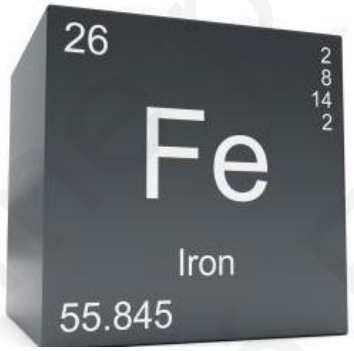
Métabolisme du fer et syndromes myélodysplasiques

Léon Kautz, PhD

IRSD, Toulouse

Iron is life. No iron = no life!

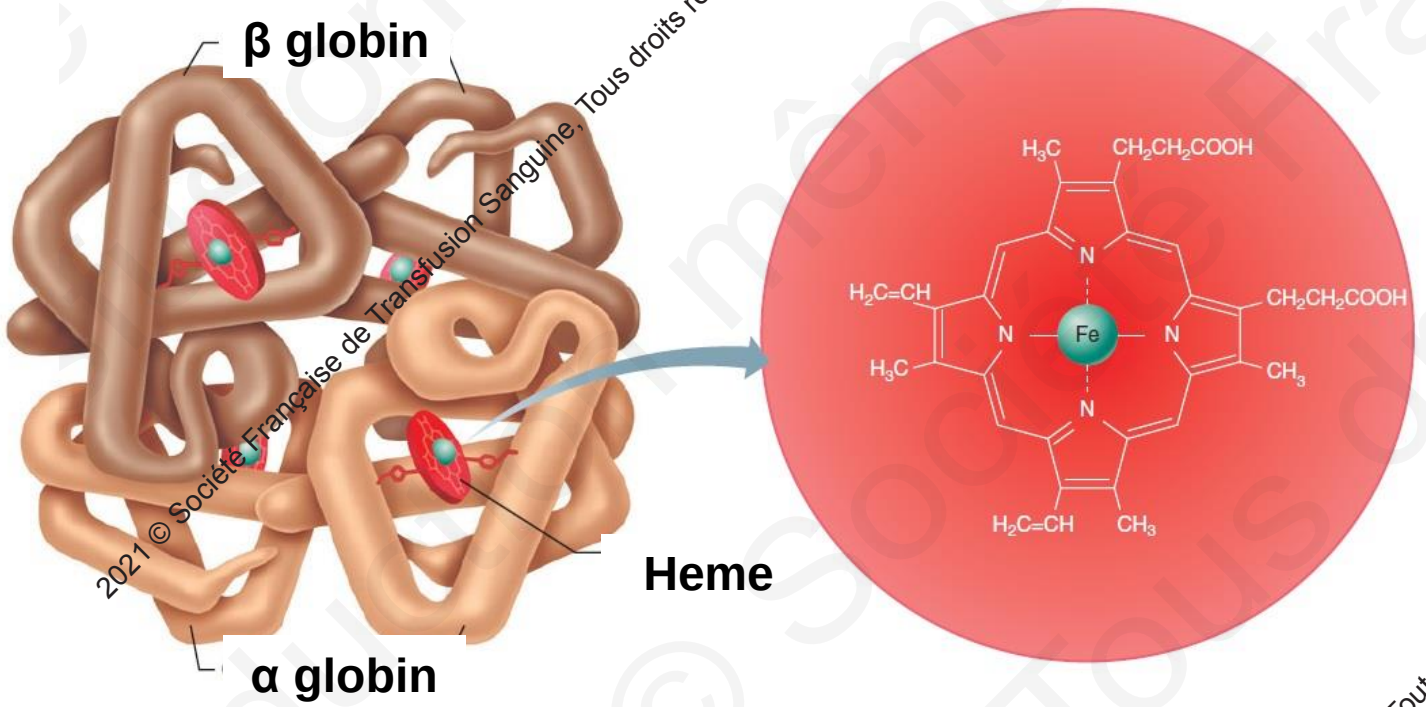


3-5 g of iron in an adult human body

2/3

O_2 transport

Iron binds oxygen to ensure its transport and storage



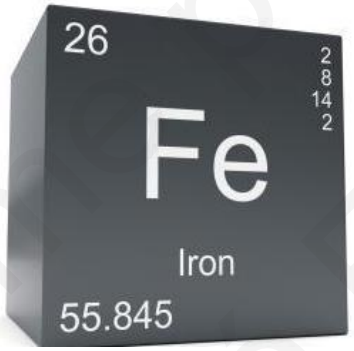
270 millions of hemoglobin / RBC

1080 Atoms of iron / RBC

2,4 million RBCs / second

Over 2 billion atoms of iron required per second in the bone marrow (>5µg)

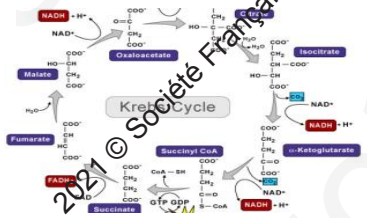
Iron is life. No iron = no life!



3-5 g of iron in an adult human body

1/3

2/3



Metabolism

DNA replication synthesis

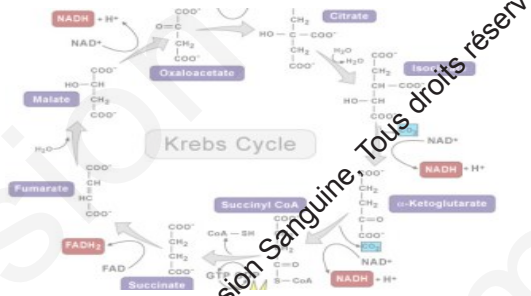
e- transfer

Production of free radicals

O_2 transport

The iron paradox

Essential



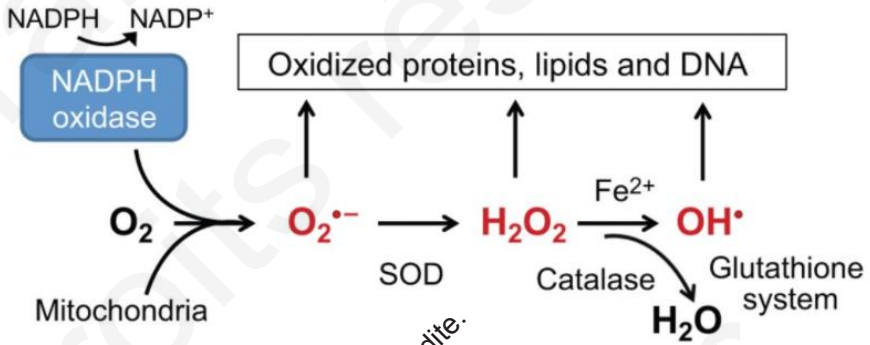
Metabolism

- Hemoprotein (Redox enzymes, cytochromes, catalases….)
- Iron-sulfur cluster proteins (NADH or succinate deshydrogenase…)

DNA replication synthesis

- Ribonucleotide reductase

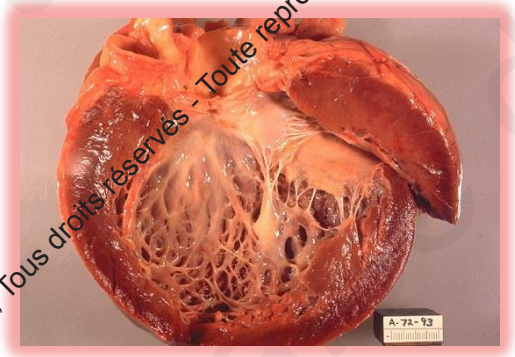
But toxic



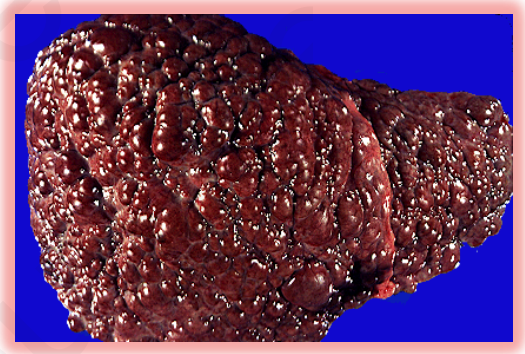
Fenton reaction

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + OH^{\bullet} + H_2O$$

Free radicals

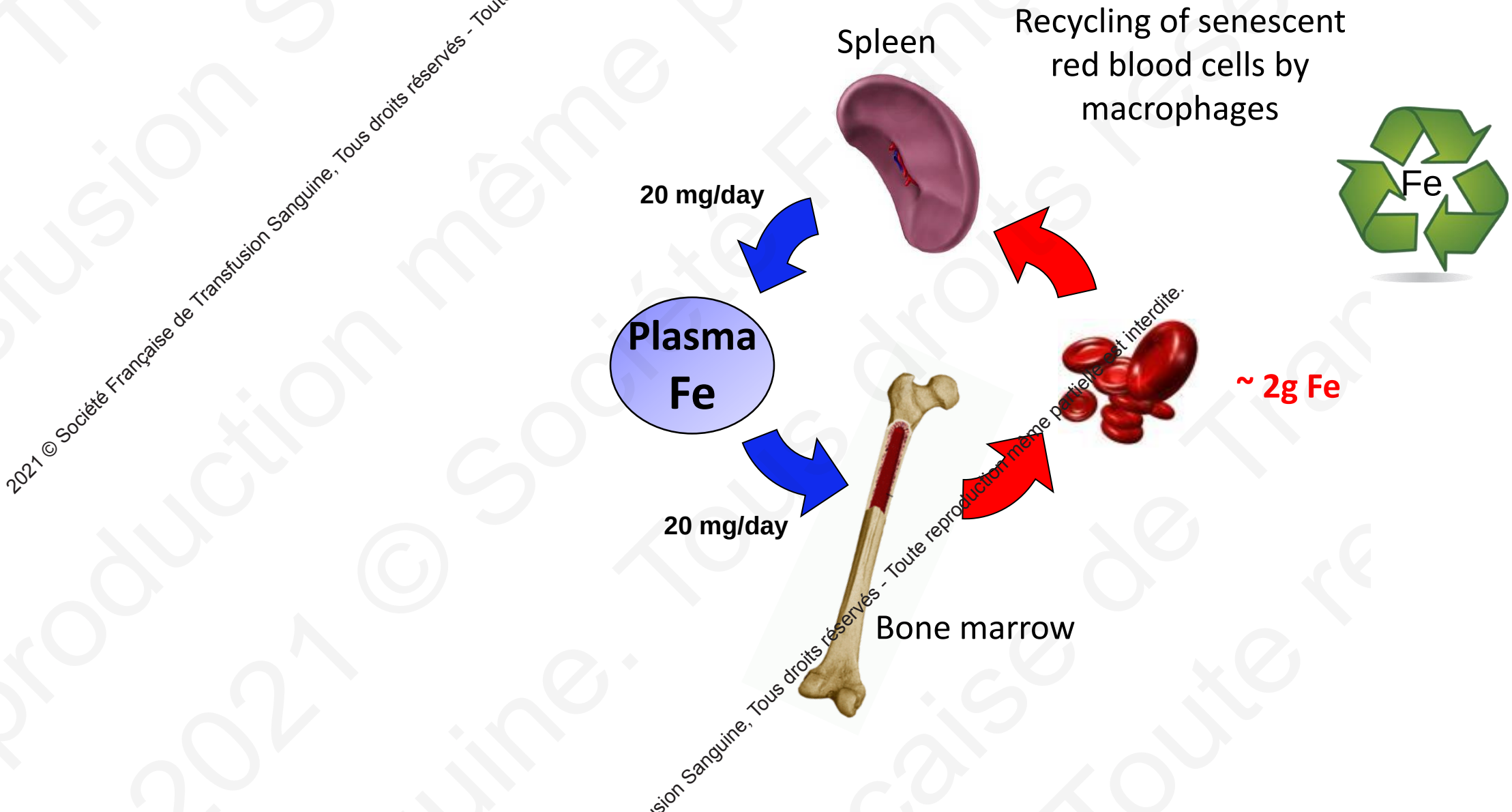


Cardiomyopathy



Cirrhosis

Iron is essential for fundamental biological processes



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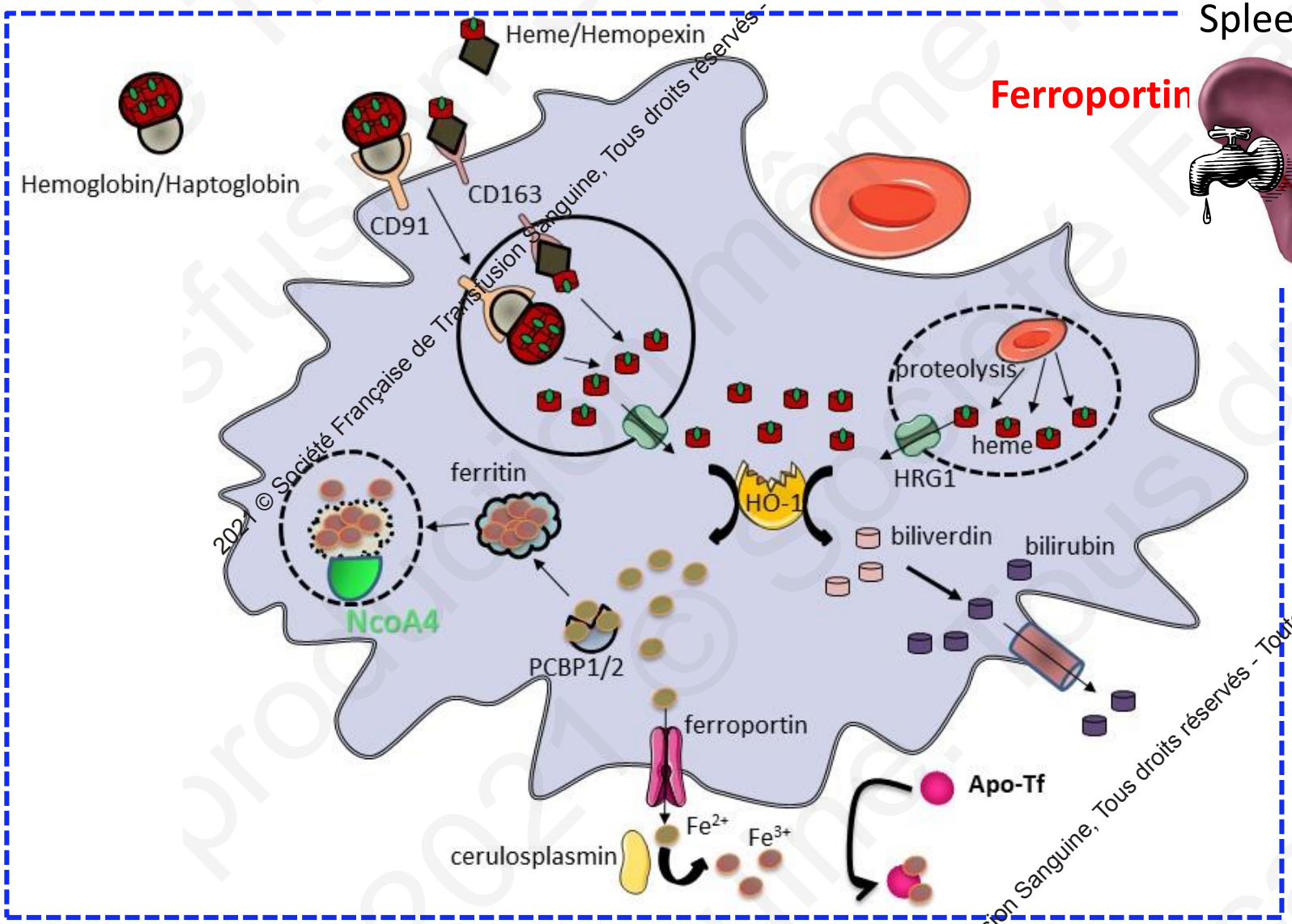


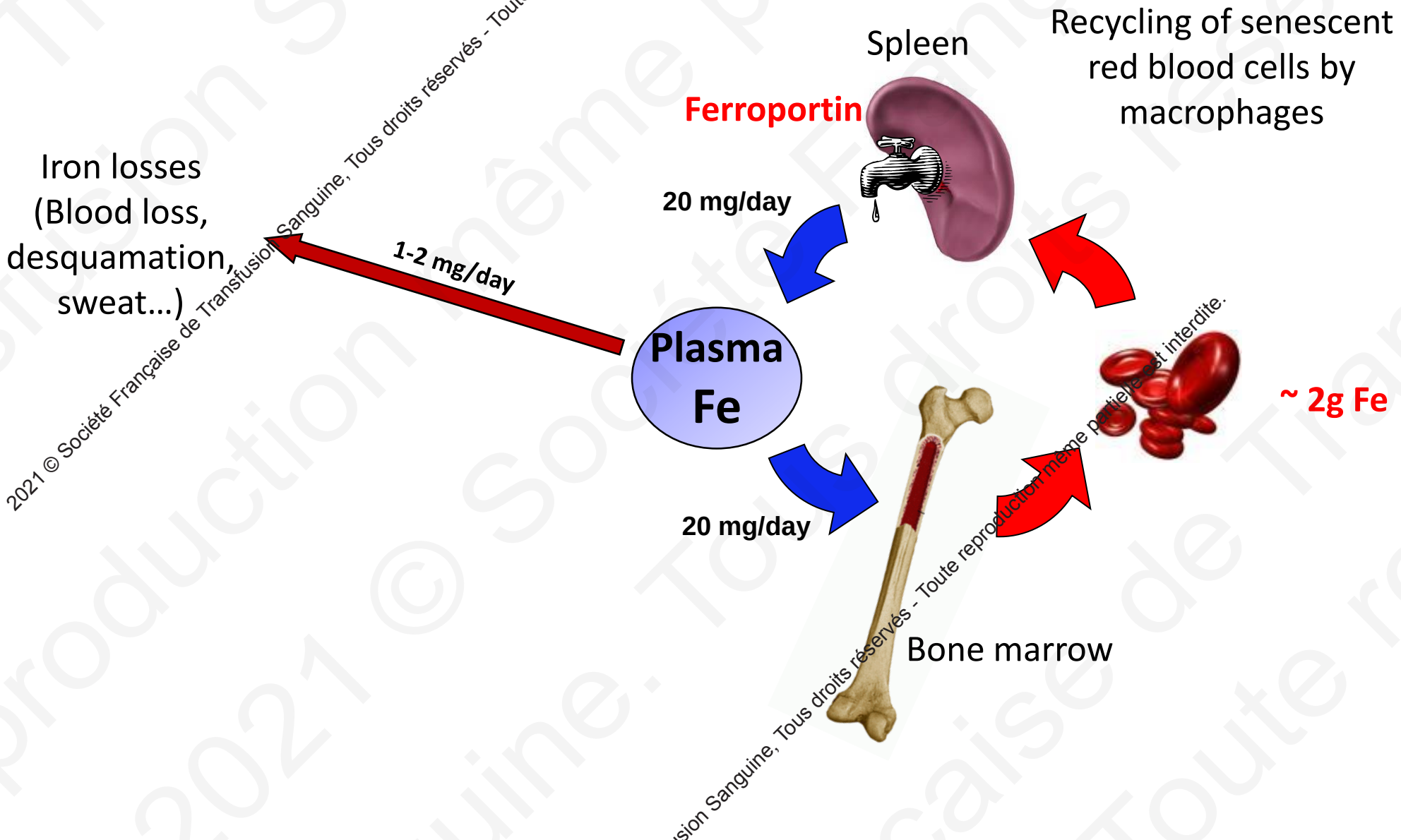
Spleen

Ferroportin

Recycling of senescent red blood cells by macrophages

~ 2g Fe

Iron is essential for fundamental biological processes



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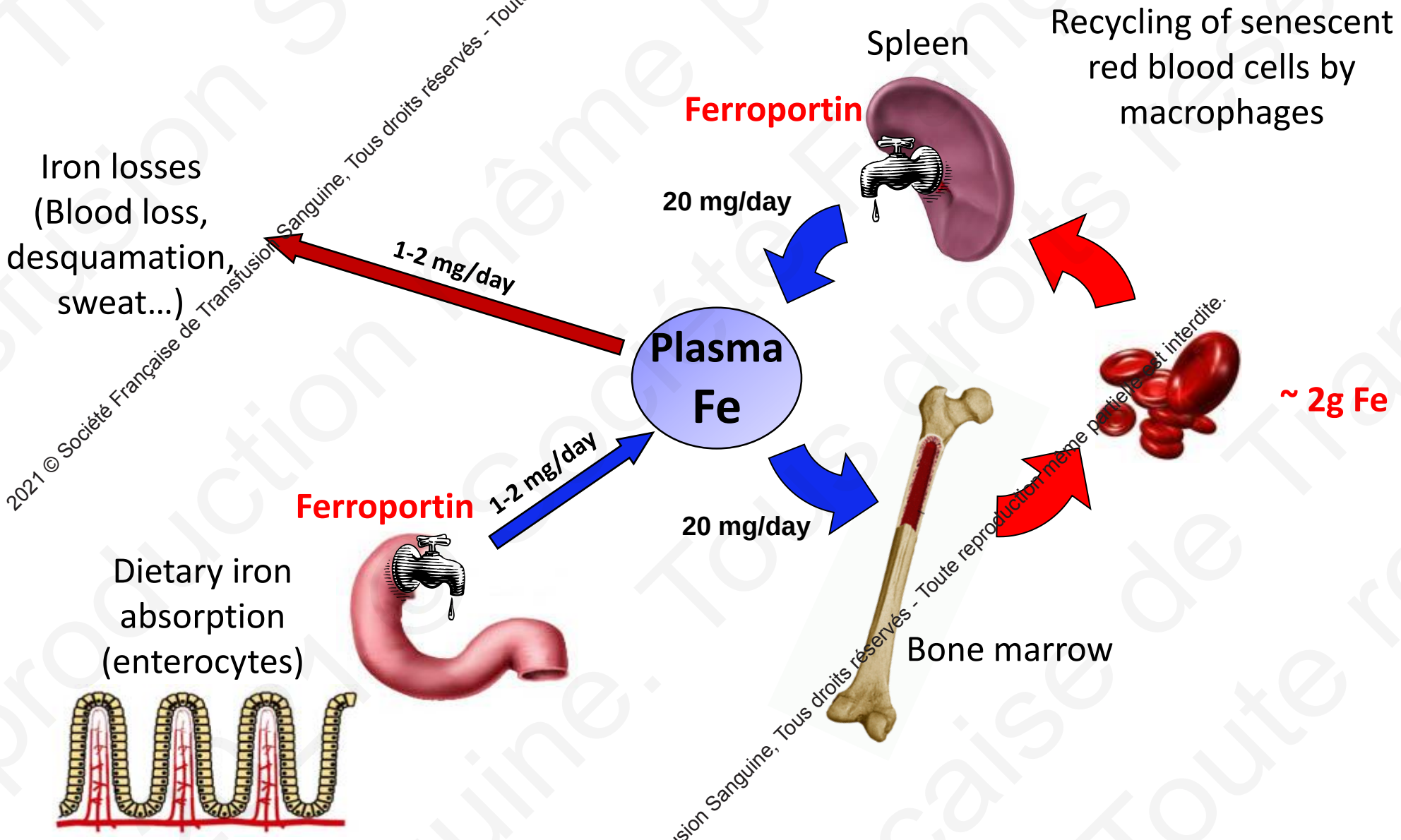


Iron is essential for fundamental biological processes

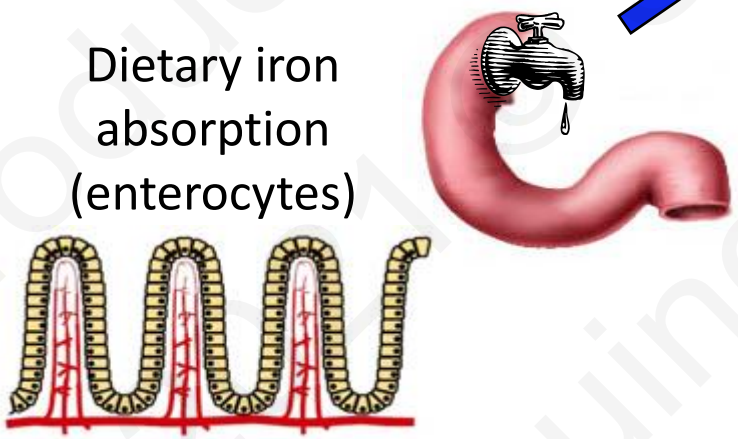
Iron losses (Blood loss, desquamation, sweat…)

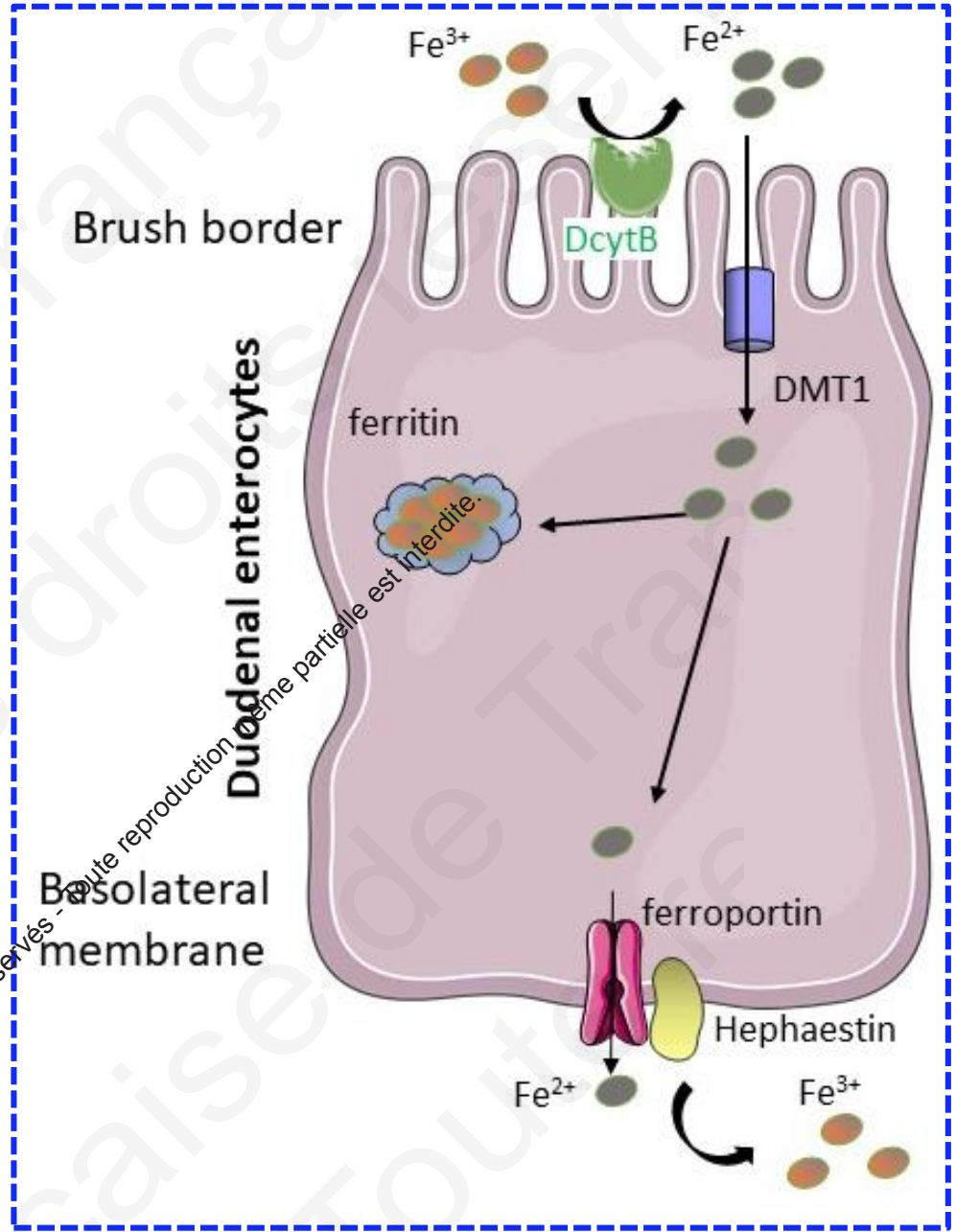
1-2 mg/day

Plasma Fe

Ferroportin 1-2 mg/day

Dietary iron absorption (enterocytes)





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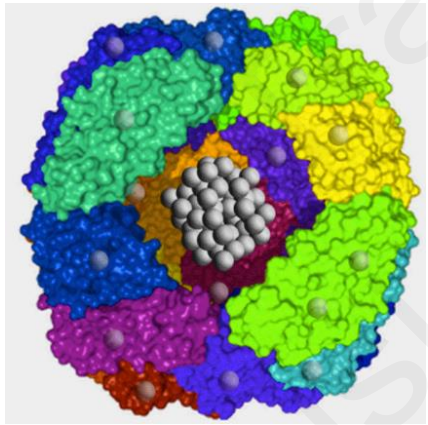
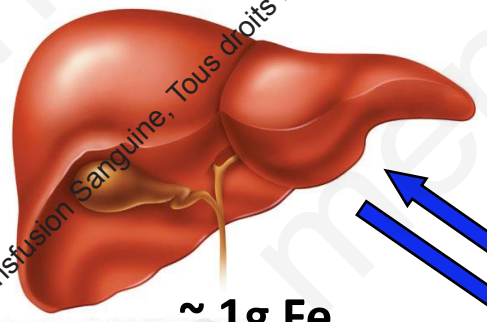


Ferritin
4500 Fe

Liver



~ 1g Fe

Storage

Spleen

Ferroportin

20 mg/day

Recycling of senescent red blood cells by macrophages

Plasma
Fe

Ferroportin

1-2 mg/day

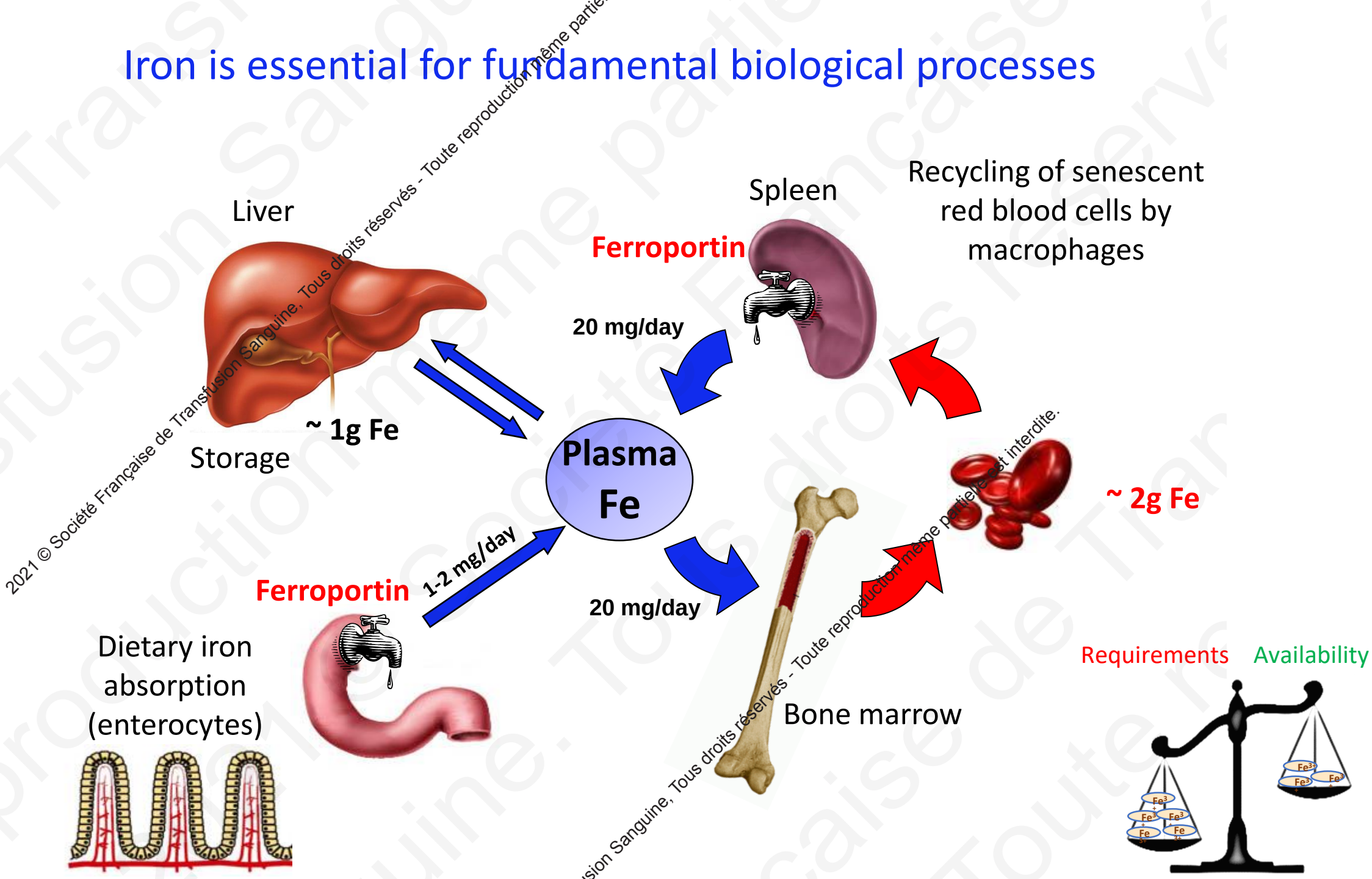
Dietary iron absorption (enterocytes)

20 mg/day

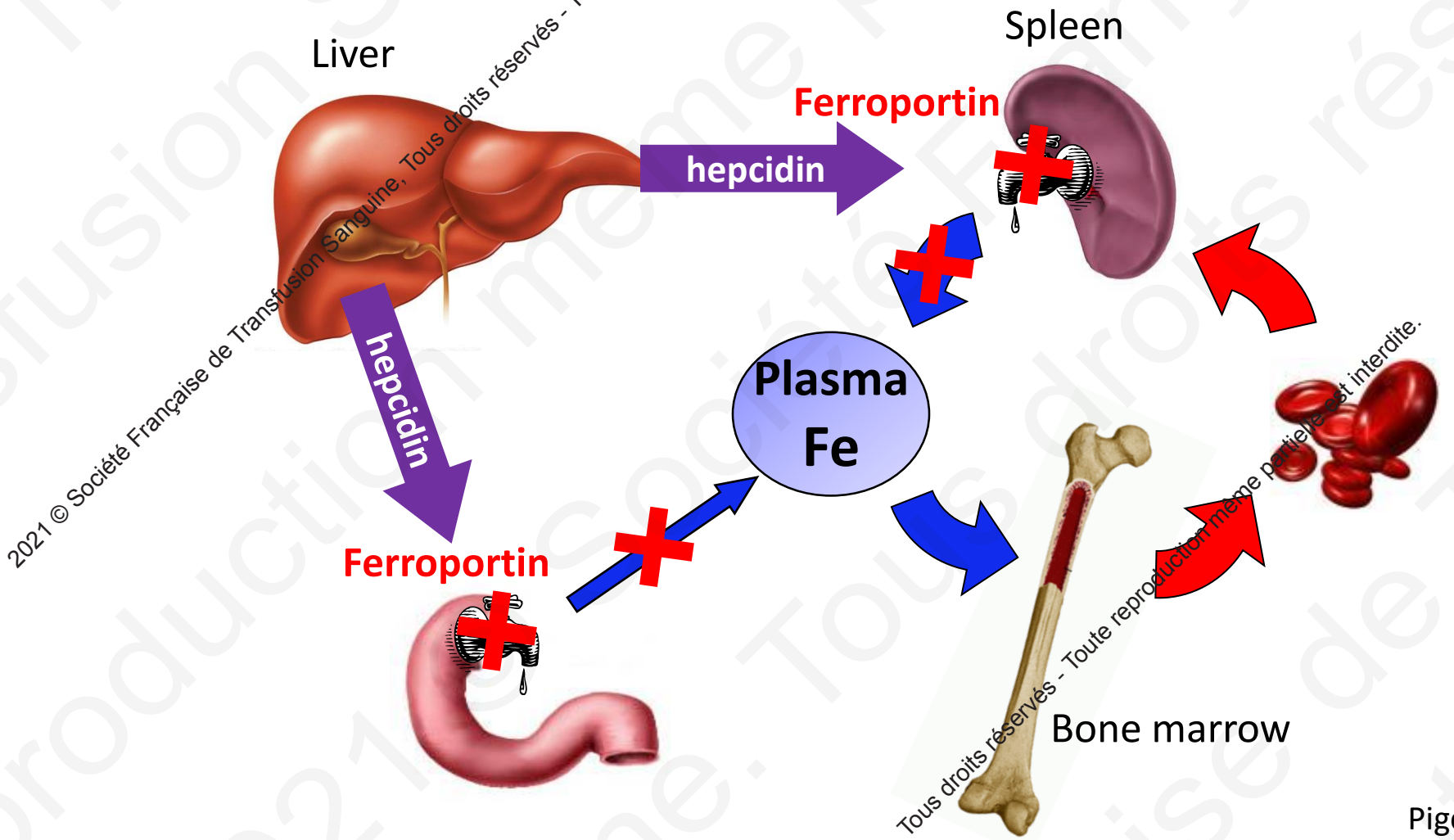
Bone marrow

~ 2g Fe

Iron is essential for fundamental biological processes



Hepcidin is the main regulator of iron homeostasis



Pigeon *et al.*, JBC, 2001
Nicolas *et al.*, PNAS, 2001
Nemeth *et al.*, Science, 2004

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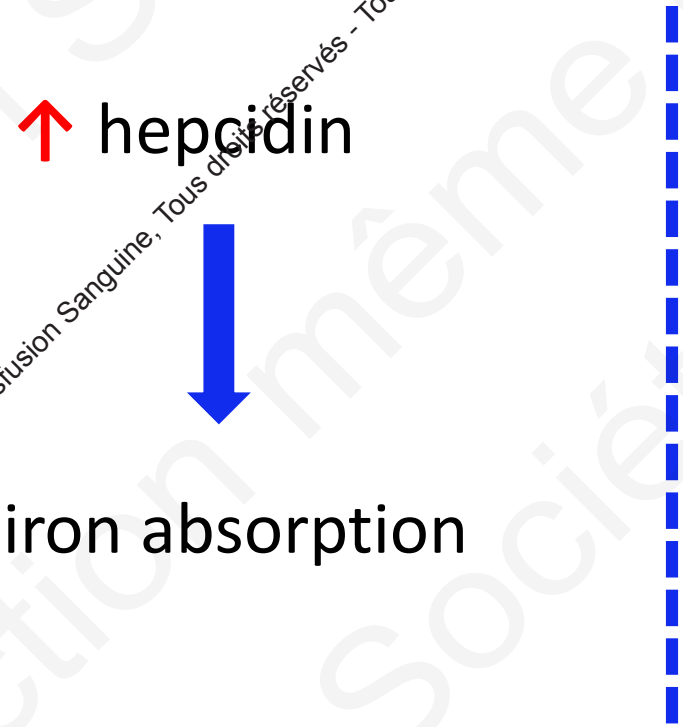

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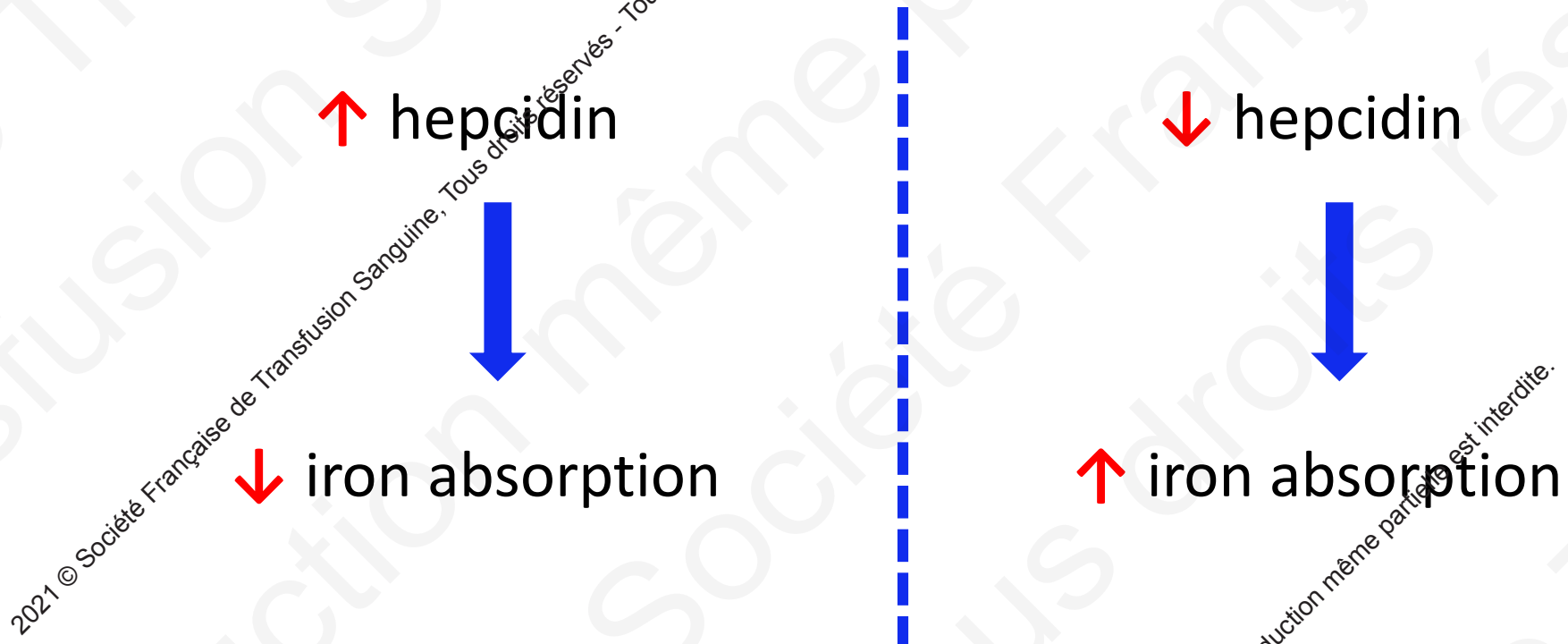

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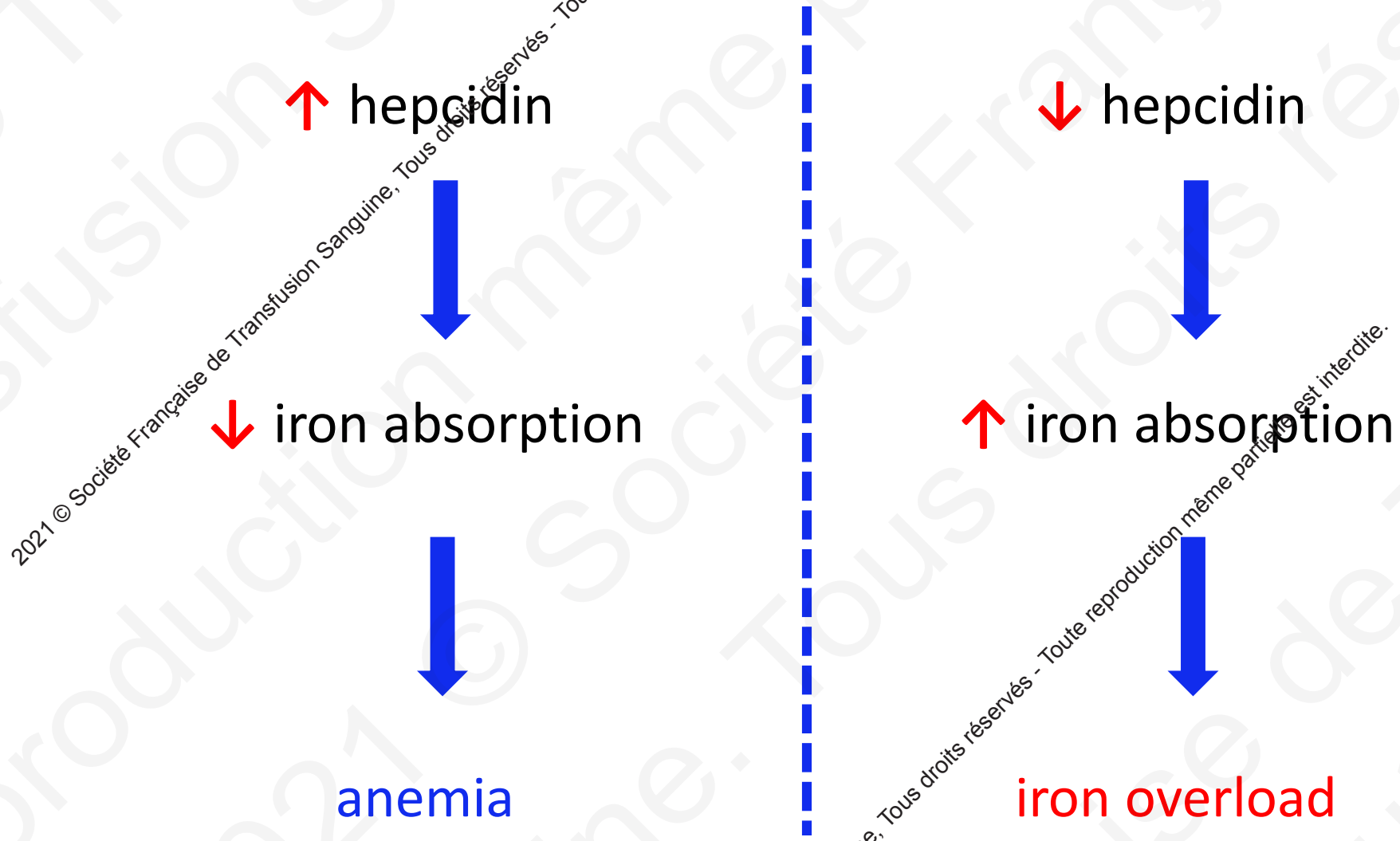
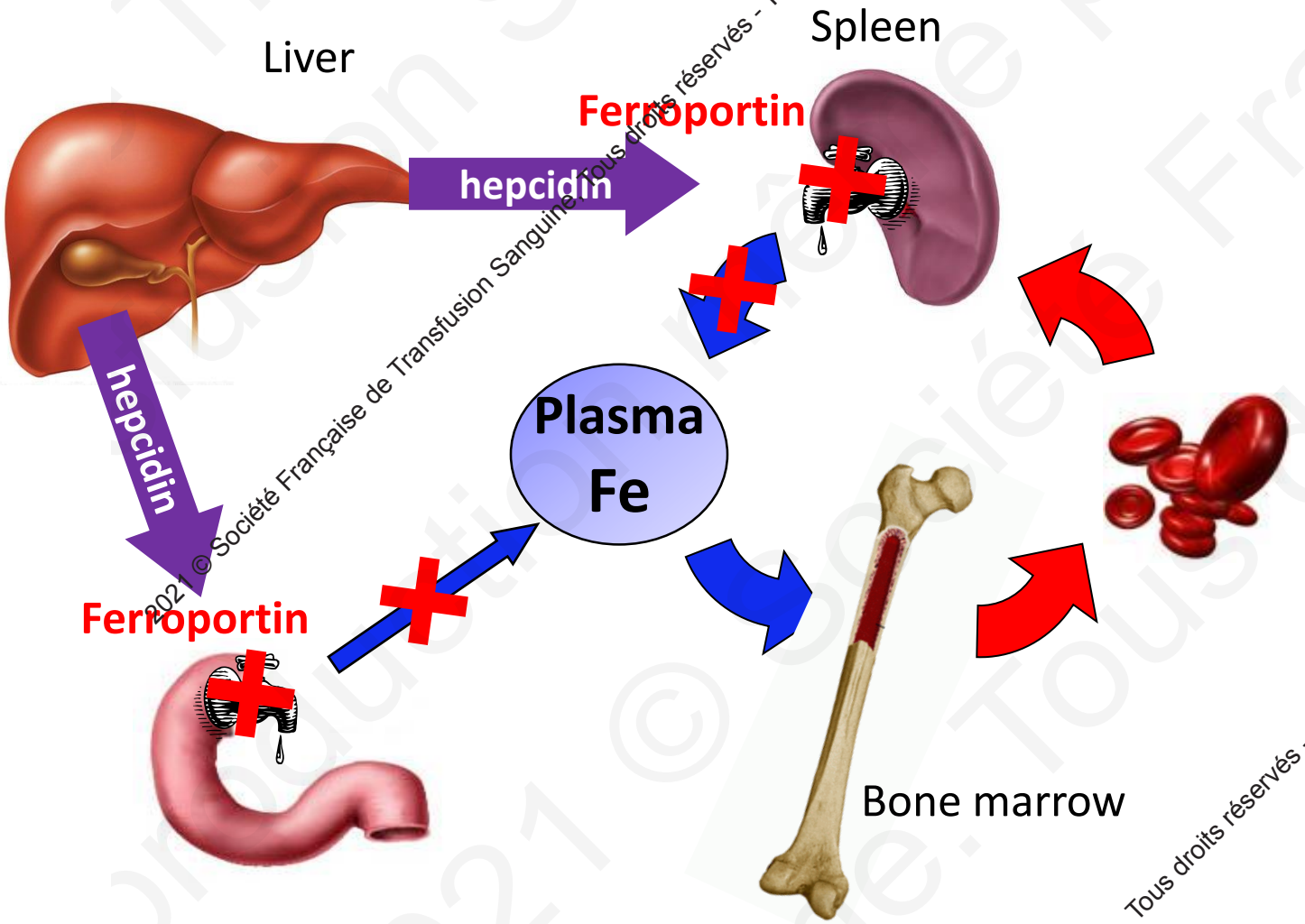


Signals directing the production of hepcidin



↑ HEPCIDIN

Iron

Inflammation

ER stress

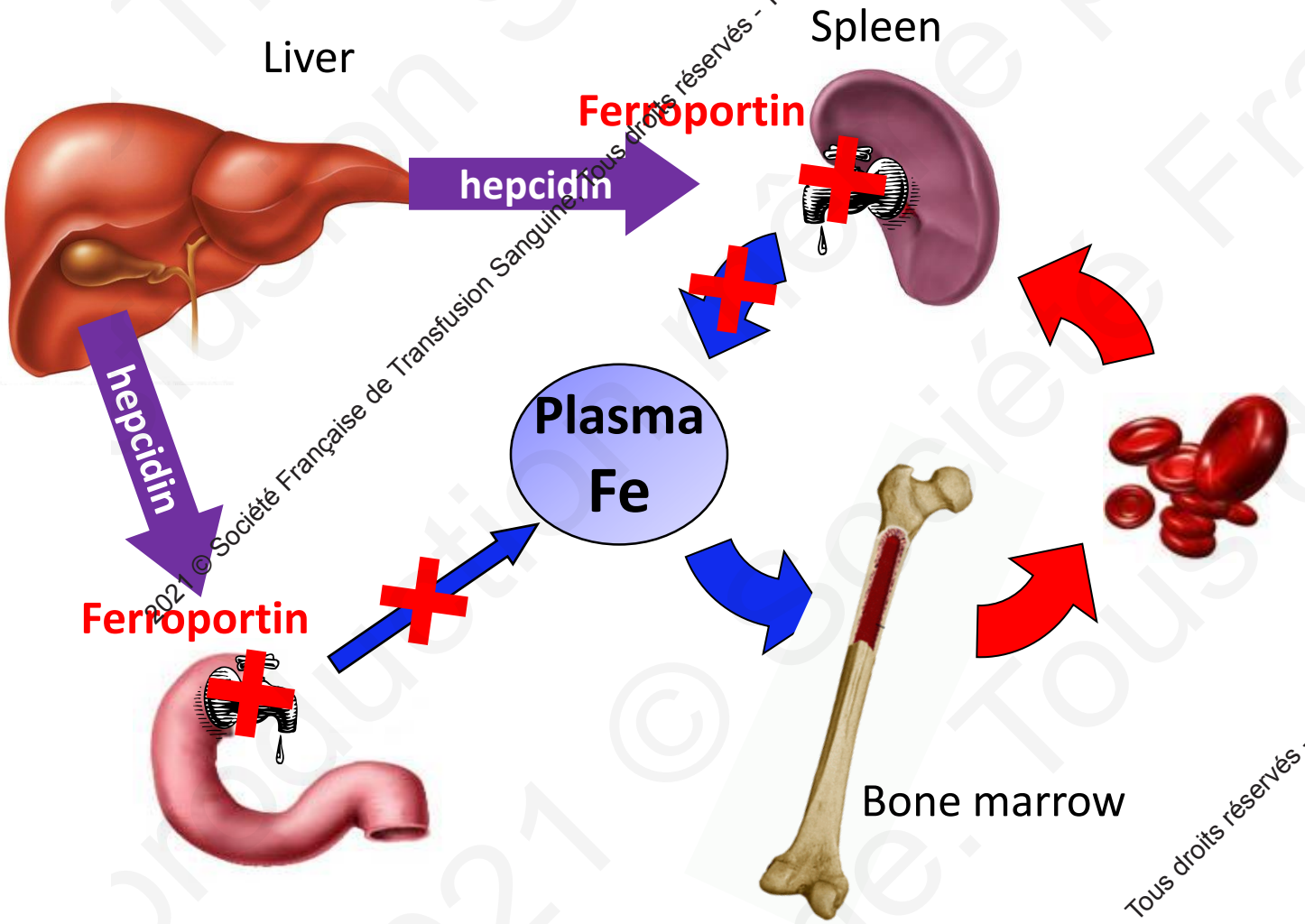
↓ HEPCIDIN

Iron deficiency

Hypoxia

Increased erythropoietic activity

Signals directing the production of hepcidin



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Iron

Inflammation

ER stress

↓ HEPCIDIN

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Hypoxia

Increased erythropoietic activity

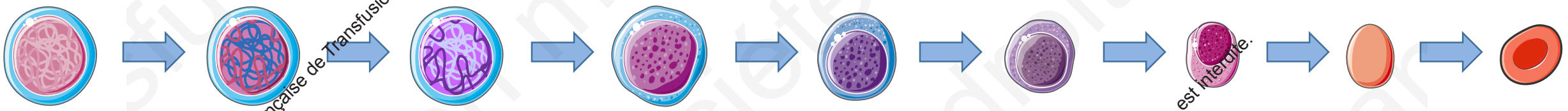
Erythropoiesis

Bone marrow

Blood

Erythroblasts

HSC → BFU-E → CFU-E → ProE → Basophilic → Polychromatic → Acidophil → Reticulocyte → Erythrocyte

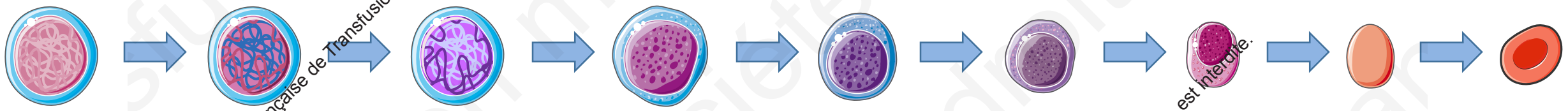


Erythropoiesis

Bone marrow | Blood

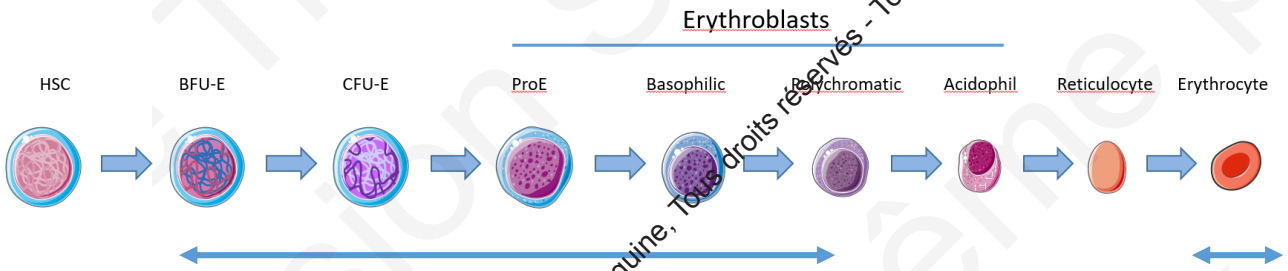
Erythroblasts

HSC → BFU-E → CFU-E → ProE → Basophilic → Polychromatic → Acidophil → Reticulocyte → Erythrocyte

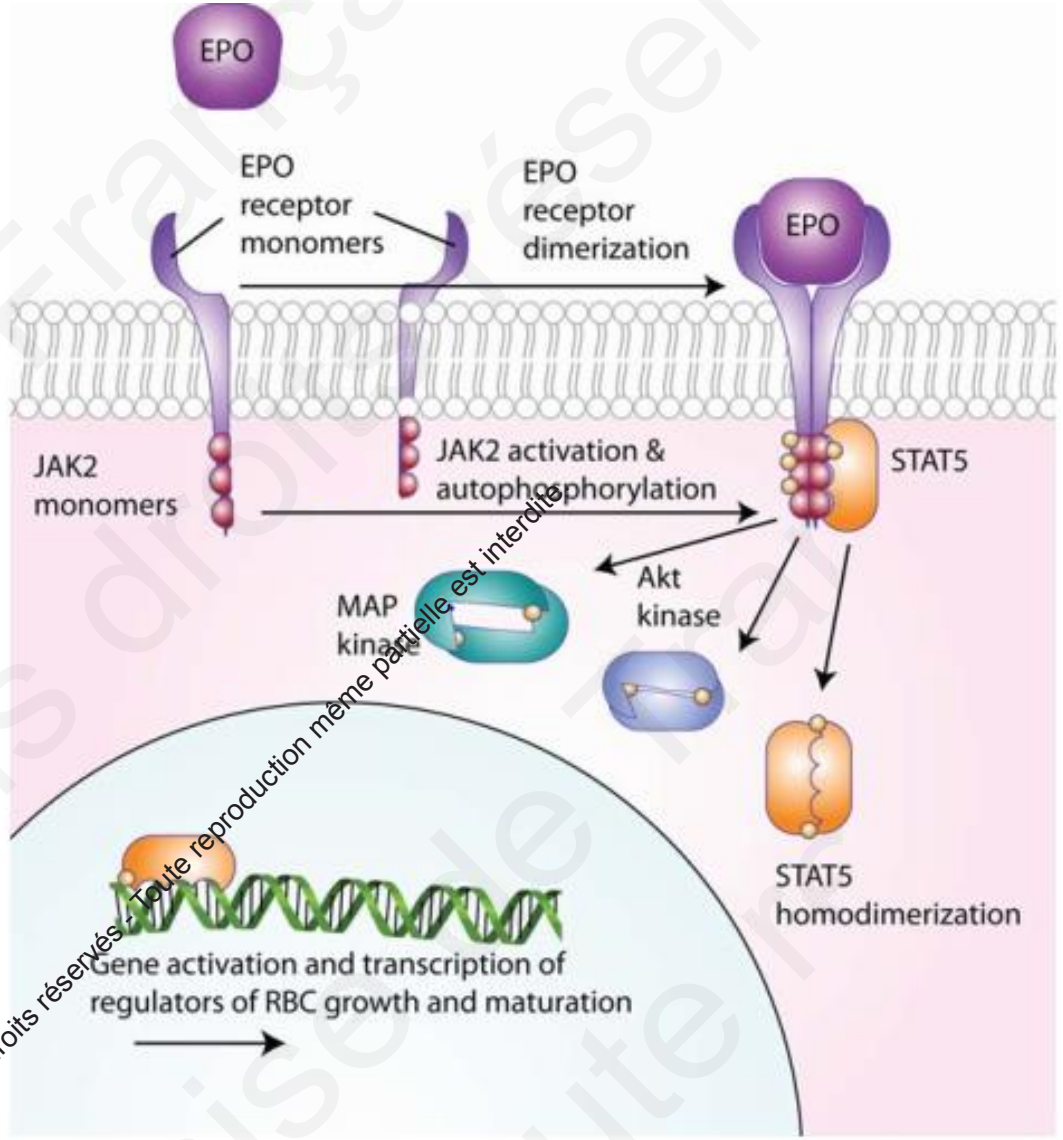


Erythropoietin (EPO) dependance

EPO is the master regulator of erythropoiesis



- Erythroid differentiation
- Erythroid proliferation
- Red cells survival



Sankaran VG, 2013

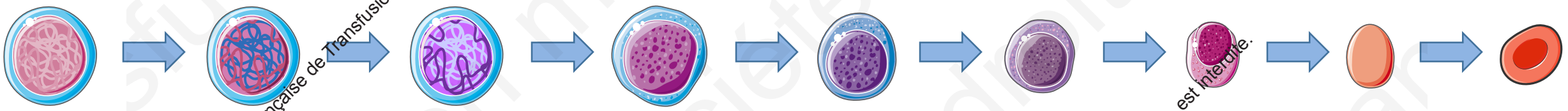
Erythropoiesis

Bone marrow

Blood

Erythroblasts

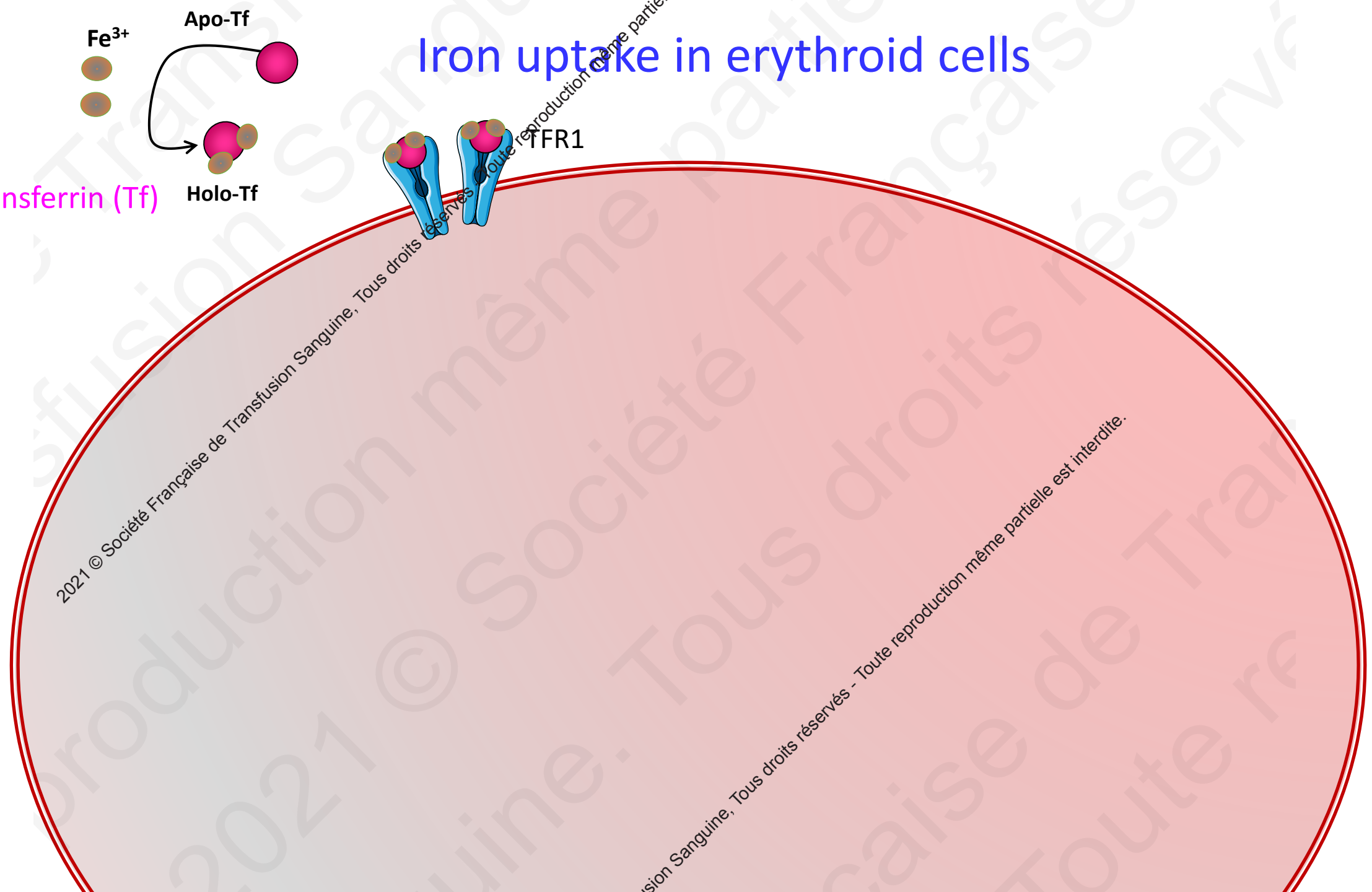
HSC BFU-E CFU-E ProE Basophilic Polychromatic Acidophil Reticulocyte Erythrocyte



EPO dependance

Iron dependance (~25mg/day)

Iron uptake in erythroid cells



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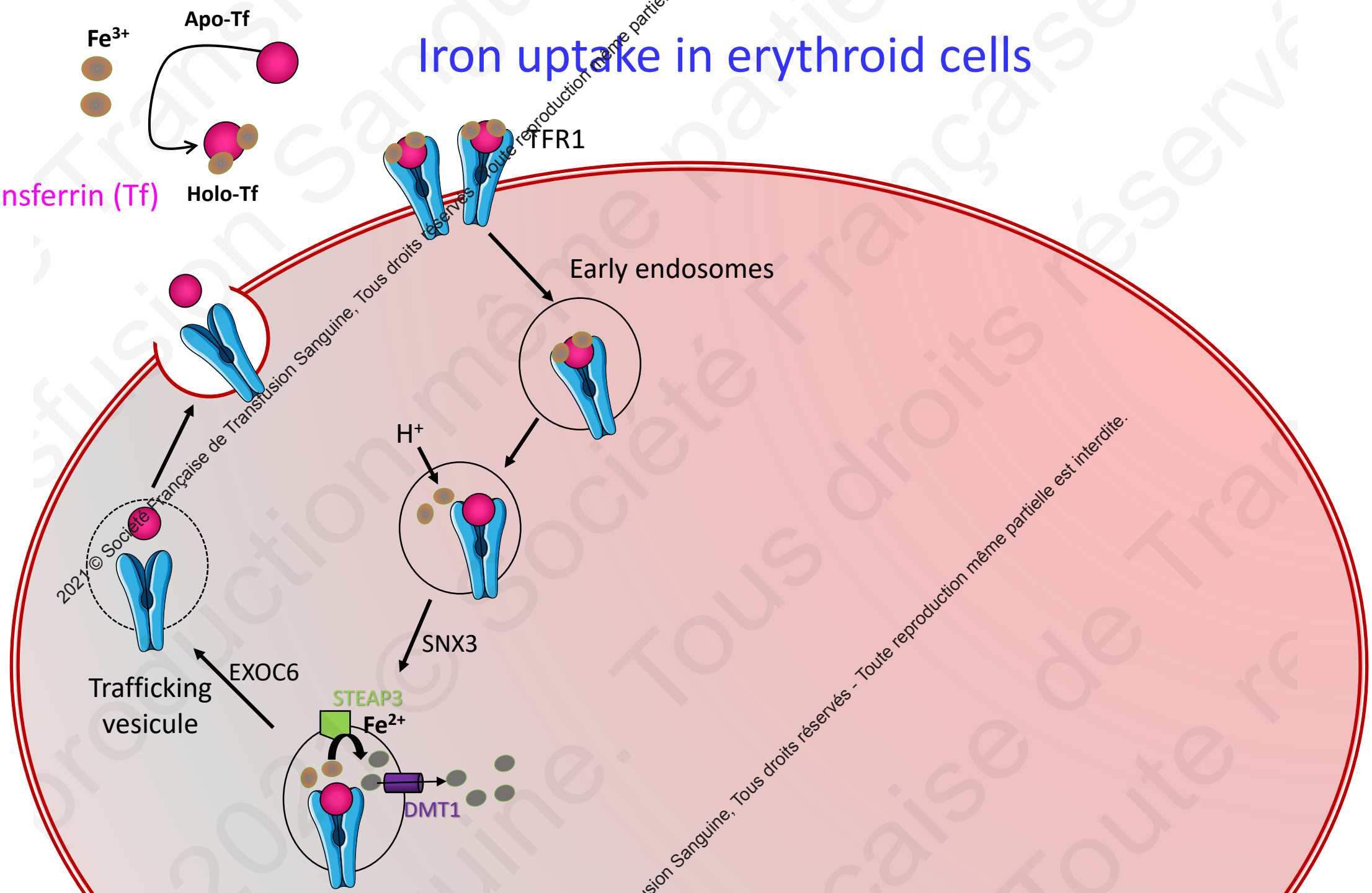
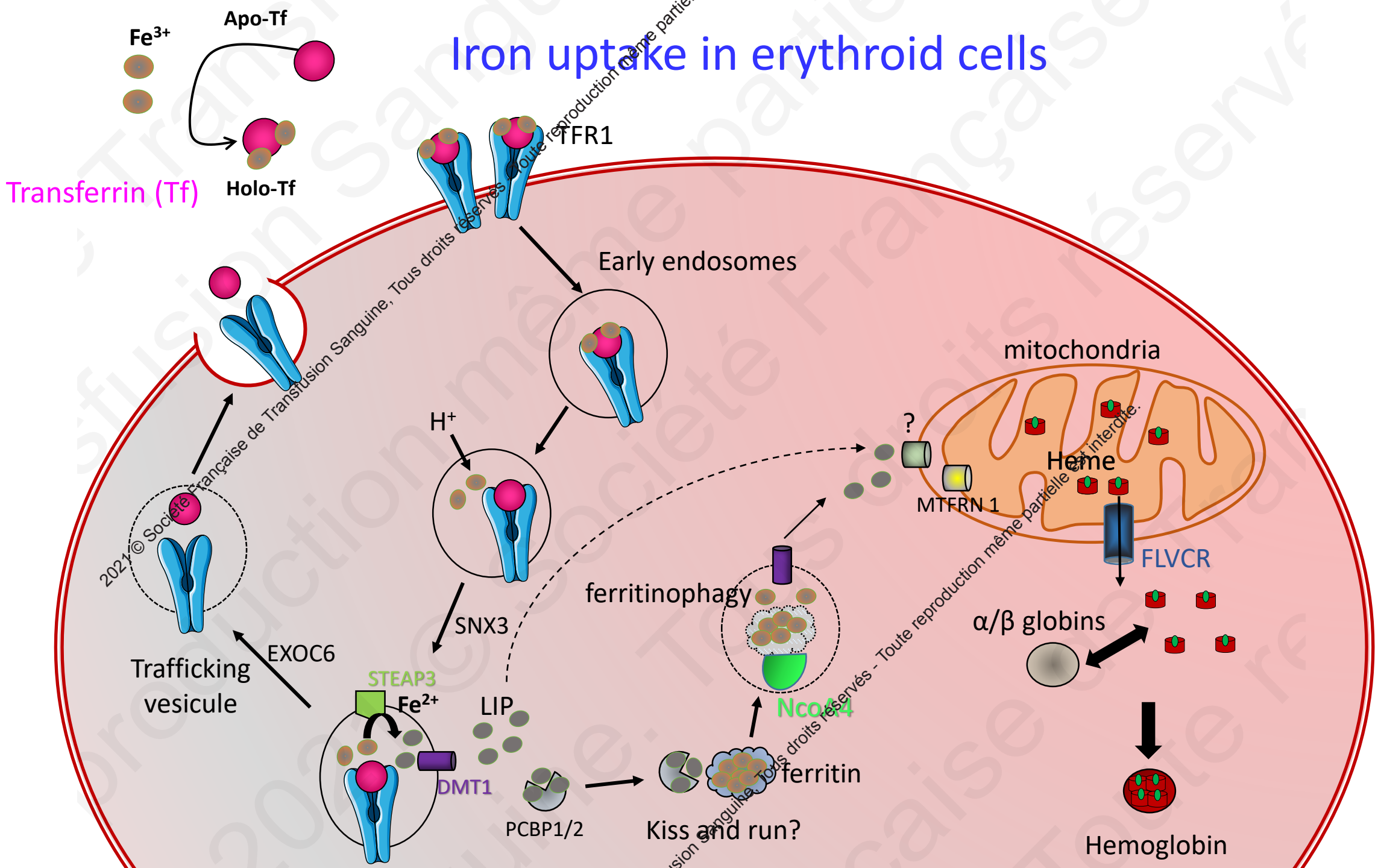


Iron uptake in erythroid cells



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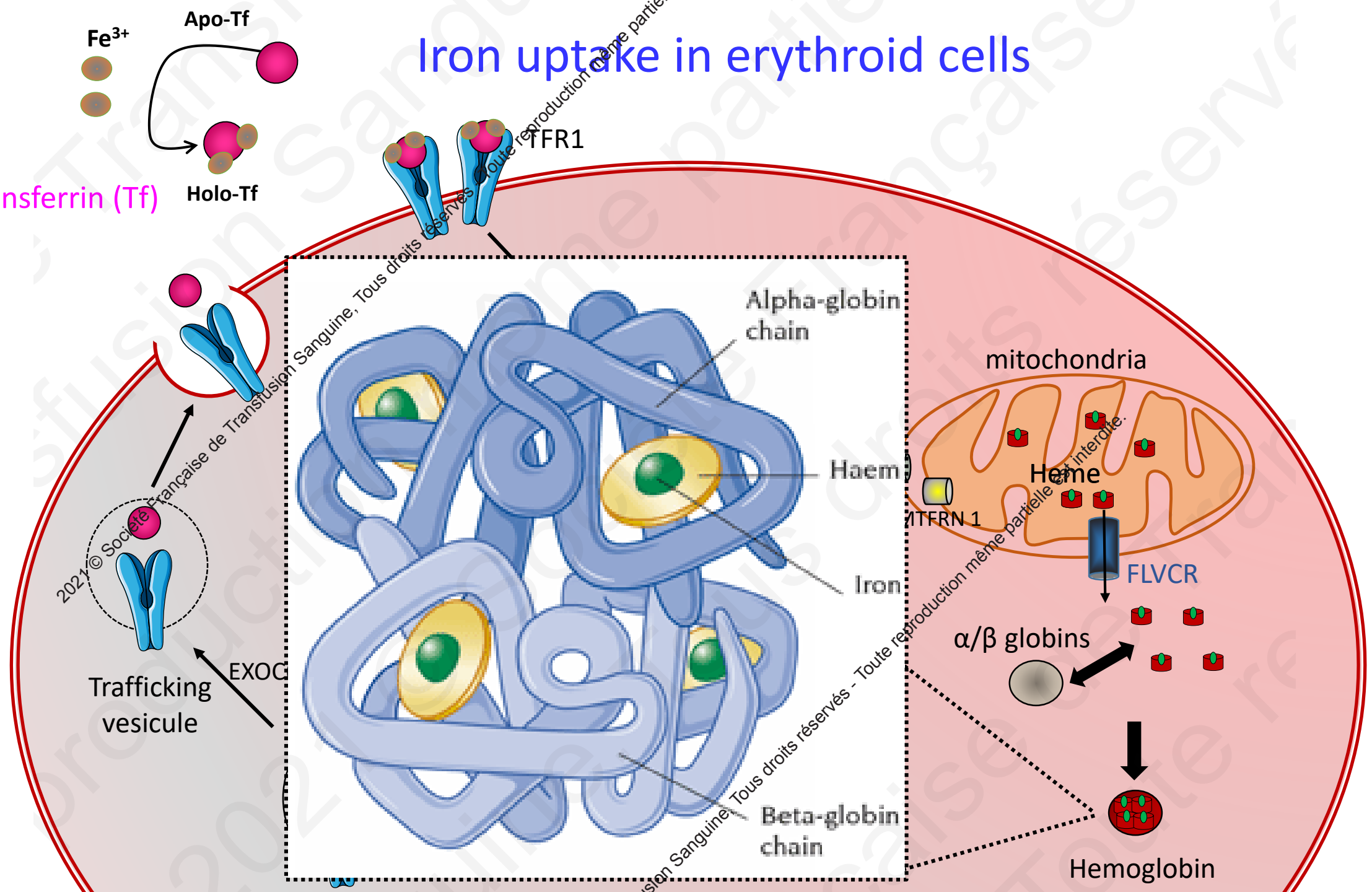


The Nobel Prize in Physiology or Medicine 2019

Ill. Niklas Elmehed. © Nobel Media.
William G. Kaelin Jr
Prize share: 1/3

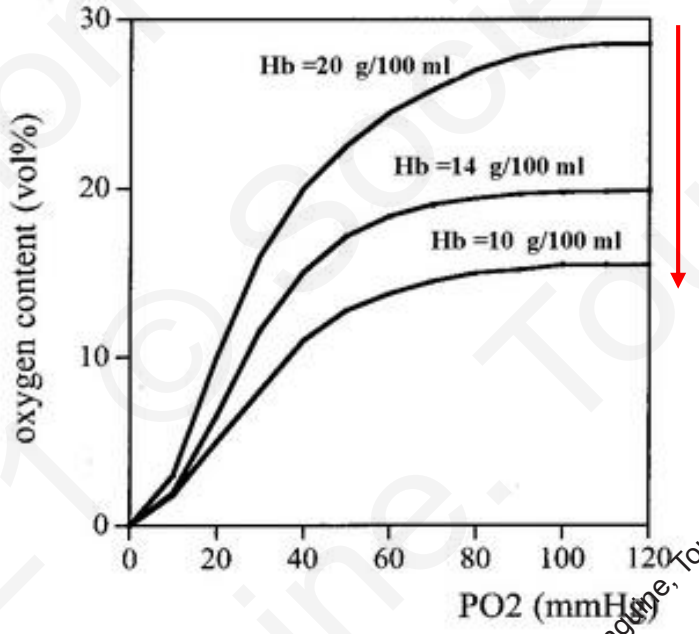
Ill. Niklas Elmehed. © Nobel Media.
Sir Peter J. Ratcliffe
Prize share: 1/3

Ill. Niklas Elmehed. © Nobel Media.
Gregg L. Semenza
Prize share: 1/3

Hypoxia

- Decreased tissue oxygen supply (generalized or local)
- Physiological response: altitude, strenous effort…
- Pathological condition: anemia (hemorrhage, hemolysis…), ischemia, ↓ hemoglobin concentration, ↓ Hb oxygen-carrying capacity, ↓ Hb-O_2 dissociation, genetic mutations

Description	pO2
Physiological	10%
Modest Hypoxia	2.5%
Moderate Hypoxia	0.5%
Severe Hypoxia	0.1%
Anoxia	0



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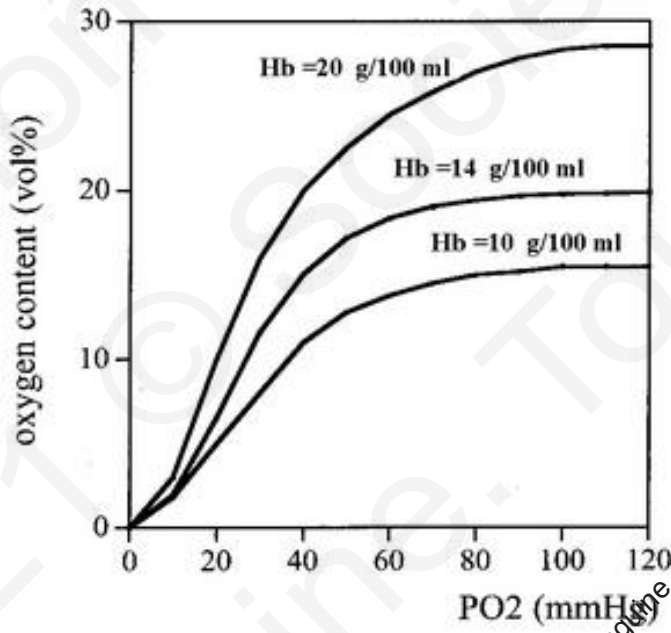
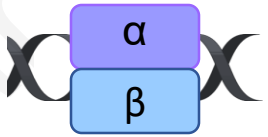


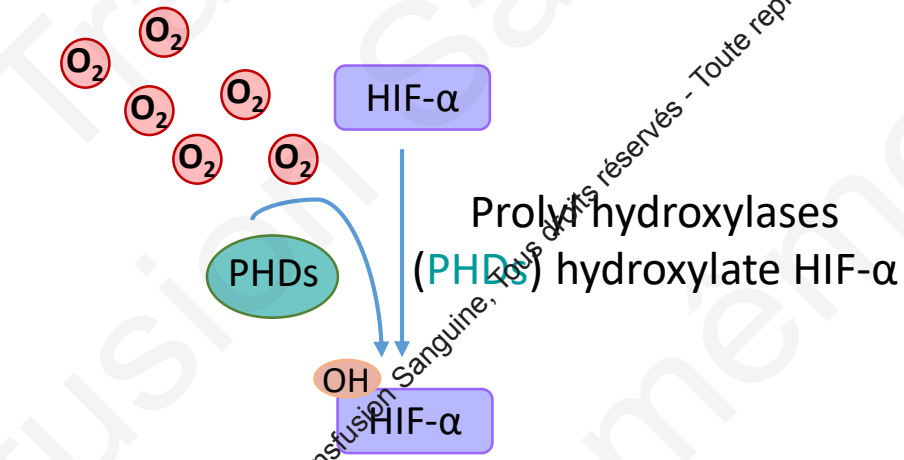
Hypoxia Inducible Factor
HIF



α sub-unit: specificity
HIF-1α
HIF-2α
HIF-3α

β sub-unit: constitutively expressed
HIF-1β (a.k.a ARNT)

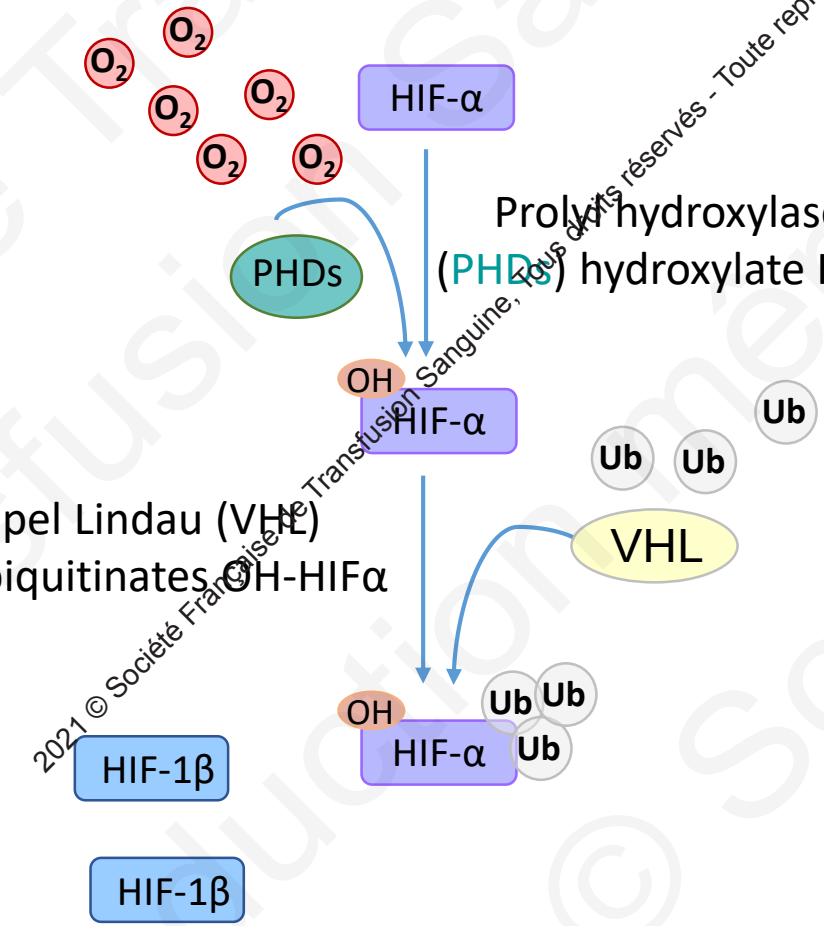
Normoxia



Prolyl hydroxylases (PHDs) hydroxylate HIF-α

Hypoxia

Normoxia

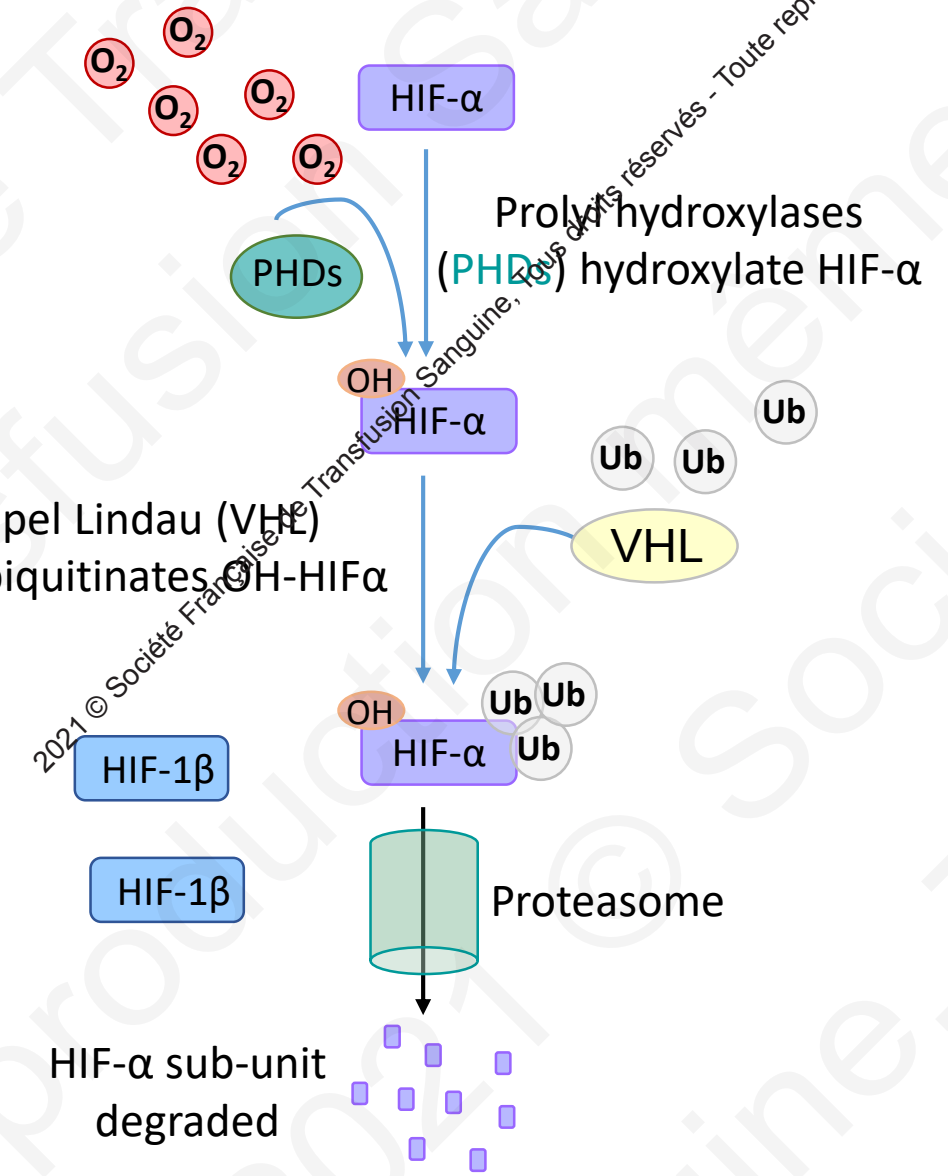


Prolyl hydroxylases (PHDs) hydroxylate HIF-α

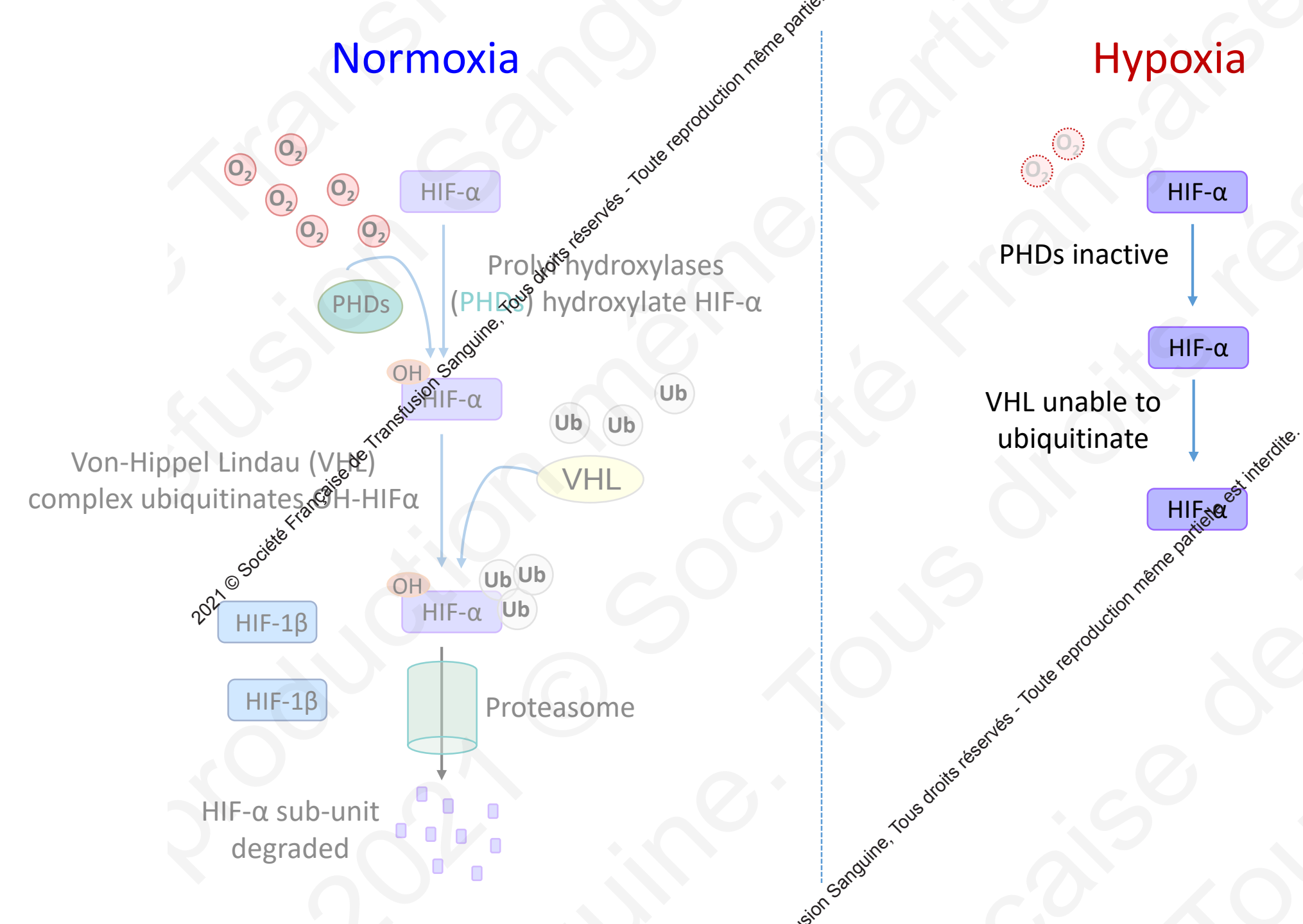
Von-Hippel Lindau (VHL) complex ubiquitinates OH-HIFα

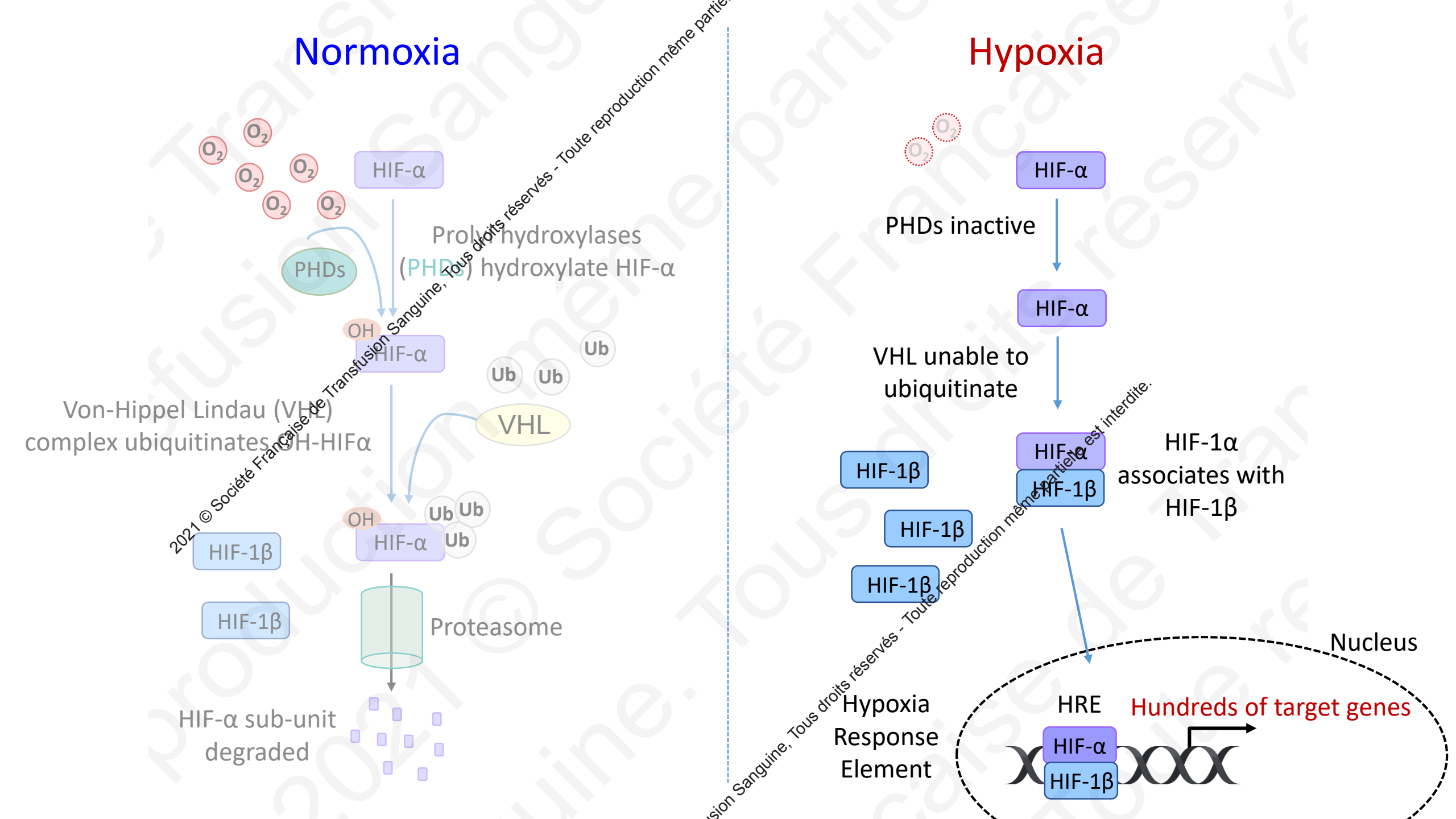
Hypoxia

Normoxia

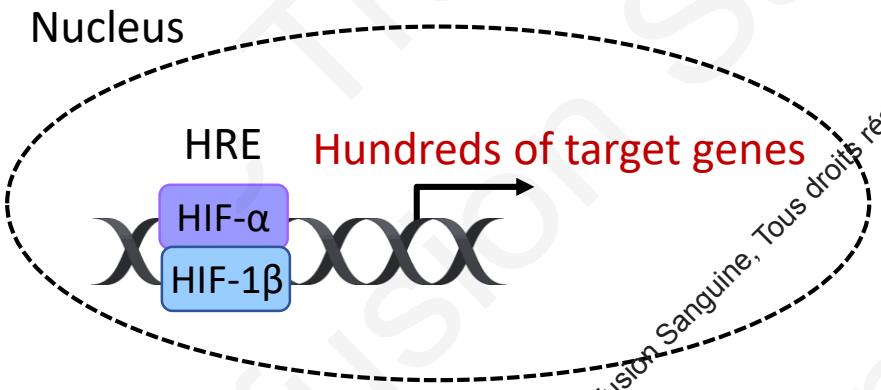


Hypoxia





Hypoxia-regulated genes



Genes controlling cellular oxygen homeostasis

Oxygen consumption

Erythrocyte production

Angiogenesis

Mitochondrial metabolism

Hallmarks of cancer

Metabolic reprogramming

Cell proliferation

Invasion and metastasis

Apoptosis

Resistance to therapies

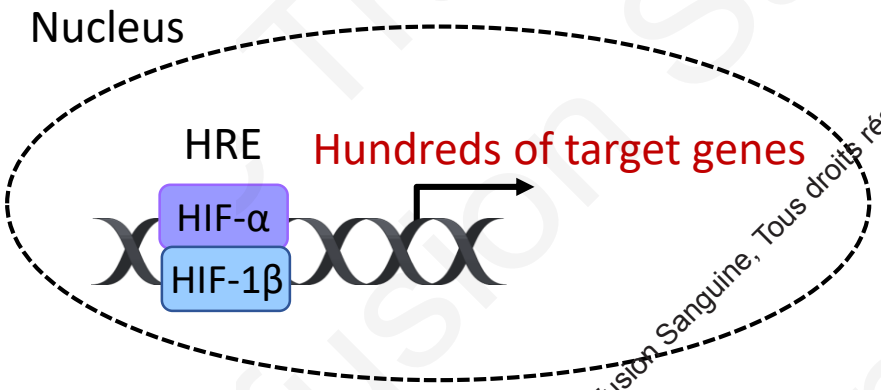
Epigenetic (methylation and acetylation)

Non coding RNAs (miRNAs, lncRNAs)

Biological clock

Cellular vesicles

Hypoxia-regulated genes



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Cellular vesicles

Physiological response to hypoxia

↓ O2

HIF2α

Increased iron absorption and recycling

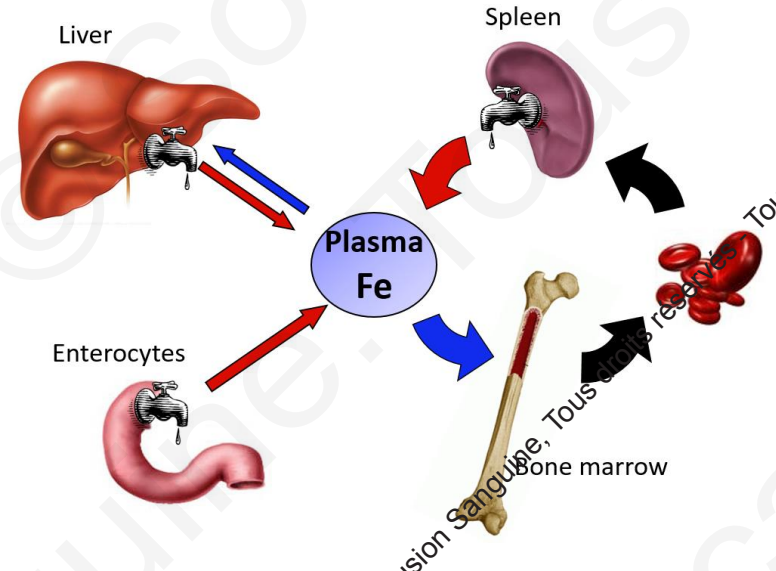
↑ Iron transporters

HRE

HIF-α

HIF-1β

gut



Physiological response to hypoxia

↓ O2

HIF2α

Increased iron absorption and recycling

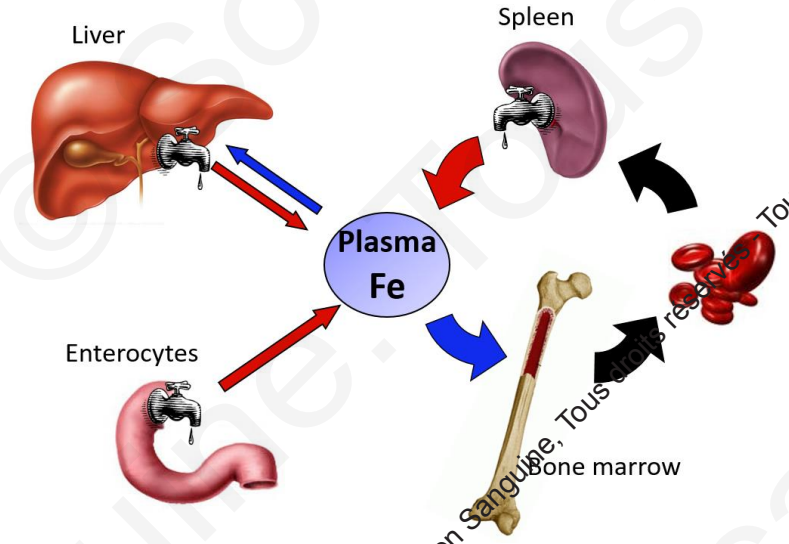
↑ Iron transporters

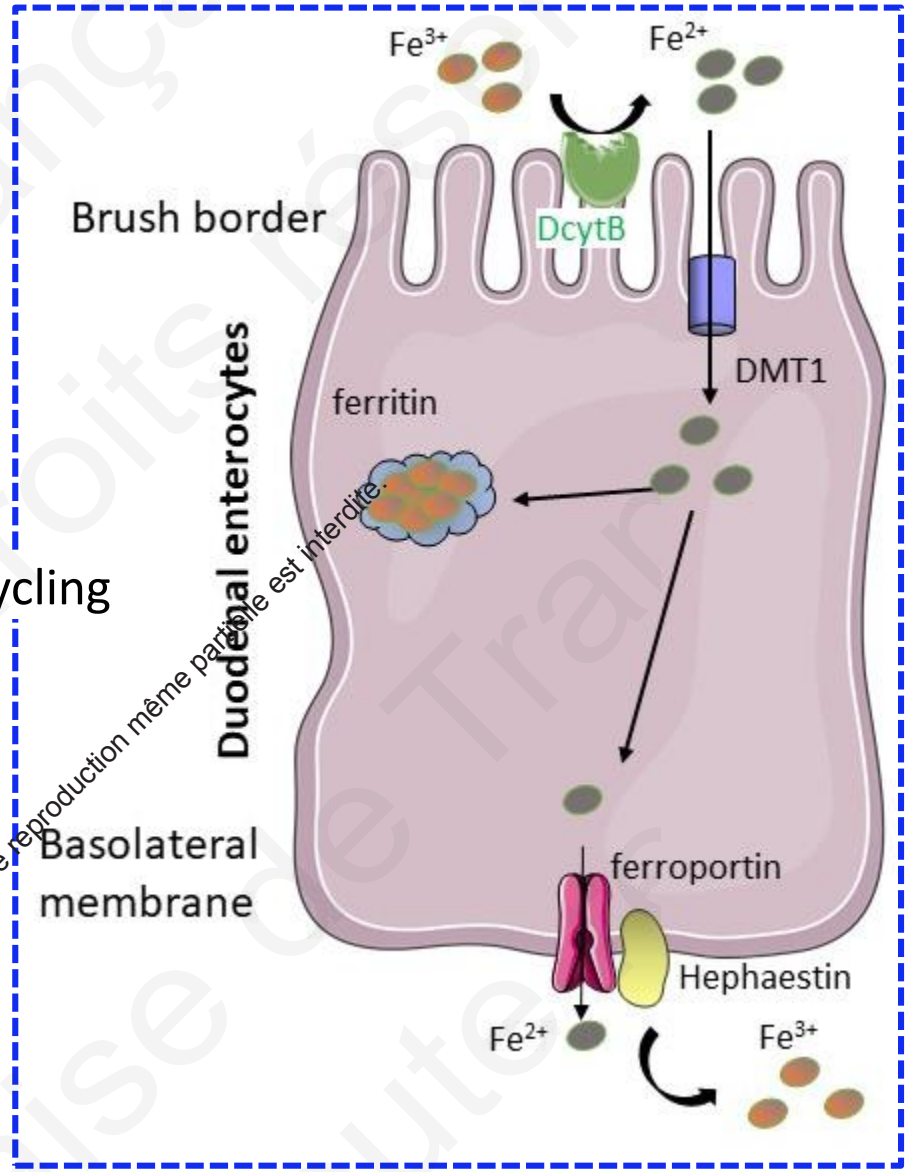
HRE

HIF-α

HIF-1β

gut





Physiological response to hypoxia

Kidney

HRE

HIF-α

HIF-1β

↑ EPO

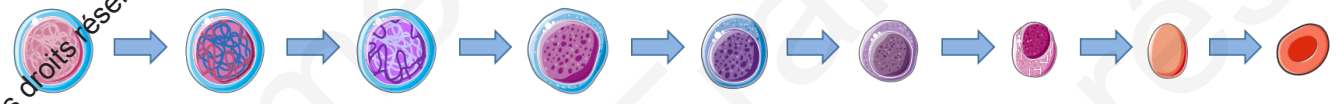
↓ O2

HIF2α

HIF2α

Erythroblasts

HSC BFU-E CFU-E ProE Basophilic Polychromatic Acidophil Reticulocyte Erythrocyte



Erythroid proliferation

Increased iron absorption and recycling

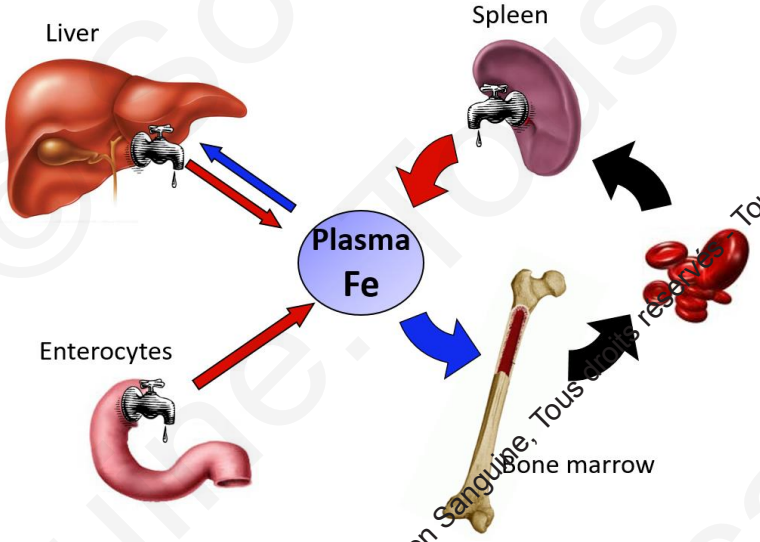
HRE

HIF-α

HIF-1β

↑ Iron transporters

gut



Physiological response to hypoxia

Kidney

HRE

HIF-α

HIF-1β

↑ EPO

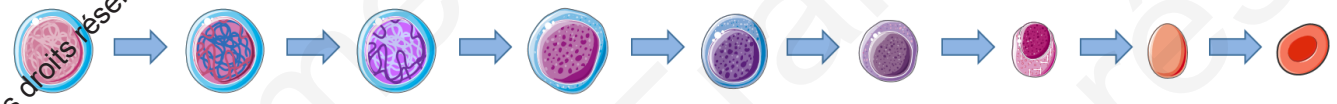
↓ O2

HIF2α

HIF2α

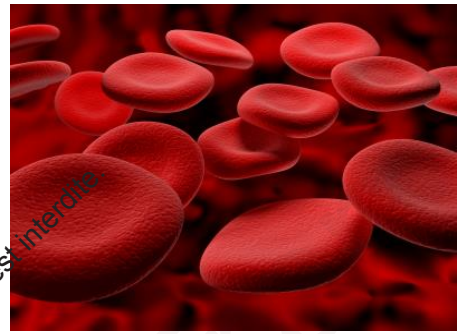
Erythroblasts

HSC BFU-E CFU-E ProE Basophilic Polychromatic Acidophil Reticulocyte Erythrocyte



Erythroid proliferation

Increased iron absorption and recycling



↑ O2

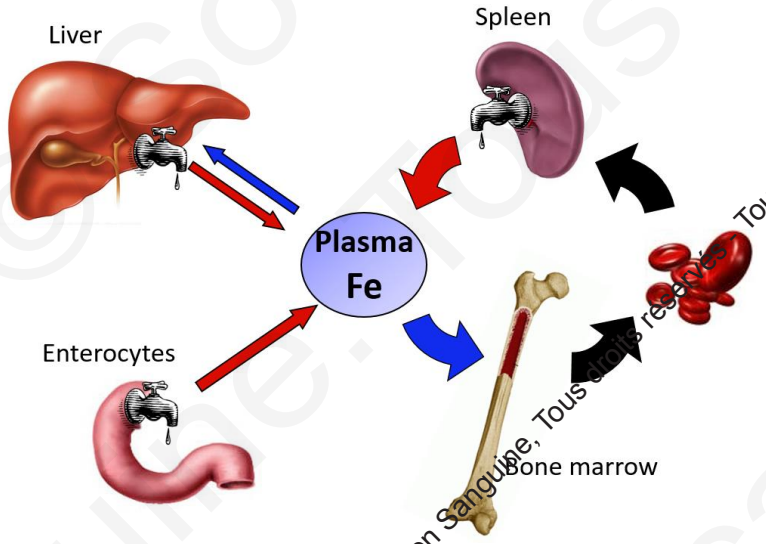
HRE

HIF-α

HIF-1β

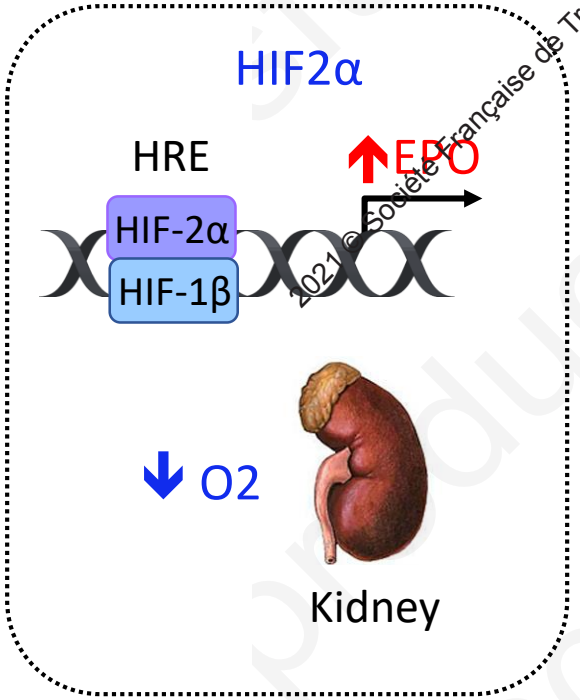
↑ Iron transporters

gut



Hypoxia stimulates EPO production and erythropoiesis

Erythropoietic stimulation (anemia, hypoxia)



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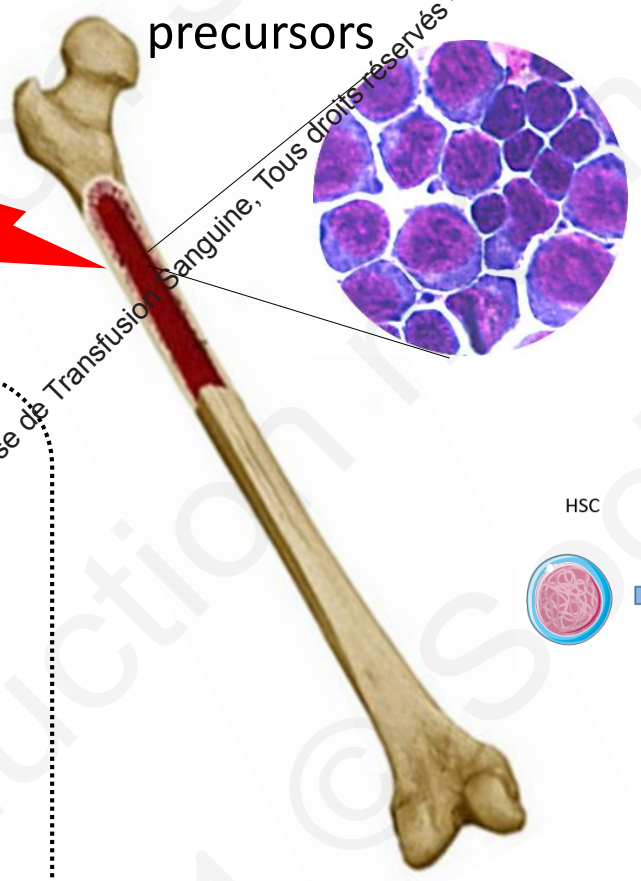
EPO

Erythroid precursors



HIF2α

HRE

↑EPO

HIF-2α

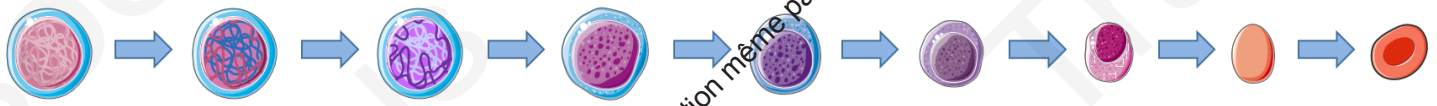
HIF-1β

↓ O2

Kidney

Erythroblasts

HSC → BFU-E → CFU-E → ProE → Basophilic → Polychromatic → Acidophil → Reticulocyte → Erythrocyte



Erythroid proliferation

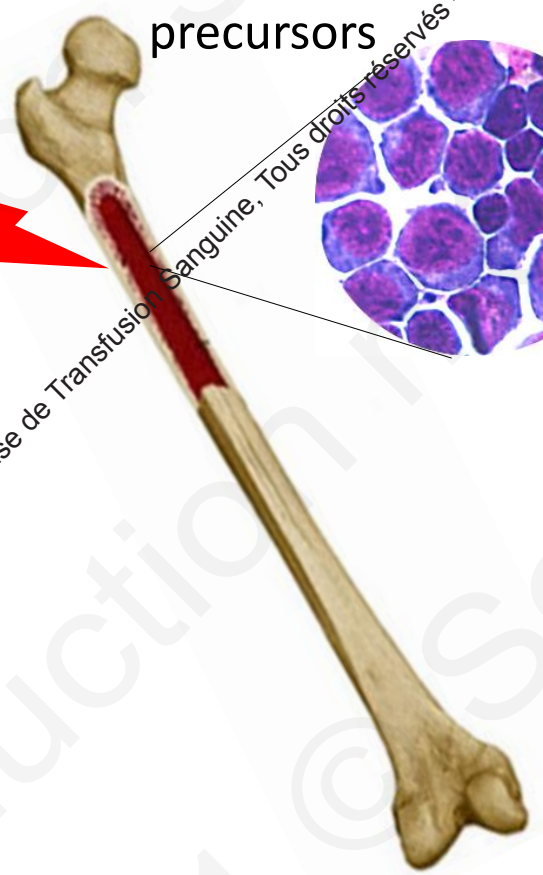
The “erythroid regulator” erythroferrone

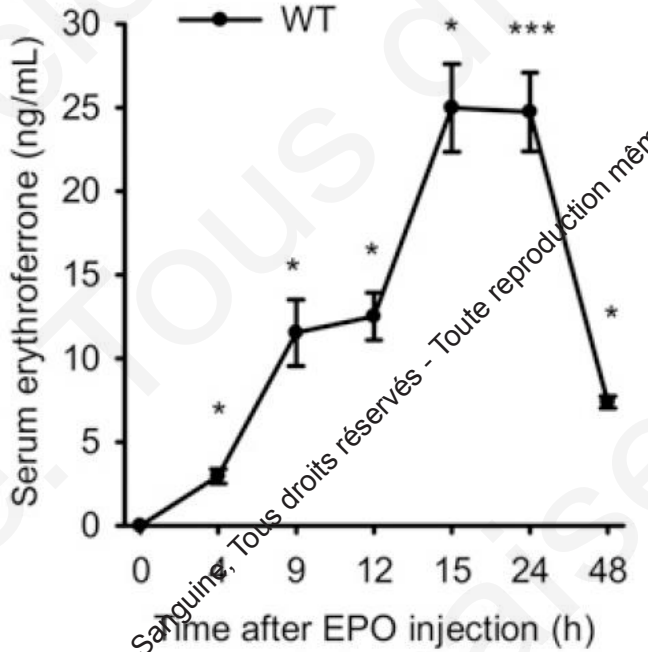
Erythropoietic stimulation (anemia, hypoxia)

EPO

Erythroid precursors

Erythroferrone (ERFE)





Kautz, Nat Genet 2014

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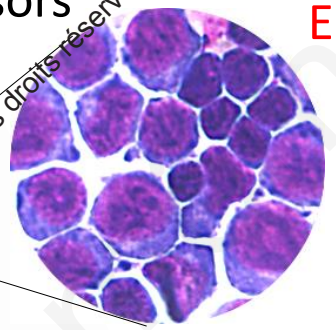
Erythropoietic stimulation (anemia, hypoxia)

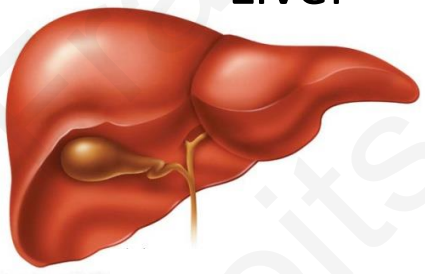
EPO

Erythroid precursors

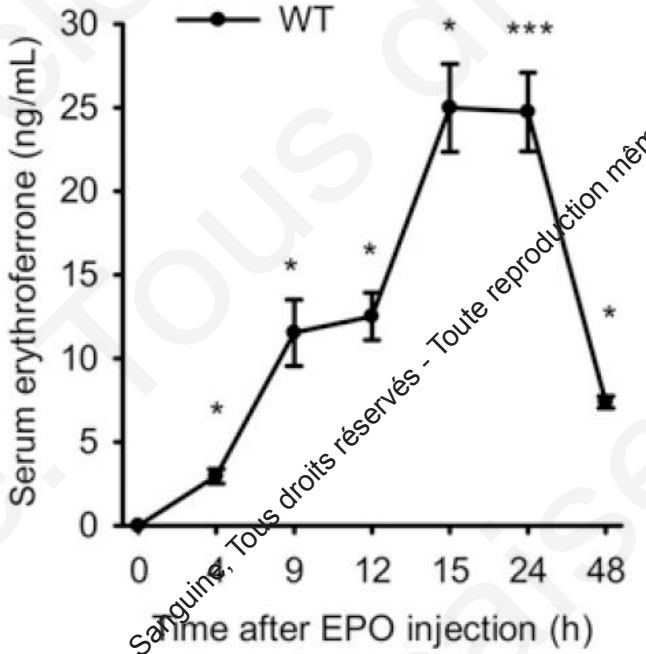


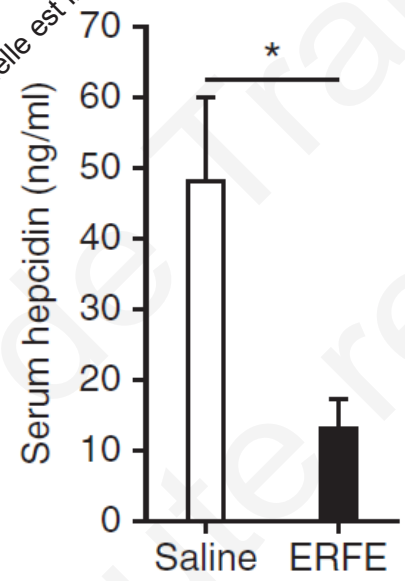
Erythroferrone (ERFE)

Liver



↓ Hepcidin





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Erythropoietic stimulation (anemia, hypoxia)

EPO

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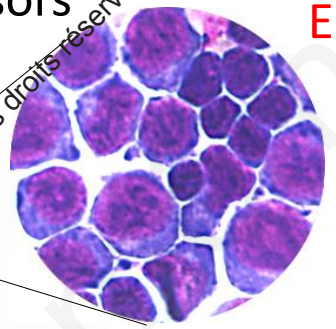
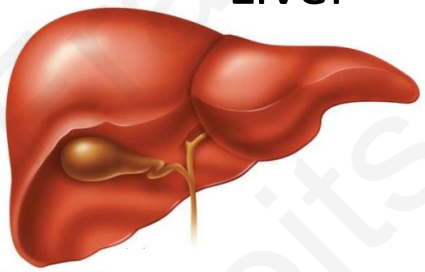


Erythroferrone (ERFE)

Liver



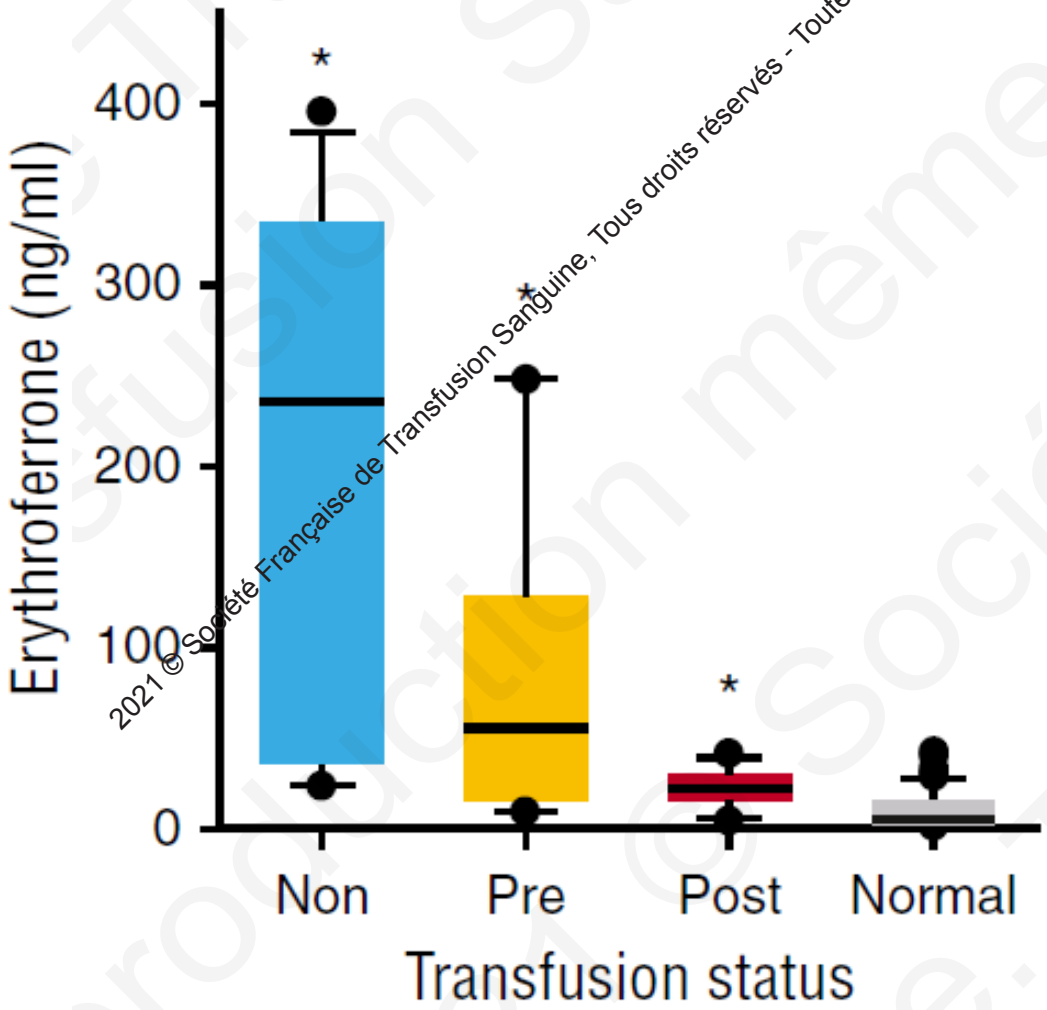
Hepcidin

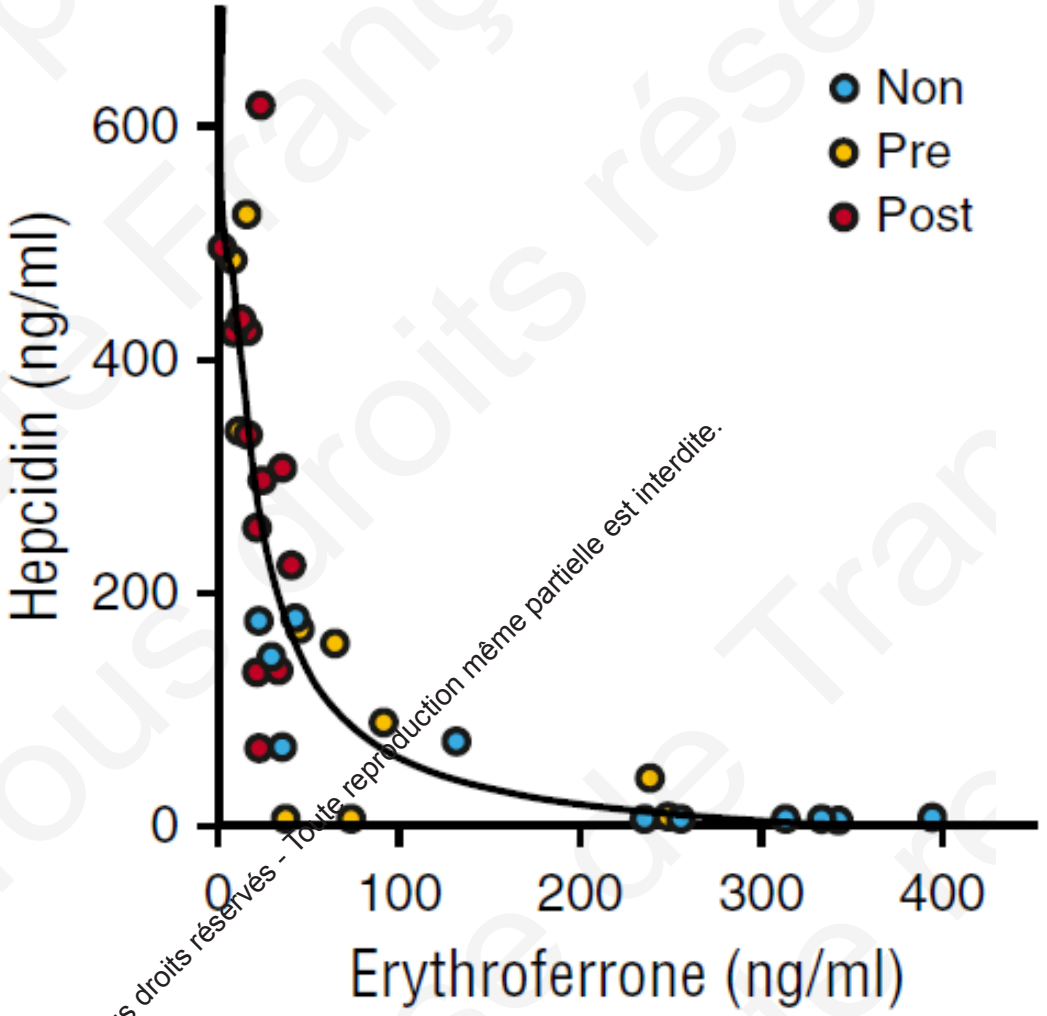
New red blood cells synthesis

iron availability

Kautz, Nat Genet 2014

Circulating ERFE levels are elevated in patients with β-thalassemia





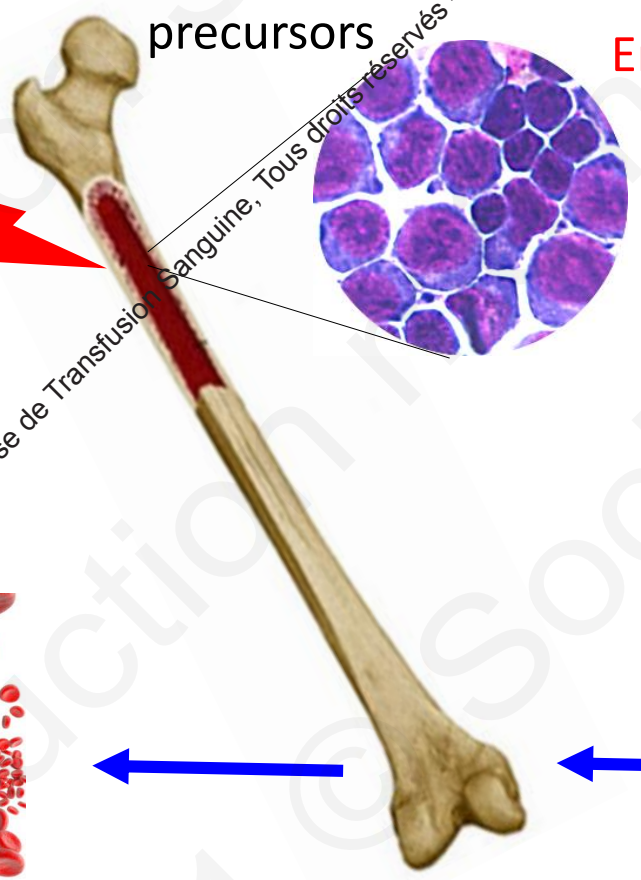
Ganz et al., Blood 2008

Hypoxia stimulates iron absorption through the inhibition of hepcidin

Erythropoietic stimulation (anemia, hypoxia)

EPO

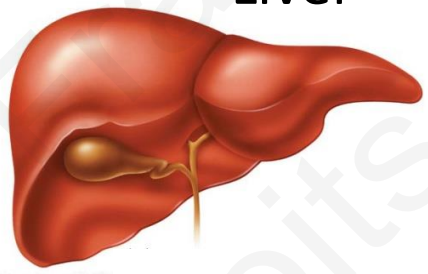
Erythroid precursors



Erythroferrone (ERFE)

GDF15
TWSG1

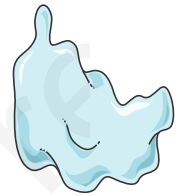
Liver



Hepcidin

PDGF-BB

Platelets



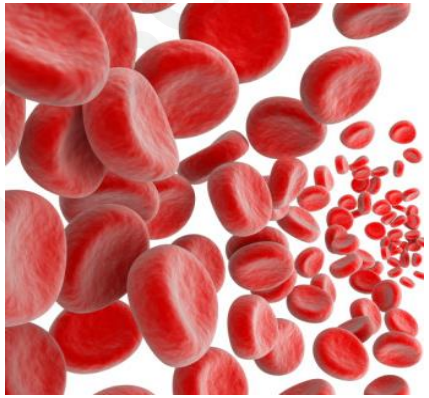
iron availability

New red blood cells synthesis

Tanno, Nat Med 2007
Tanno, Blood 2009
Sonnweber, Gut 2014

Disorders of iron homeostasis are common

- Iron restriction (iron insufficiency in tissues)
  - Anemia of inflammation
    - infections, inflammatory bowel disease, cancer…
  - Anemia of chronic kidney disease



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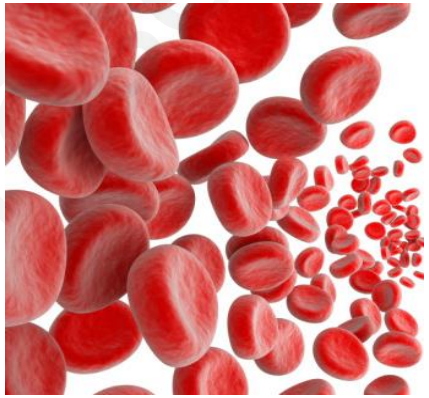

- Iron overload (toxicity from excess iron)
  - Hereditary hemochromatosis (1:300)
  - Iron-loading anemias (e.g. β-thalassemia, myelodysplastic syndromes)

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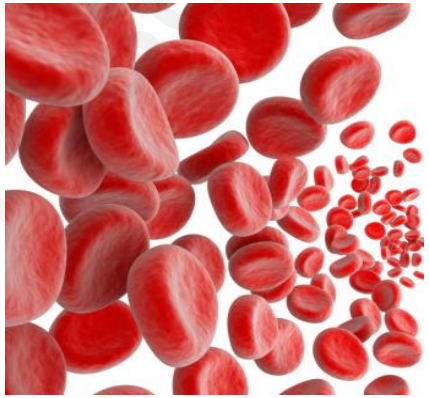

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Current treatments ineffective, costly and burdensome for the patients

→ New therapeutic strategies are much needed

Myelodyplastic syndromes (MDS)

Hematopoietic stem cells

Aging

Mutations

Clonal Expansion

Bone marrow

Aging

Progenitors

Precursors

Differentiation defects
« dysplasia »

Differentiated cells

Erythrocytes Platelets PN Monocytes Lymphocytes

cytopenia

80% of MDS patients present with anemia

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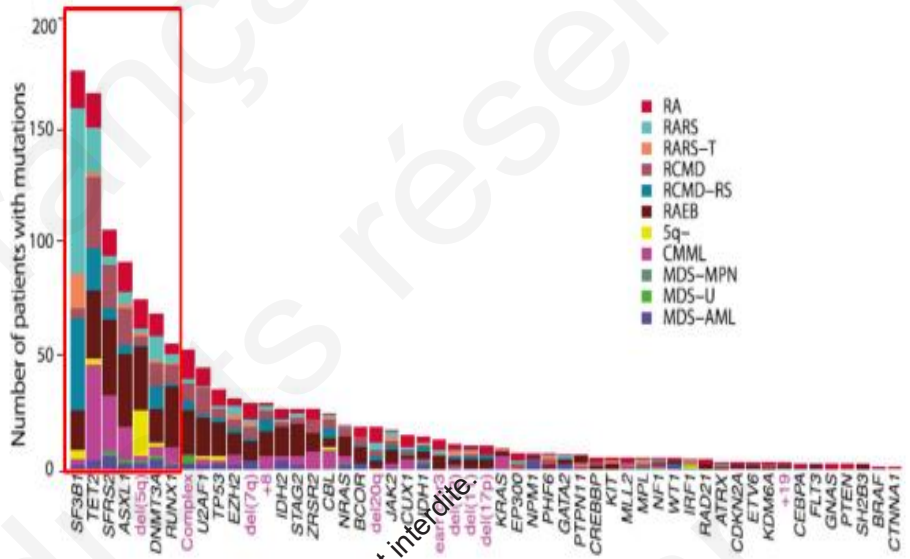
80% of MDS patients present with anemia

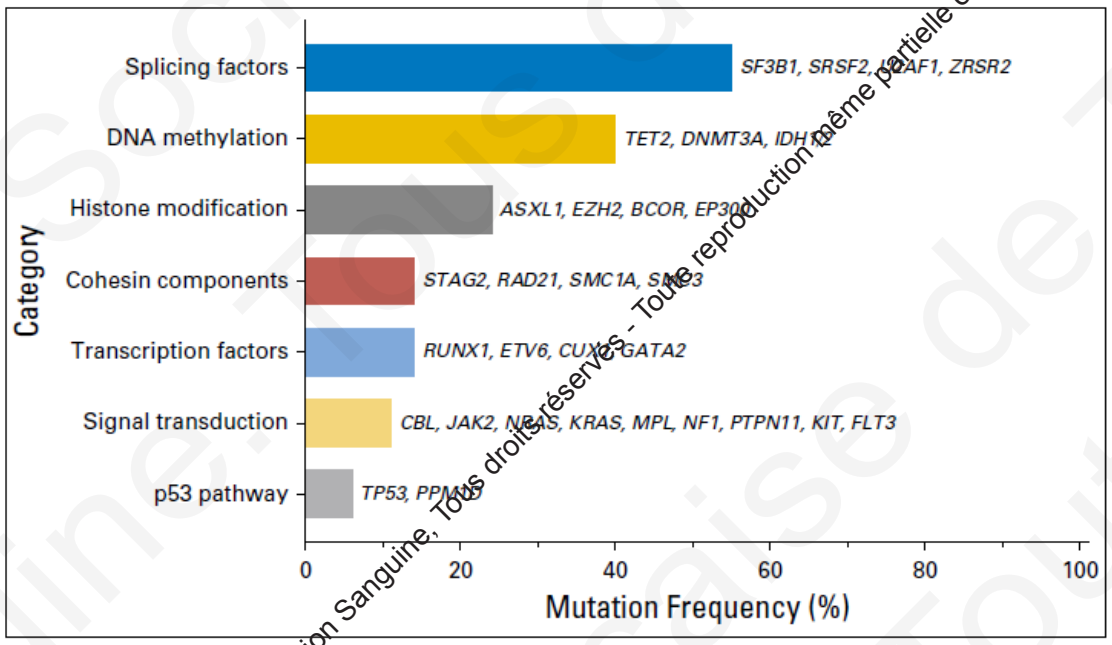
Classification of MDS (WHO 2016)

6 groups of MDS depending on the type and number of cytopenia

- MDS single lineage
- MDS multi lineage
- MDS with ring sideroblasts (single or multi lineage)
- MDs with isolated del(5q)
- MDS with excess blasts
- MDS unclassified



Papaemmanuil, Blood, 2013



Kennedy and Ebert, JCO, 2017

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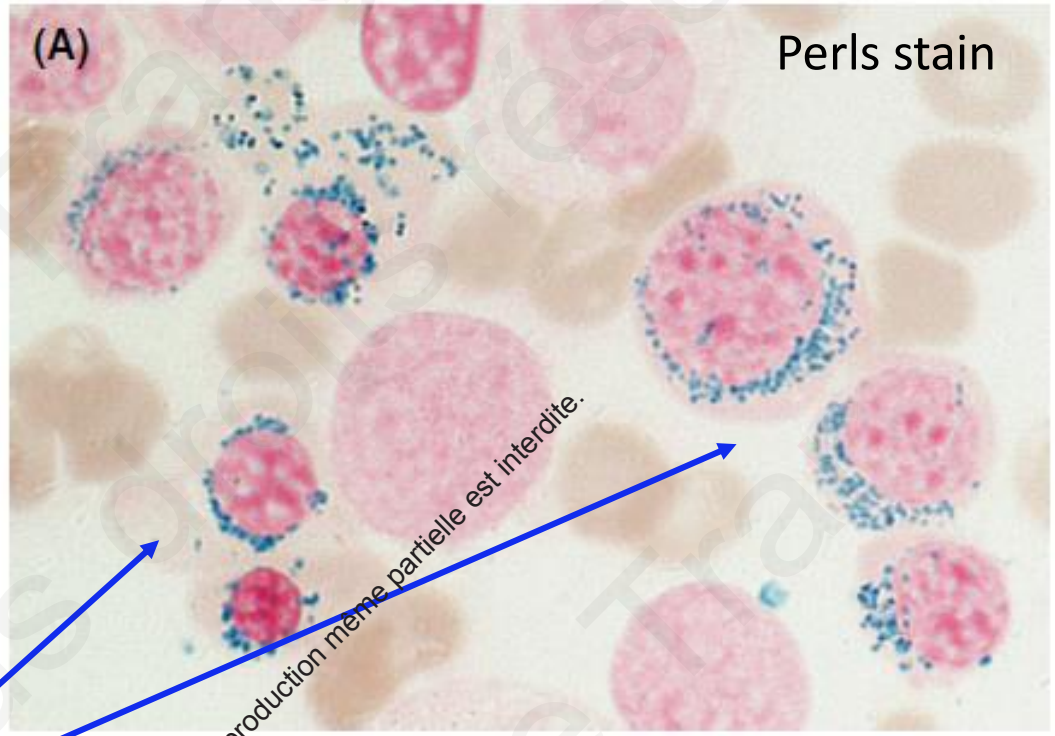


Malcovati, Blood 2016

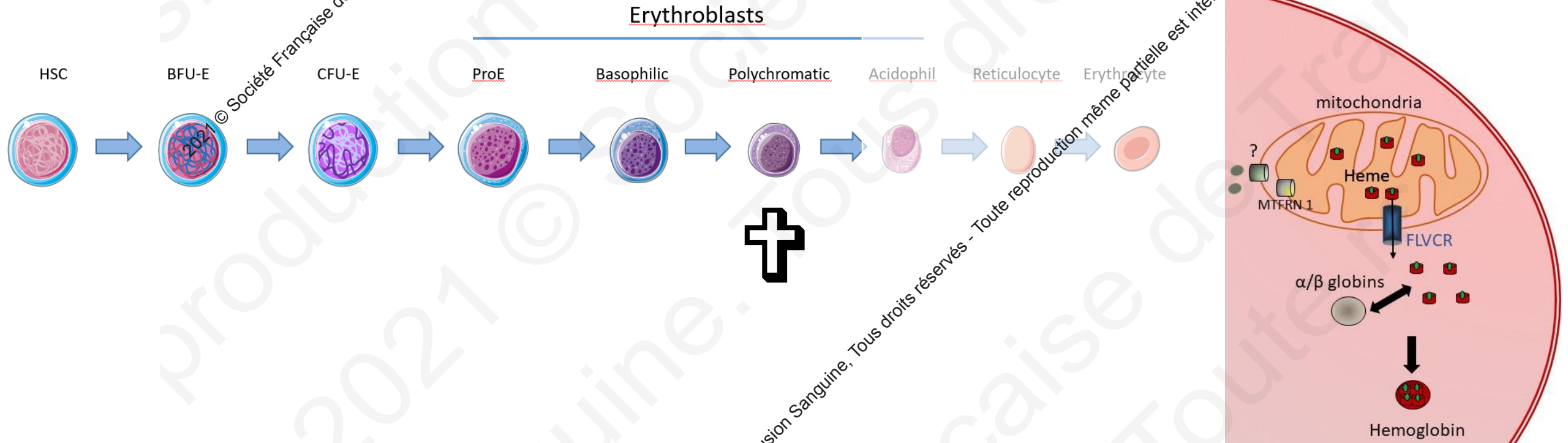
Ring sideroblasts
(sidéroblastes en couronne)
90% of patients carry mutations in SF3B1 gene

Hb < 8 g/dL

Pathophysiology of MDS with ring sideroblasts

- Iron accumulation in mitochondria
- Premature cell death of erythroid precurors

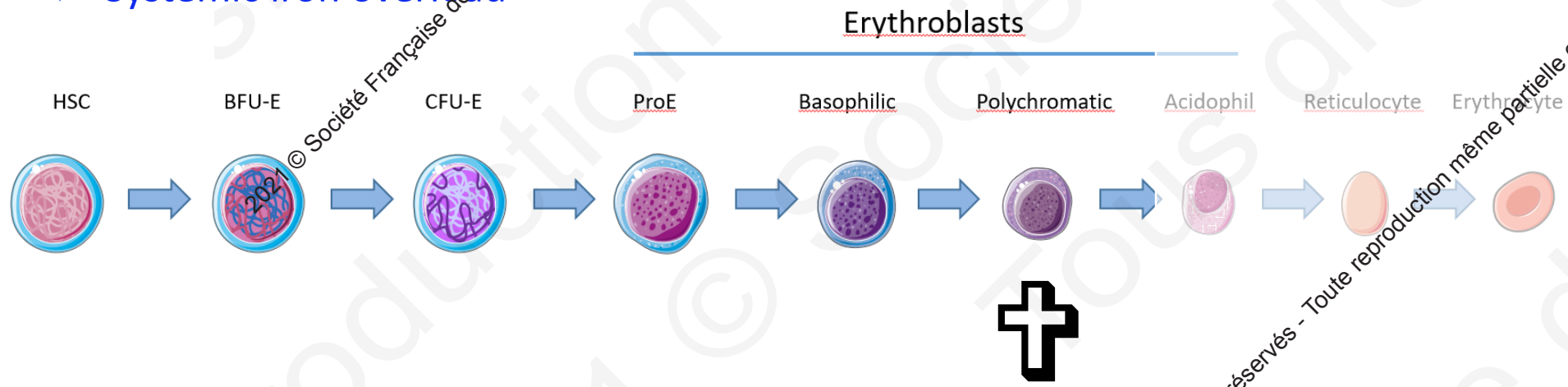
→ Insufficient production of red blood cells - anemia

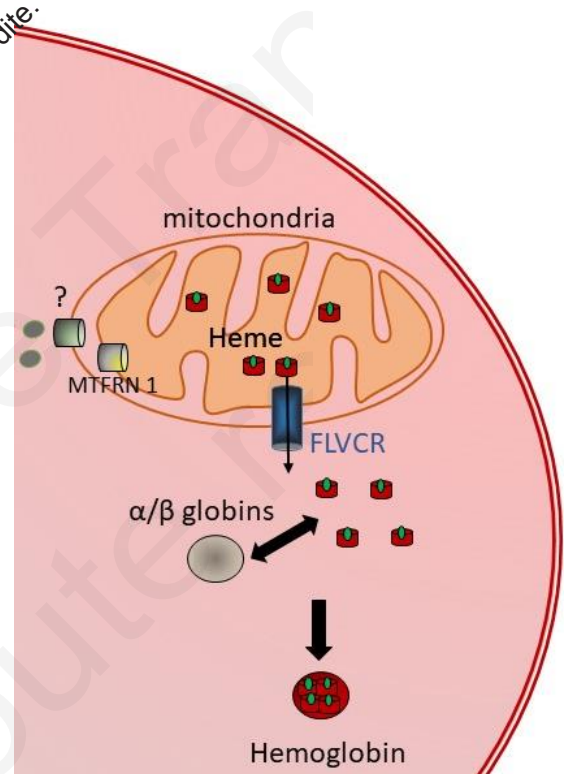


Pathophysiology of MDS with ring sideroblasts

- Iron accumulation in mitochondria
- Premature cell death of erythroid precurors
- Ineffective erythropoiesis
- Systemic iron overload

→ Insufficient production of red blood cells - anemia





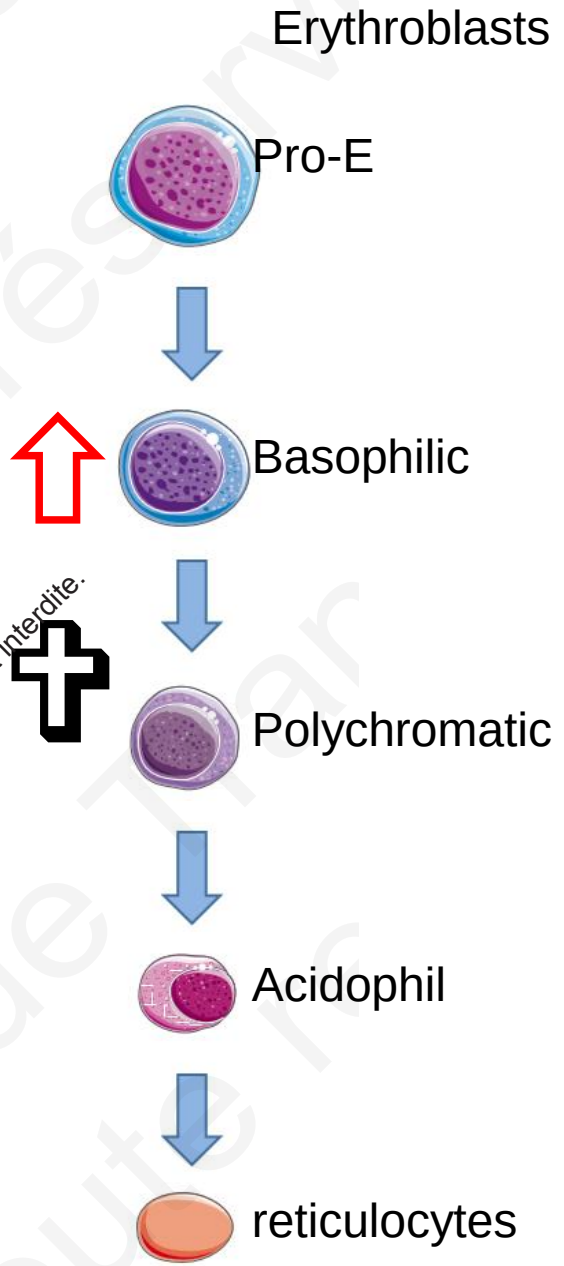
Ineffective erythropoiesis in MDS

- Cytopenia caused by apoptosis of early erythroid progenitors

↑ Apoptosis of early erythroid progenitors

→ Anemia / hypoxia

→ HIF stabilized



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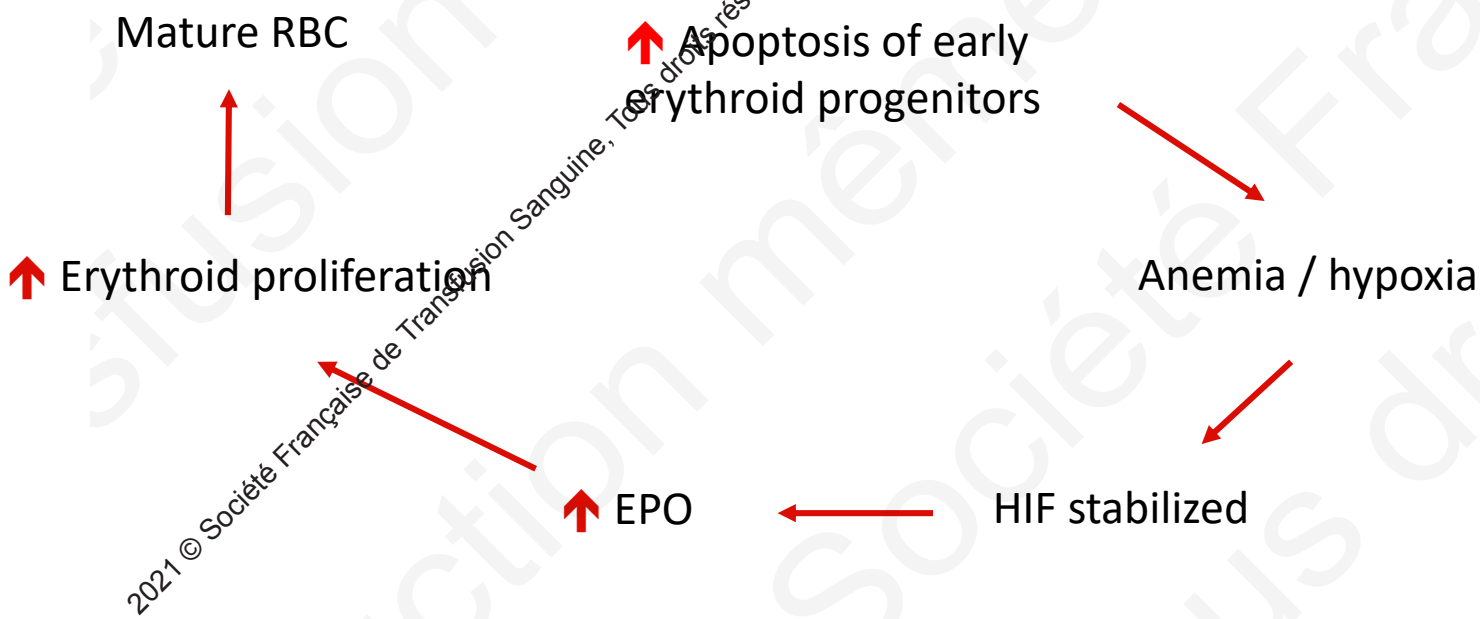

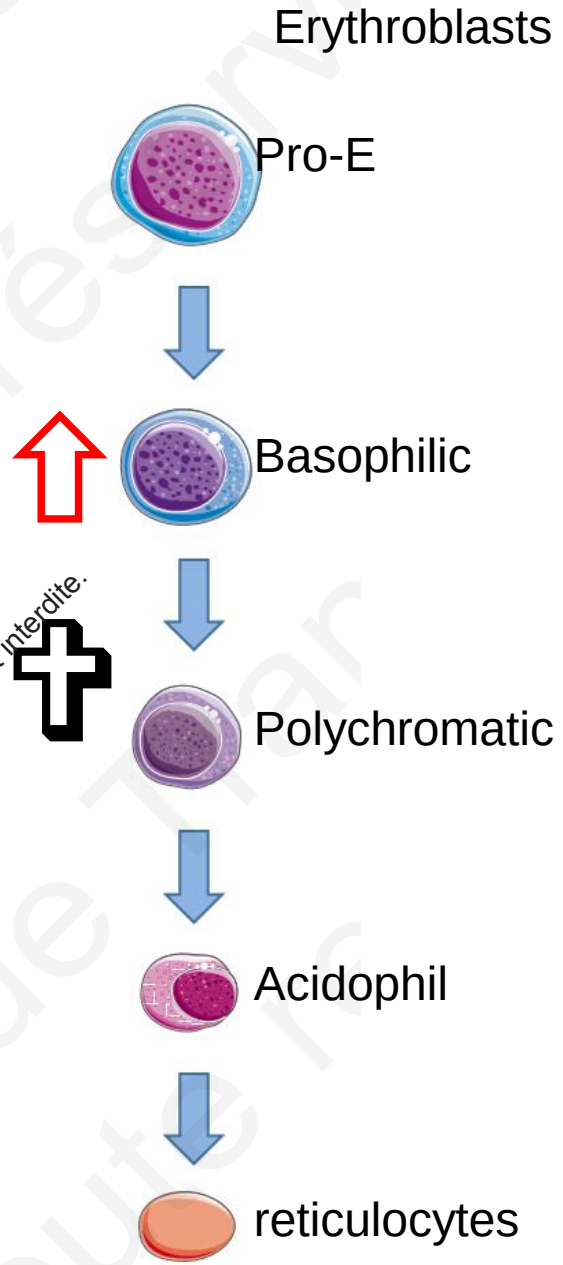

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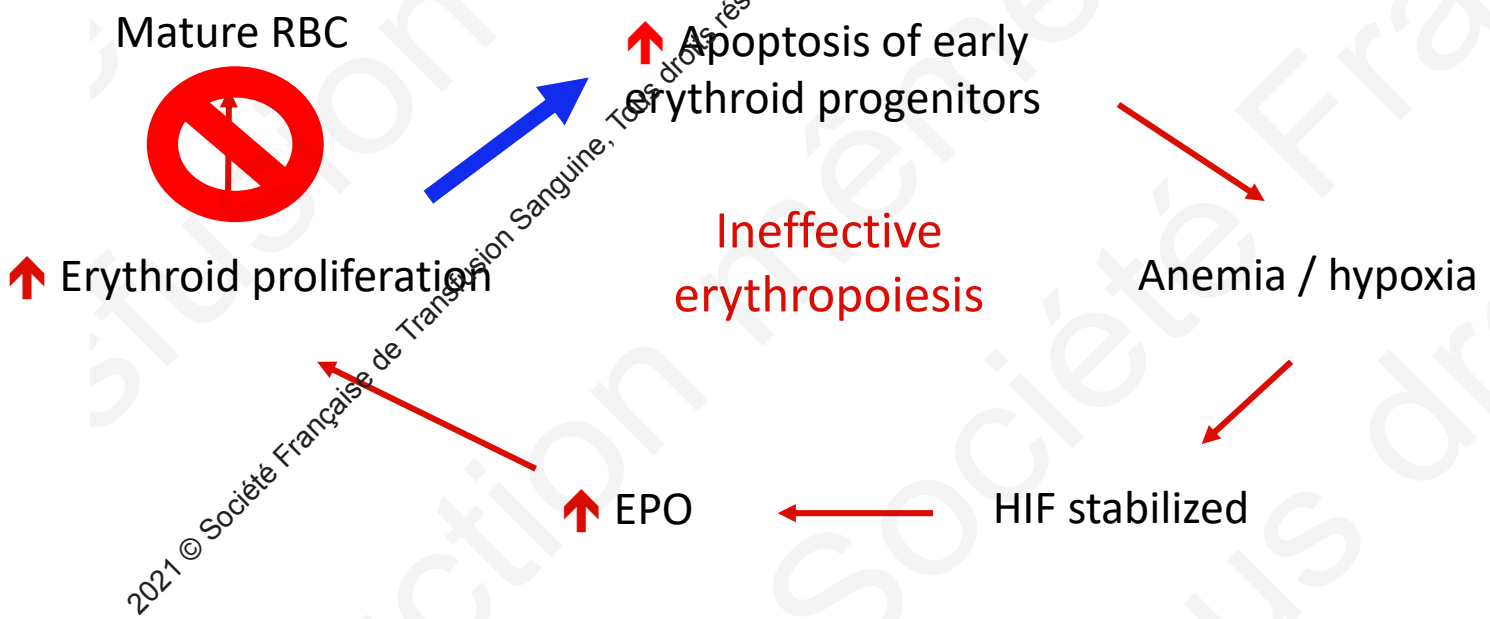

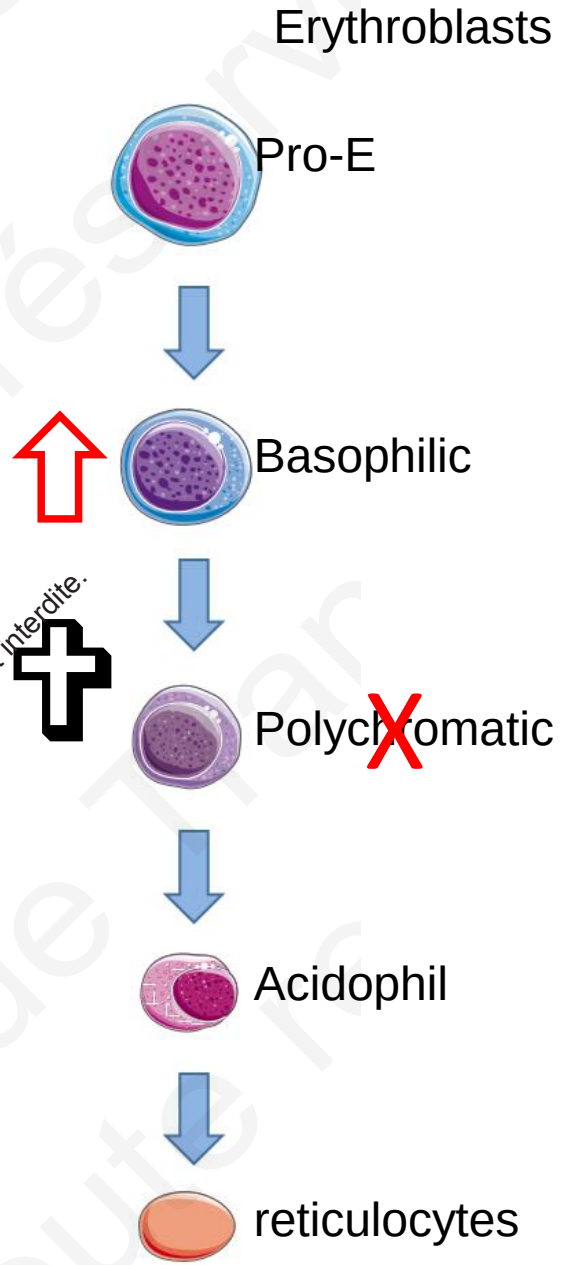

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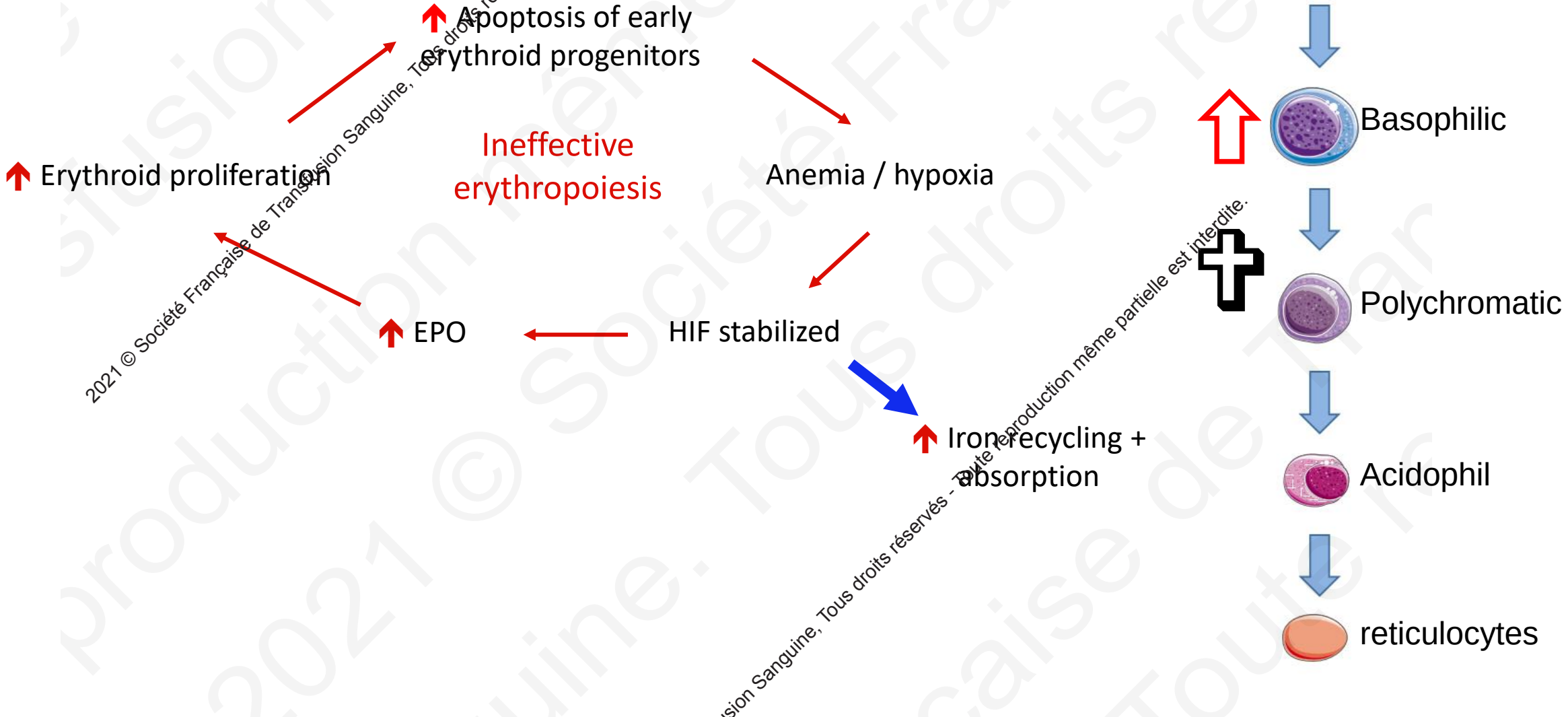

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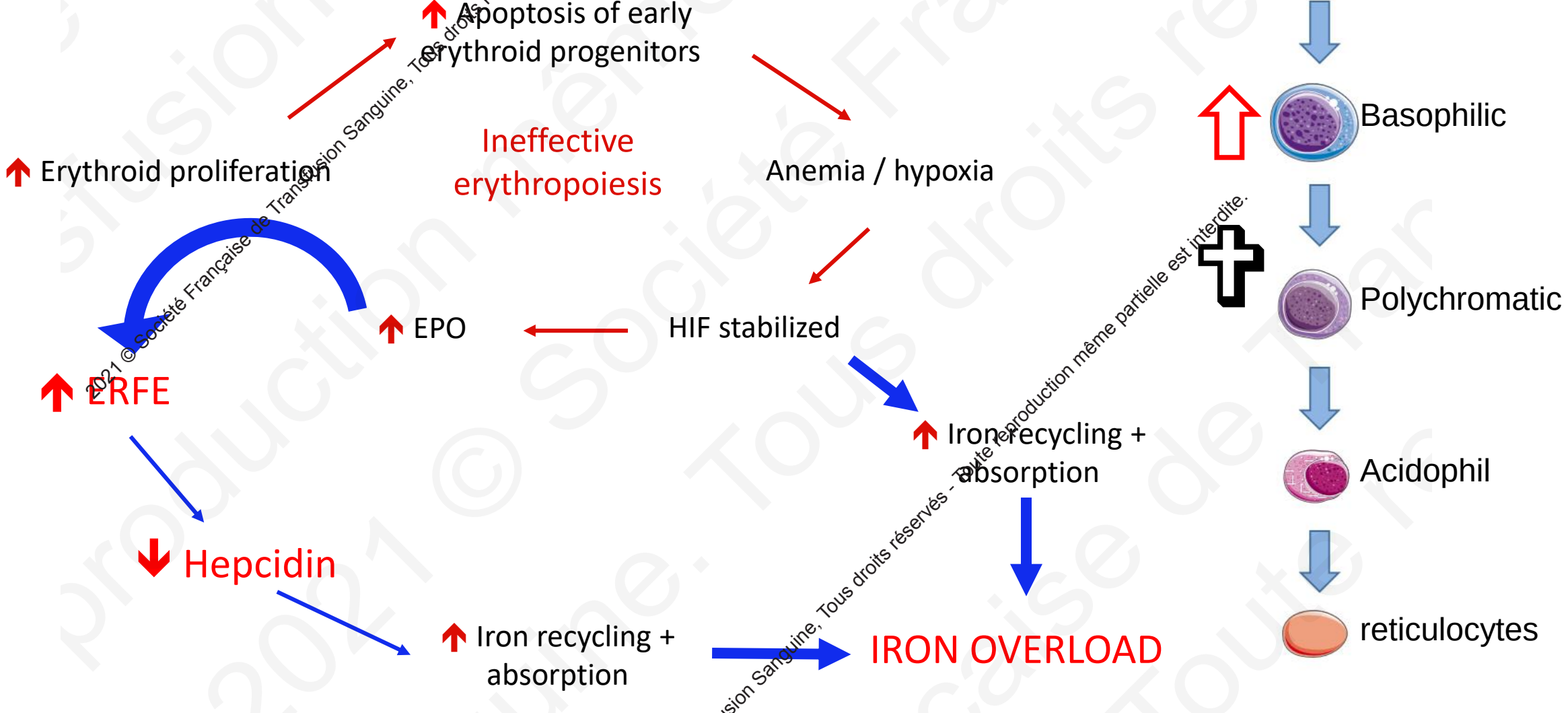
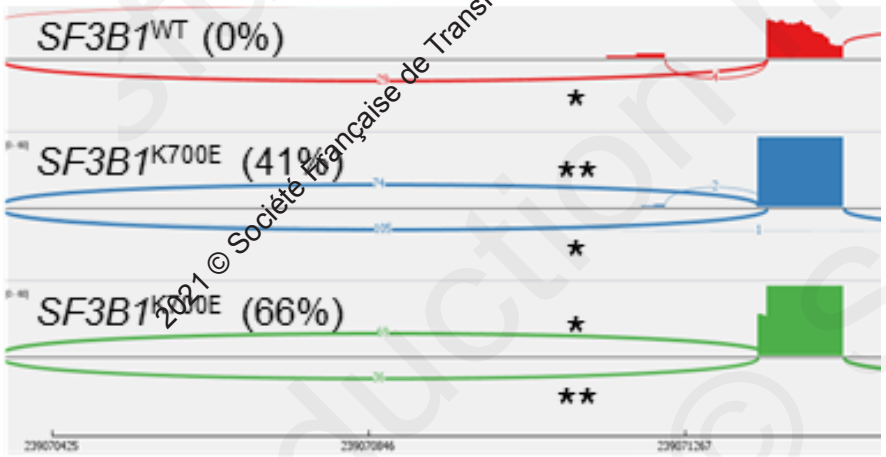


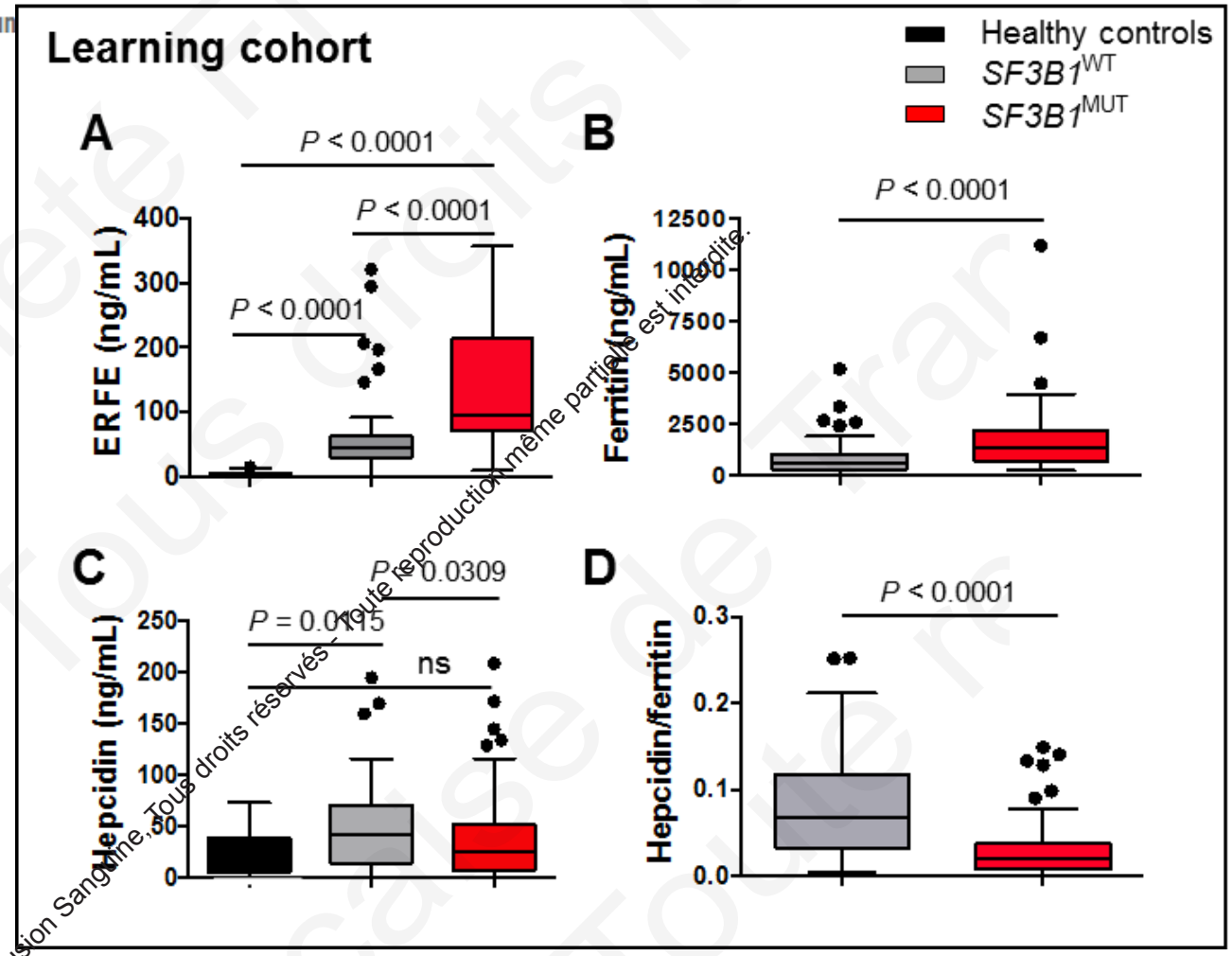
Circulating ERFE levels are elevated in patients with MDS

RESEARCH ARTICLE | MYELODYSPLASTIC SYNDROME

A variant erythroferrone disrupts iron homeostasis in *SF3B1*-mutated myelodysplastic syndrome

Sabrina Bondu[1,2,3,4,*], Anne-Sophie Alary[1,2,3,4,5,*], Carine Lefèvre[1,2,3,4,6], Alexandre Houy[7], Grace Jun





Bondu, Alary et al, Sc Trans Med, 2019

ERFE^{VPFQ}: an ERFE variant specific of clonal hematopoiesis

N ERFE^{WT} C

MLFVRQSDKGVNGKKRSRGKAKKLK FGLPGPPGPPGPQGPPGP IIPPEALLKEFQLLLKG

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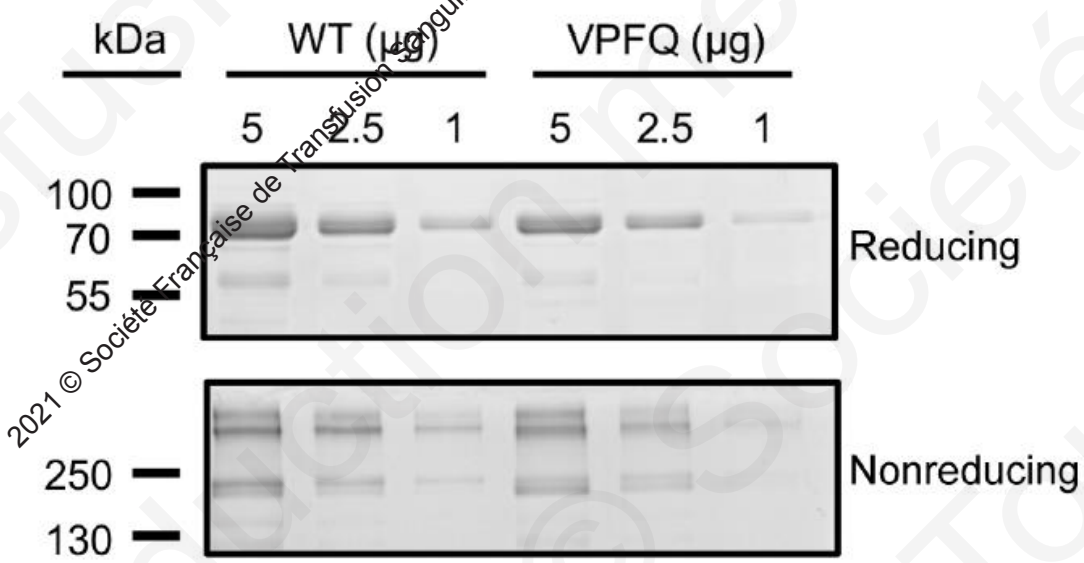

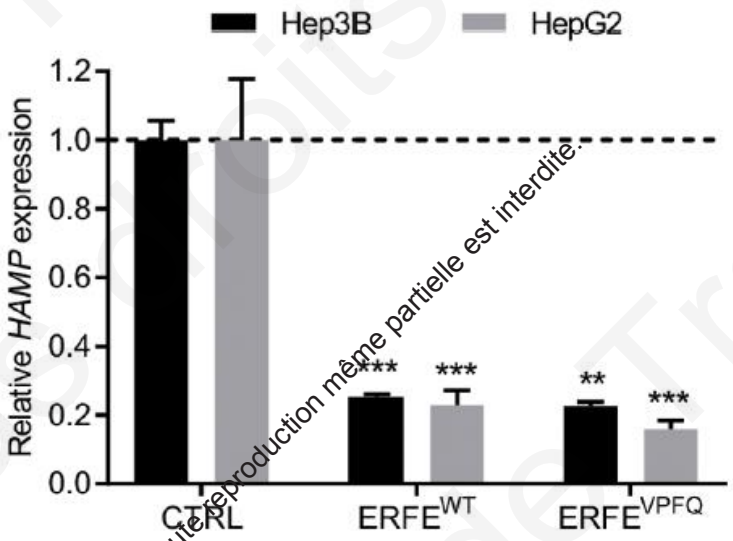

Bondu et al., Sci Transl Med 2019

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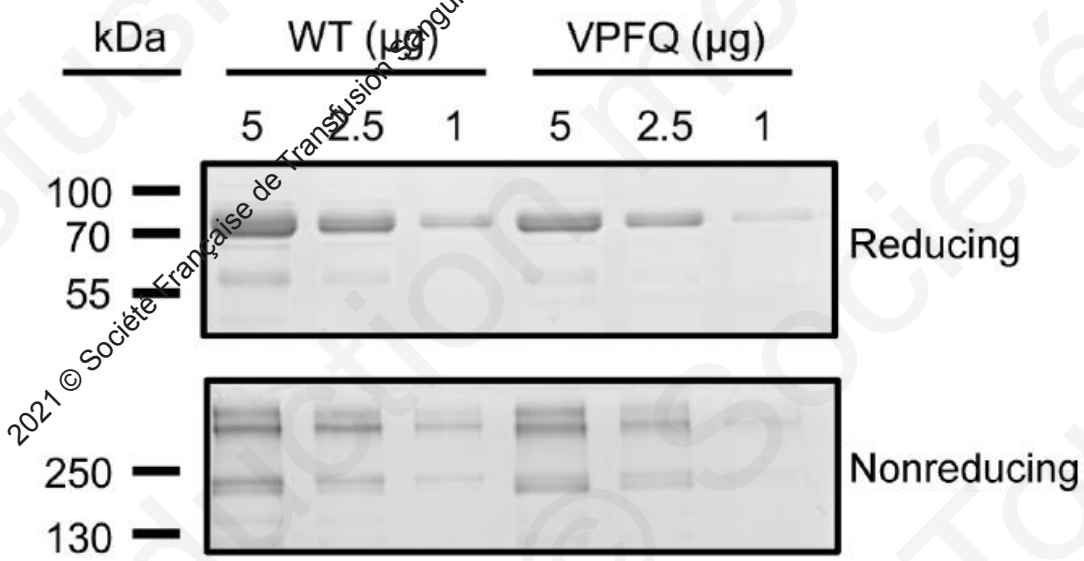
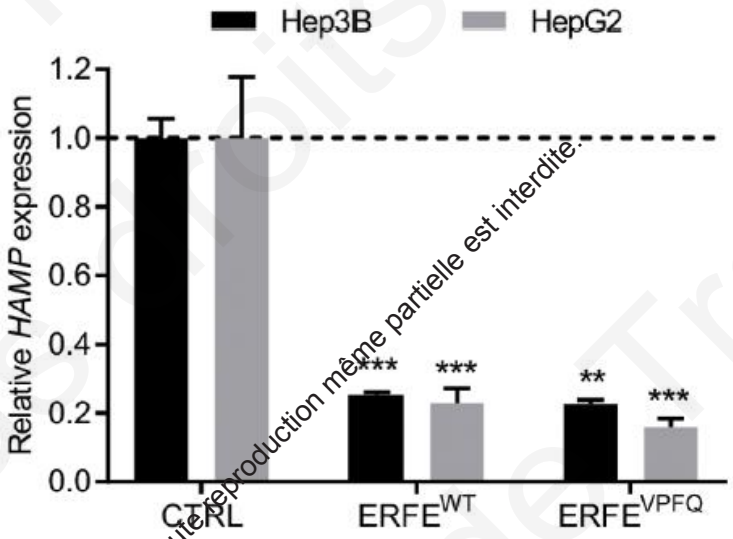




Bondu et al., Sci Transl Med 2019

Development of a diagnostic tool based on the monitoring of ERFE^{VPFQ}

institut cochin CENTRE DE RECHERCHE

O. Kosmider
M. Fontenay

Systemic iron overload in MDS

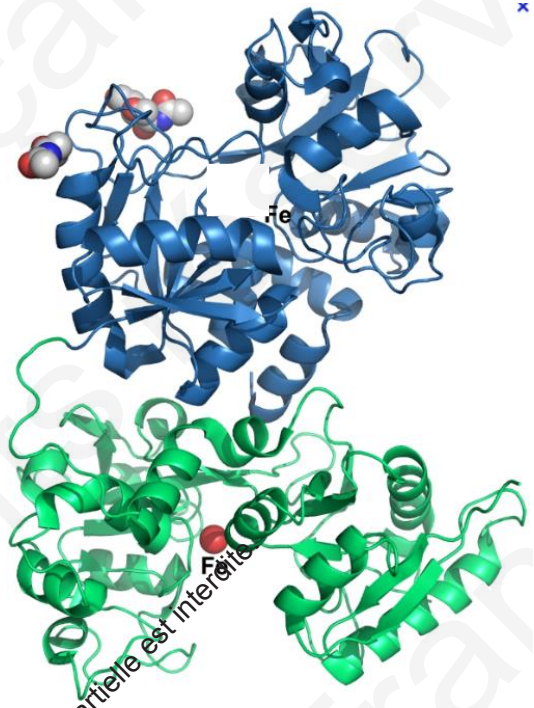
Increased iron absorption and recycling

↓

Transferrin binding capacity is exceeded

↓

Accumulation of NTBI (Non Transferrin Bound Iron)



Normal transferrin saturation: 30-40%

Tf sat > 50% = iron overload

Tf sat > 60-70% = NTBI

Systemic iron overload in MDS

Increased iron absorption and recycling

↓

Transferrin binding capacity is exceeded

↓

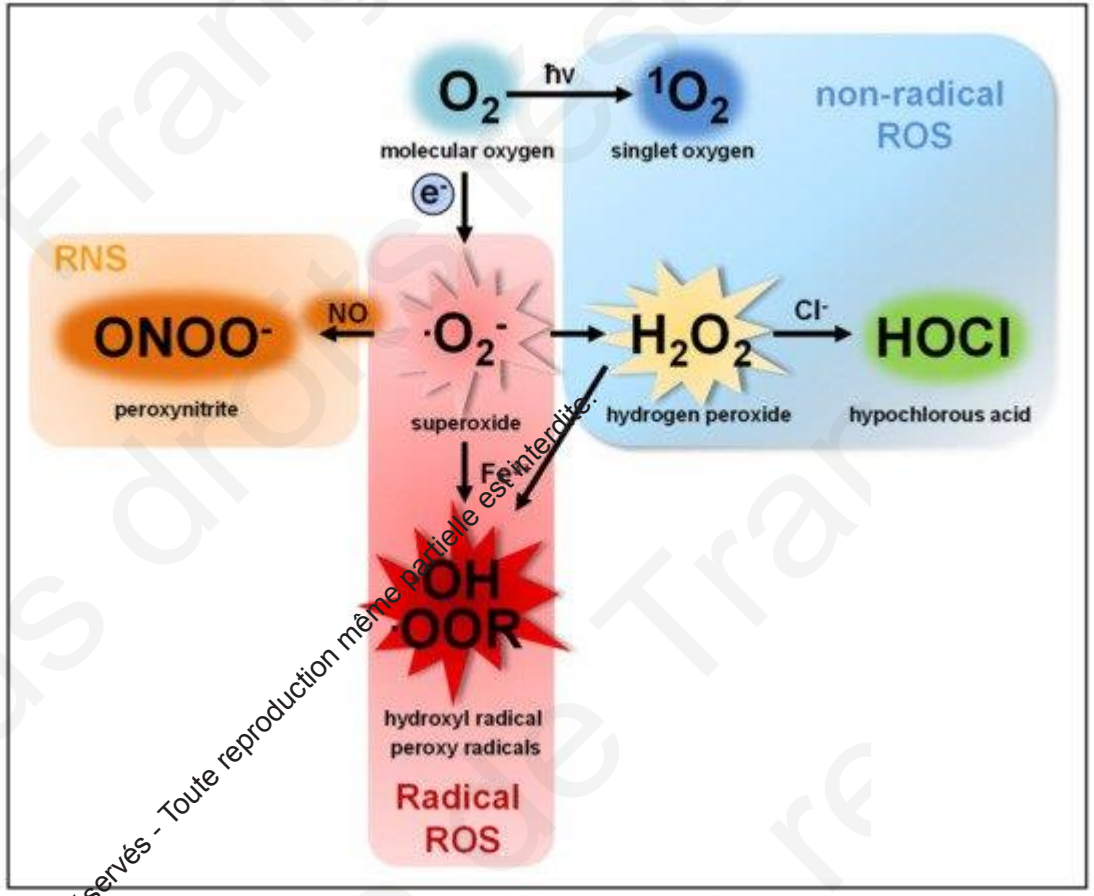
Accumulation of NTBI (Non Transferrin Bound Iron)

↓

Generation of Reactive Oxygen Species (ROS)

↓

Oxidative stress



Systemic iron overload in MDS

Increased iron absorption and recycling

↓

Transferrin binding capacity is exceeded

↓

Accumulation of NTBI (Non Transferrin Bound Iron)

↓

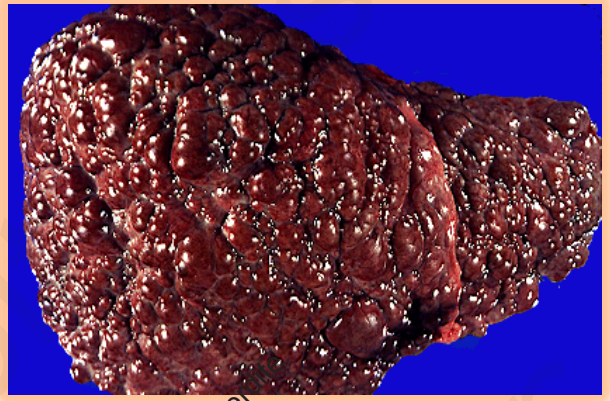
Generation of Reactive Oxygen Species (ROS)

↓

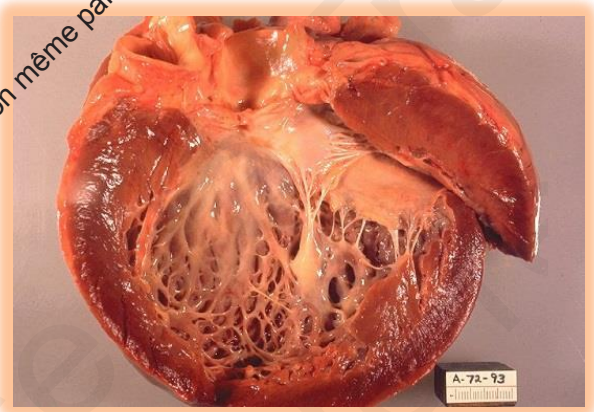
Oxidative stress

↓

Cellular damage and organ dysfunction (liver, heart, pancreas)



Cirrhosis



Cardiomyopathy

Clinical consequences of iron overload in MDS

- Overall survival – increased risk of death every 500µg/L additionnal serum ferritin above 1000µg/L
- Cardio-vascular functionality – myocardial iron overload, vascular impairment, inflammation, ROS production, LDL oxidation, atherosclerosis
- Liver functions – fibrosis, cirrhosis, hepatocellular carcinoma
- Predisposition to infections – iron promotes pathogens growth and impairs immune cell response (macrophage, neutrophils, lymphocytes), cytokine production and nitric oxide formation
- Erythropoiesis and leukemic progression – iron excess may aggravate bone marrow failure and impairs erythroblast differentiation, RBC maturation and survival, epigenetic abnormalities and telomere erosion
- Impact on bone marrow microenvironement, MSCs, Immune cells, Bone cells

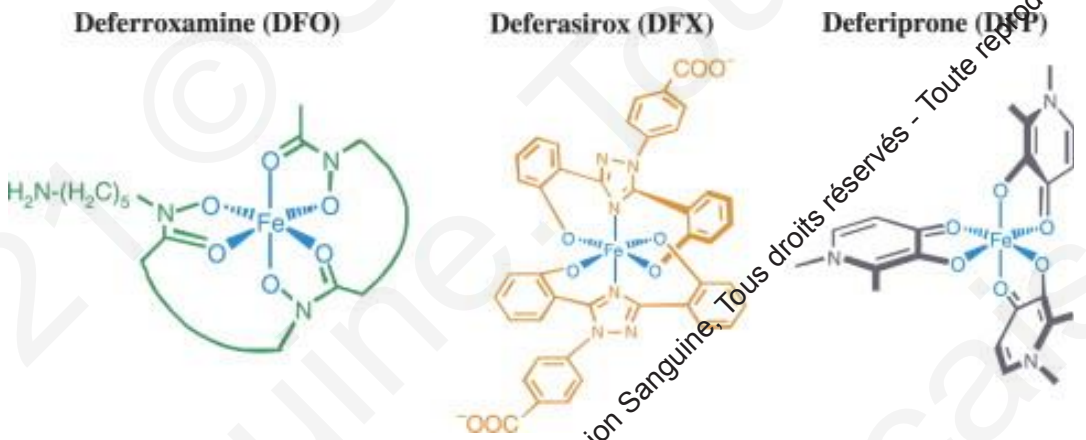
Transfusional iron overload

Anemia → RBC transfusion

1 unit of pRBC = 250 mg of iron

Daily iron requirements = 1-2 mg

- Iron chelation
  - Deferoxamine: short half life in parenteral administration, poor patient compliance
  - Deferiprone: oral chelator. Limited studies in MDS
  - Deferasirox: oral chelator. Superior efficacy but GI adverse events and renal insufficiency



Summary

- MDS are a heterogenous group of clonal hematopoesis characterized by dysplasia of at least one cell lineage and cytopenia in the bone marrow and the peripheral blood
- 80-90% of MDS patients present with anemia at diagnosis
- Premature cell death in MDS-RS results in ineffective erythropoiesis
- The erythroid hormone erythroferrone causes systemic iron overload
- Iron overload leads to tissue damage, organ dysfunction and increased morbidity and mortality

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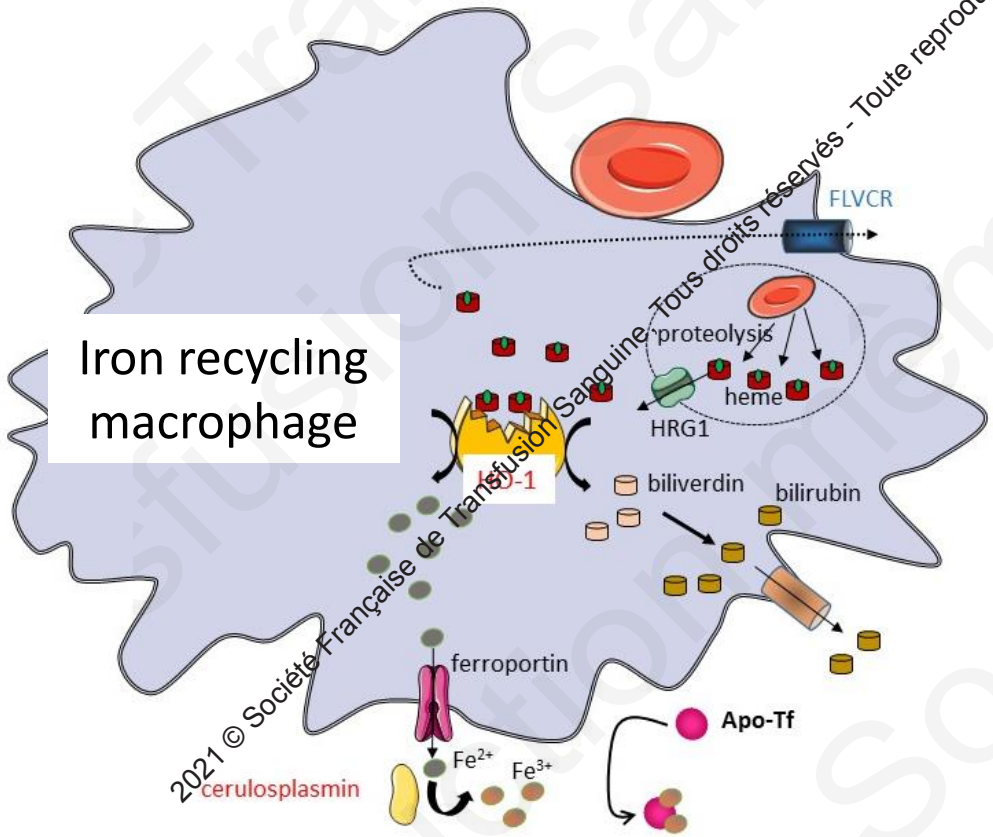
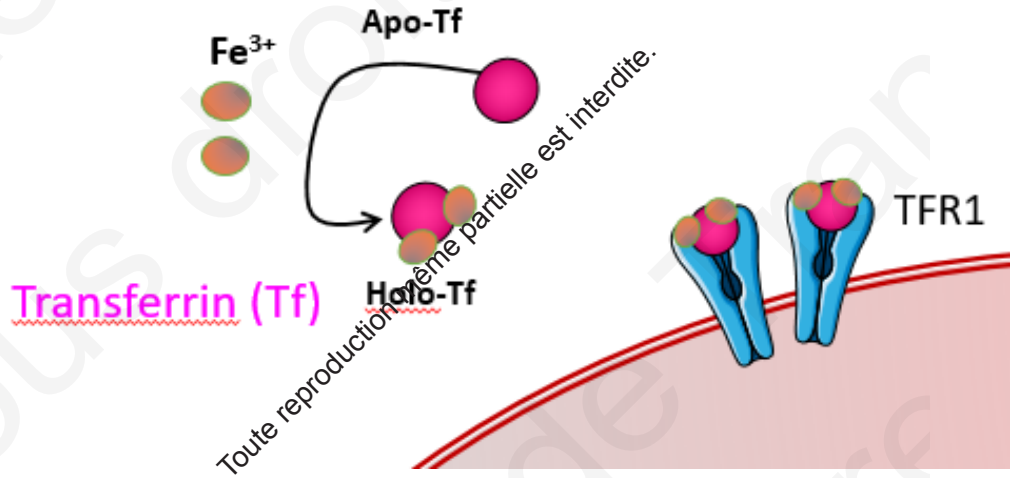
MERCI DE VOTRE ATTENTION

Regulation of iron related genes by HIF1α



- Heme oxygenase (HO-1)
- Ceruloplasmin (ferroxydase)

Increase iron recycling by macrophages



- Transferrin and TFR1: increase iron uptake into red cells

Rolfs, JBC 1997
Tacchini, JBC 1999
Mukhopadhyay, JBC 2000
Lee, JBC 1997