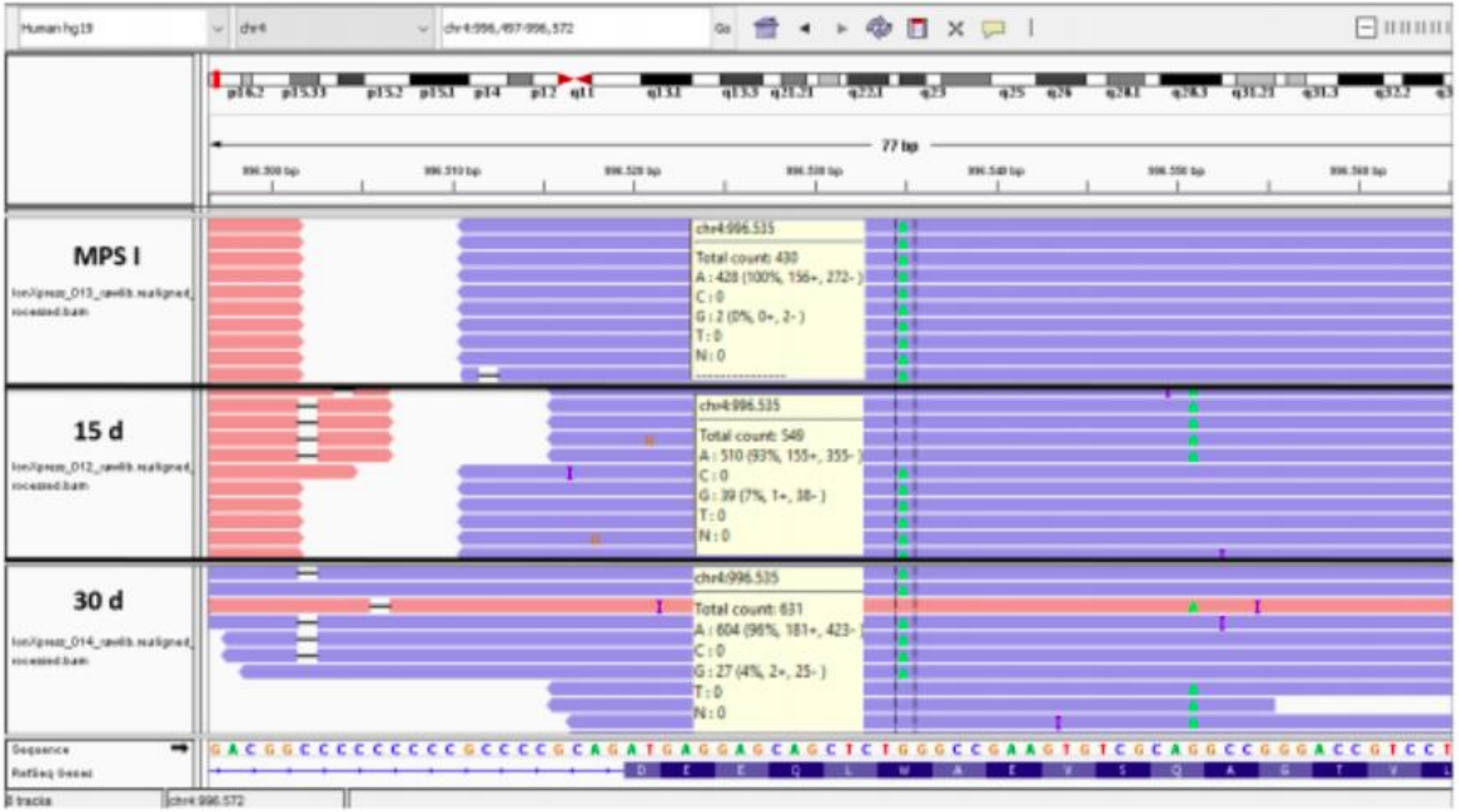
Percentual pequeno de células corrigidas (~ 7%)



Protein Cell 2015, 6(5):363–372
DOI 10.1007/s13238-015-0153-5

Protein & Cell

Terapia Gênica
CP-HCPA

RESEARCH ARTICLE

CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

Puping Liang, Yanwen Xu, Xiya Zhang, Chenhui Ding, Rui Huang, Zhen Zhang, Jie Lv, Xiaowei Xie, Yuxi Chen, Yujing Li, Ying Sun, Yaofu Bai, Zhou Songyang, Wenbin Ma, Canquan Zhou✉, Junjiu Huang✉

A

Sites	Sequence	Indel frequency (mutant embryos/total embryos)		Locus
HBD	TTGACAGCAGTCTTCTCCTCAGG	0/11	0/12	chr14:5234413

pressing need to further improve the fidelity and specificity of the CRISPR/Cas9 platform, a prerequisite for any clinical applications of CRSIPR/Cas9-mediated editing.

E

No.16 embryo

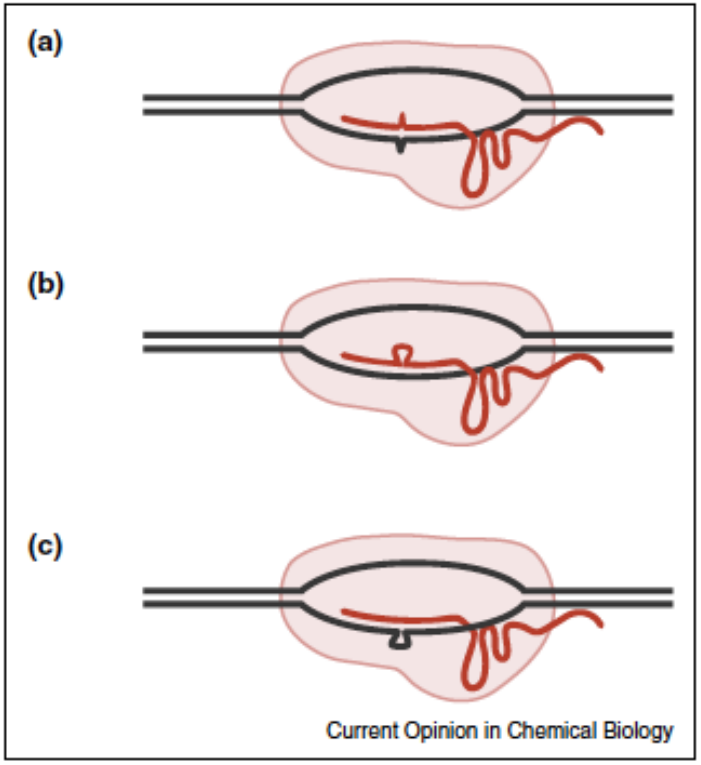
GCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAA	8/50
GCATCTGACTCCTGAAGAAAAATCCGCTGTCACTGCCCTGTGGGGCAAGGTGAA	6/50
GCATCTGACTCCTGAAGAAAAATCCGCCGTTACTGCCCTGTGGGGCAAGGTGAA	1/50
GCATCTGACTCCTGAGGAGAAGACTGCTGTCAATGCCCTGTGGGGCAAAGTGAA	21/50
GCACCTGACTCCTGAG---AAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAA	14/50

Recombinação com gene da globina delta

How specific is CRISPR/Cas9 really?

Henriette O'Geen, Abigail S Yu and David J Segal

Current Opinion in Chemical Biology 2015, **29**:72–78



Some sgRNAs allow binding or cleavage at variants of the target site. In addition to single-base mismatches **(a)**, some sgRNAs can tolerate DNA sequences with an extra base (**(b)**, DNA bulge) or a missing base (**(c)**, sgRNA bulge).

Diferenças...

... nas células alvo

... nas Cas9 das várias bactérias

… nos gRNA

… nos métodos de avaliação

Exemplo de Potenciais *Off Target*

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	A	B	C	D	E	F	G	H	I
1	guide id	chr	strand	position	sequence	# mismatches	score	ontarget	gene
2	1420923726	chr11	-1	69400873	TCGTCCATGCAGTGAGGTGATGG	0	100	True	NM_011640
3	1420923727	chr17	-1	54559100	TCGTCCATGGAGTGAGGTGATGG	1	92.1	False	None
4	1420923728	chr3	-1	153891676	TTGTCCATGCAGTGAGGTTAAAG	2	5.541666667	False	None
5	1420923729	chr15	-1	66551400	TCATGCATGCAGTGAGGTGAGGG	2	5.383333333	False	NM_009375
6	1420923730	chr13	1	81457506	AAGTCCATGAAGTGAGGTGAAGG	3	2.525108225	False	None
7	1420923731	chr7	1	51226124	CCCTCCCTGCAGTGAGGTGAAGG	3	1.712894511	False	NM_010645
8	1420923732	chr7	1	51371725	CCCTCCCTGCAGTGAGGTGAAGG	3	1.712894511	False	NM_010114
9	1420923733	chr1	-1	166786002	GCTGCCAGGCAGTGAGGTGAAGG	4	1.366780156	False	None
10	1420923734	chr3	1	92800388	TAGCTCAAGCAGTGAGGTGATAG	4	1.364942529	False	None
11	1420923735	chr9	1	72973159	CAGCACATGCAGTGAGGTGAAAG	4	1.324349442	False	None
12	1420923736	chr7	1	51200730	CTCTCCTTGCAGTGAGGTGATGG	4	0.919204167	False	NR_033120
13	1420923737	chr11	-1	96844769	TCTTCCCTGCATTGAGGTGATGG	3	0.908412471	False	None
14	1420923738	chr2	-1	165245332	GAGTCCAGGCTGTGAGGTGAAAG	4	0.807015306	False	None
15	1420923739	chr1	-1	154013777	CCTTCTATGAAGTGAGGTGATAG	4	0.786045066	False	None
16	1420923740	chr7	1	19730487	TCATTCATGCAGTGTGGTGACAG	3	0.785713928	False	NM_026111
17	1420923741	chr14	-1	119066196	TCTCTGATGCAGTGAGGTGAAAG	4	0.778438874	False	None
18	1420923742	chr14	1	64142666	TCATATATGTAGTGAGGTGACAG	4	0.761576737	False	NM_177594

Predição *in silico* baseada na sequencia

The NEW ENGLAND JOURNAL of MEDICINE

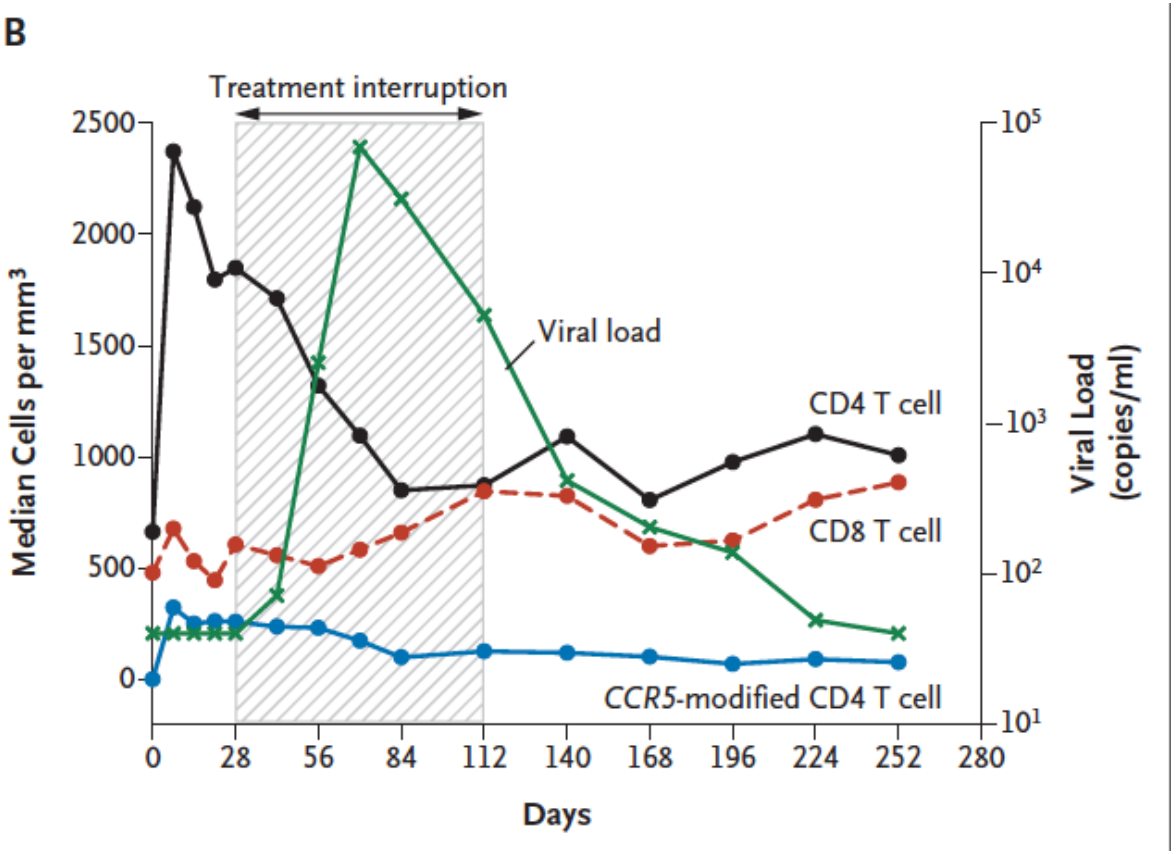
ESTABLISHED IN 1812 MARCH 6, 2014 VOL. 370 NO. 10

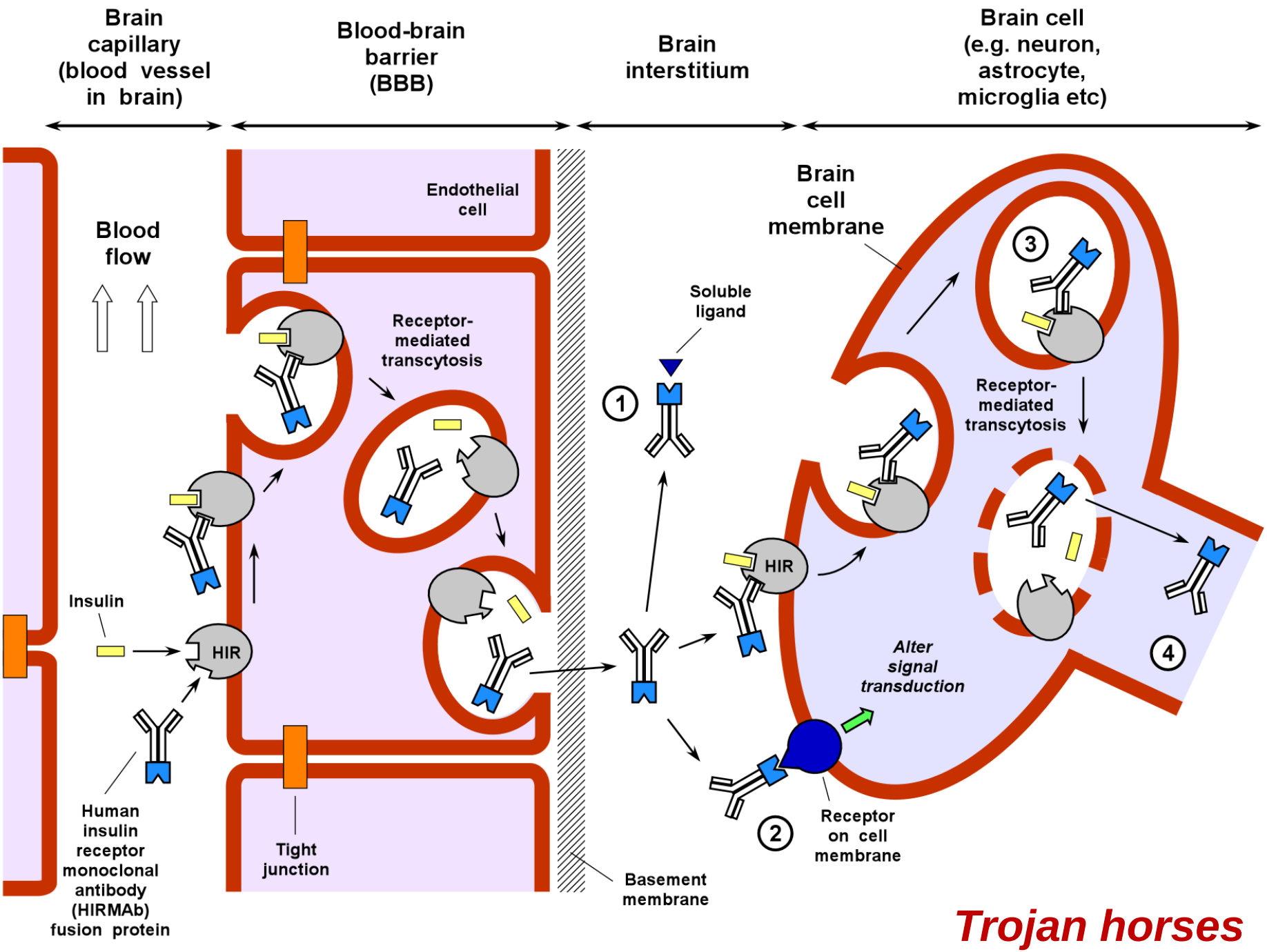
Terapia Gênica
CP-HCPA

Gene Editing of *CCR5* in Autologous CD4 T Cells of Persons Infected with HIV

Pablo Tebas, M.D., David Stein, M.D., Winson W. Tang, M.D., Ian Frank, M.D., Shelley Q. Wang, M.D., Gary Lee, Ph.D., S. Kaye Spratt, Ph.D., Richard T. Surosky, Ph.D., Martin A. Giedlin, Ph.D., Geoff Nichol, M.D., Michael C. Holmes, Ph.D., Philip D. Gregory, Ph.D., Dale G. Ando, M.D., Michael Kalos, Ph.D., Ronald G. Collman, M.D., Gwendolyn Binder-Scholl, Ph.D., Gabriela Plesa, M.D., Ph.D., Wei-Ting Hwang, Ph.D., Bruce L. Levine, Ph.D., and Carl H. June, M.D.

Uma única injeção de células T modificadas.





Trojan horses

William Pardridge – propôs o uso de Trojan horses para MPS em 2001

Ruben Boado – publicou a prova de conceito (*in vitro*) em 2008
e o estudo em modelo animal (*in vivo*) em 2011

armagen

Armagen – empresa que Pardridge criou e onde ambos trabalham iniciou o ensaio clínico em 2014

Falha na interação com empresas e no empreendedorismo na Universidade.

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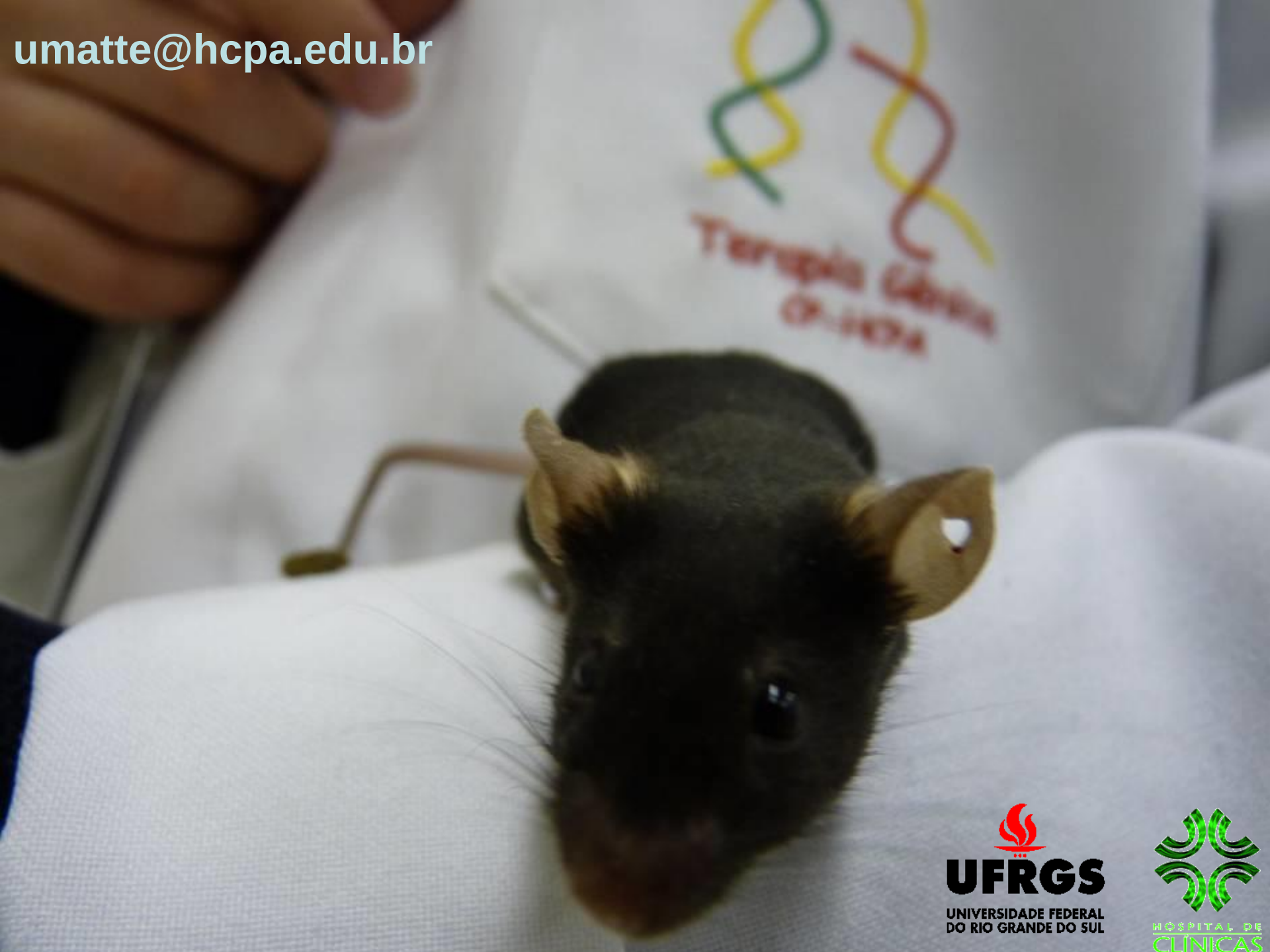
Gabriela Pasqualim – doutorado
Esteban Gonzalez – doutorado
Edina Poletto – mestrado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