“INFLAMAÇÃO E ADESÃO CELULAR NA ANEMIA FALCIFORME E SEU REFLEXO NO TRATAMENTO”

FAPESP

Simpósio Regional sobre Medicina Translacional

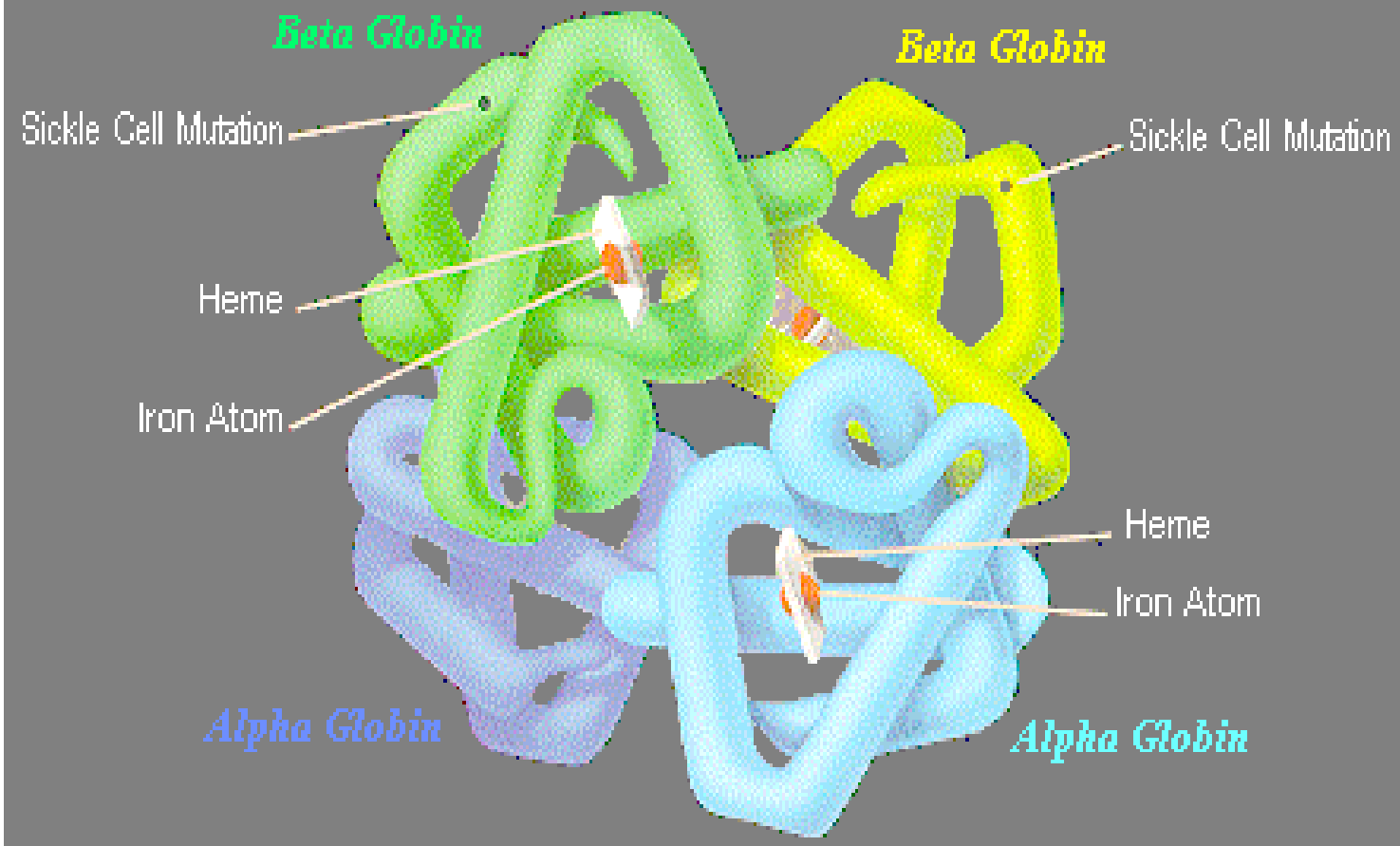
02/12/2011- FAPESP - São Paulo

HEMÁCIA

- Durável
- Flexível
- Elevada concentração de hemoglobina
- Máxima eficiência no transporte de oxigênio
- Vida média : 120 dias
- 550,6 quilômetros

A Molecule To Breathe With

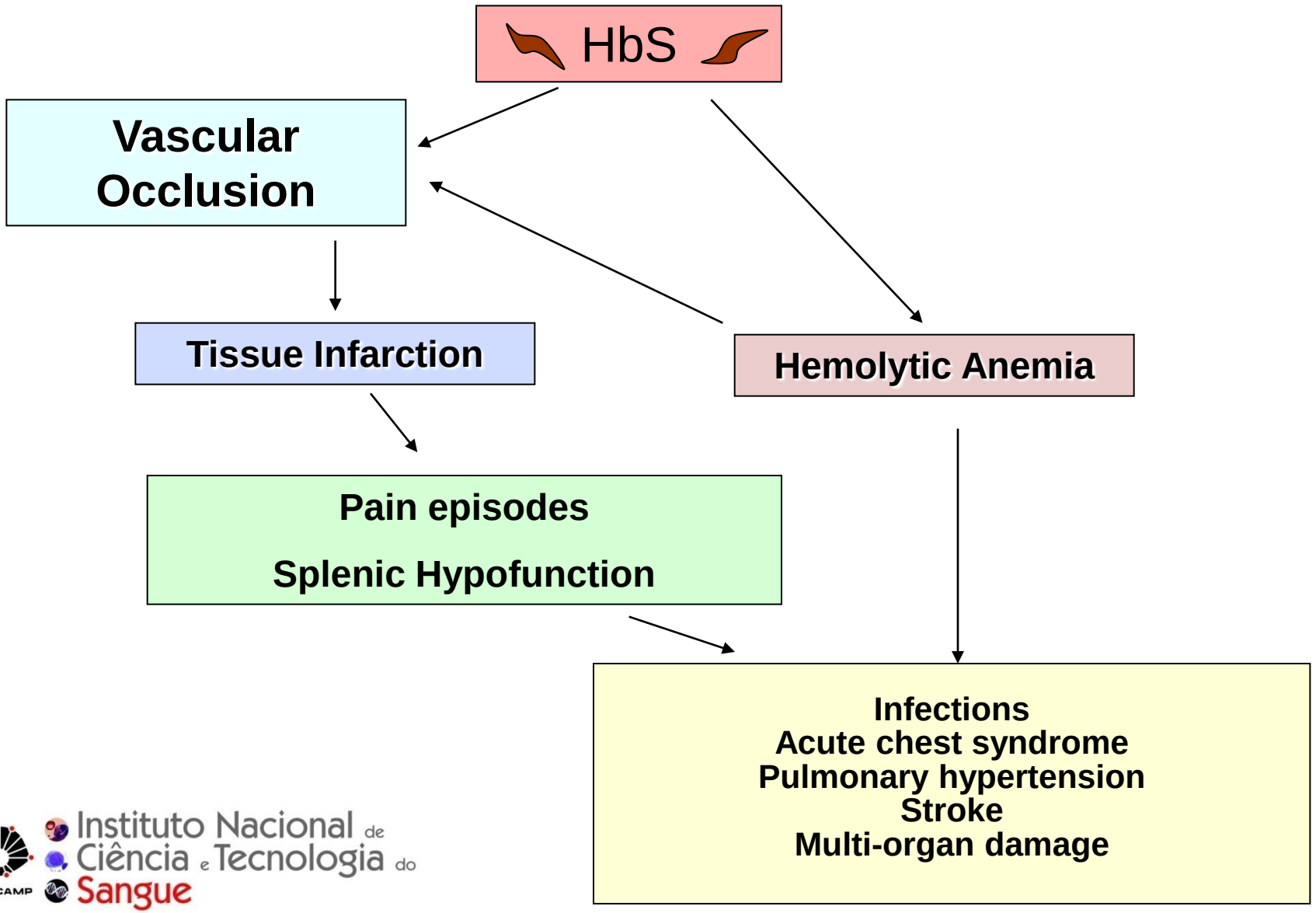
HEMOGLOBIN



The Pathophysiology of Sickle Cell Anemia

Costa: Hematologia, Fundamentos e Prática 2001

Vaso-occlusion in Sickle Cell Disease



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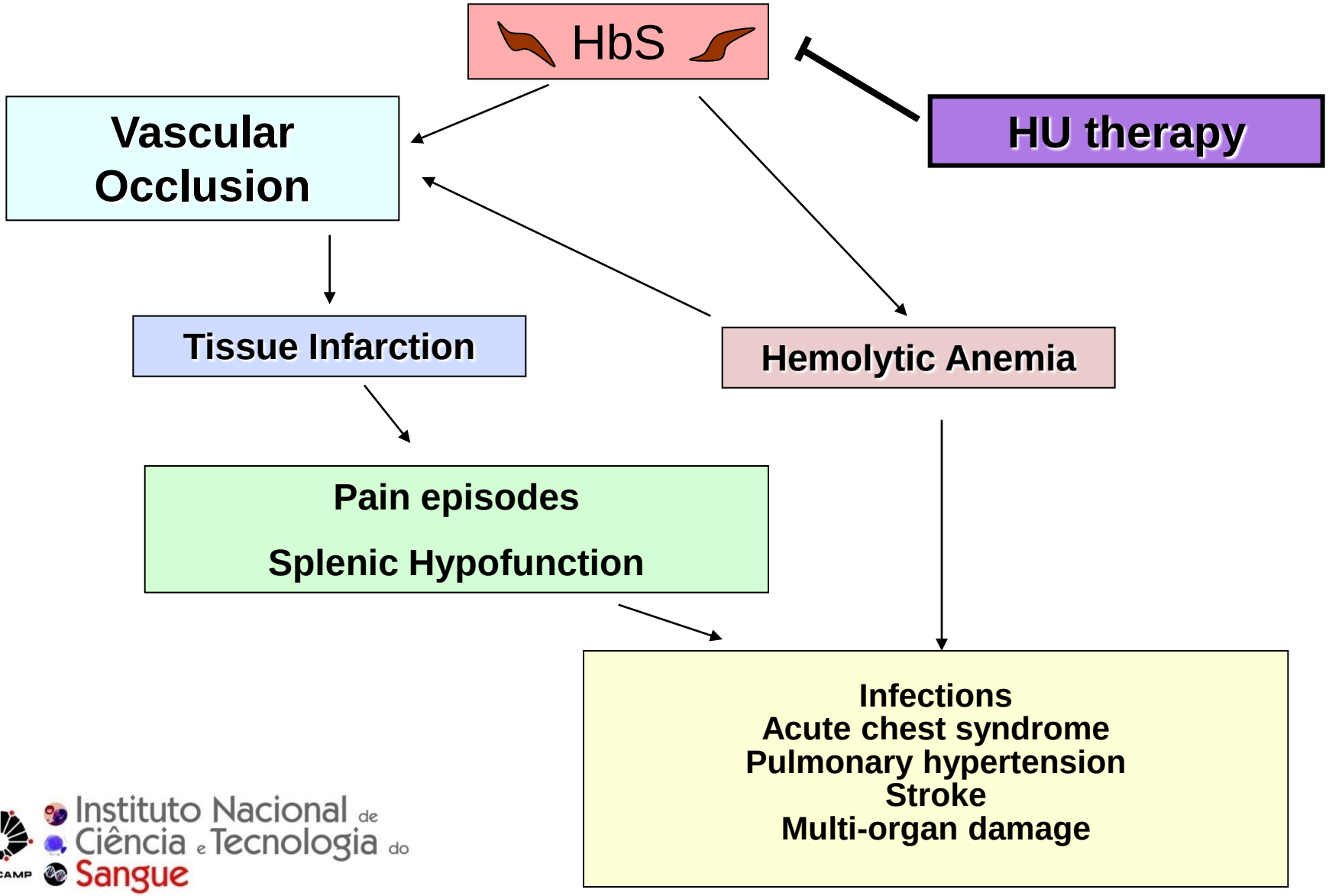

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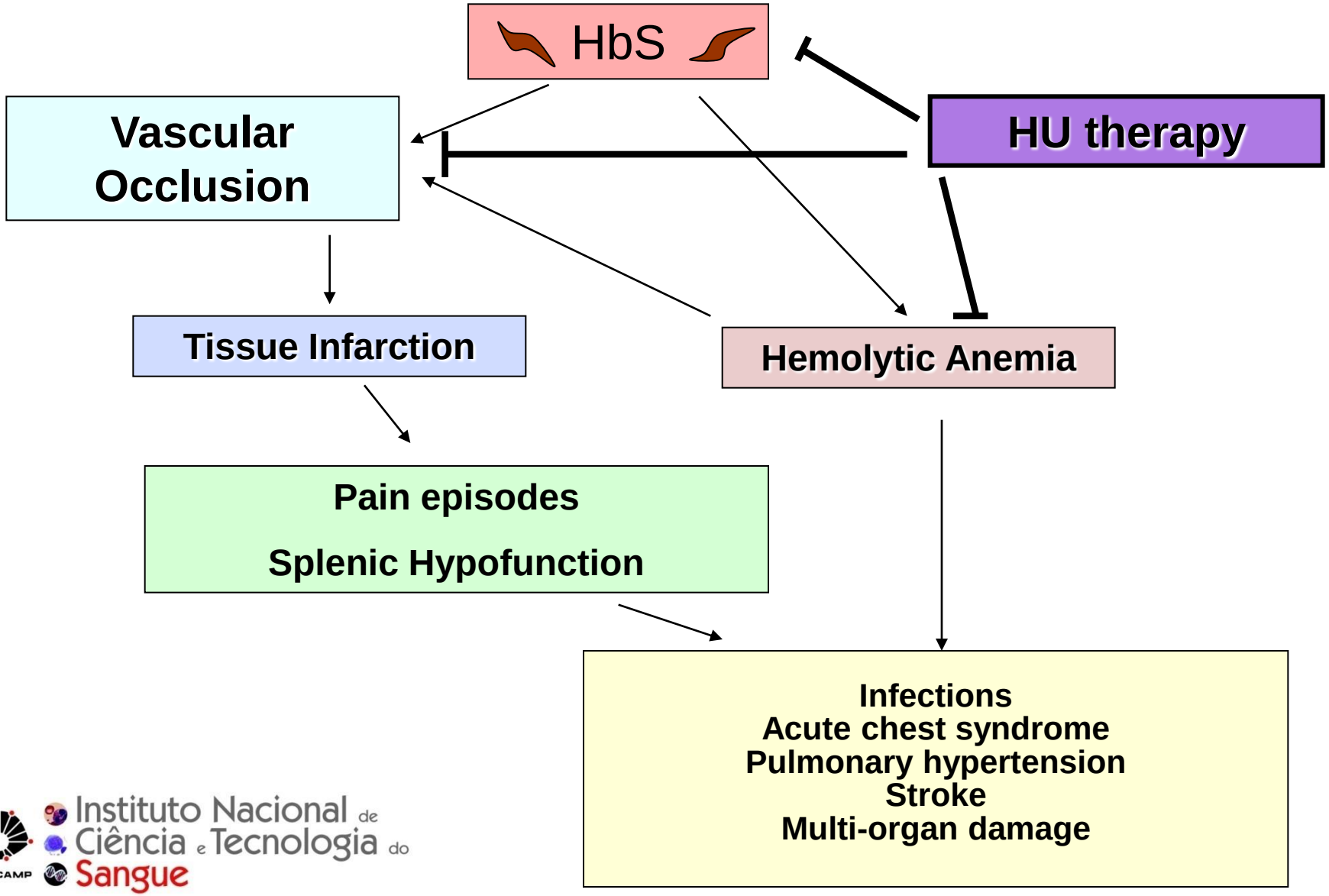
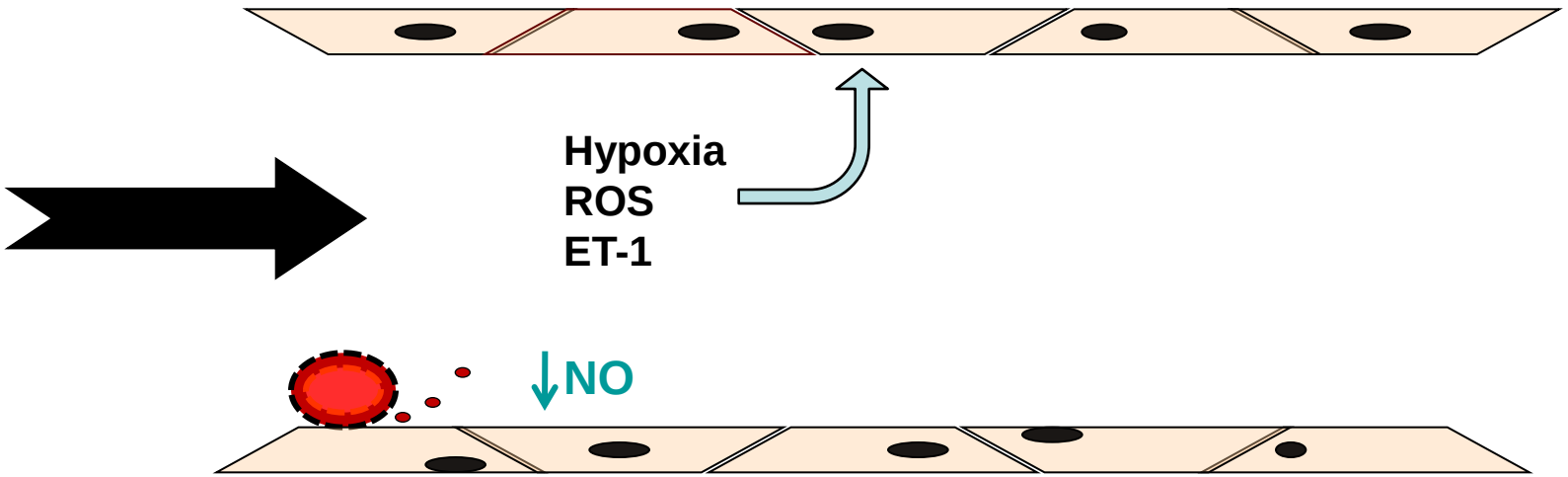


Vaso-occlusion pathophysiology: Role of endothelial activation, vascular inflammation and cell adhesion



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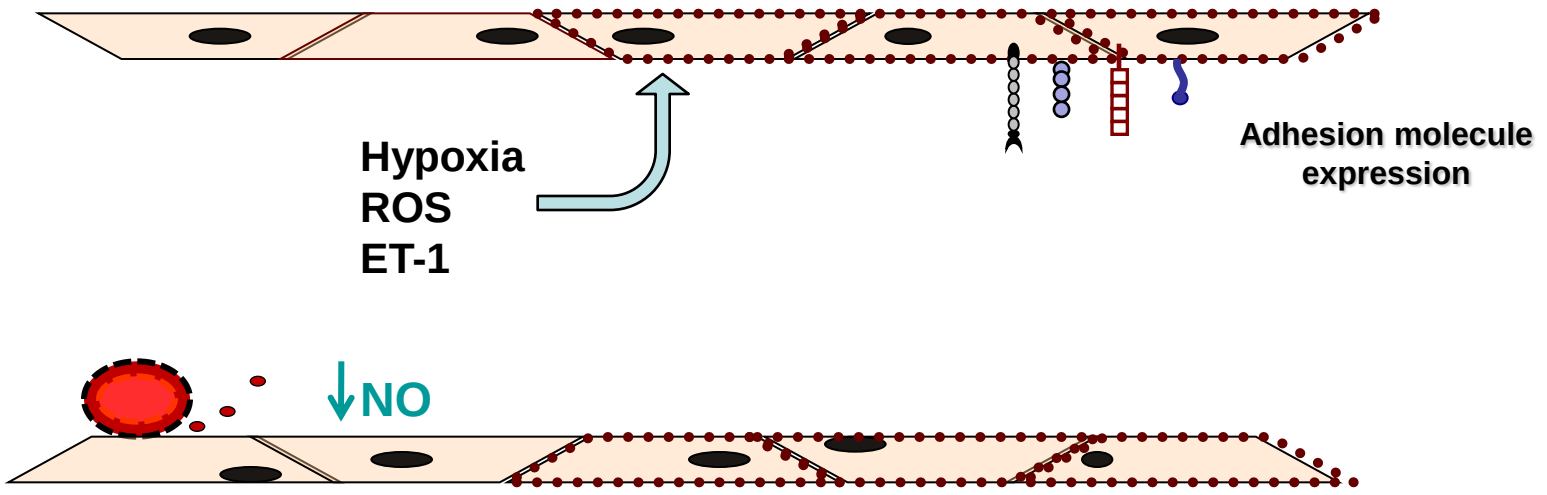

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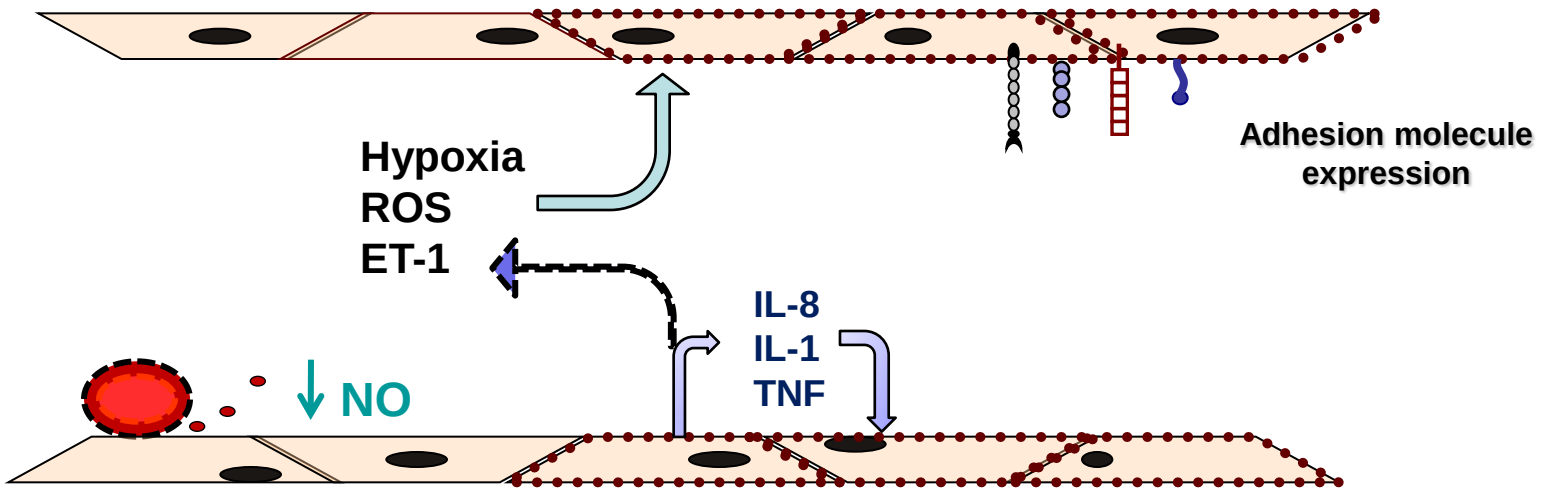

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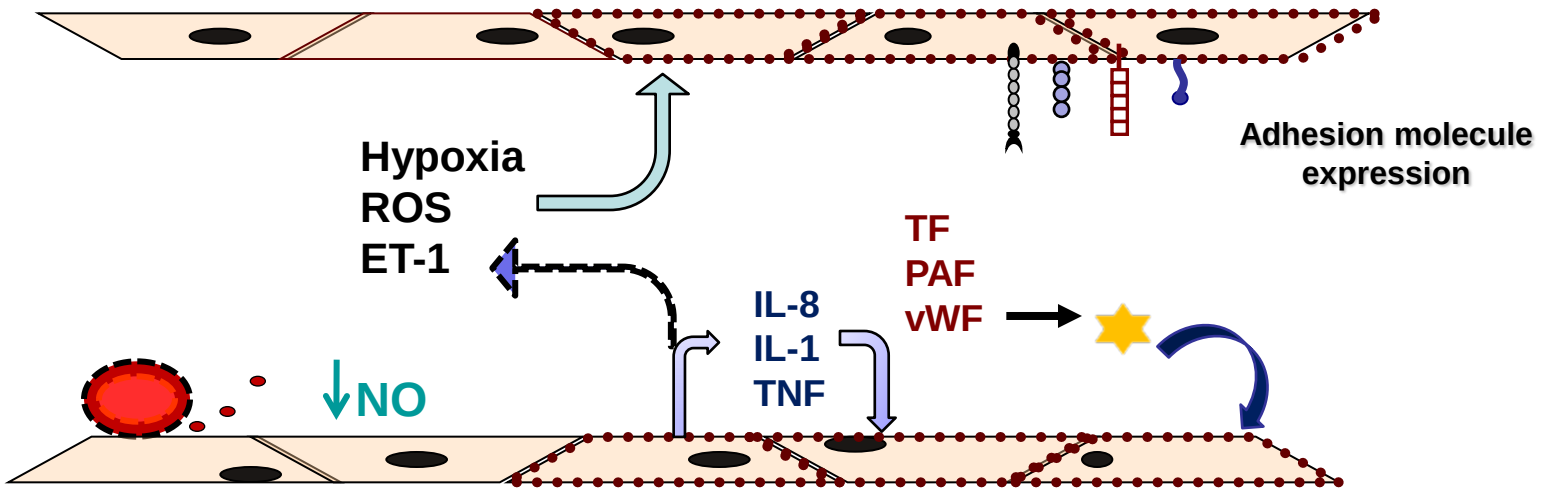

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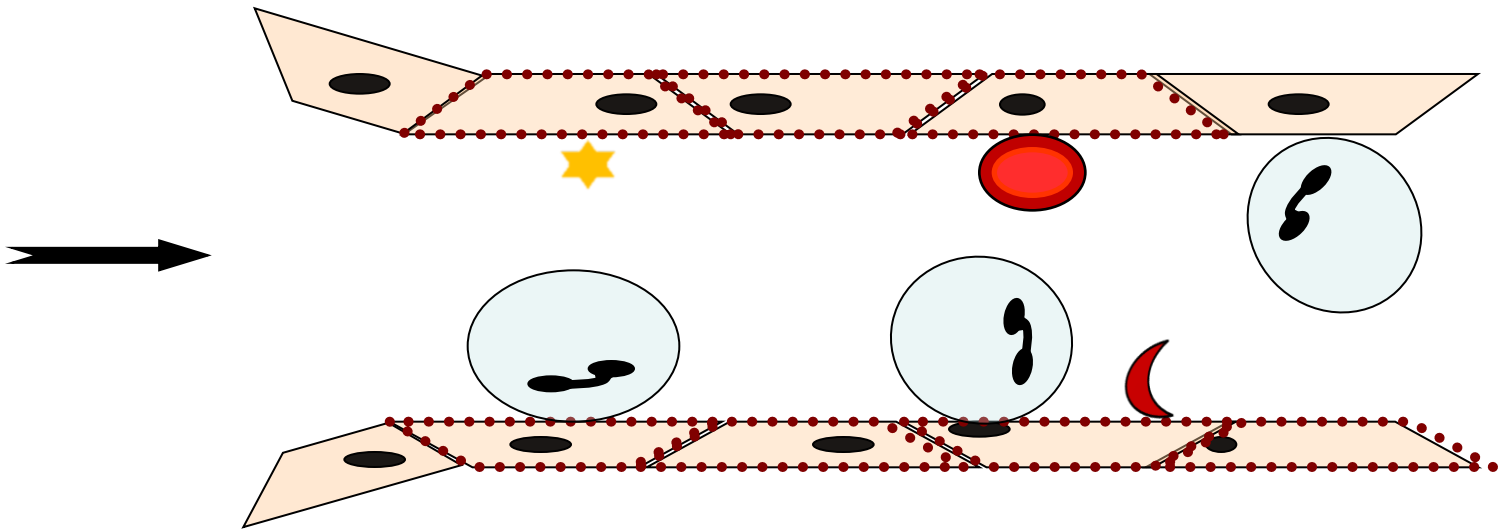

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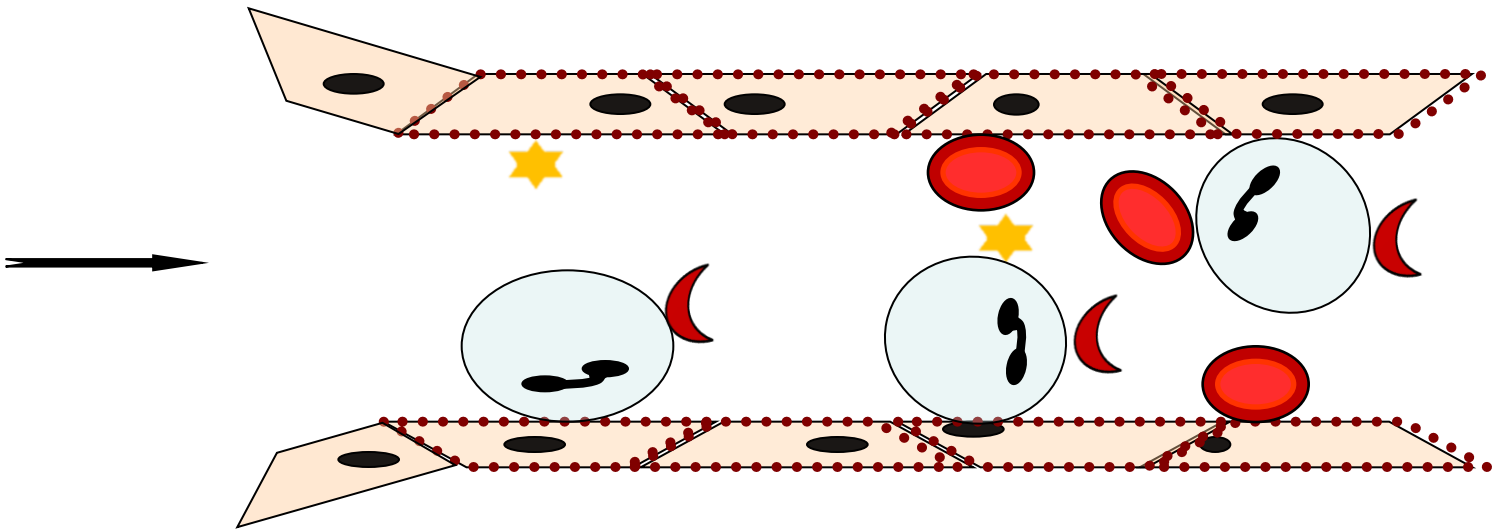

Targeting Vaso-occlusion:

Reduce Hemolysis

Reduce Inflammation

Reduce Endothelial Activation

Reduce Red Cell Adhesion

Reduce Neutrophil Adhesion

Increase NO Bioavailability

Reduce Oxidative Stress

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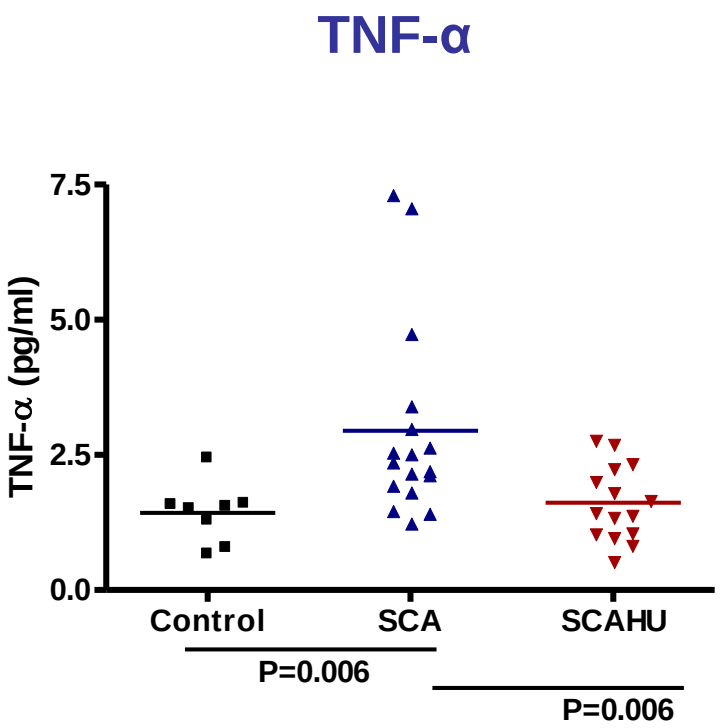
Targeting Vascular Inflammation:

Plasma Inflammatory Proteins in SCD



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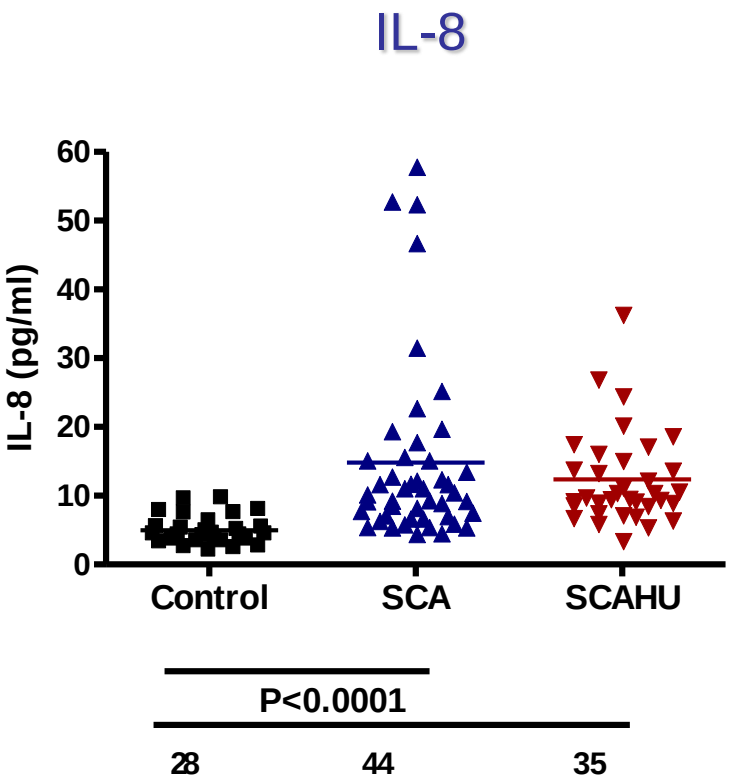
Lanaro et al., 2009

Targeting Vascular Inflammation:

Plasma Inflammatory Proteins in SCD



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Lanaro et al., 2009; Garrido, *in submissão*

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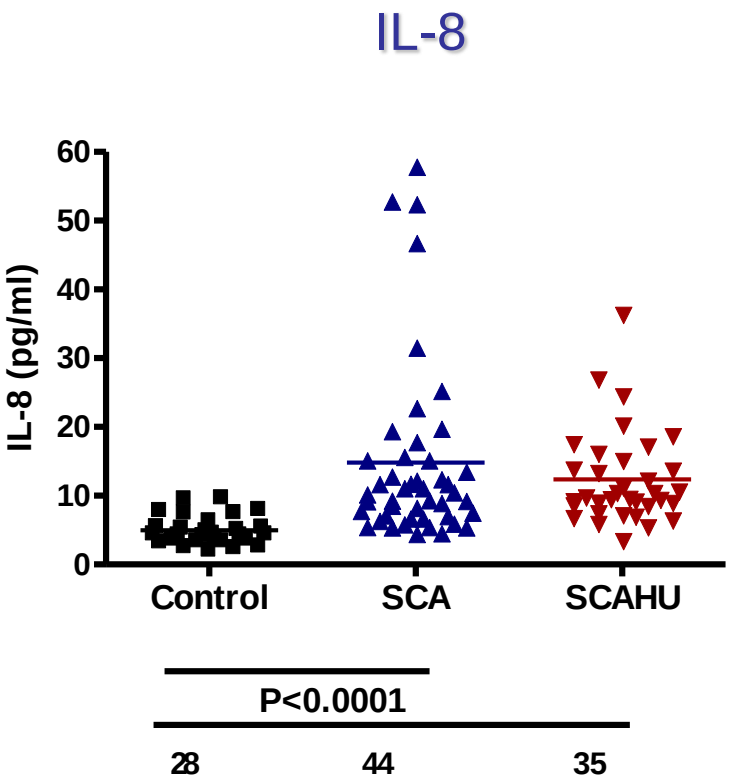

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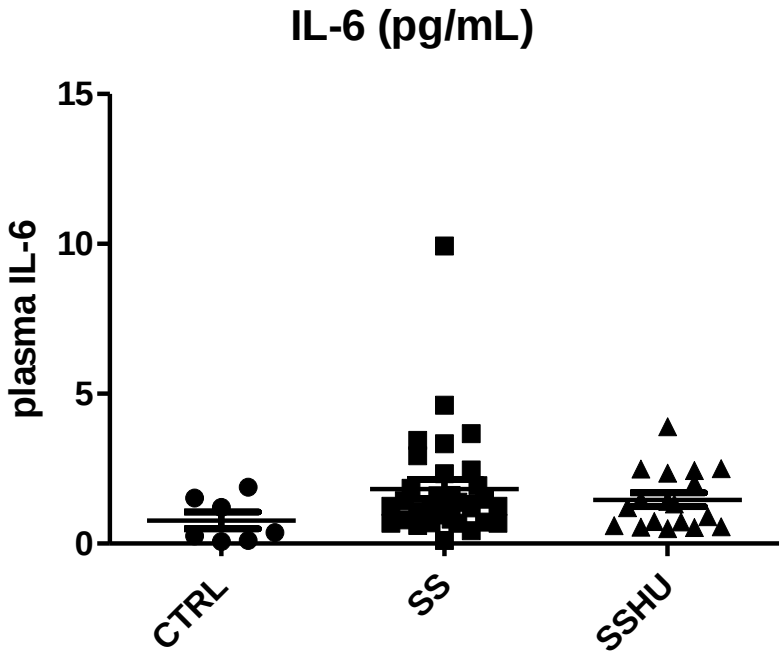

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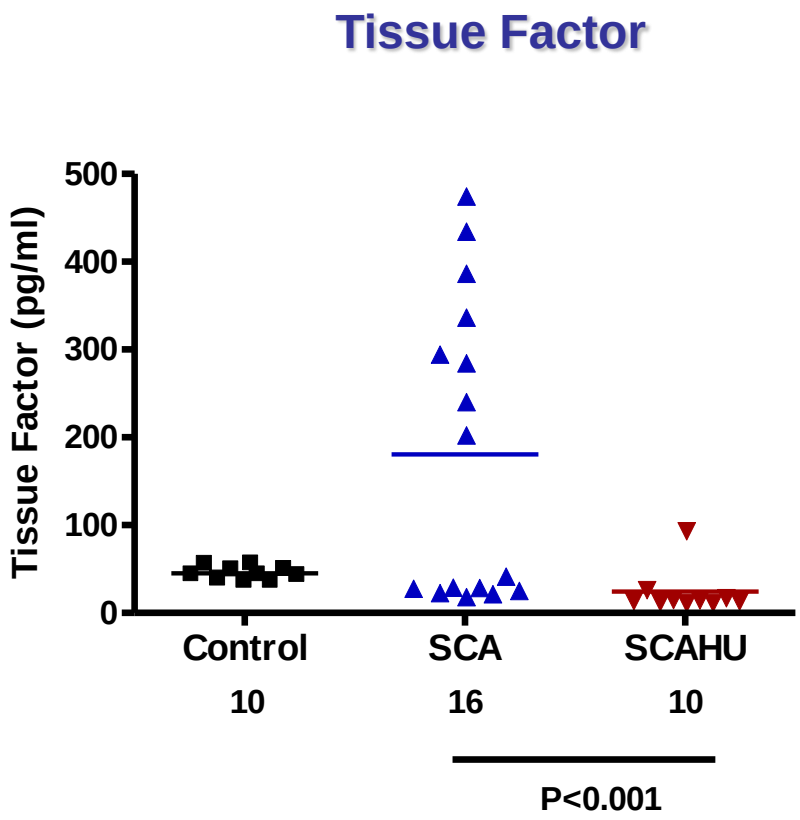
Fertrin, *unpublished*

Targeting Vascular Inflammation:

Pro-coagulant factors in SCD



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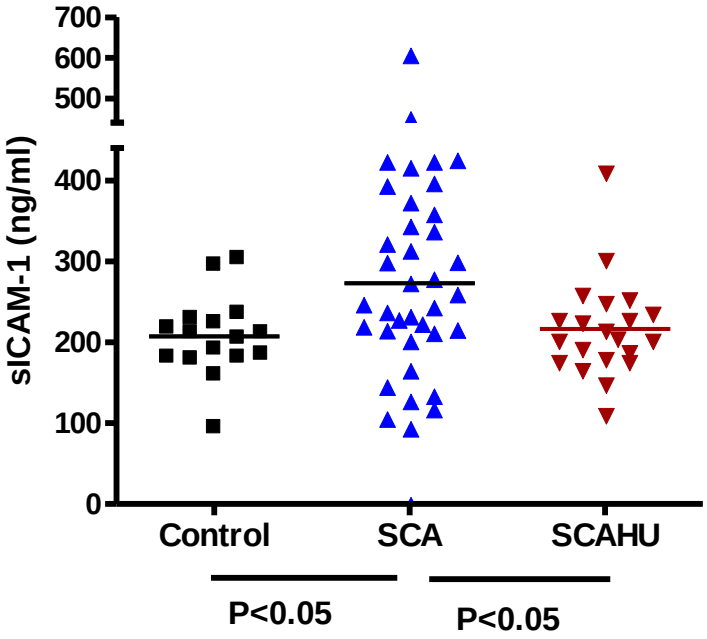
Garrido, *in submission*

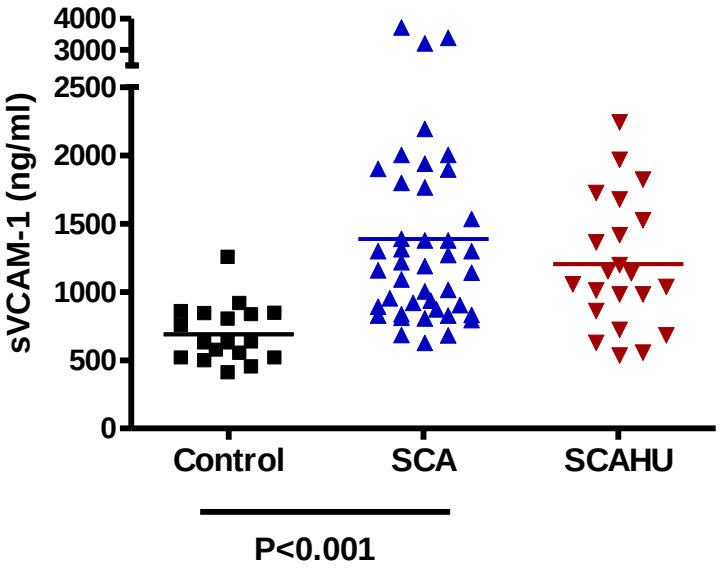
Targeting Vascular Inflammation:

Endothelial Adhesion Molecules

Plasma ICAM-1



Plasma VCAM-1



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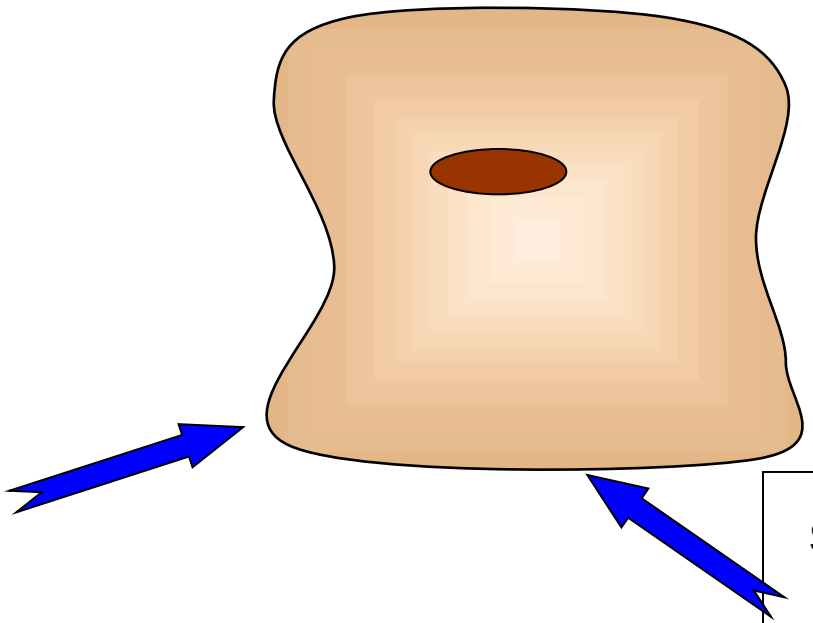
Garrido, *in submission;* Conran, 2004

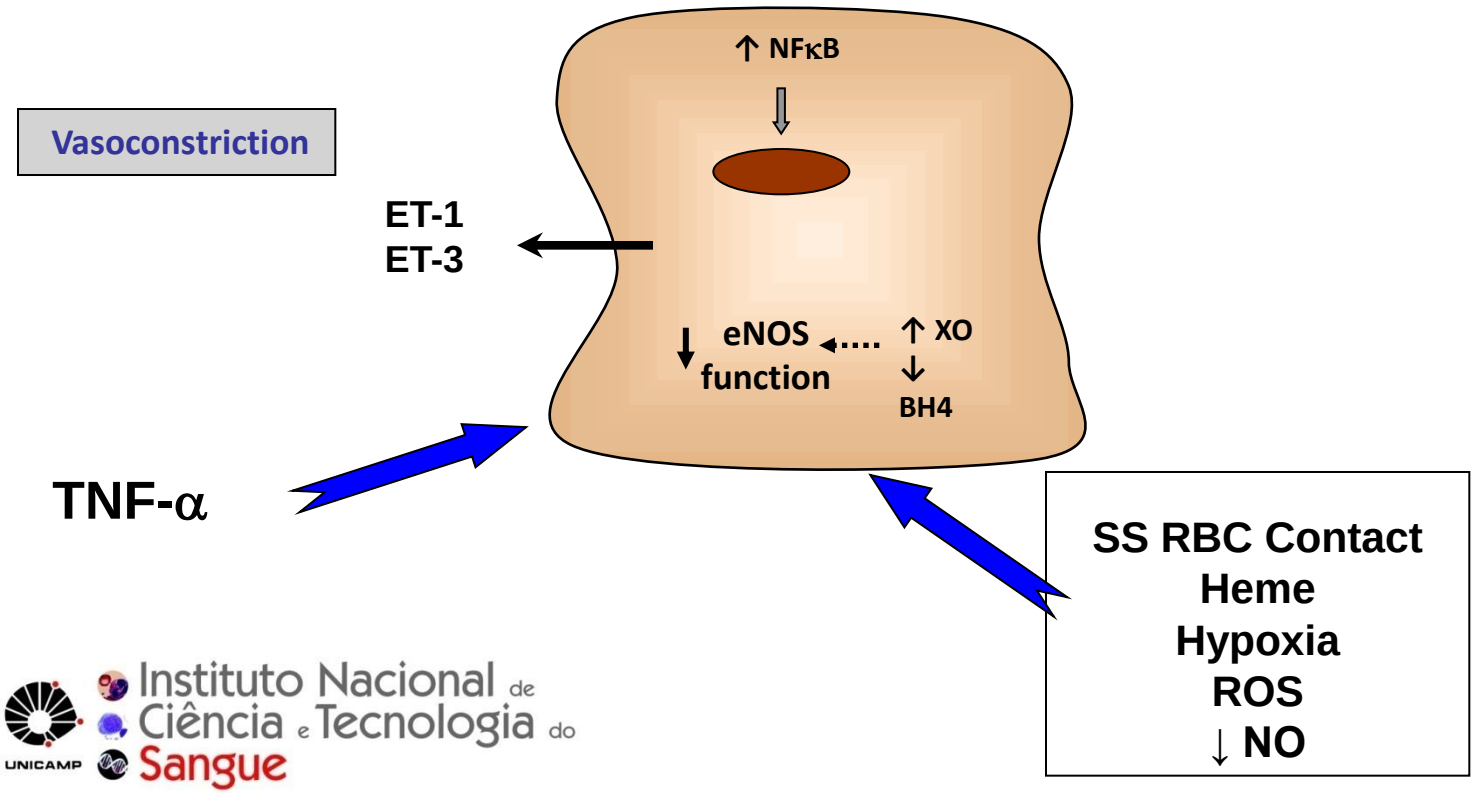
The Endothelium and Vascular Inflammation



TNF-α

SS RBC Contact
Heme
Hypoxia
ROS
↓ NO

The Endothelium and Vascular Inflammation



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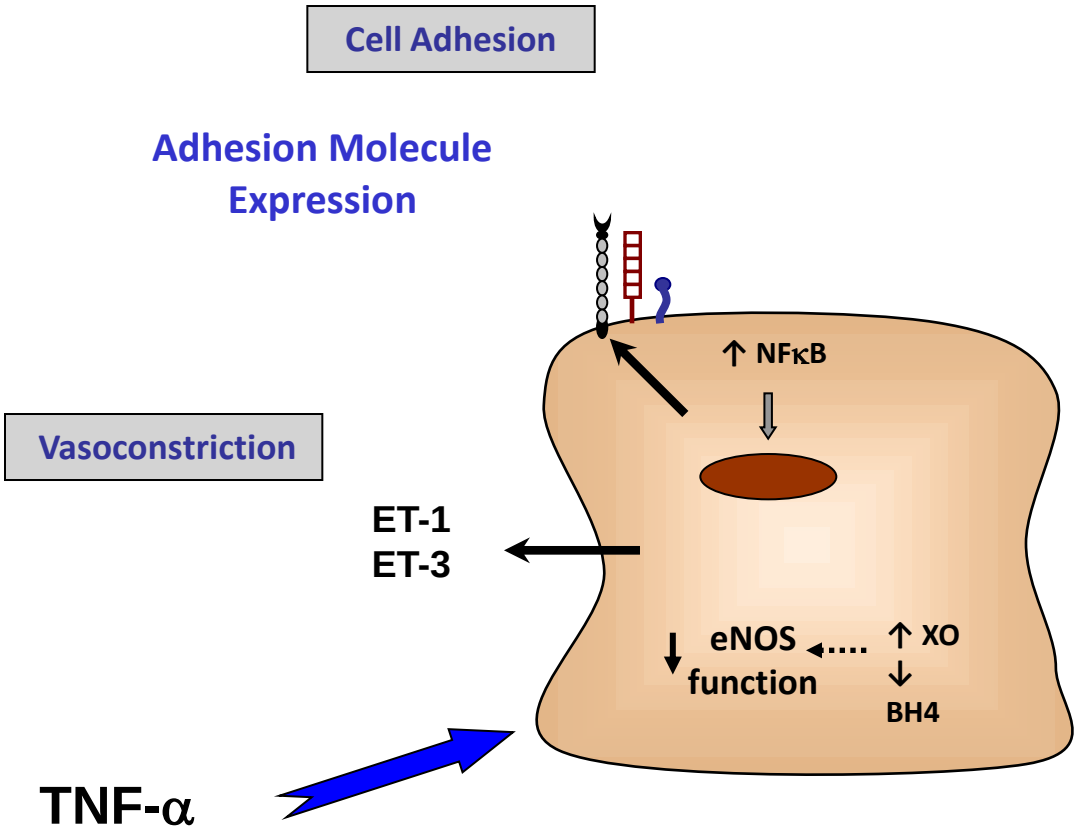

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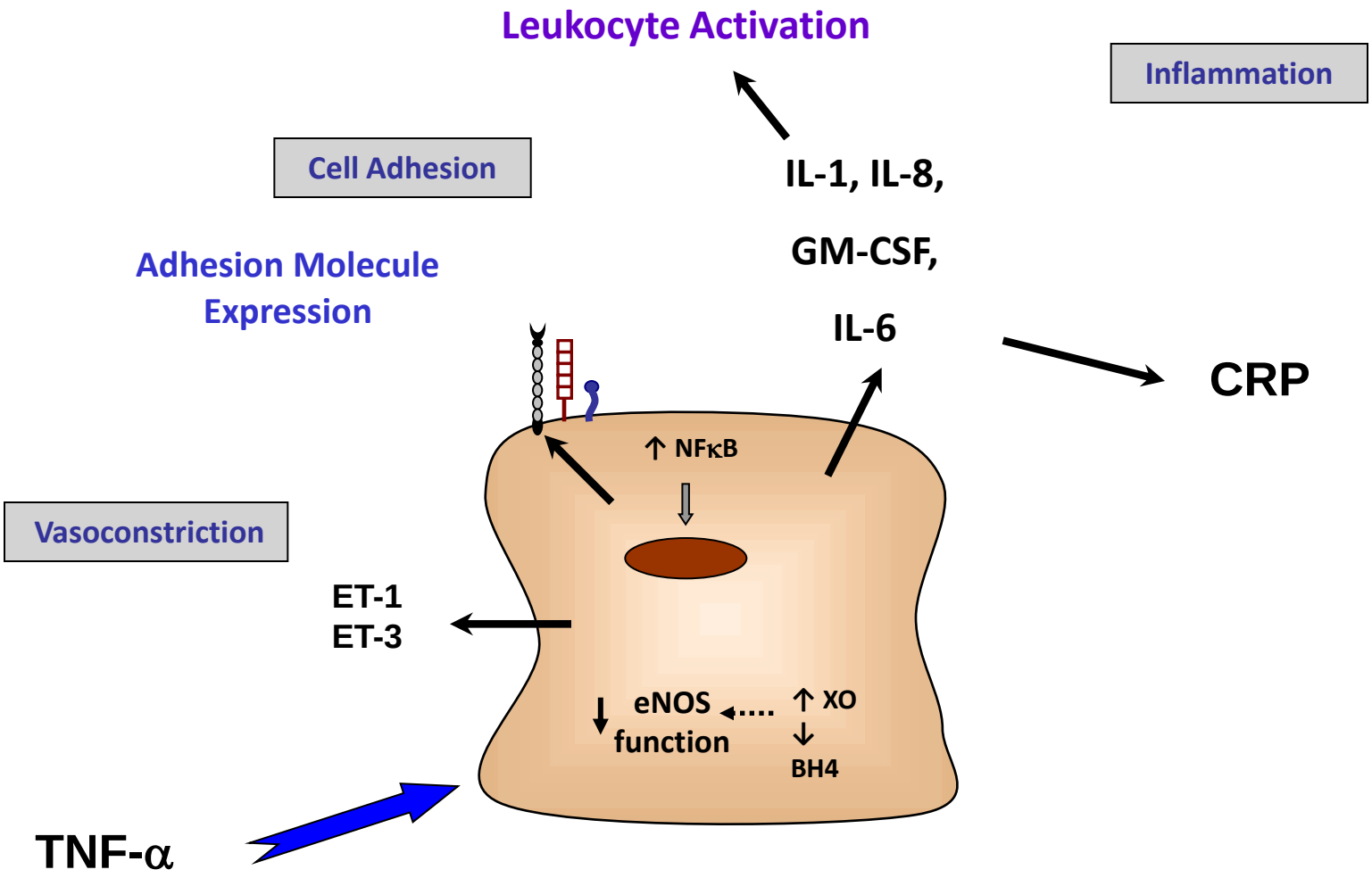

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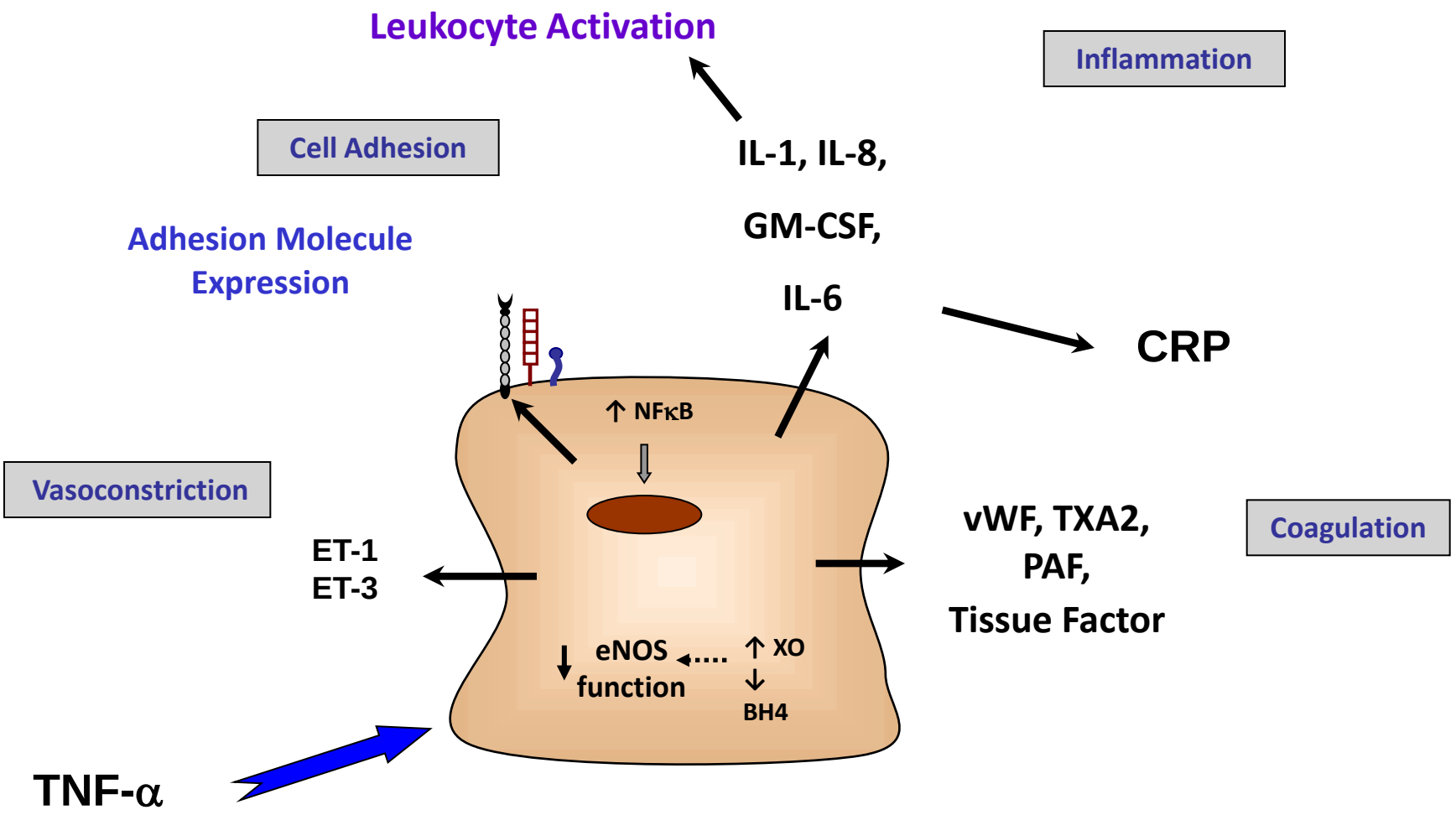

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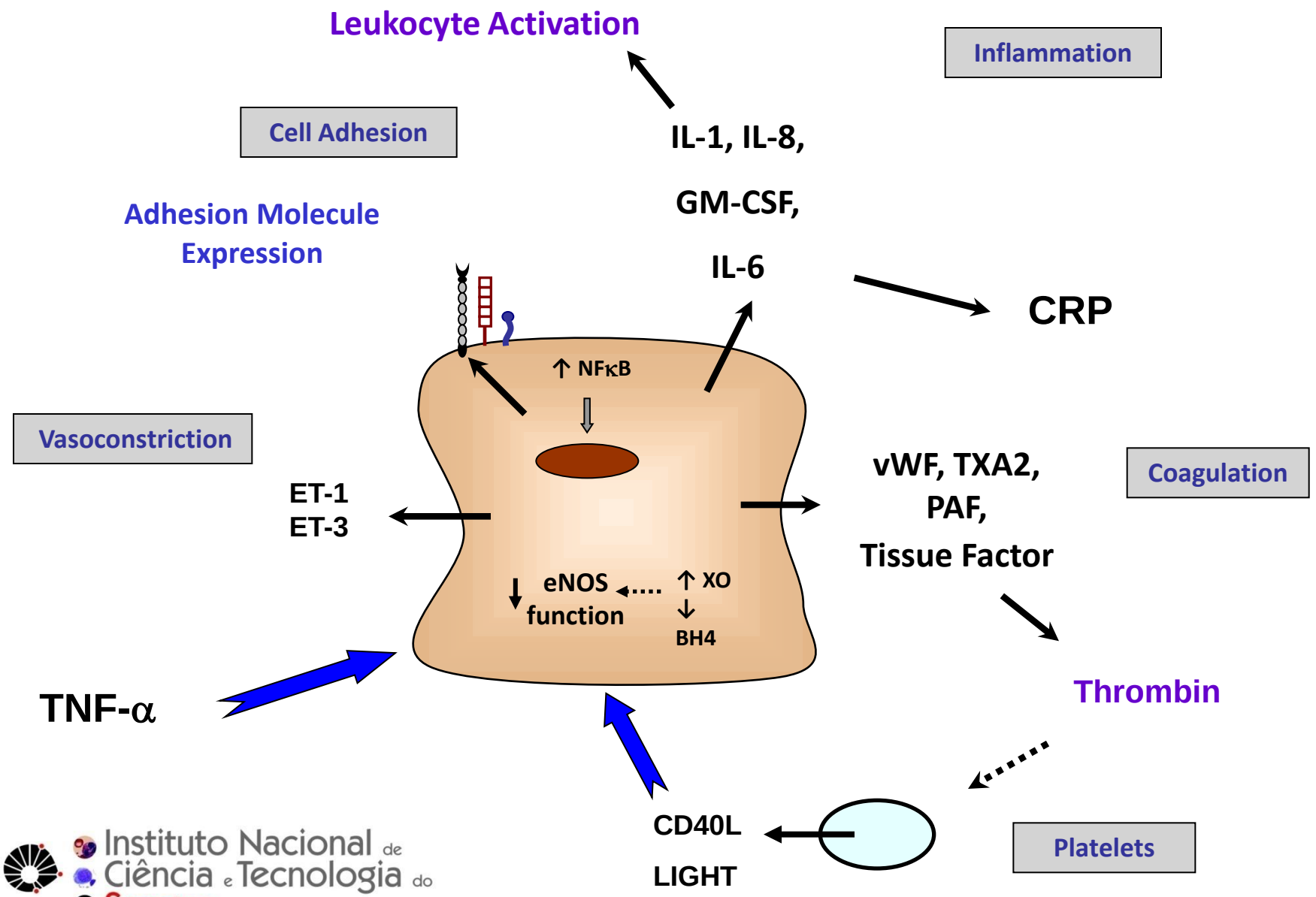

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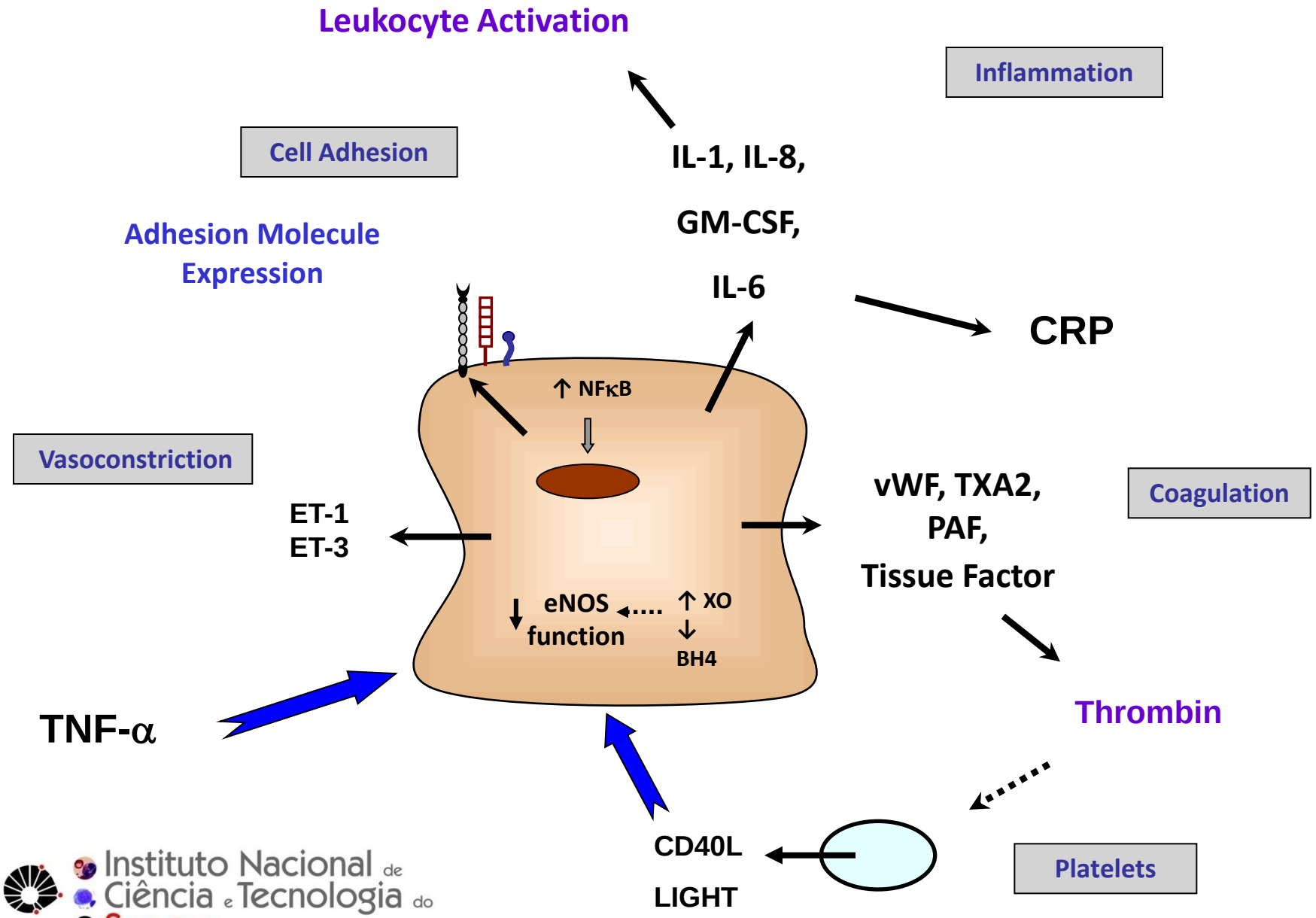

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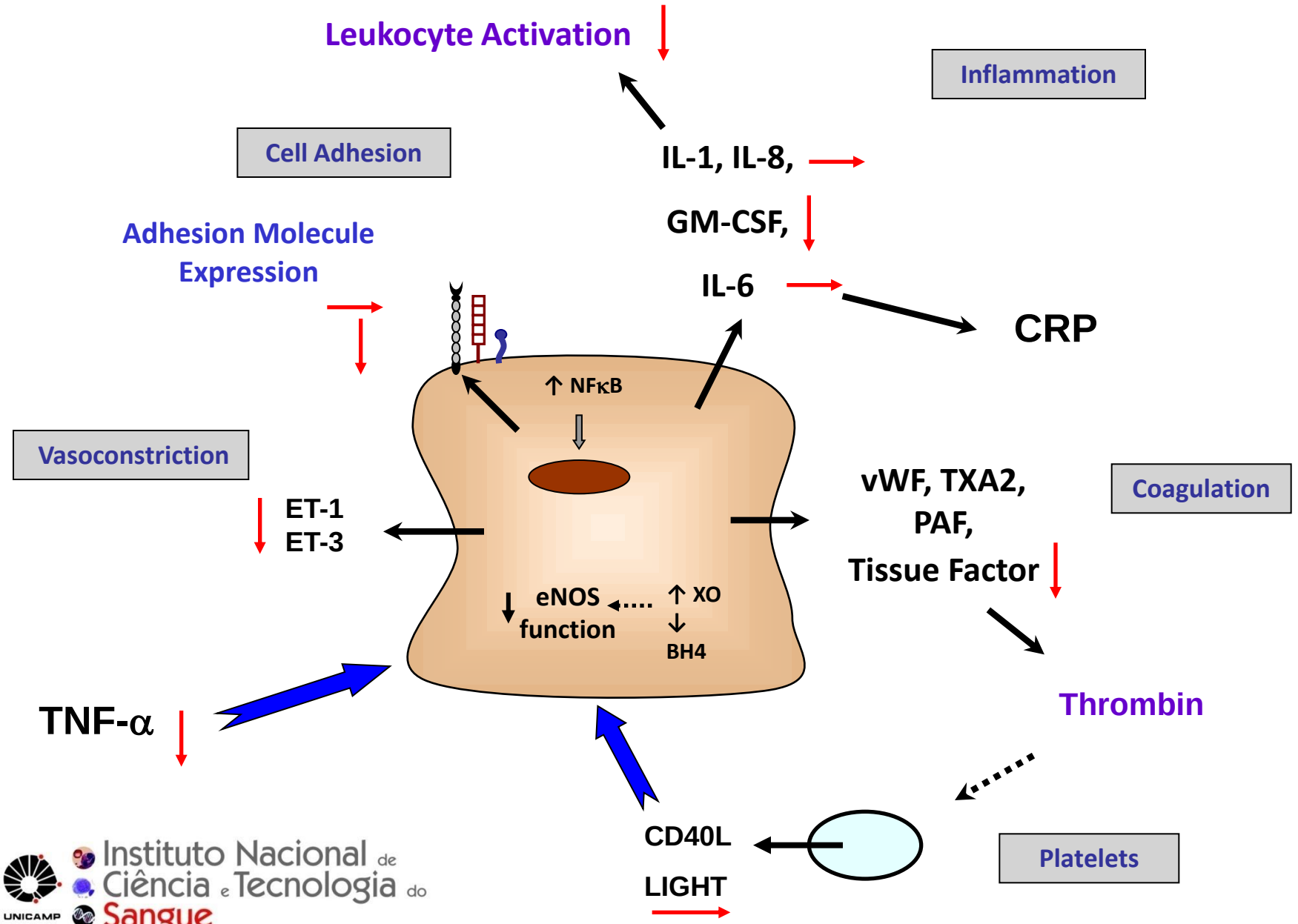
Hydroxyurea Therapy and Vascular Inflammation



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Targeting Vaso-occlusion:

Reduce Hemolysis

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Reduce Endothelial Activation

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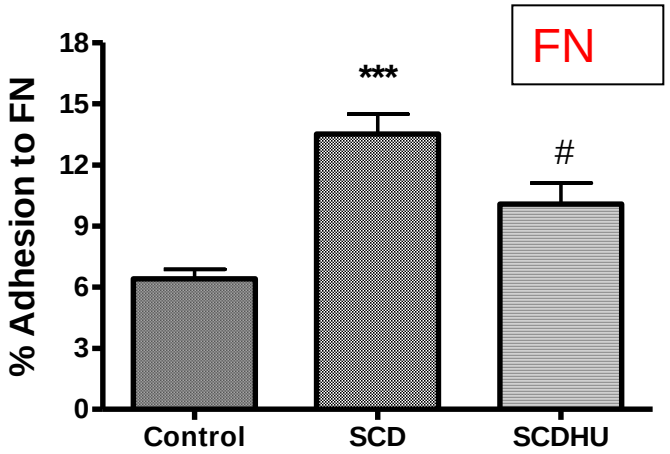
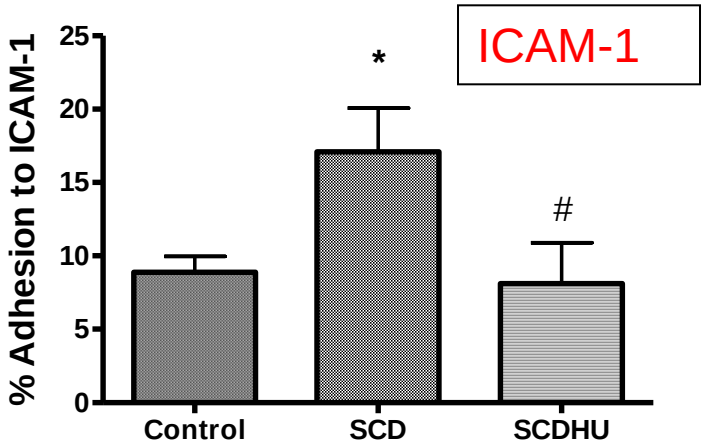
Reduce Neutrophil Adhesion

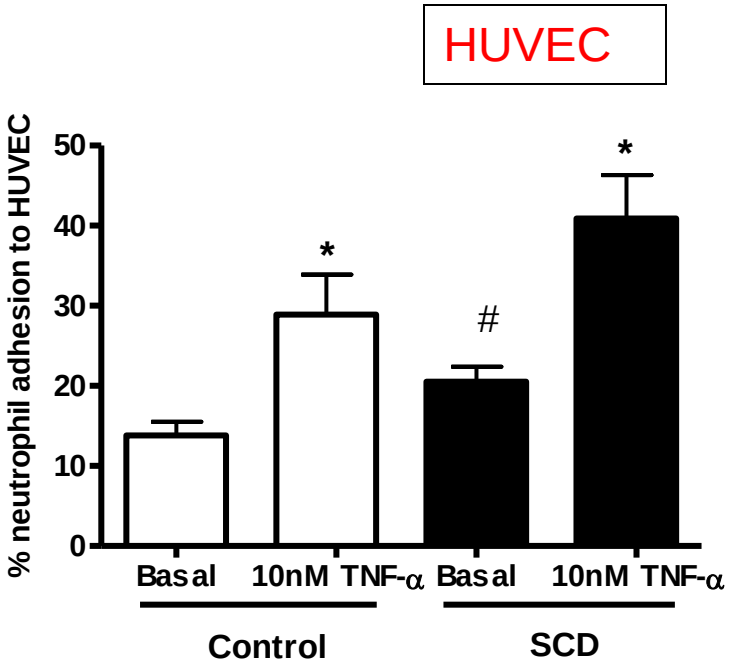
Increase NO Bioavailability and cGMP signalling

Reduce Oxidative Stress

SCA Neutrophil adhesion properties are increased *in vitro*







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Canalli et al., 2008; 2011

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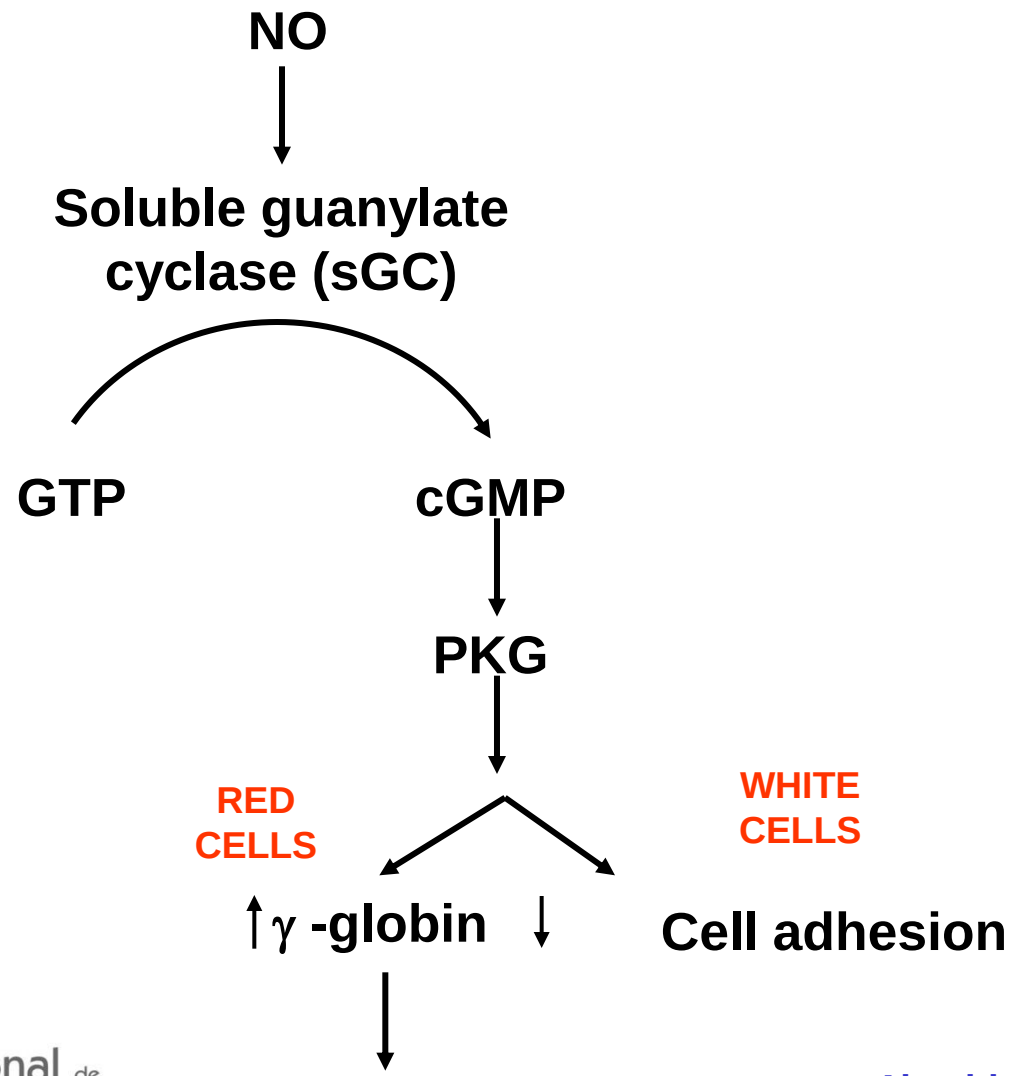
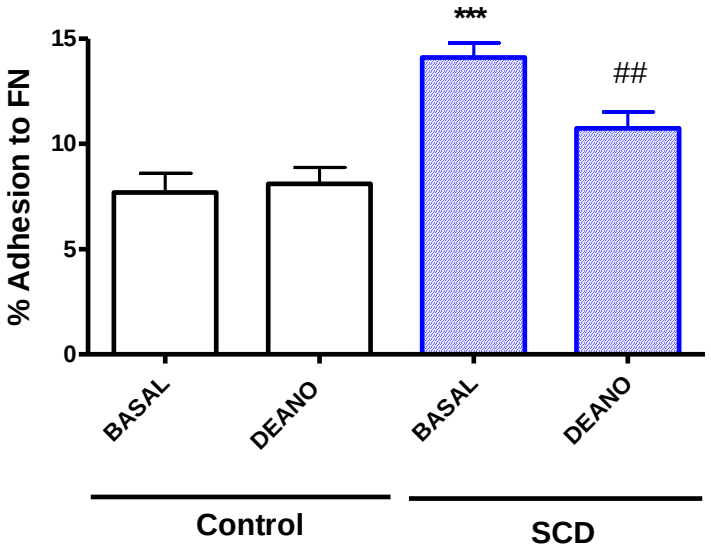
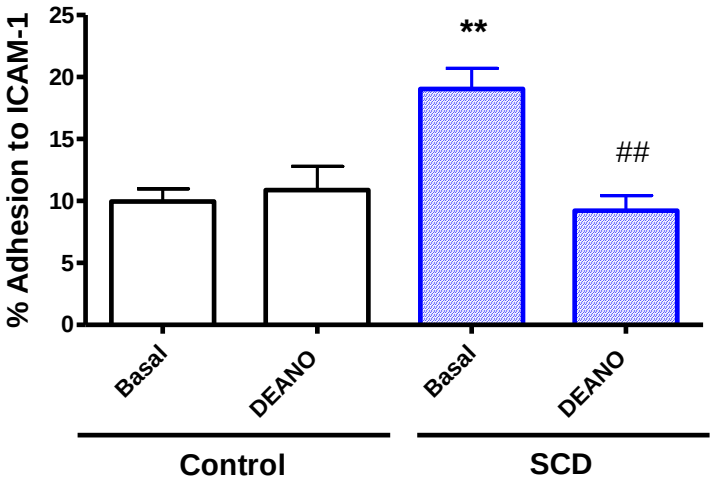
NO-cGMP pathway in SCD



Almeida *et al*., 2008; Cokic et al., 2003; Ikuta et al., 2001

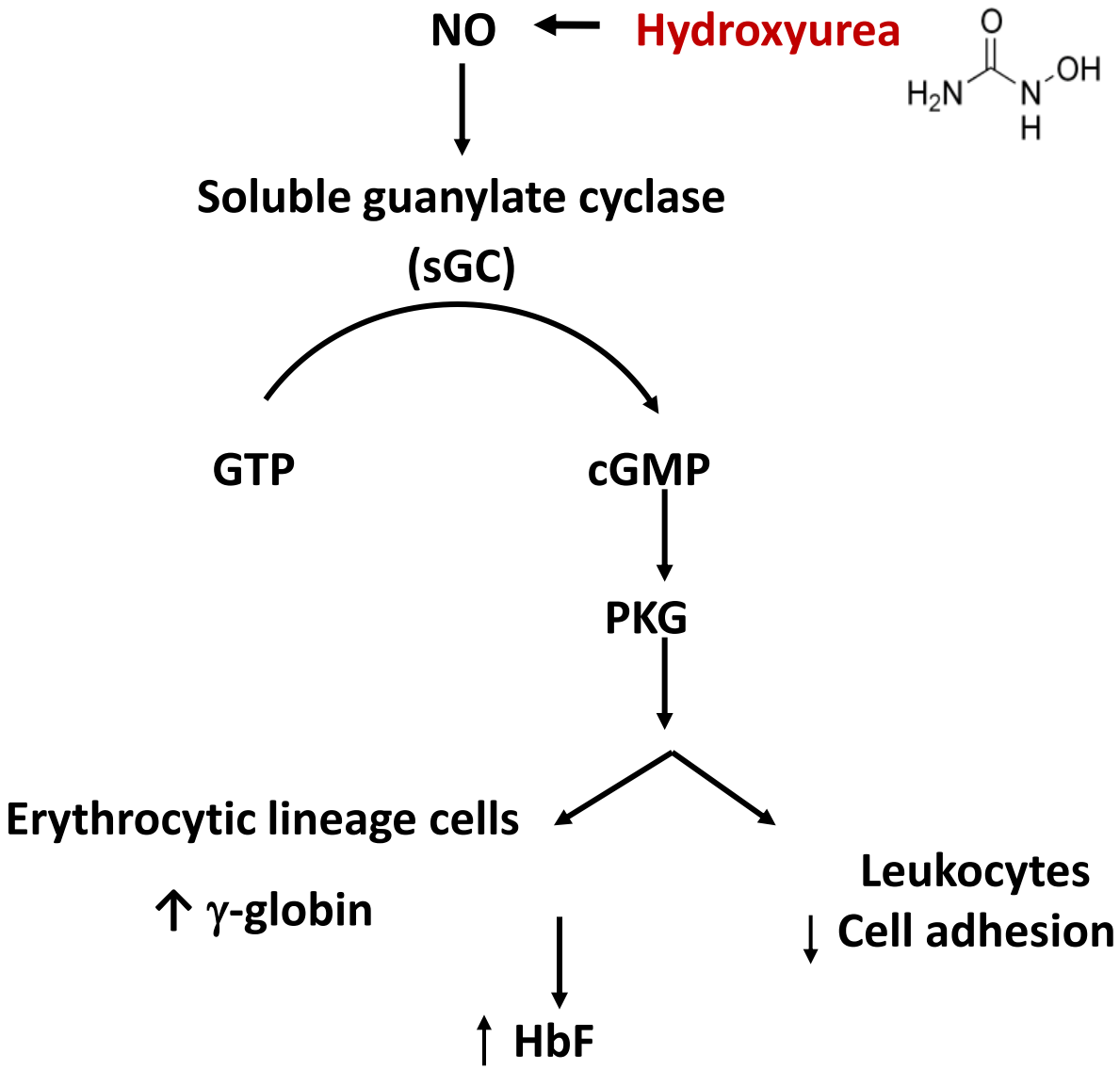
NO donors reduce SCD neutrophil adhesive properties *in vitro*





Canalli et al., 2008

The cGMP-dependent pathway as a potential therapeutic target for SCA vaso-occlusion



King, 2004

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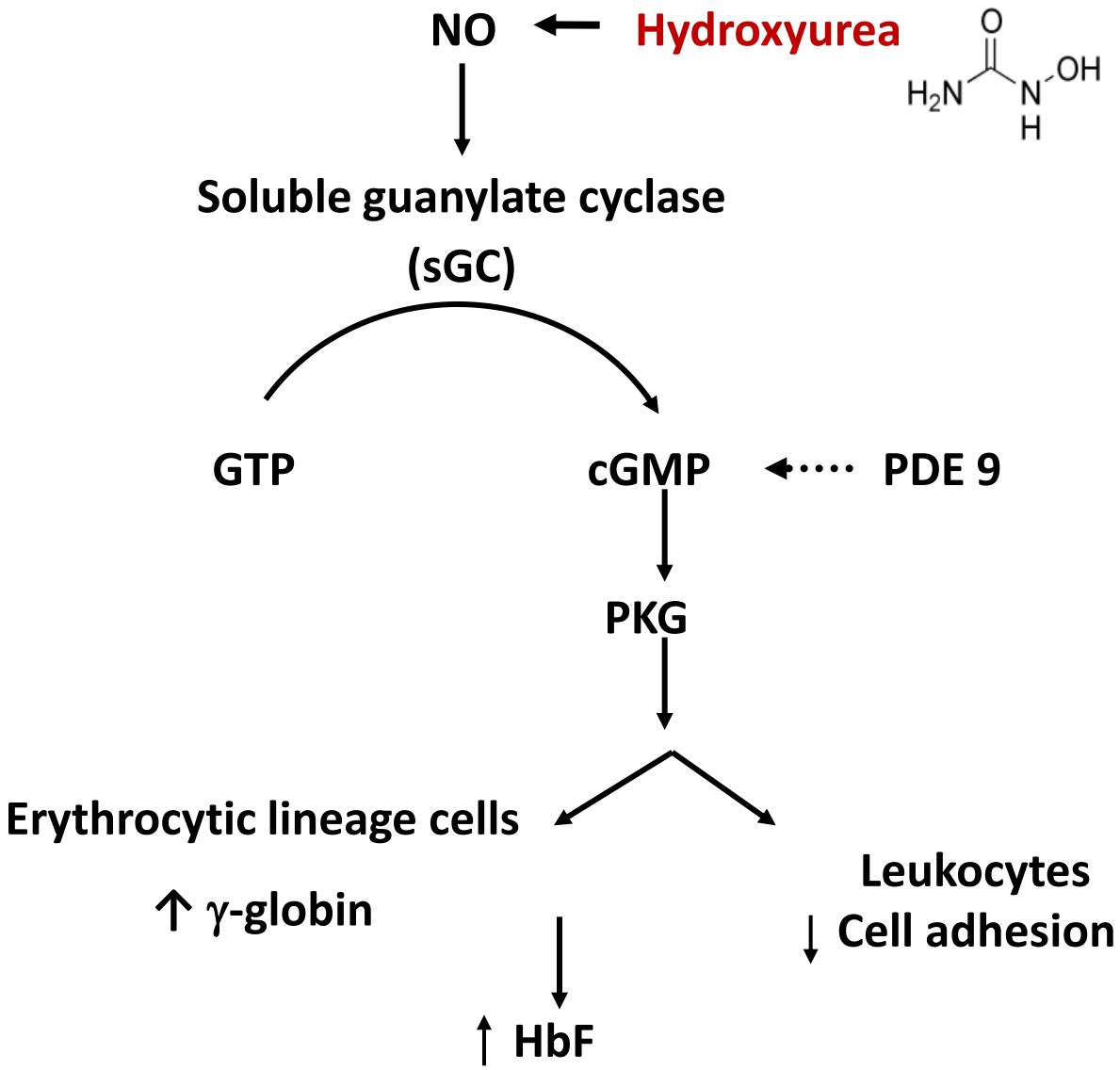

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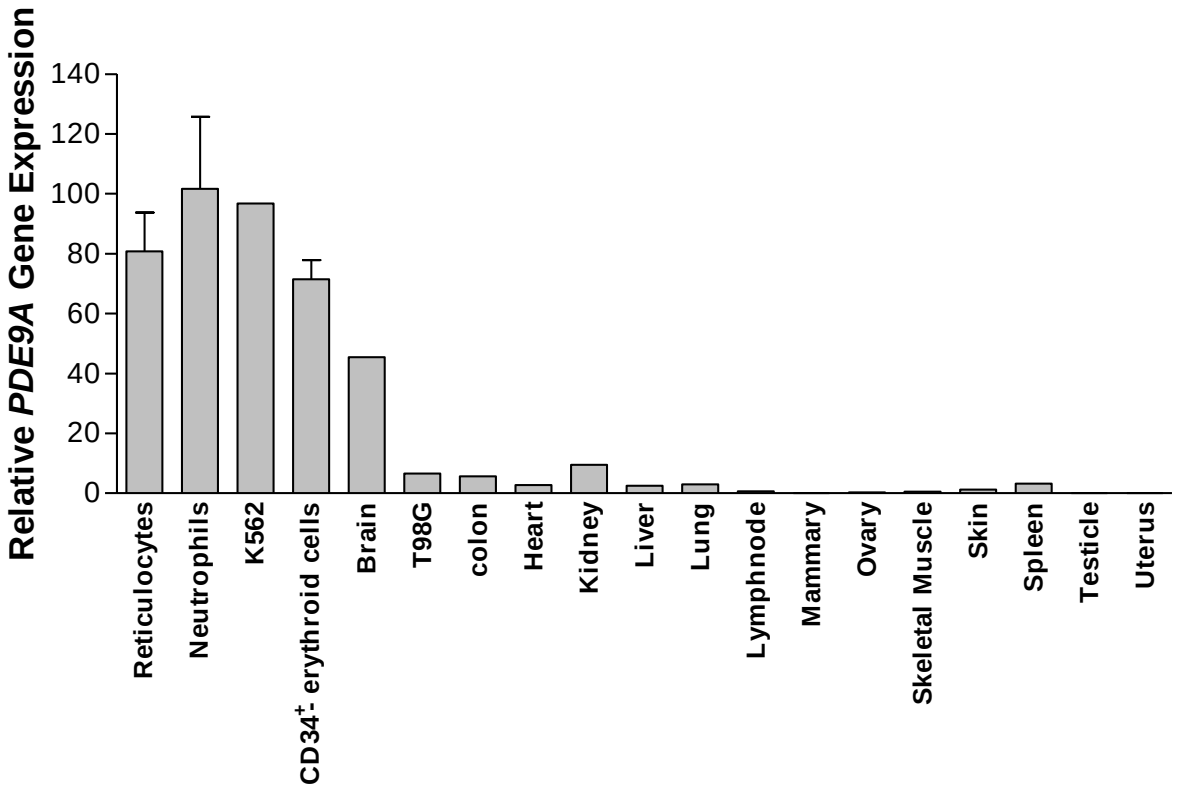

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Almeida et al., 2008

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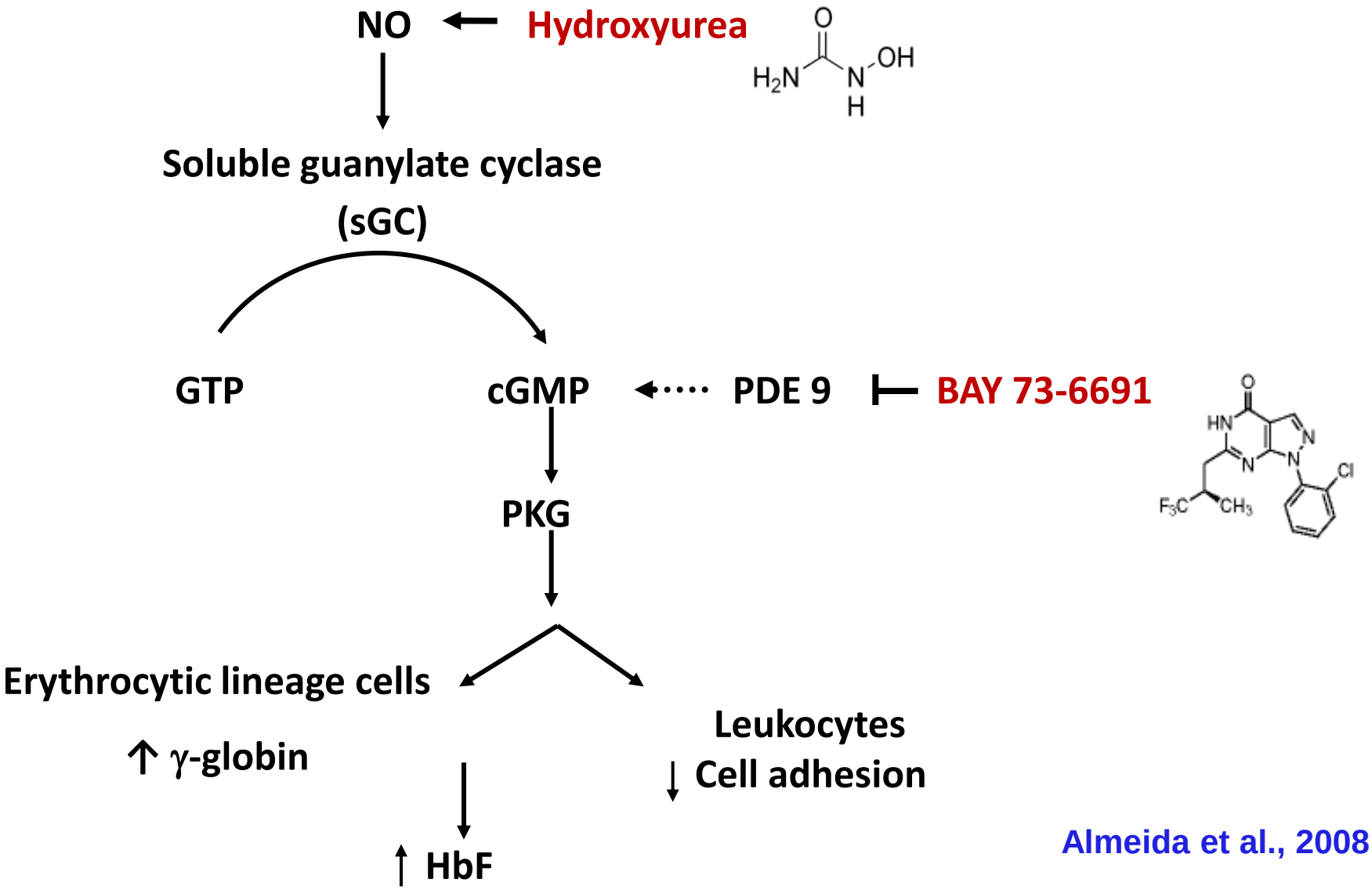
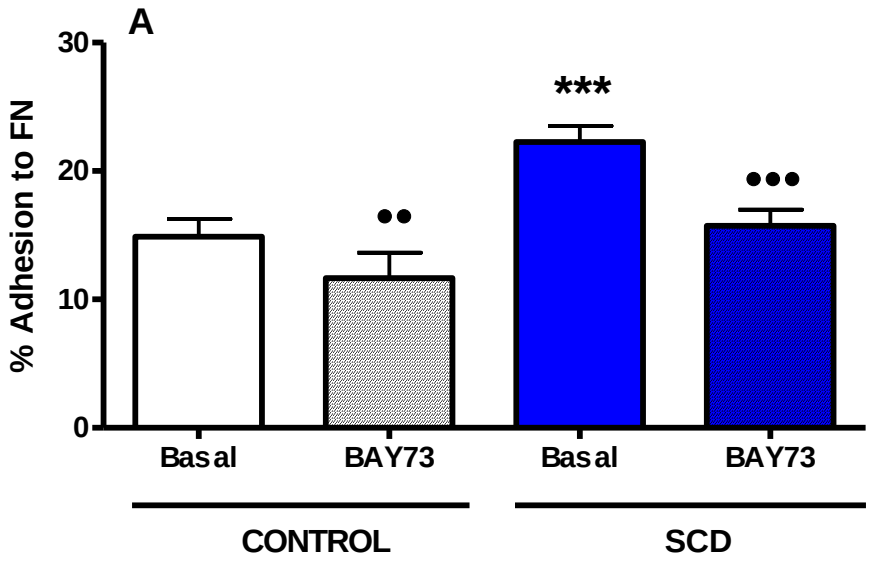


Almeida et al., 2008

Inhibition of PDE9 decreases neutrophil adhesion to FN, *in vitro*



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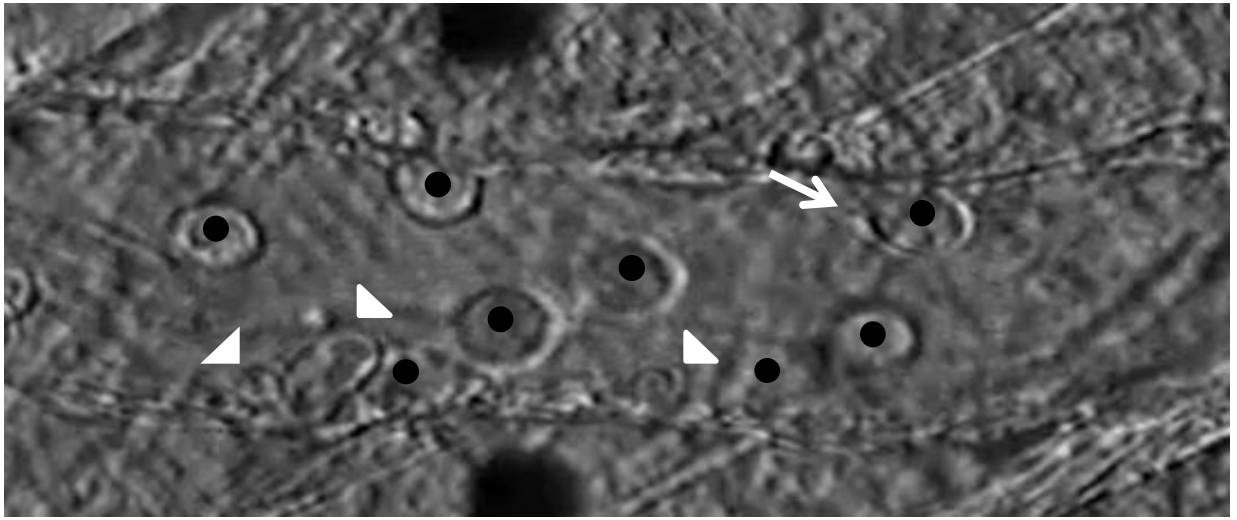
Miguel et al., 2011

Sickle Cell Mouse Inflammatory Vaso-occlusive Model

Blood flow



3h post -TNF-α

Cremaster Muscle

Measure: leukocyte Adhesion, rolling and extravasation

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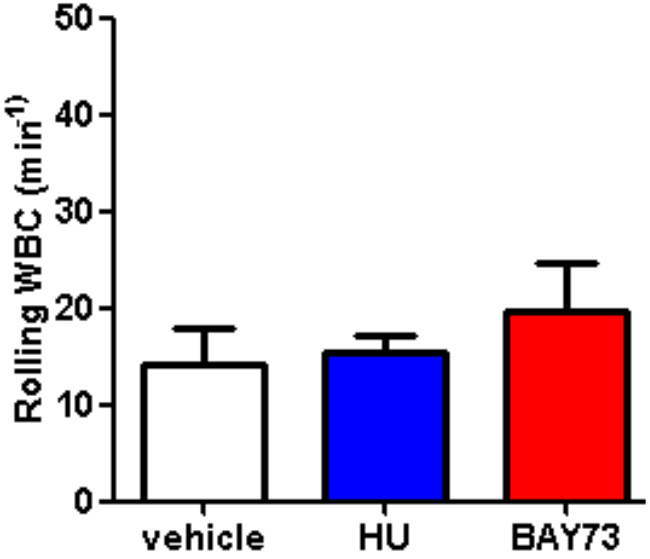
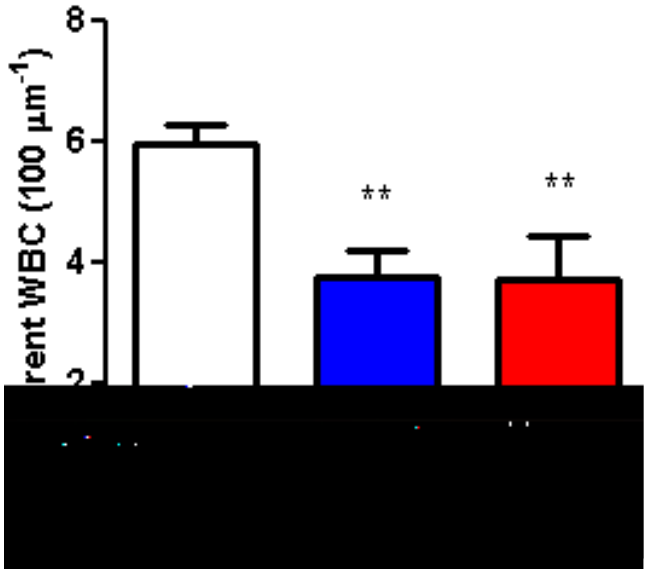
Albert Einstein College of Medicine, NY

Effects of HU and BAY73-6691 on leukocyte recruitment in TNF-α-treated SCD mice





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Albert Einstein College of Medicine, NY

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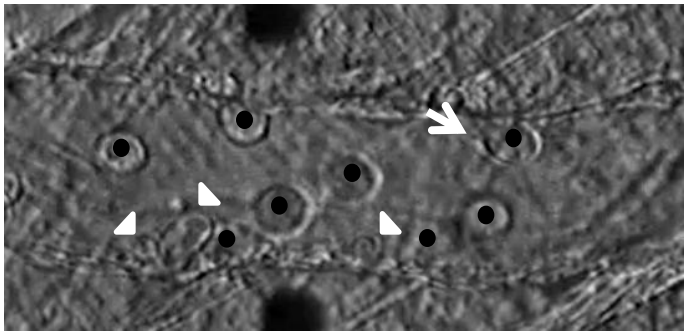
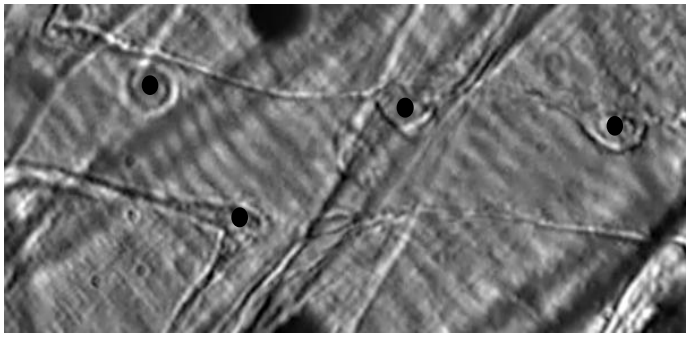
Vehicle



BAY73-6691

HU



HU + BAY73-6691

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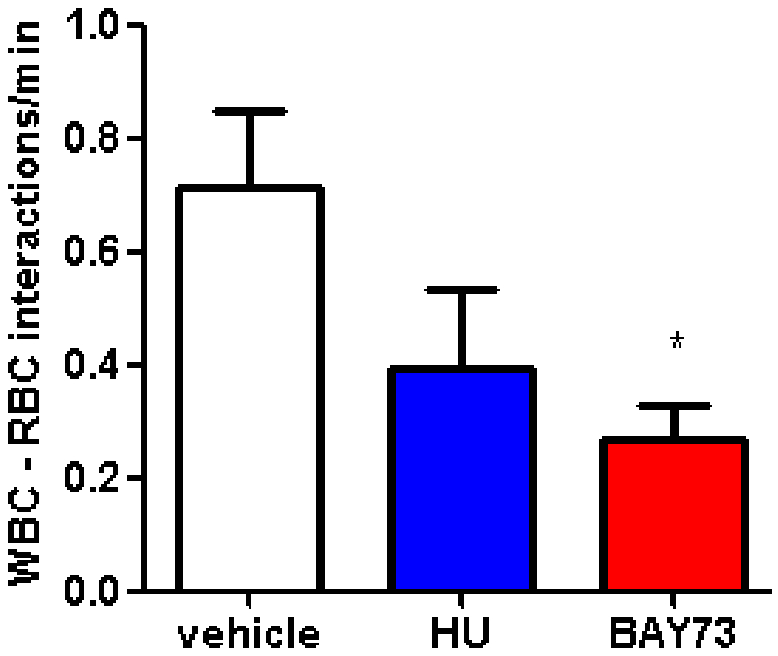
Albert Einstein College of Medicine, NY

10μm

Blood flow

BAY73-6691 decreases WBC-RBC interactions



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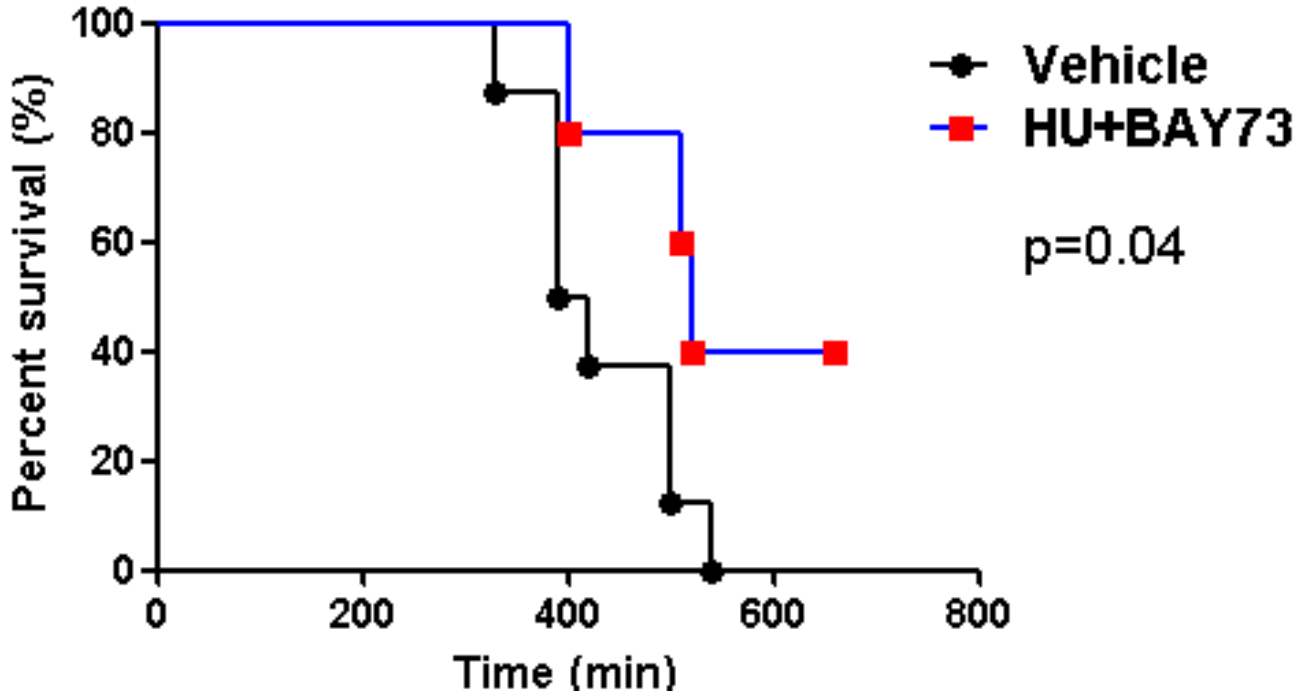
Co-administration of HU and BAY73-6691 improves SCD mouse survival in the setting of vaso-occlusion



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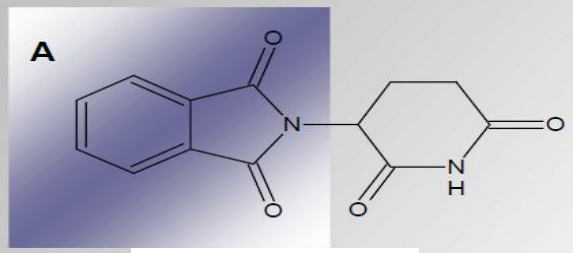
NOVEL HYBRID OF HYDROXYUREA AND THALIDOMIDE BASED PHARMACOPHORES INDUCE FETAL HEMOGLOBIN AND BLOCK MONOCYTE ACTIVATION

Thalidomide

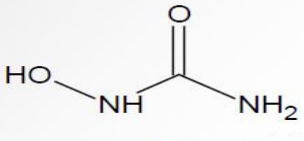
✓ Thalidomide and its recently developed IMiD (immunoodulatory derivates of thalidomide) derivatives (such as pomalidomide and lenalidomide) potently inhibit cytokine release from activated monocytes and suppress adhesion molecule expression on vascular endothelium;

✓ These properties are critically linked to the phthalimide ring in the thalidomide molecule.

Molecular Hybridization



Thalidomide



1

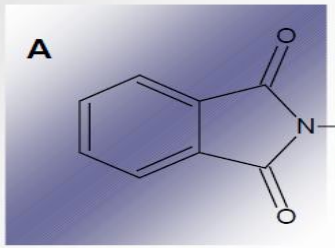
Hydroxyurea

Oxidação
3 e⁻

Molecular Hybridization

A + B

Nitric oxide

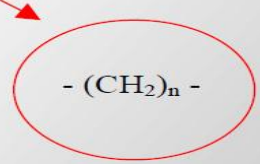


Spacer

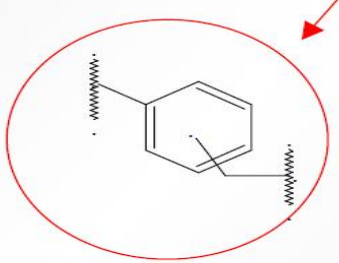
arílico

arilsulfonamídica

alifático

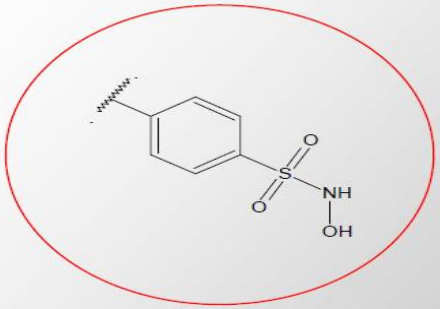


n = 1 compostos I
n = 2 composto II



regioisômeros *o*, *m* e *p*

m = composto III
p= comspoto V



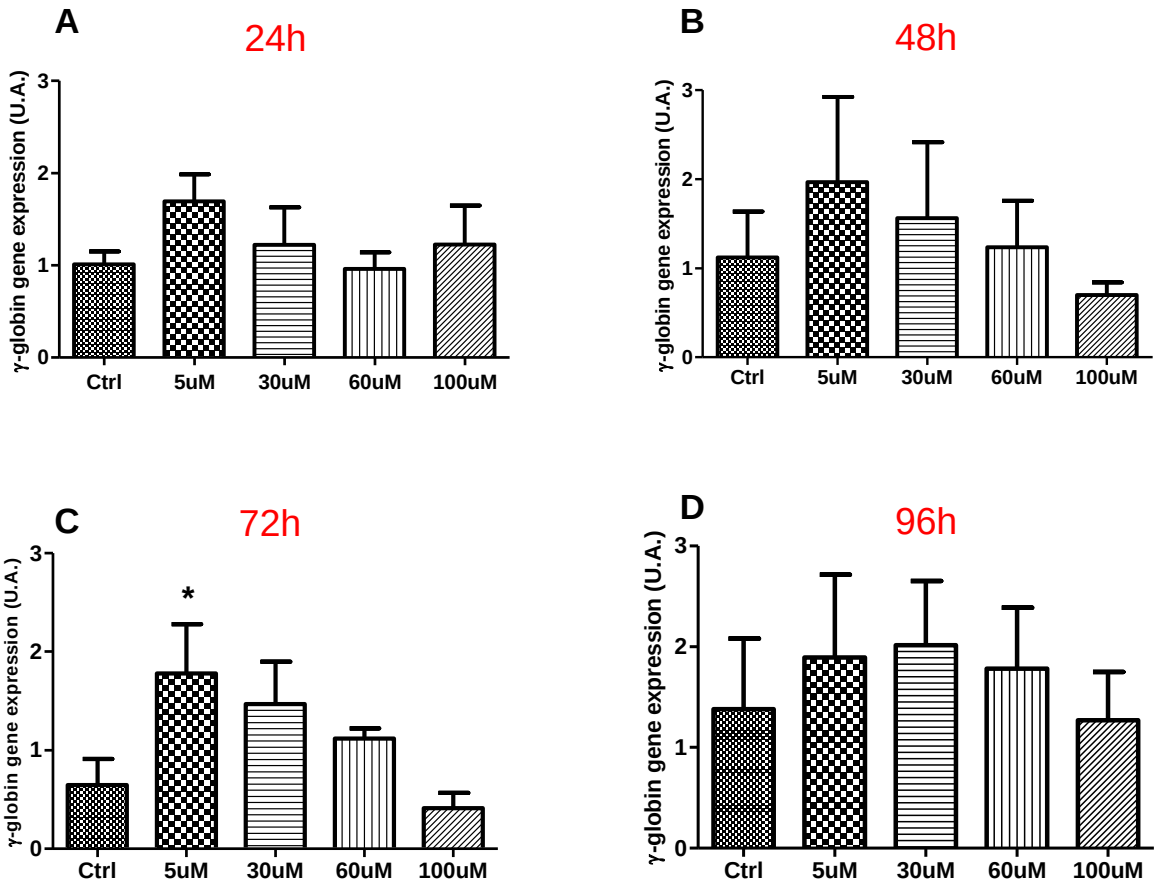
Aim

The aim of this study was to evaluate the effects of a novel hybrid compound *Lap*desf 1 (2-[4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)phenyl]ethyl nitrate) on γ-globin gene expression in cells culture, fetal hemoglobin levels in sickle cell transgenic mice and cytokine release from activated monocytes.

Results

K562 Cell Culture

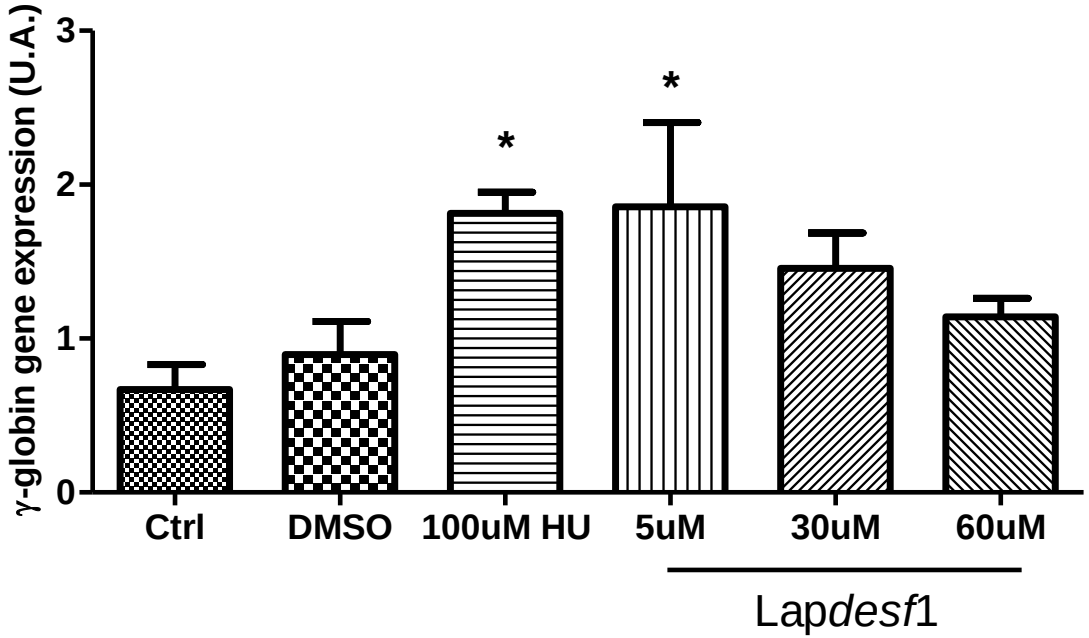
Human K562 cells were maintained in DMEM with 10% FBS, Pen/Strep, in humidified air (5% CO_2, 37°C). γ-globin gene expression was analyzed by qRT-PCR and quantified using the Gnorm program.



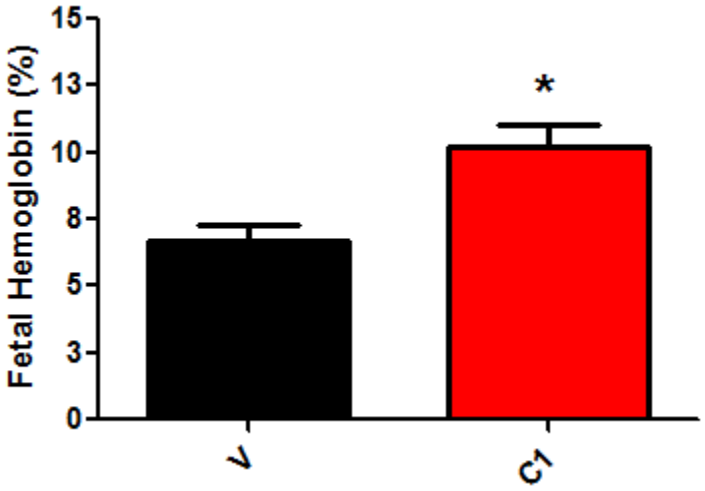
K562 Cell Culture incubated with Lap*desf 1, n≥3, * P<0.05, compared to control.*

Erythroid Cell Culture

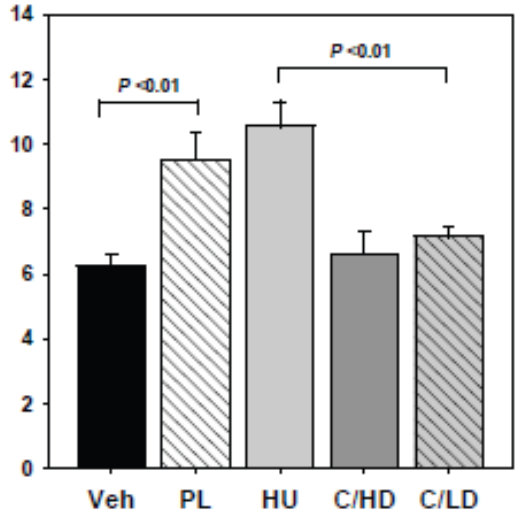
CD34^{+} cells were isolated from healthy donors and cultived with EPO, Stem cell factor and IL-3. The cells were treated on day 9 and collected after 4 days (day13).



Sickle Cell Transgenic Mice Chronic Treatment



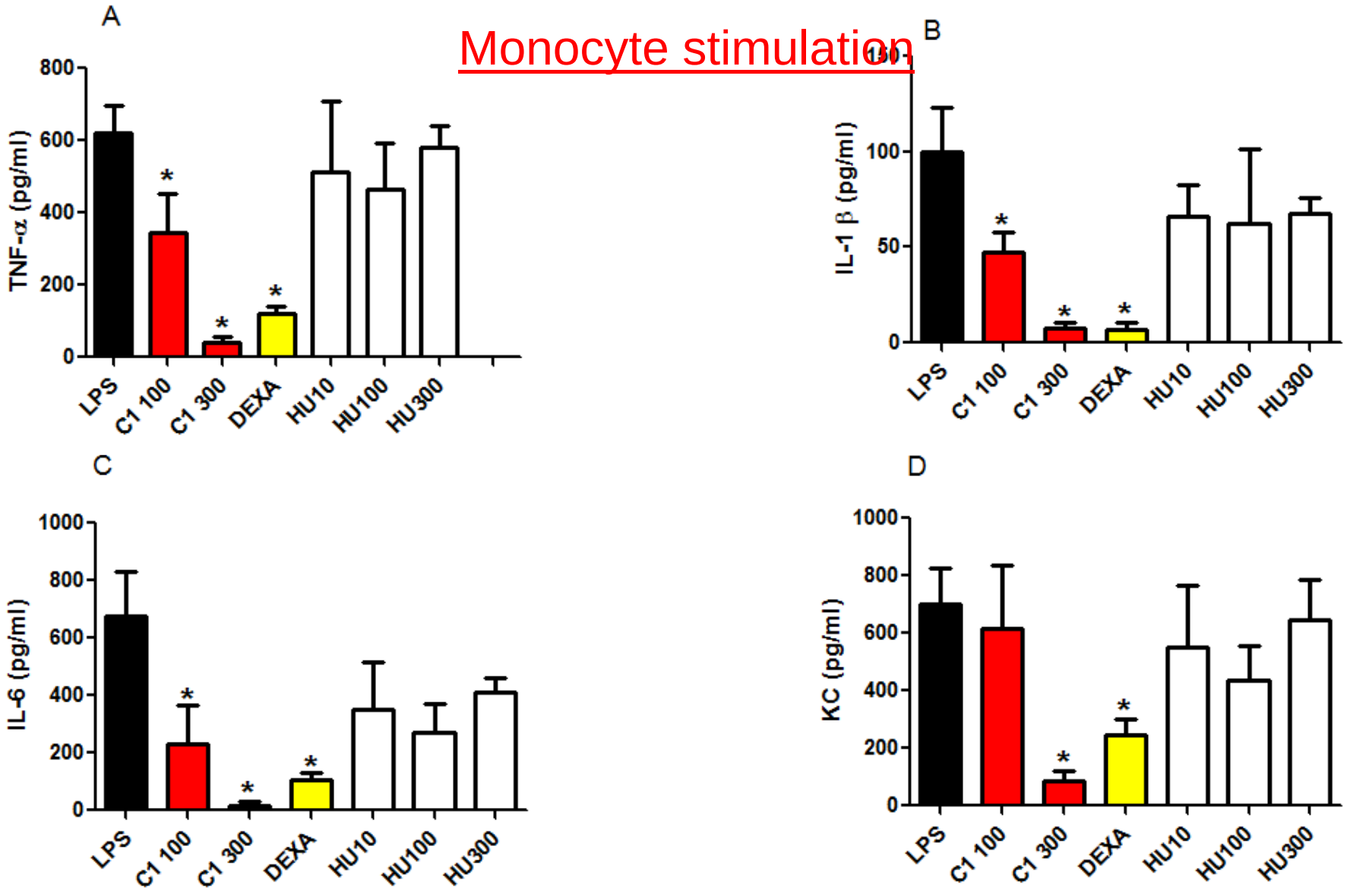
*Transgenic KT sickle mice were treated i.p. for 8 weeks with test drug and levels of fetal hemoglobin determined by HPLC, n≥6, * P<0.05.*



Veh: Vehicle
PL: Pomalidomide
HU: Hydroxyurea
C/HD: High-dose (10mg/Kg PL and 100mg/Kg HU)
C/LD: Low-dose (10mg/Kg PL and 10mg/HU)

Mieler et al.; 2011*; Blood*

Monocyte stimulation



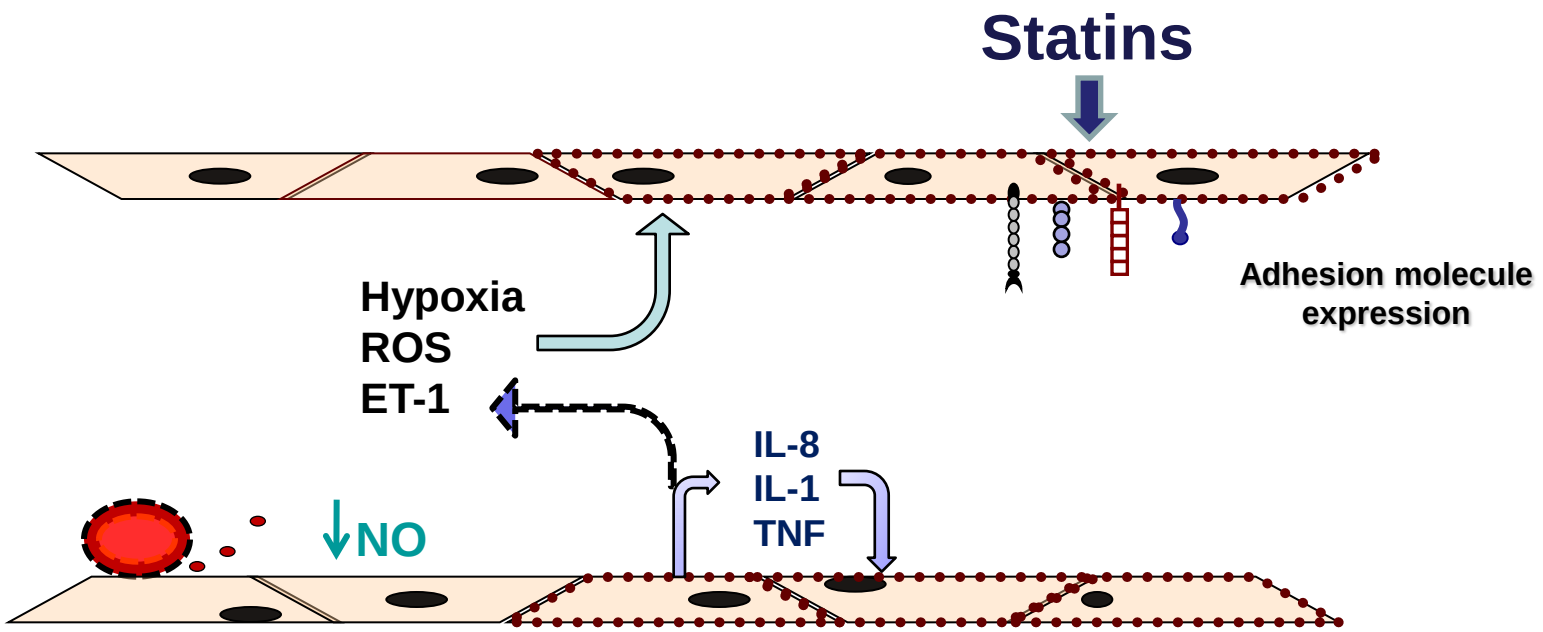
Levels of pro-inflammatory mediators determined by ELISA in the supernatant of mononuclear culture from sickle cell transgenic mice treated with LPS and co-incubated with test drugs (24H). Dexamethasone (1µM) was used a positive anti-inflammatory control (A) TNF-α. (B) IL-1β. (C) IL-6. (D) KC. n≥5, * P<0.05, compared to LPS.

Santos J.et al:J.Med.Chem,2011

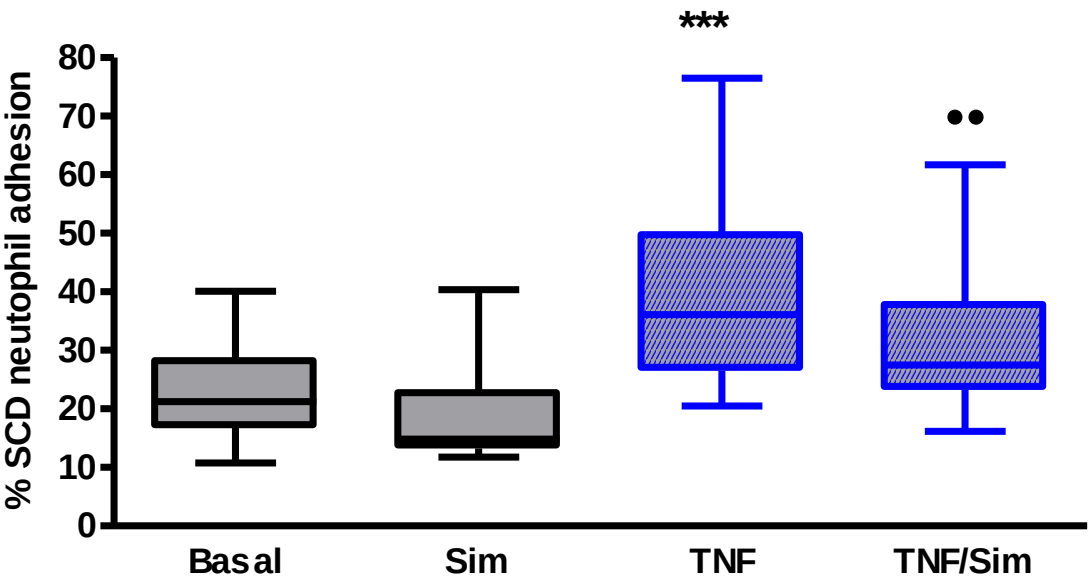
Our results support the hypothesis that Lap*desf* 1 can augment HbF synthesis. In addition, this compound has the ability to inhibit TNF-α production and other inflammatory cytokines and could help reduce chronic inflammation in sickle-cell patients, thus reducing or preventing clinical complications associated with SCD.

These data suggest that Lap*desf* 1 represents a novel drug worthy of additional study for SCD and β thalassemia and perhaps certain other diseases associated with chronic inflammation.

Decreasing Endothelial Interactions and Vascular Inflammation in SCD



Statins reduce SCD neutrophil adhesion to endothelial cells *in vitro*



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HEMOCENTRO CAMPINAS

HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN

CNPq FAPESP Instituto Nacional de Ciência e Tecnologia do Sangue

Hereditary Persistence of Fetal Hemoglobin (HPFH)

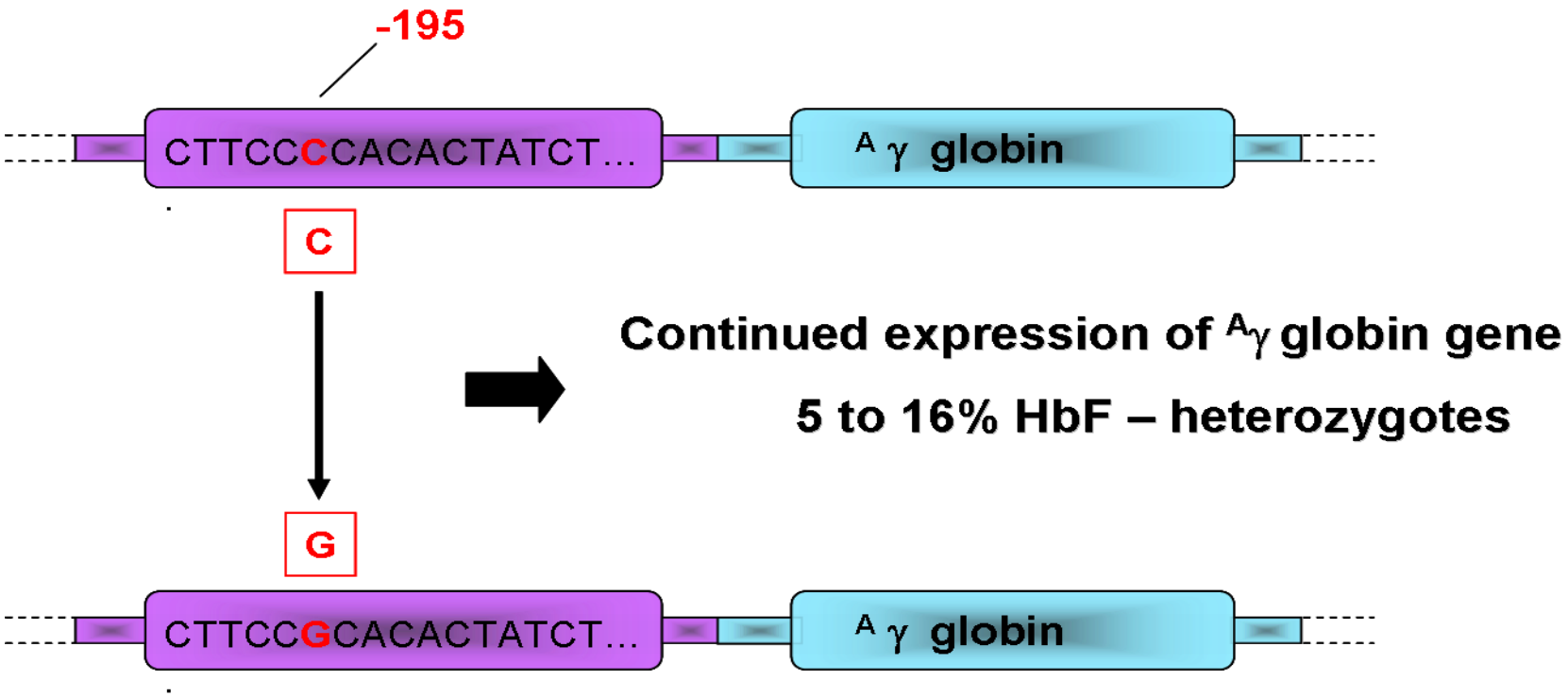
- HPFH is a genetic disorder that results in the increased levels of fetal hemoglobin in adult life.

- Types:
  - deletional - involving the beta globin gene cluster (dHPFH)

  - non-deletional – point mutations in the gamma globin gene promoter (ndHPFH)

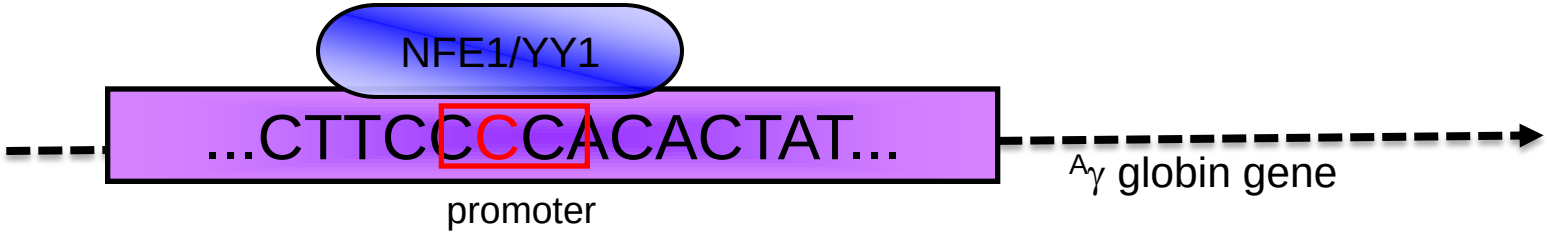
(Forget, 1998; Steinberg *et al.*, 2001).

Brazilian Type
Hereditary Persistence of Fetal Hemoglobin



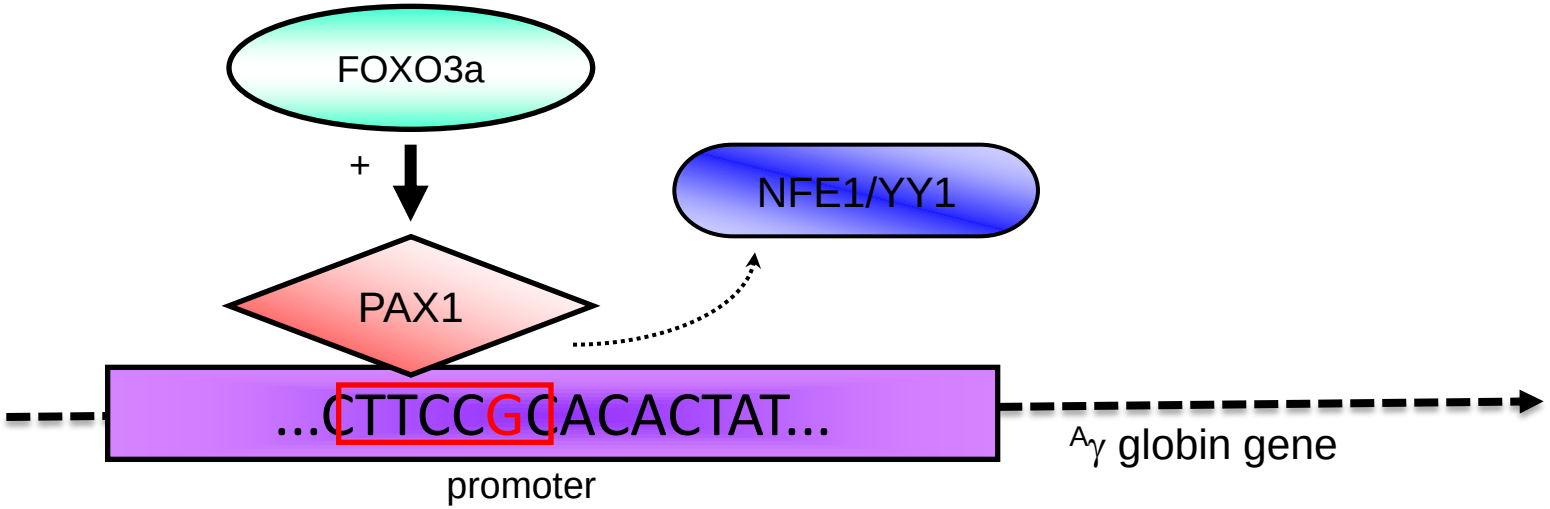
Costa *et al.*, 1990

A. Wild Type



Aγ Globin Gene Expression Repressed

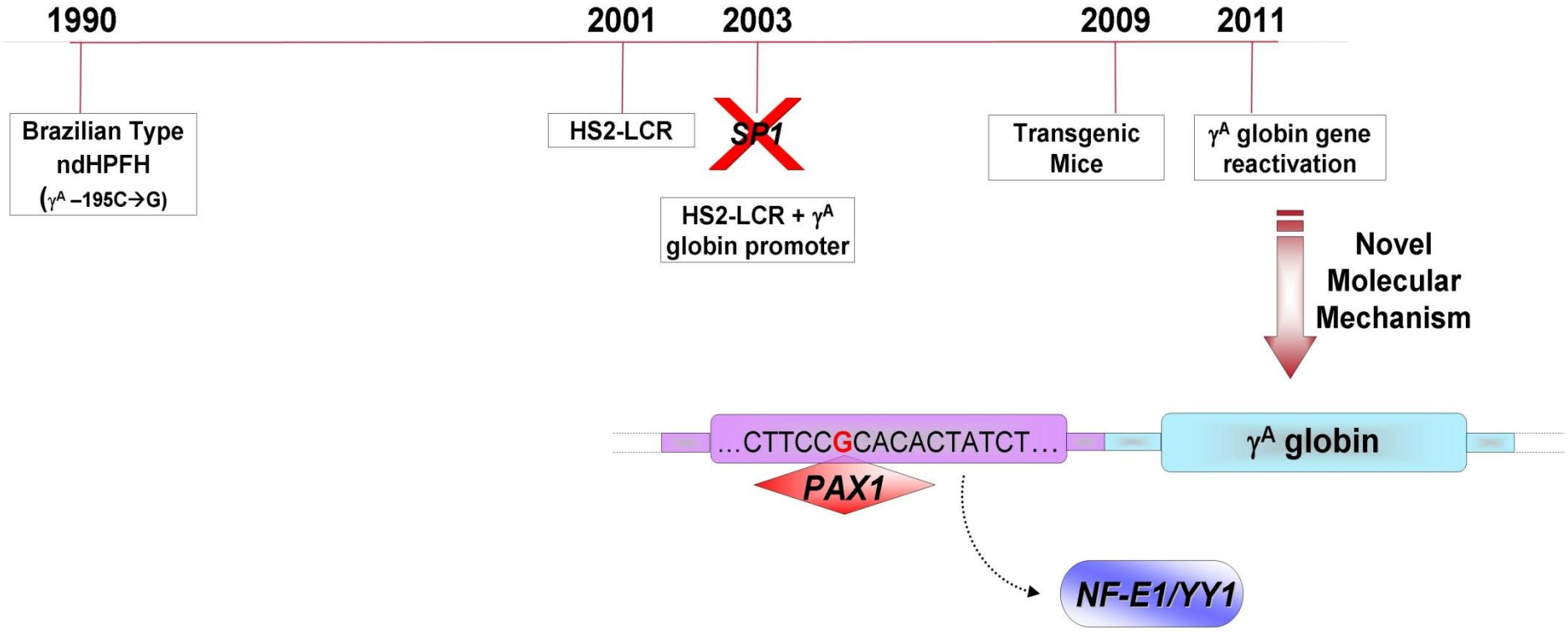
B. Brazilian Type ndHPFH



Aγ Globin Gene Expression Reactivation

Roversi *et al.*, 2011

Brazilian Type of ndHPFH



Science
AAAS

18 November 2011:
Vol. 334 - no. 6058 - pp. 993-996

Correction of sickle cell disease in adult mice by interference with fetal hemoglobin silencing.

Xu J, Peng C, Sankaran VG, Shao Z, Esrick EB, Chong BG, Ippolito GC, Fujiwara Y, Ebert BL, Tucker PW, Orkin SH.

Children's Hospital Boston
Department of Pediatric Oncology
Dana-Farber Cancer Institute
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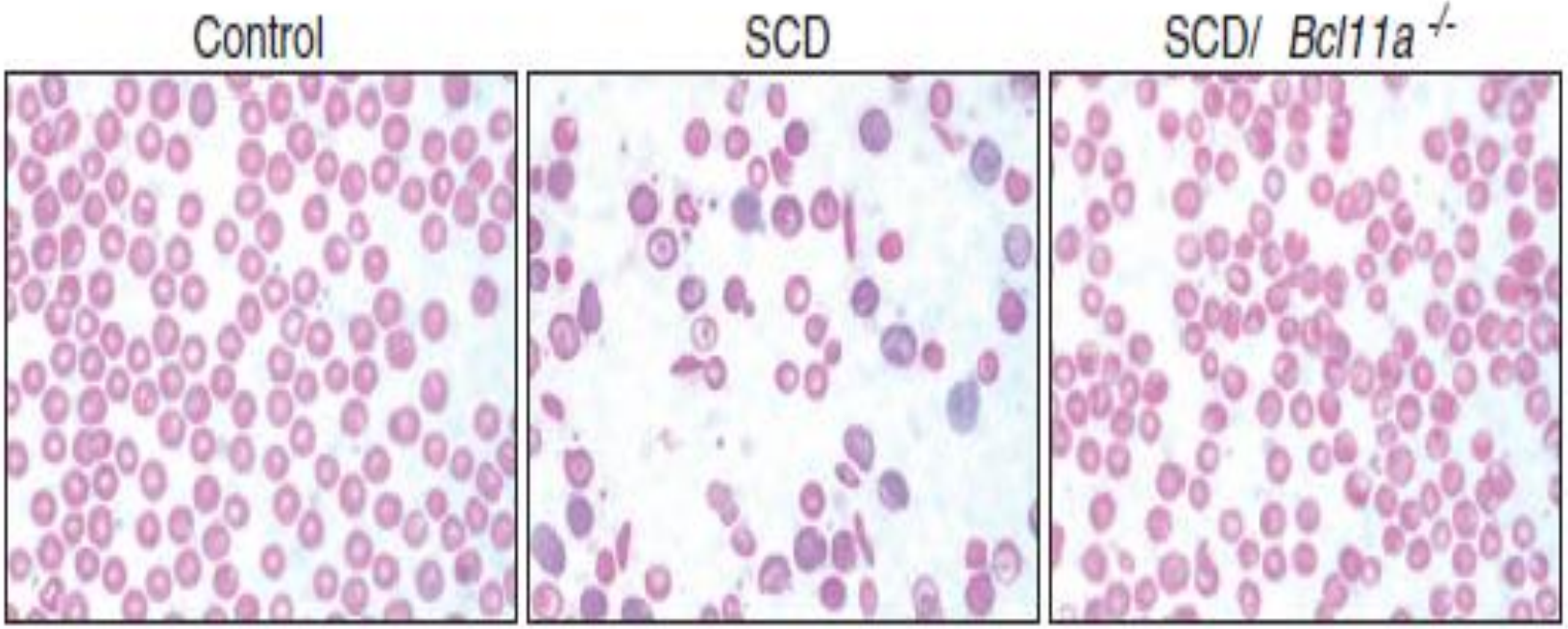
BCL11A

- Transcriptional repressor of HbF production;
- Contributes to HbF silencing in primary human erythroid cells;
- Regulator of globin switching in development.

AIMS

Investigate the contribution of BCL11A to γ-silencing in adults through conditional inactivation of this gene in mice carrying the human β-globin gene cluster.

Inactivation of BCL11A rescues sickle cell defects in humanized SCD mice.



Representative blood smears of control, SCD, and SCD/*Bcl11a* $^{-/-}$ mice are shown at 1000x magnification.

Conclusion

The correction of SCD in mice by genetic manipulation of a single component involved in globin gene regulation constitutes a requisite step in translating new insights in HbF silencing into mechanism-based, improved therapy for the major hemoglobin disorders.

Summary

• Vascular inflammatory molecule production may contribute to vaso-occlusion in SCD, by activating the endothelium and blood cells;

• Approaches to reduce vascular inflammation should be further investigated;

•Inhibition of leukocyte adhesion to the vessel wall may be an important approach for the prevention of sickle cell vaso-occlusion;

- STATINS

- Selectin Inhibitors (GMI-1070)

Summary

• HU may have important HbF-independent effects that could play a role in the prevention of leukocyte adhesion and vaso-occlusion;

• Drugs that amplify the cGMP-dependent effects of HU should be further studied;

• Inhibition of the PDE9 enzyme may represent a relatively tissue-specific drug target in SCD, with potential for increasing both HbF production and decreasing leukocyte adhesion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Lediana Miguel
Carol Lanaro
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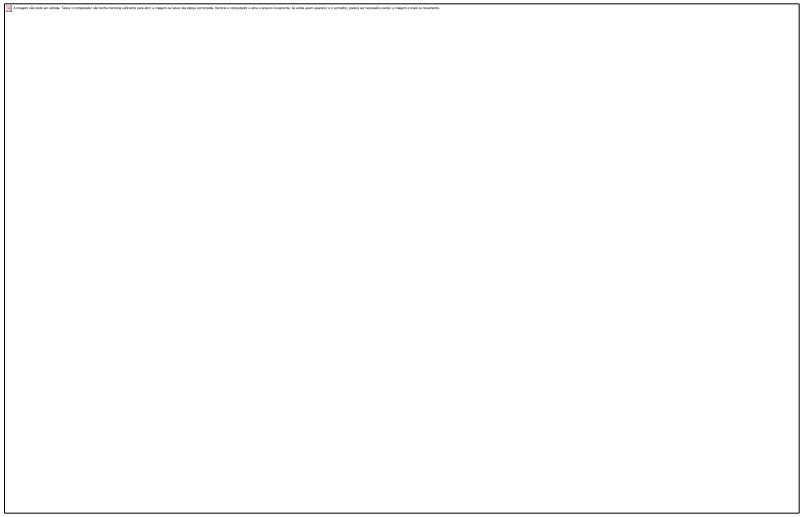
Paul Frenette
Christoph Scheiermman
Jungan Jang
Camila Almeida

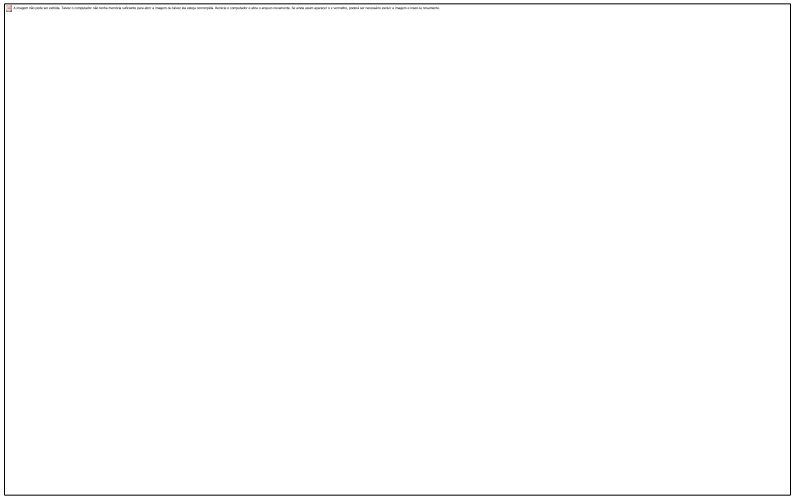
Targeting Vascular Inflammation:

Plasma Inflammatory Modulators in SCD

PGE_1



PGE_2



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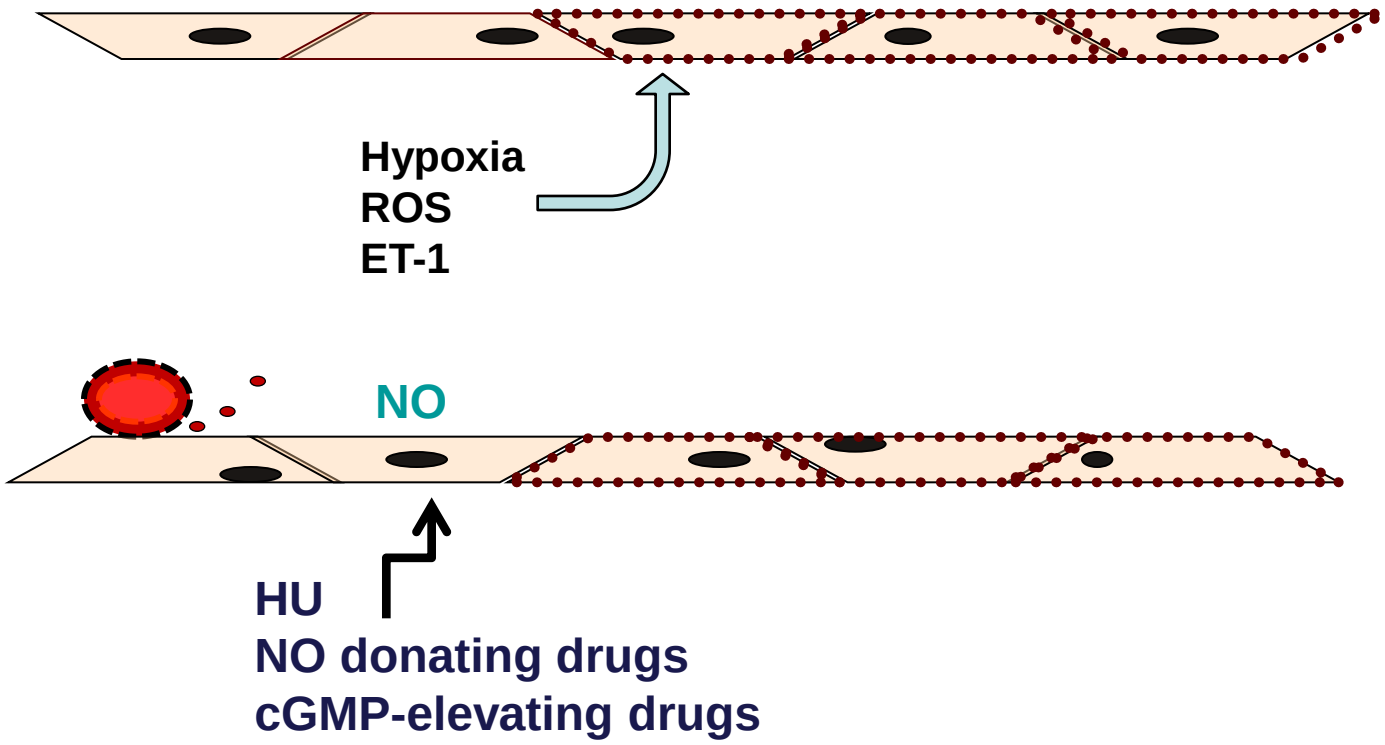
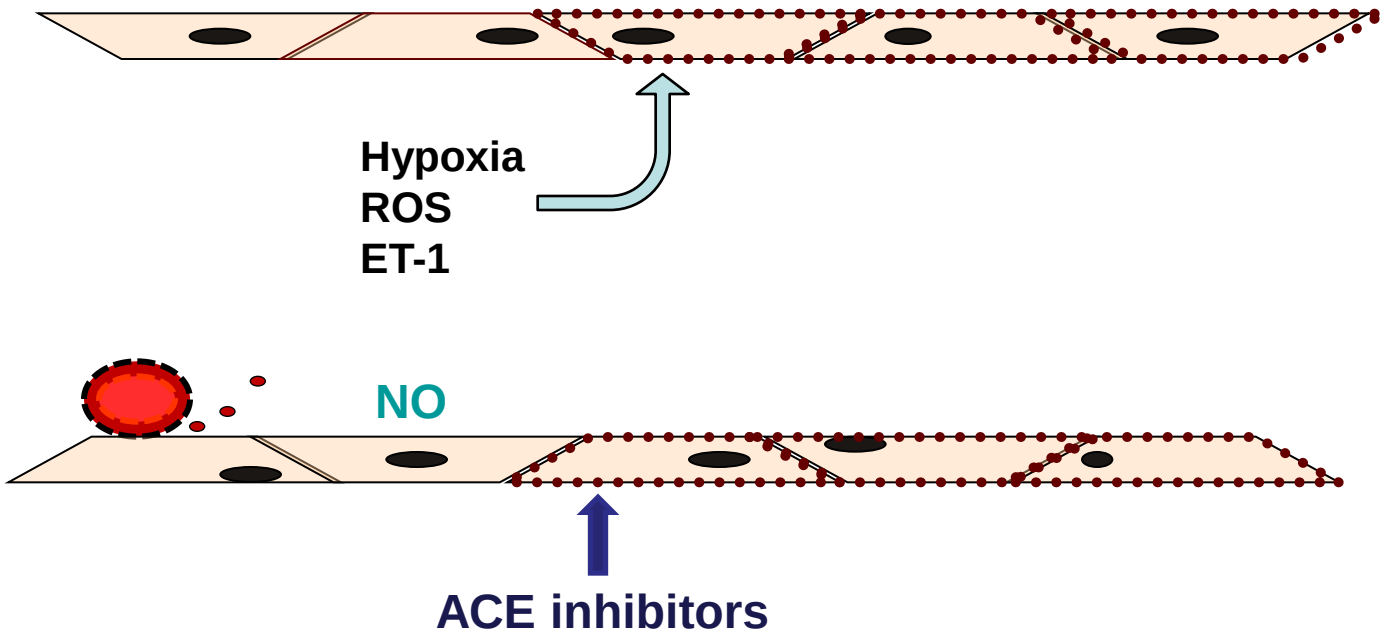
Lanaro et al., 2009; Conran et al., 2007

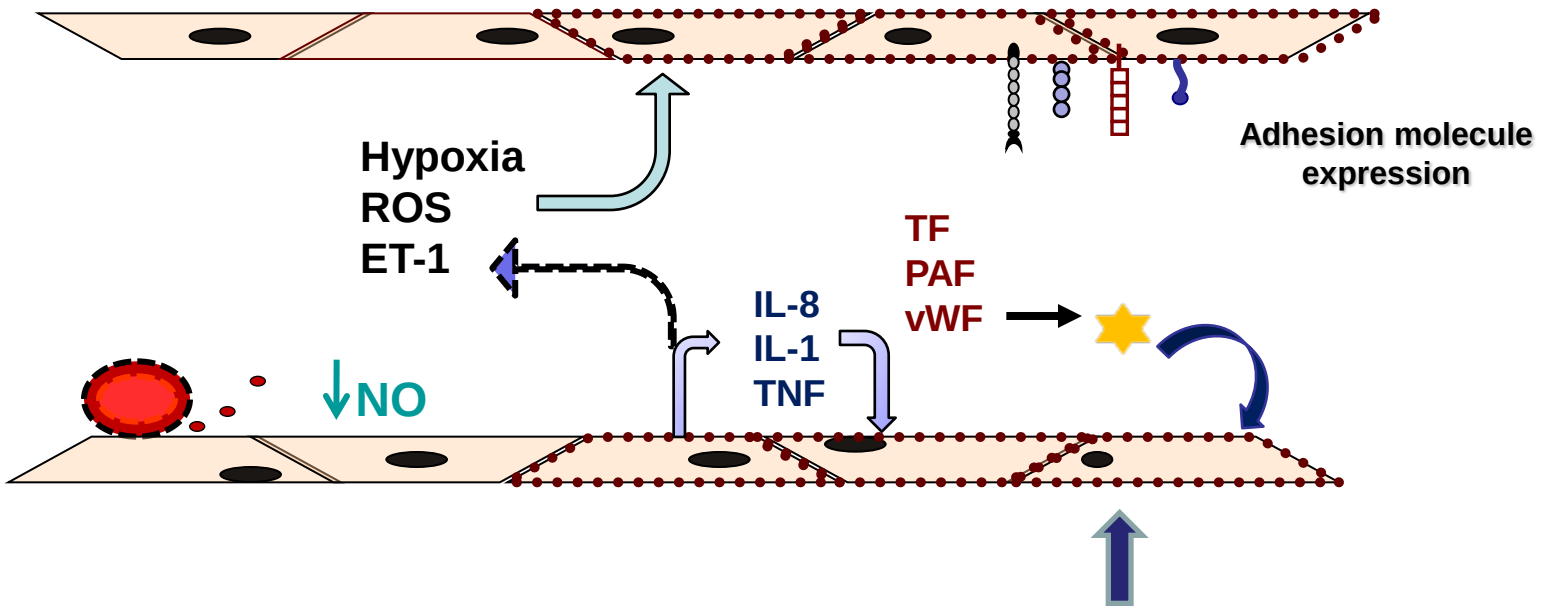
Decreasing Endothelial Interactions and Vascular Inflammation in SCD



Decreasing Endothelial Interactions and Vascular Inflammation in SCD



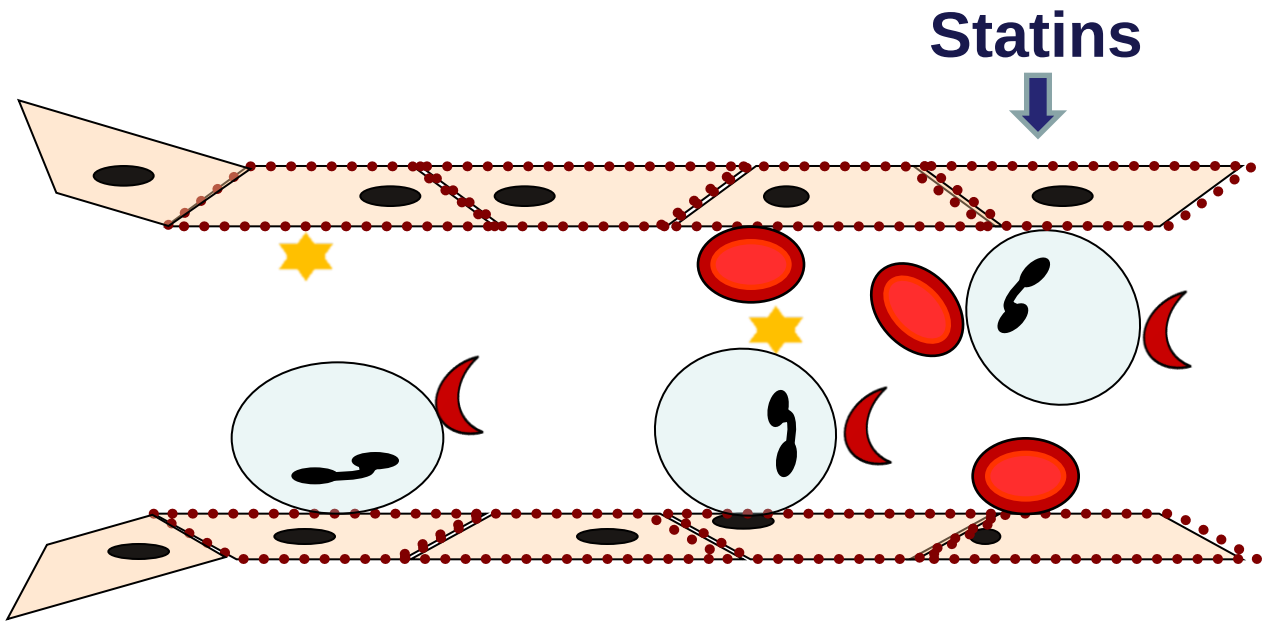
Decreasing Endothelial Interactions and Vascular Inflammation in SCD



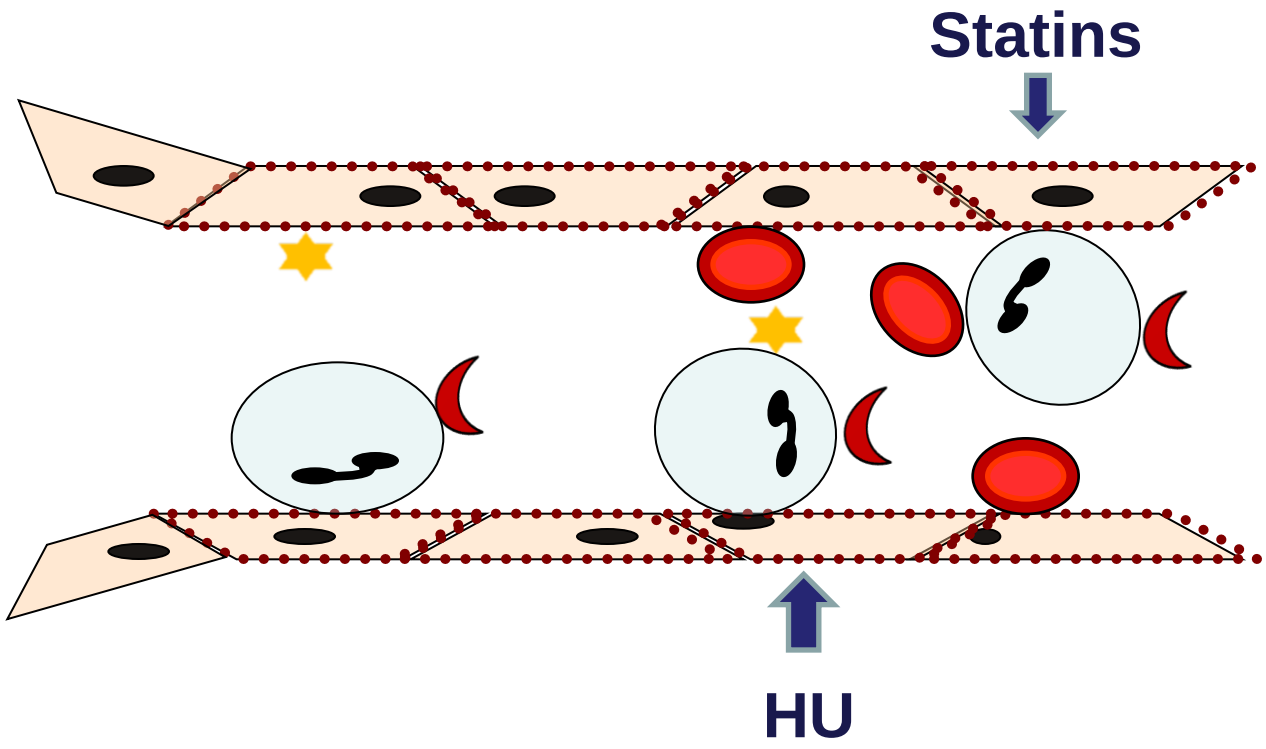
Anti-platelet drugs

- Eptifibatide

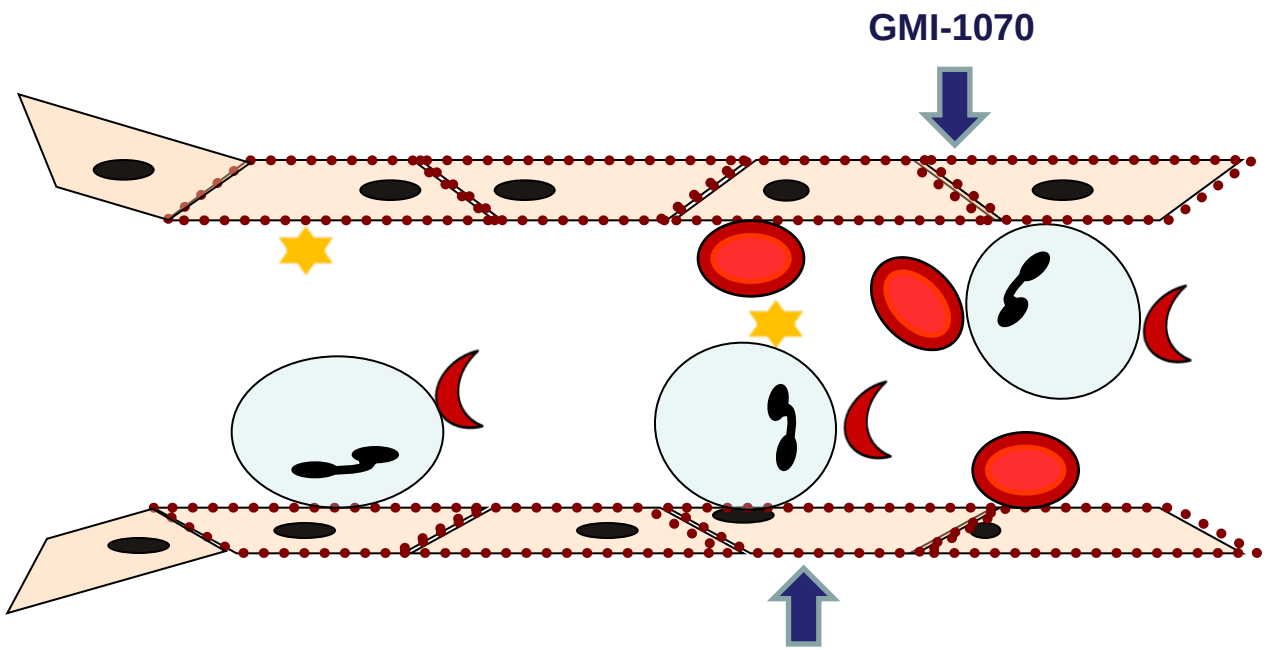
Decreasing Endothelial Interactions and Vascular Inflammation in SCD



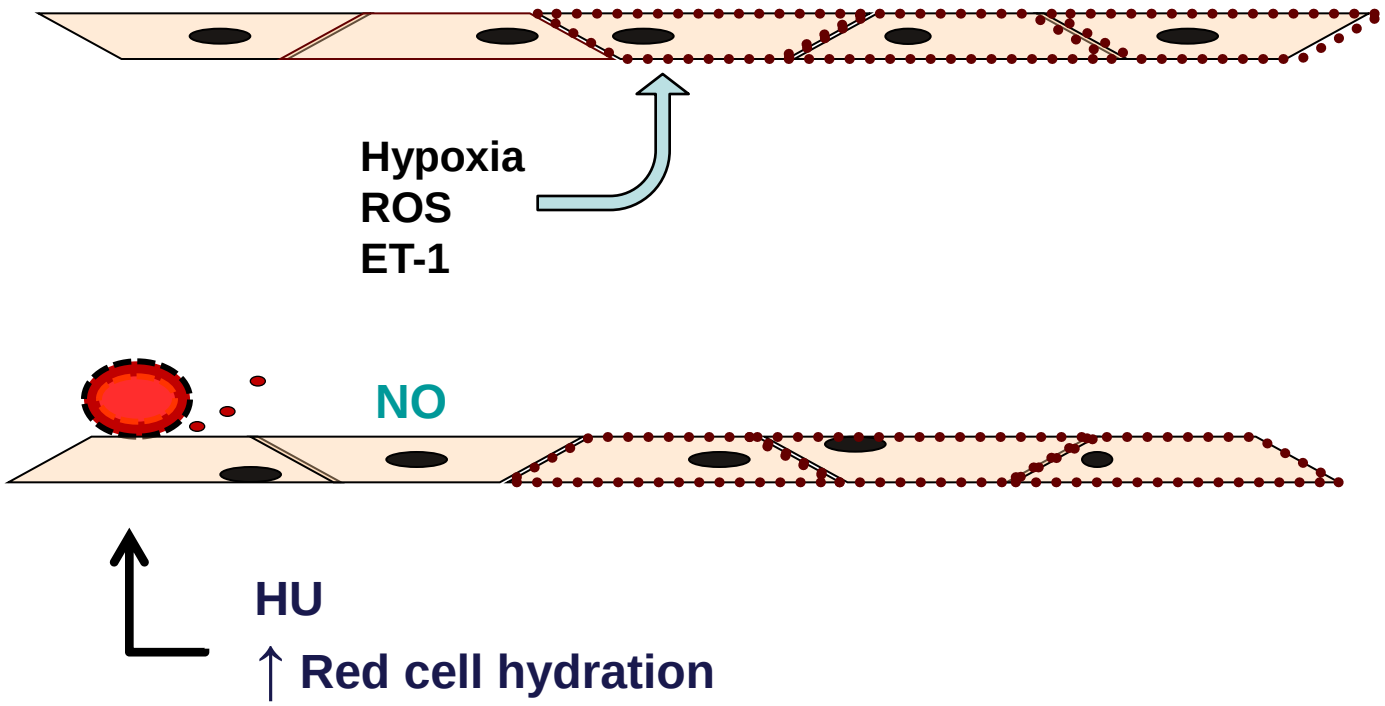
Decreasing Endothelial Interactions and Vascular Inflammation in SCD



Decreasing Endothelial Interactions and Vascular Inflammation in SCD



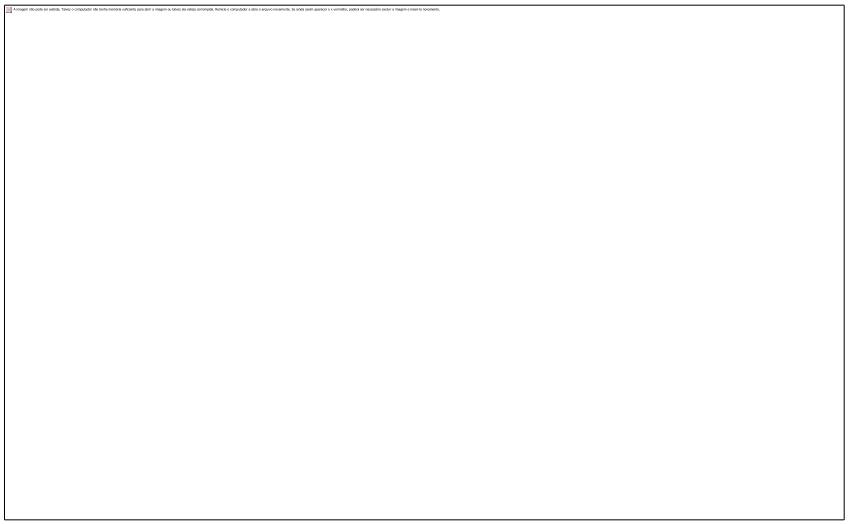
Decreasing Endothelial Interactions and Vascular Inflammation in SCD – possible approaches



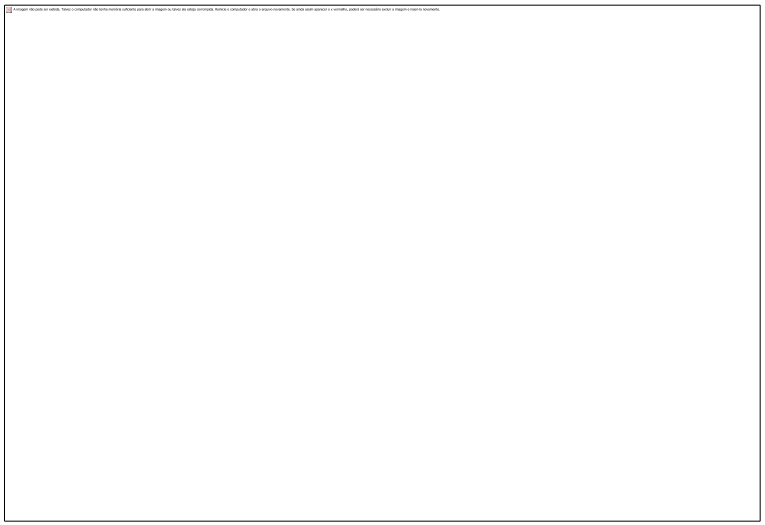
Statins reduce SCD neutrophil adhesion to endothelial cells *in vitro*

Role for Adhesion Molecule Expression

ICAM-1 expression on HUVEC



VCAM-1 expression on HUVEC



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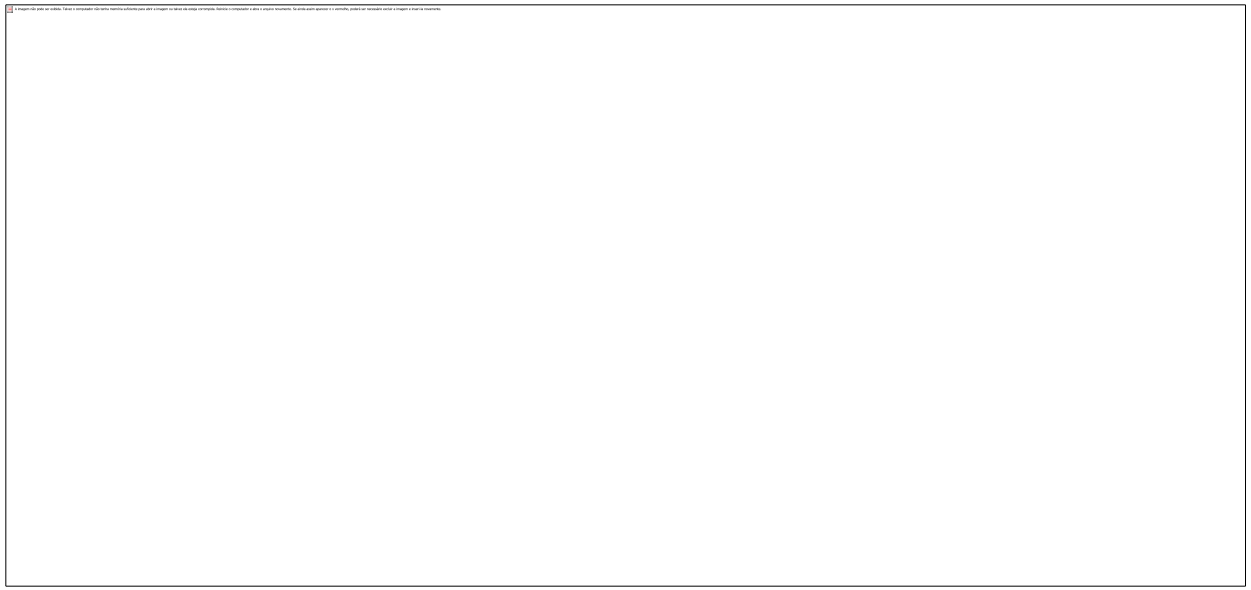
Canalli et al., 2011

Statins reduce SCD neutrophil adhesion to endothelial cells *in vitro*

Role for NO production



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Disclosures

No affiliations to declare

Targeting Vascular Inflammation:

HC and Plasma Inflammatory Proteins in SCD

IL-8 →

TNF-α ↓

PGE_1 →

PGE_2 →

GM-CSF ↓

Tissue Factor ↓

Soluble adhesion molecules ↓ →

IL-6

C-reactive protein

Endothelin-1

Angiogenic markers

CD40L →

LIGHT →

NT-proBNP

Anti-inflammatory Proteins

IL-10 ↑

HO-1

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Lanaro et al., 2009; Lampoumeroulie et al., 2005; Conran et al., 2004

Targeting Vascular Inflammation:

Plasma Inflammatory Proteins in SCD

IL-8

TNF-α

IL-6

PGE_2

PGE_1

GM-CSF

C-reactive protein

Endothelin-1

TF

NT-proBNP

Soluble adhesion molecules

Angiogenic markers

CD40L

LIGHT

Anti-inflammatory Proteins

HO-1

Lanaro et al., 2009; Brittain et al., 2007; Lee et al., 2006; Makis et la., 2006; Mohan et al., 2005; Rybicki 1998; Graido Gonzalez et al., 1998