2019

Progetto Ematologia Romagna

ERITROPOIESI EFFICACE ED INEFFICACE
QUADRI CLINICI DI RIFERIMENTO

FEDERICA PILO

OSPEDALE ONCOLOGICO A. BUSINCO

FAENZA 19 Ottobre 2019

AGENDA

Efficacy erythropoiesis

Dyserythropoiesis mechanisms

Overview of hematologic diseases characterized by dyserytropoiesis

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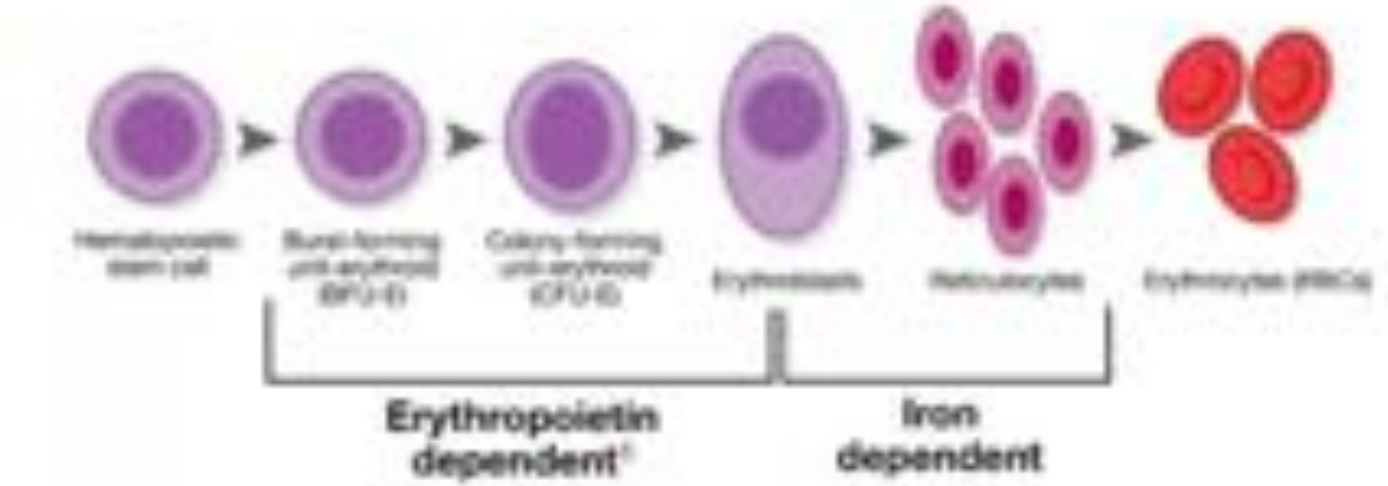
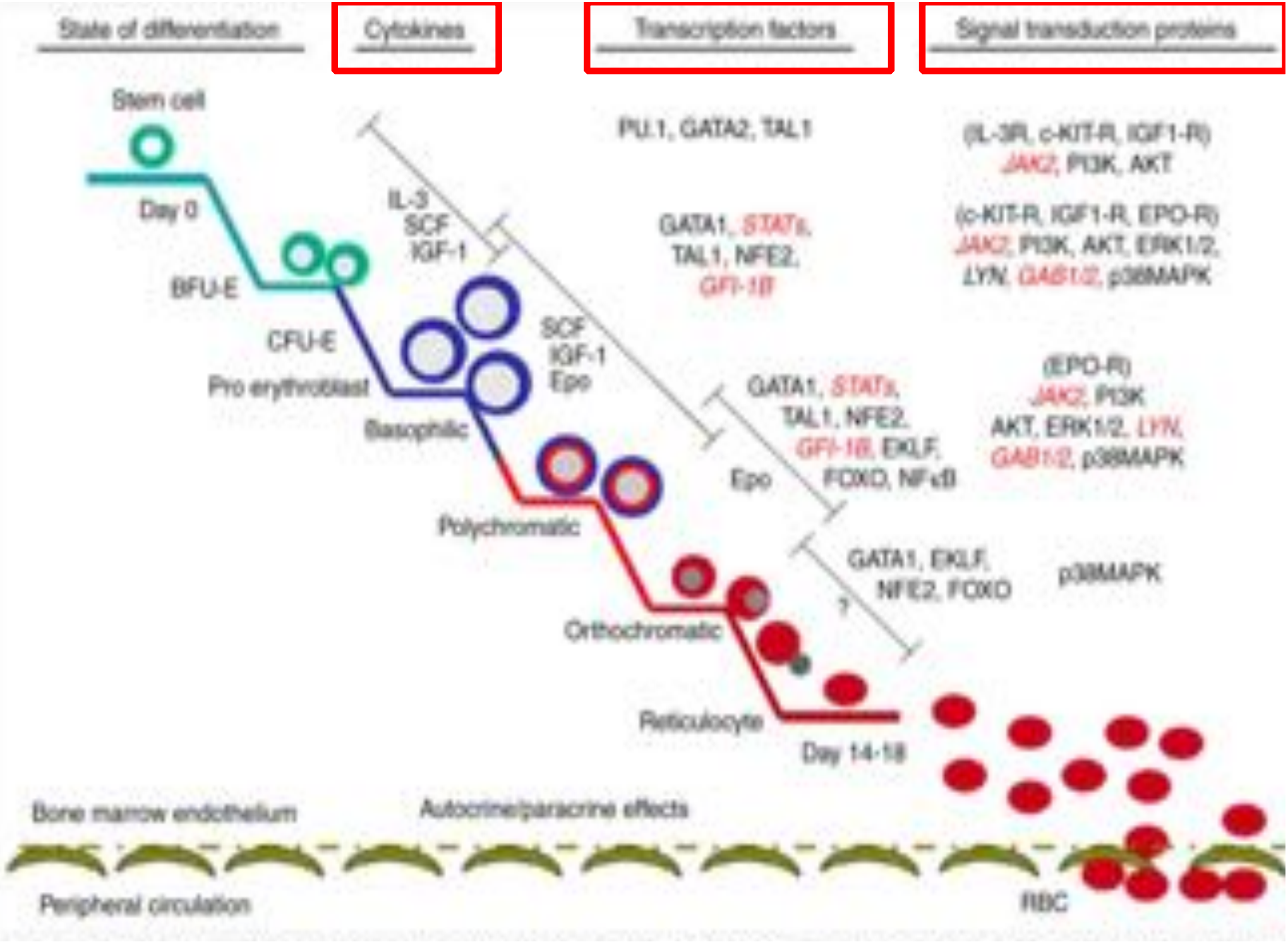
Overview of hematologic diseases characterized by dyserytropoiesis

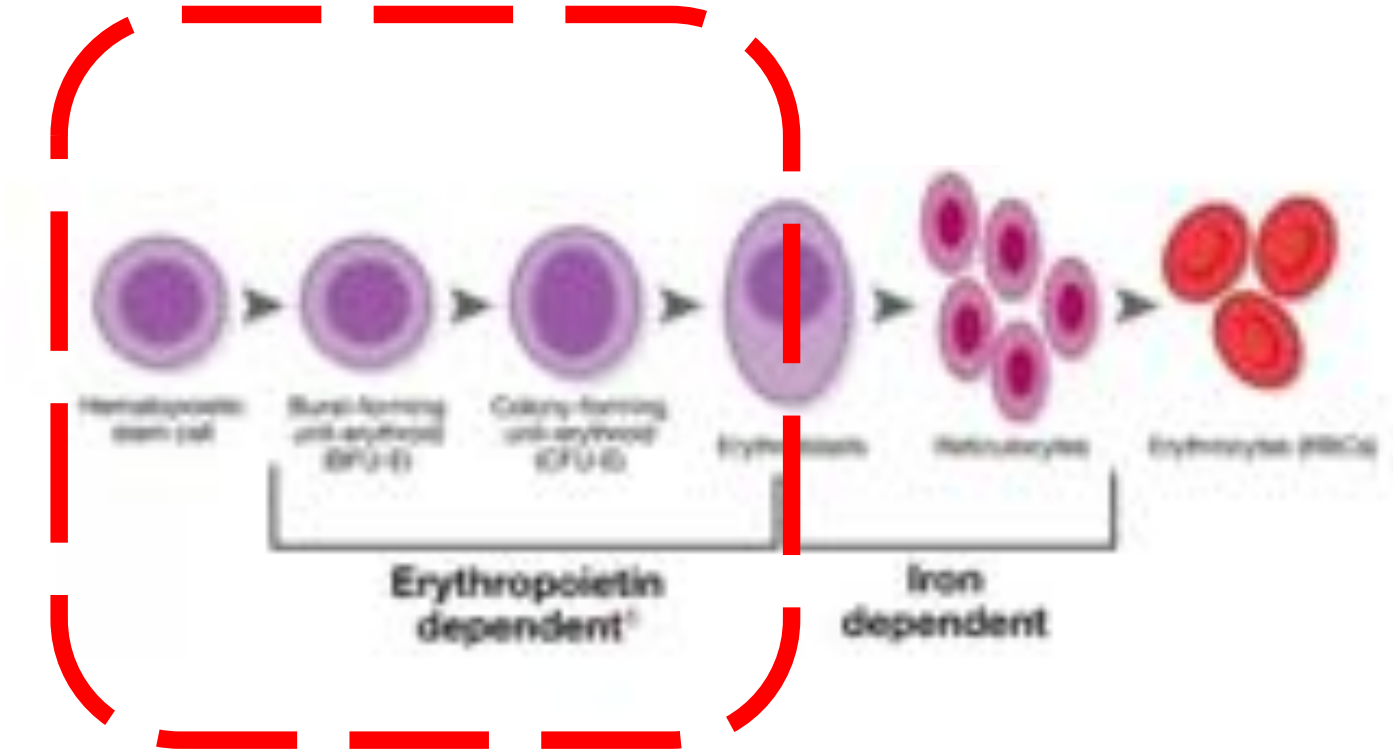
Efficacy erythropoiesis



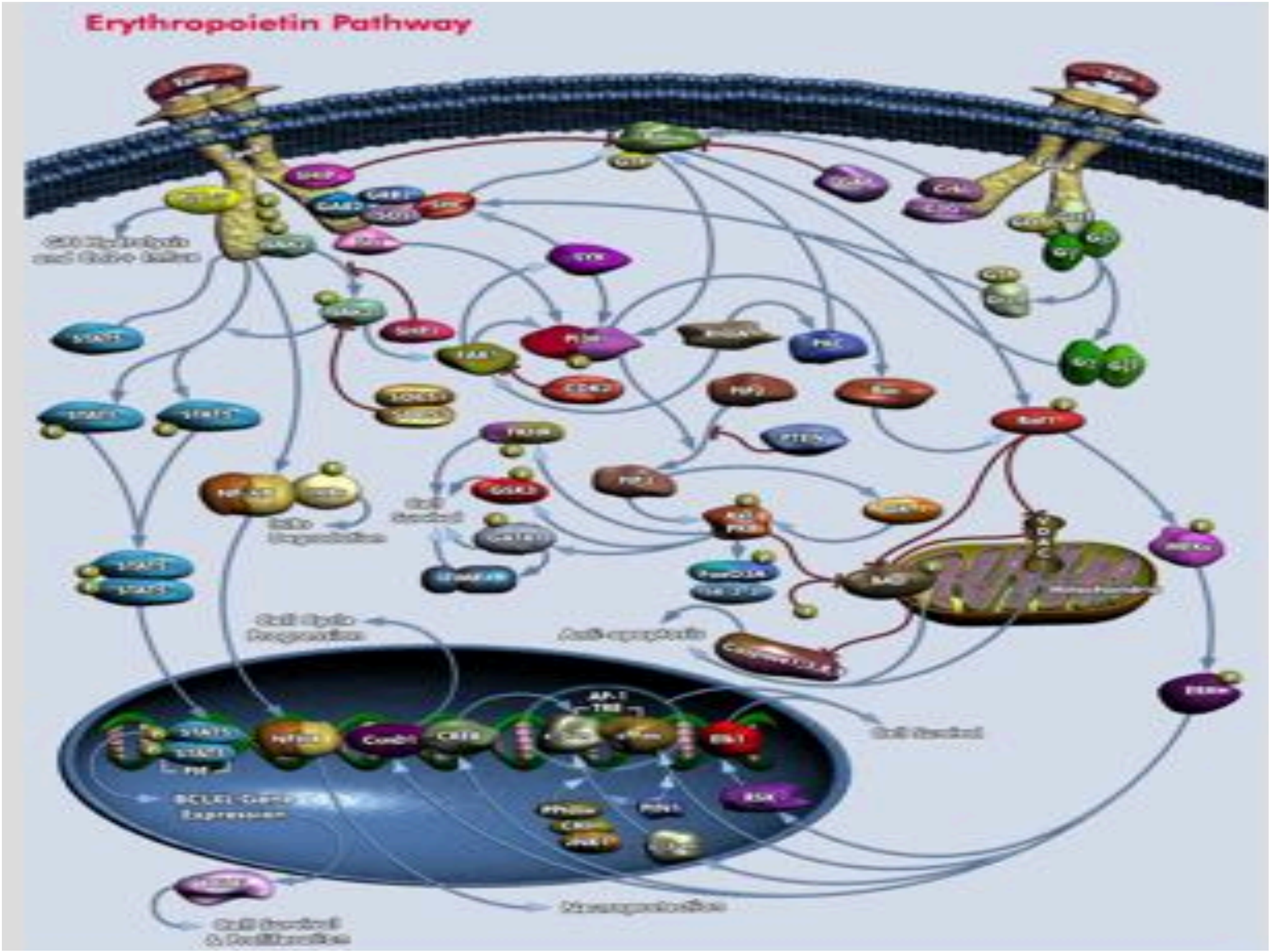
- Adult humans produce approximately 200 billion erythrocytes daily
- 20mg of iron molecules are utilizeddaily for erythropoiesis


State of differentiation
Cytokines
Transcription factors
Signal transduction proteins
Stem cell
Day 0
BFU-E
CFU-E
Pro erythroblast
Basophilic
Polychromatic
Orthochromatic
Reticulocyte
Day 14-18
IL-3
SCF
IGF-1
SCF
IGF-1
Epo
Epo
PU.1, GATA2, TAL1
GATA1, STATs,
TAL1, NFE2,
GFI-1B
GATA1, STATs,
TAL1, NFE2,
GFI-1B, EKLF,
FOXO, NFκB
GATA1, EKLF,
NFE2, FOXO
?
(IL-3R, c-KIT-R, IGF1-R)
JAK2, PI3K, AKT
(c-KIT-R, IGF1-R, EPO-R)
JAK2, PI3K, AKT, ERK1/2,
LYN, GAB1/2, p38MAPK
(EPO-R)
JAK2, PI3K
AKT, ERK1/2, LYN,
GAB1/2, p38MAPK
p38MAPK
Bone marrow endothelium
Autocrine/paracrine effects
Peripheral circulation
RBC

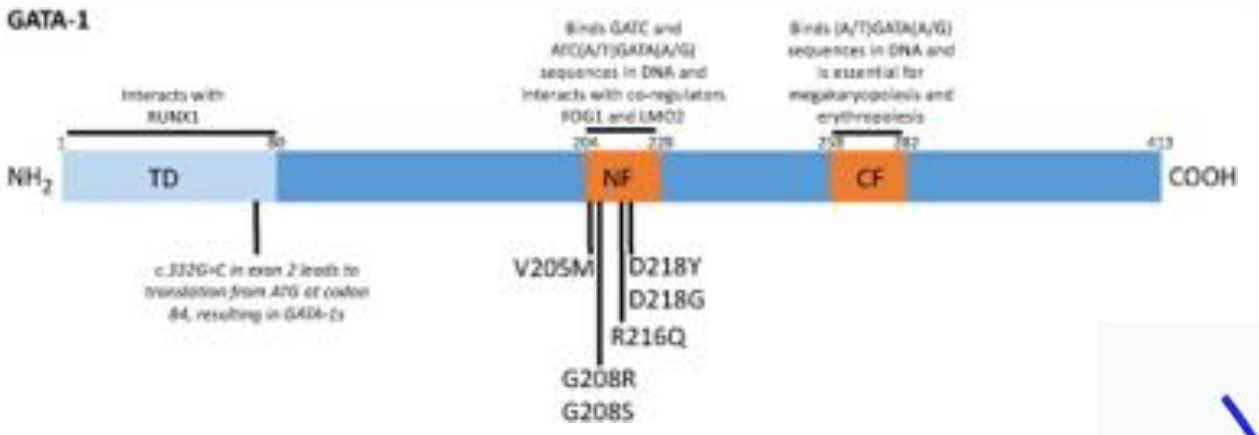
Efficacy erythropoiesis

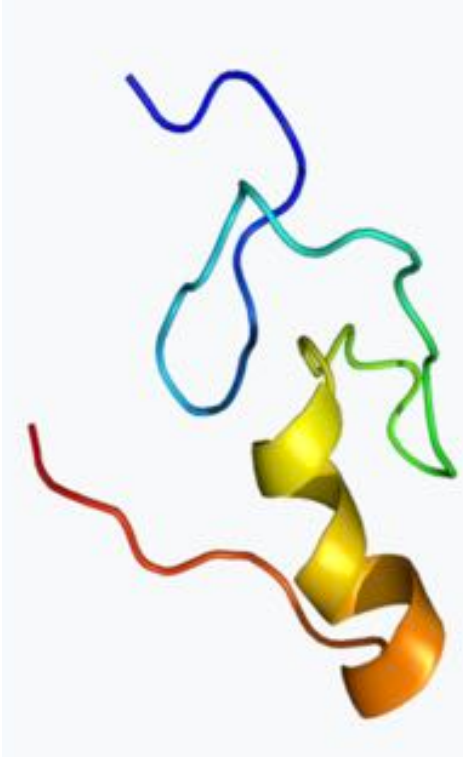


Int. J. Mol. Sci. **2016**, *17*(5), 677


Erythropoietin Pathway

GATA1: master transcription factor of erythropoiesis





Promotes: cell survival e.g Bcl-X,EpoR

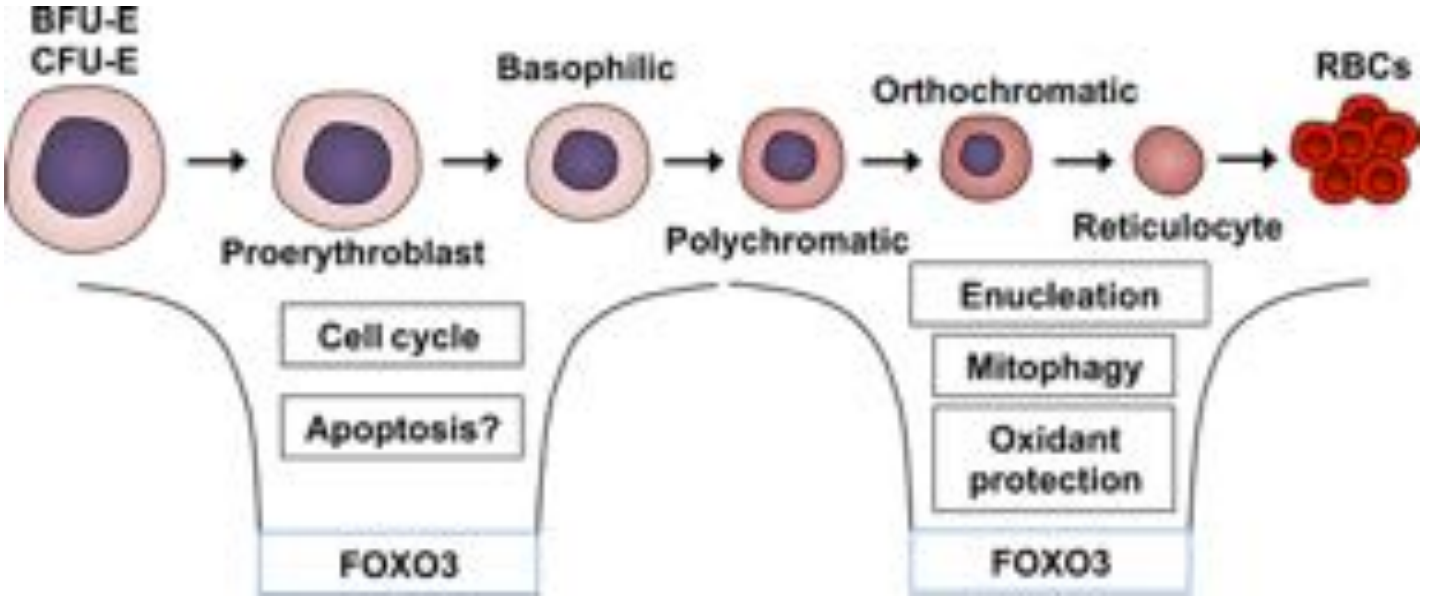
Upregulates: other essential erythroid transcription factors, KLF1,FOG-1 ecc

Activates: ery-specific transcrption program (globin,heme synthesis etc)

Suppress: hemopoietic multipotentiality and alternative cell fate e.g GATA-2

Suppress: cell proliferation

FOXO3 regulation of terminal erythroblast maturation

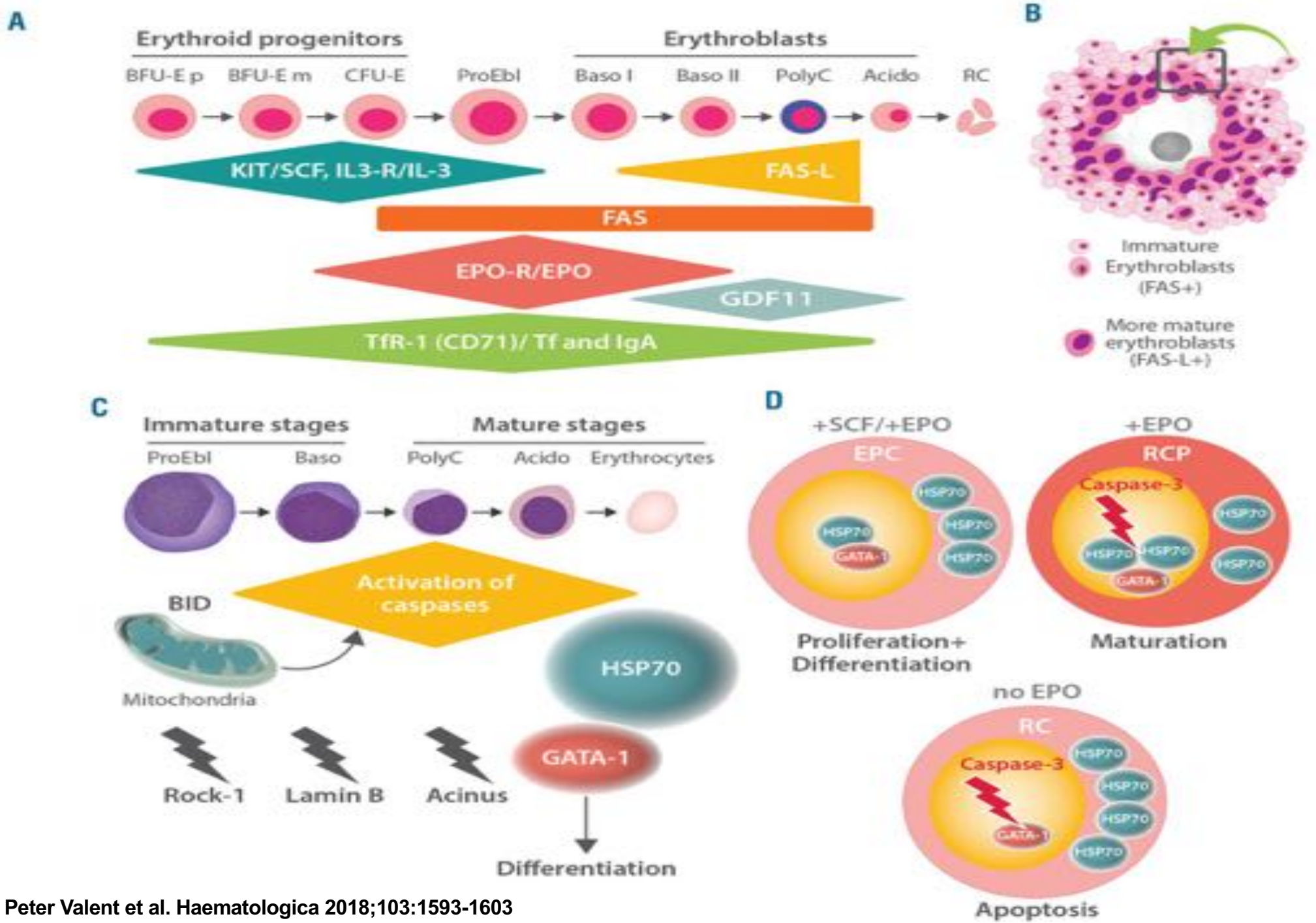


In early stage: regulates cell cycle progression and potentially has a role in modulating apoptosis

In late stage: regulates genes responsible for erythroblast maturation, enucleation, mitophagy and antioxidant defence.

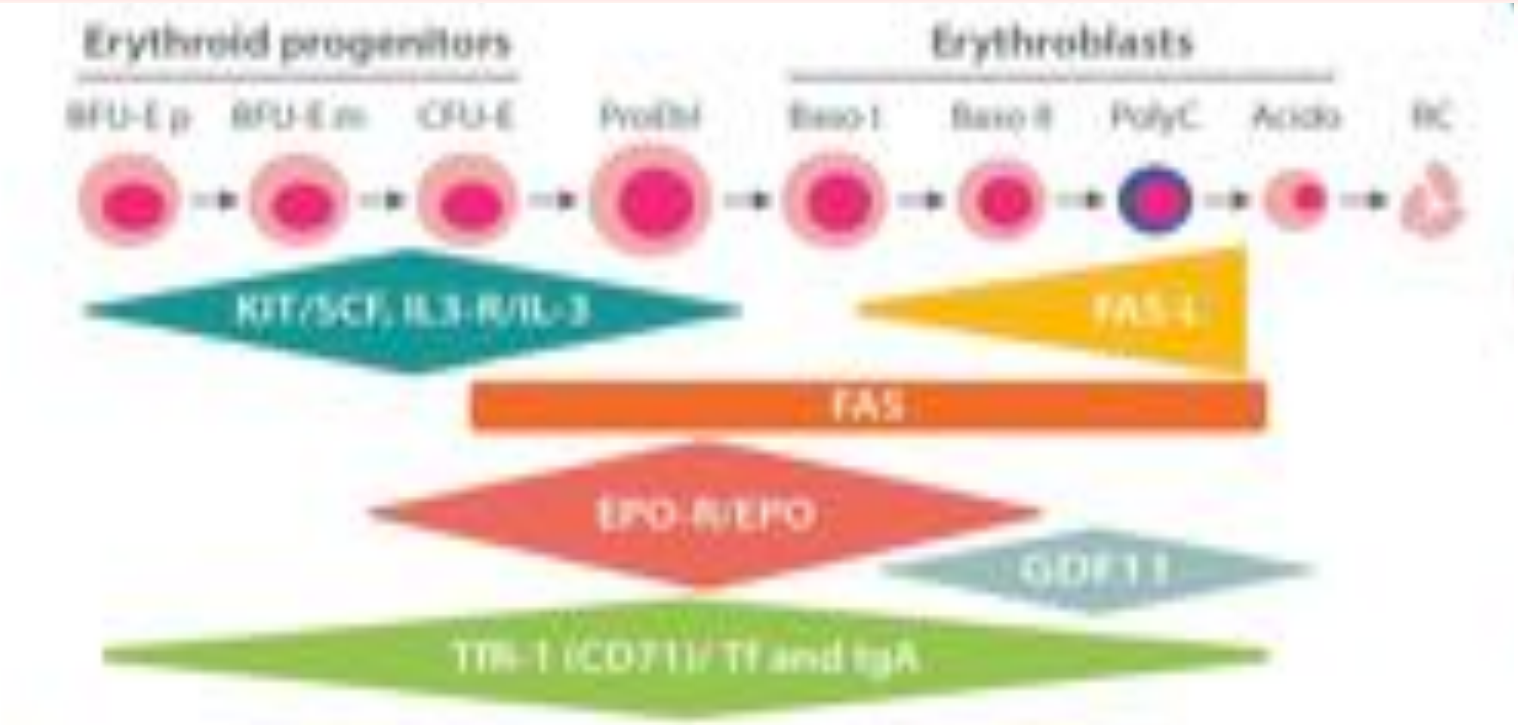
British Journal of Haematology, Volume: 174, Issue: 5, Pages: 661-673, First published: 21 July 2016, DOI: (10.1111/bjh.14194)

Major pathways and molecules involved in the regulation of erythropoiesis.



Peter Valent et al. Haematologica 2018;103:1593-1603

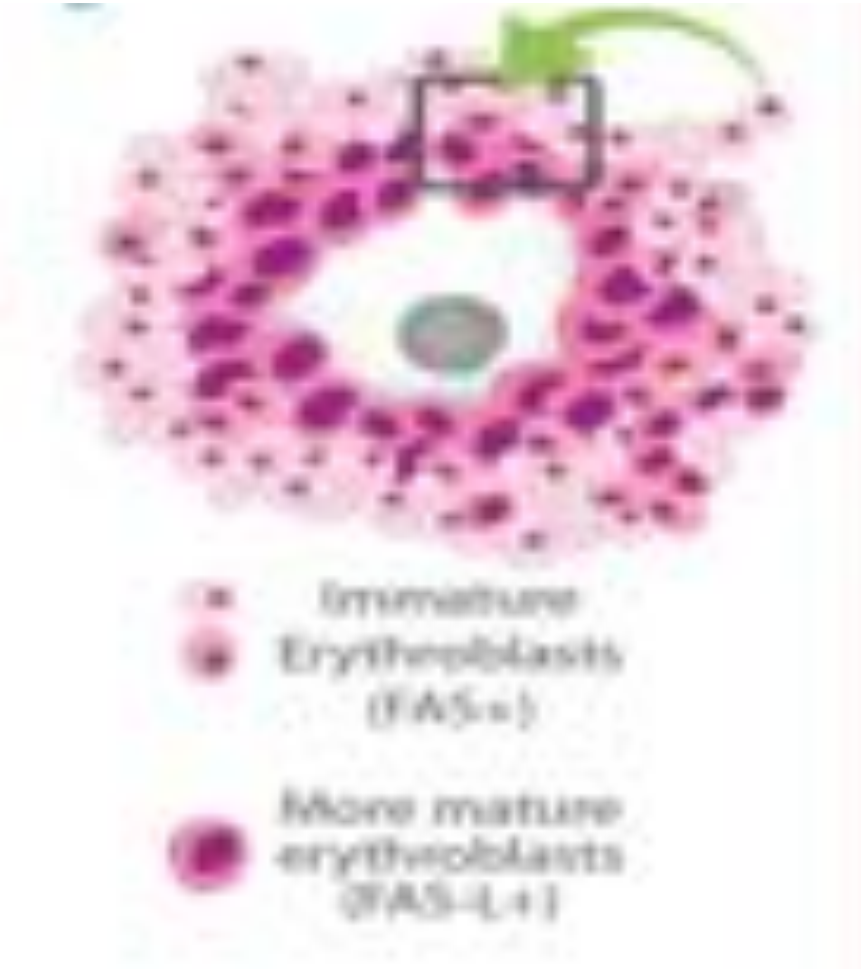
Development of erythropoietic progenitor cells and erythroblasts.



(p) primitive
(m) mature
(BFU-E) burst-forming unit erythroid cells
(CFU-E) colony-forming unit erythroid cells
(ProEbl) proerythroblasts
(Baso) basophilic erythroblasts (type I and II),
(PolyC) polychromatic erythroblasts
(Acido) acidophilic erythroblasts

(SCF) stem cell factor
(IL-3) interleukin-3 and their receptor (R),
(KIT) SCF receptor
(EPO) Erythropoietin and EPO-R
(FAS) death receptor and its ligand (FAS-L).
(Tf) Transferrin and Tf-receptor-1 (TfR-1)
(GDF11) growth differentiating factor 11
(IgA) polymeric immunoglobulin A

Erythroid blood island

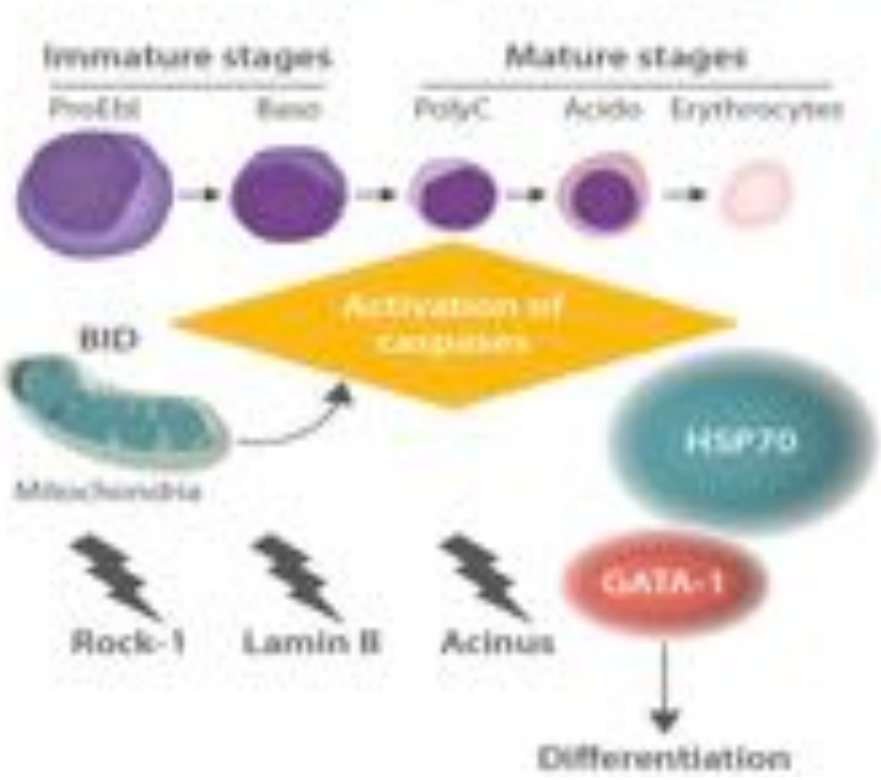


- Late erythroid precursor cells express FAS ligand (FAS-L) which may interact with early erythroid precursors that express FAS, resulting in caspase activation, which in turn triggers apoptosis and maturation arrest

- In case of a substantial need to produce more erythroid cells (e.g. after blood loss by bleeding or hemolysis), EPO protects ery from this activation allowing the cells to survive and differentiate, even if late erythroid precursors are abundant.

Peter Valent et al. Haematologica 2018;103:1593-1603

Caspase activation during terminal erythroid differentiation

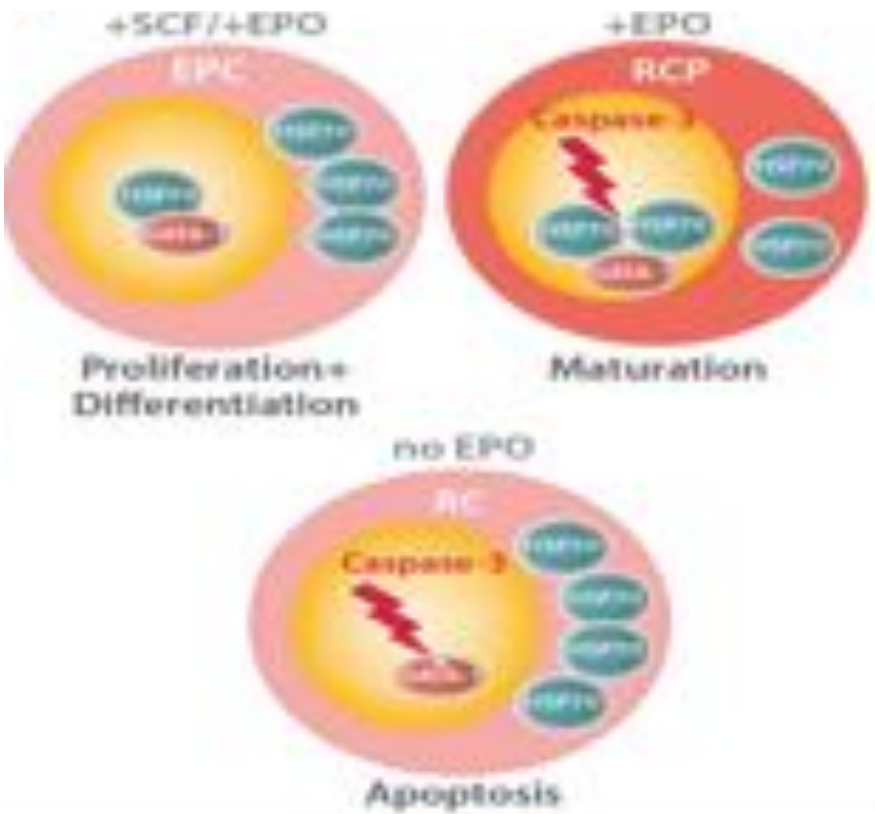


- Caspases are activated during erythroid differentiation
- This activation is absolutely required to prepare the cells for nucleation and thus formation of mature red cells
- In this process, GATA-1 is not cleaved by caspases, ensuring that erythroid maturation is preserved.
- During erythroid differentiation HSP70 enters the nucleus at the time of caspase activation, and, at this level, interacts with GATA-1 to protect it against cleavage

Peter Valent et al. Haematologica 2018;103:1593-1603

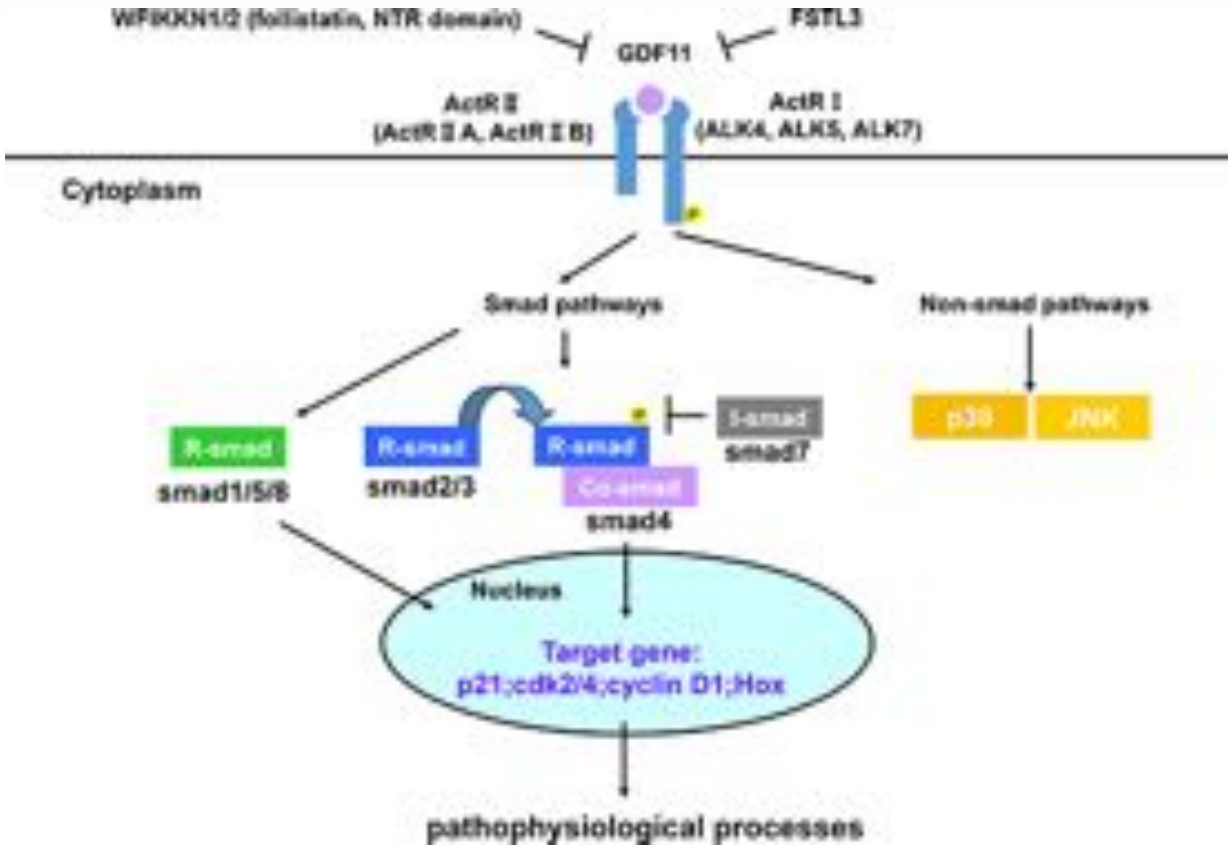
Model of terminal erythroid differentiation and apoptosis regulated by the nuclear localization of HSP70.

- It has been suggested that in the BM, erythroid precursors exhibit differential sensitivity against EPO
- The less sensitive cells undergo apoptosis upon caspase activation when the EPO level is low, whereas at higher EPO levels, most cells will survive and differentiate
- EPO induces maturation as HSP70 translocates into the nucleus to protect GATA-1 from caspase-induced degradation
- In the absence of EPO, caspase-3 induces the cleavage of GATA-1 as HSP70 cannot translocate to the nucleus, and, as a result, apoptosis occurs.



Peter Valent et al. Haematologica 2018;103:1593-1603

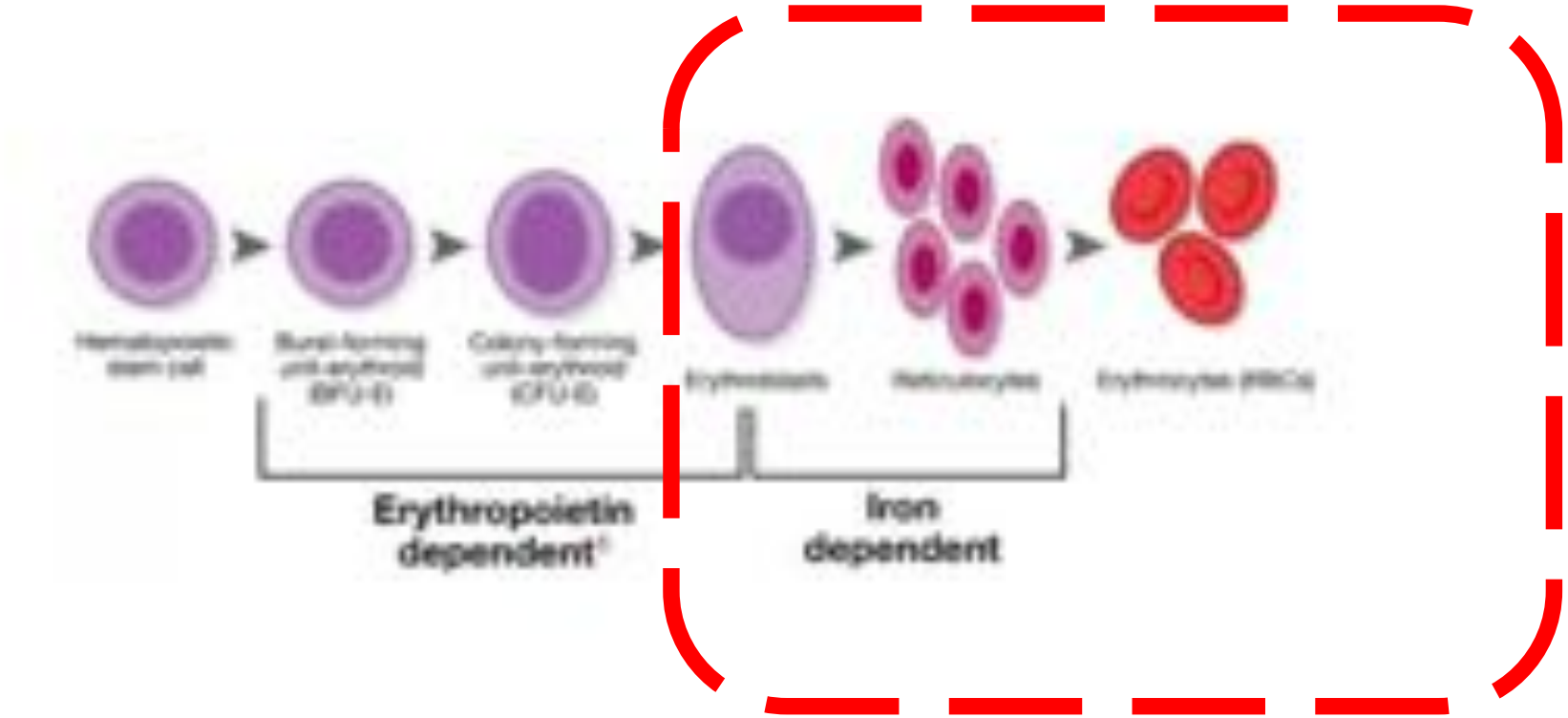
TGF-β superfamily may modulate late erythropoiesis



GDF11 and other members of the TGF-β superfamily binded to transmembrane ligands (ACVR2A-ACVR2B) throwgh the SMAD 2/3 signal activates transcription of target gene for aberrant erythroid late differentiation

Rivella Blood January 2019

Efficacy erythropoiesis



Iron homeostasis is fundamental for erythropoiesis

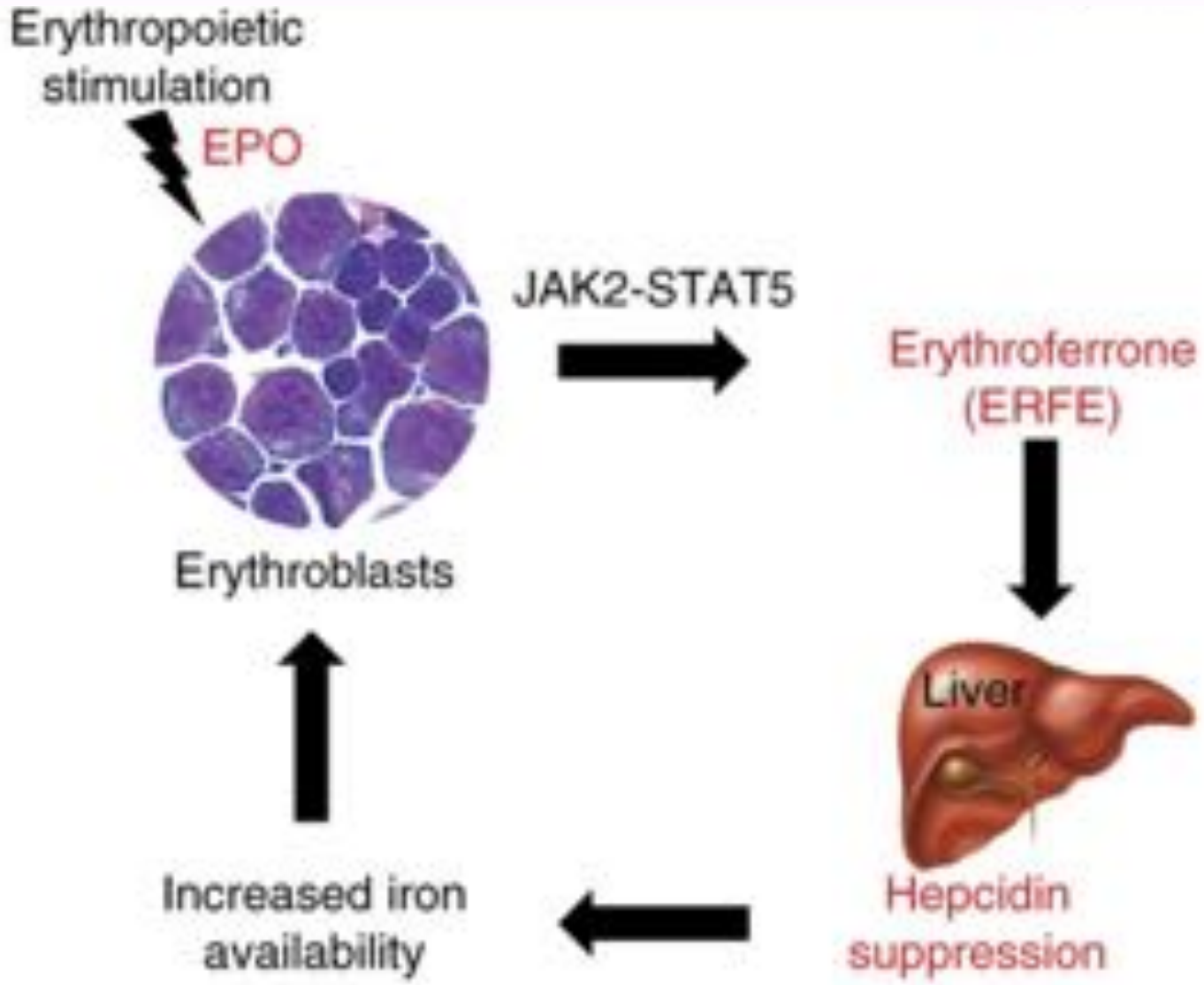
- The amount of iron contained in RBC hemoglobin accounts for about two-thirds of the total iron in the human body
- ~2 million newly synthesized erythrocytes are released into the circulation every second
- The bone marrow is thus the prime iron consumer in the body, with erythropoiesis requiring approximately 25 mg iron daily
- The vast majority of iron is provided by macrophages, which recycle hemoglobin-derived iron by phagocytosing senescent erythrocytes

Iron homeostasis is of paramount importance for erythropoiesis since erythropoiesis affects the iron metabolism, and in turn, iron homeostasis feeds back into the regulation of erythroid development

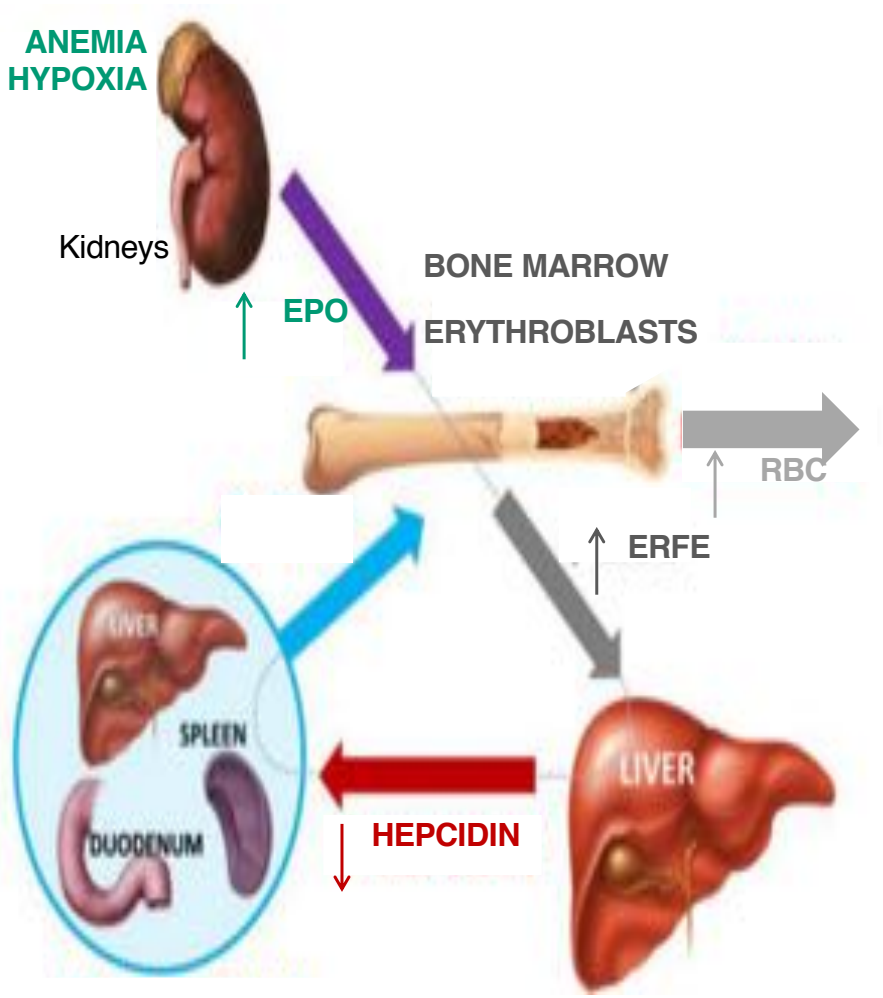
Yiannikourides A, Latunde-Dada GO. Medicines (Basel). 2019;6:E85.

RBC, red blood cell.

Erythroferrone regulation



Physiologic erythroid regulation



During stress erythropoiesis (e.g. hemorrhage), anemia causes increased EPO secretion by the kidneys. EPO-stimulated ERFE secretion inhibits hepcidin transcription, and provides adequate iron for erythropoiesis and other body requirements

Hepcidin suppression, in turn, cause

- Increased intestinal iron absorption
- Increased iron recycling from splenic macrophages
- Increased iron release from hepatic stores

As a result, circulating iron increases, allowing increased iron availability for erythropoiesis

Ganz T. Free Radic Biol Med. 2019;133:69-74.

EPO, erythropoietin; ERFE, erythroferrone.

AGENDA

Efficacy erythropoiesis

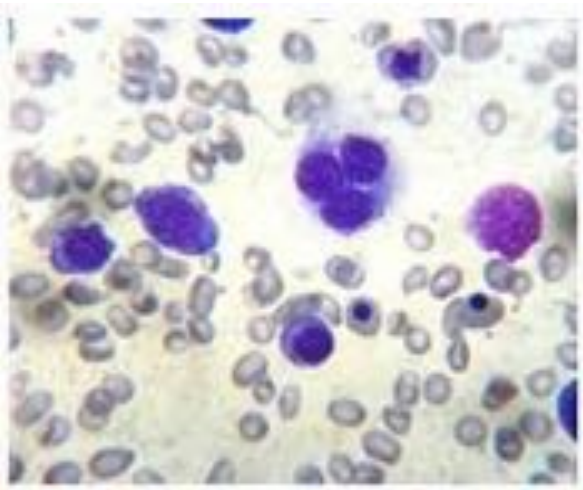
Dyserythropoiesis mechanisms

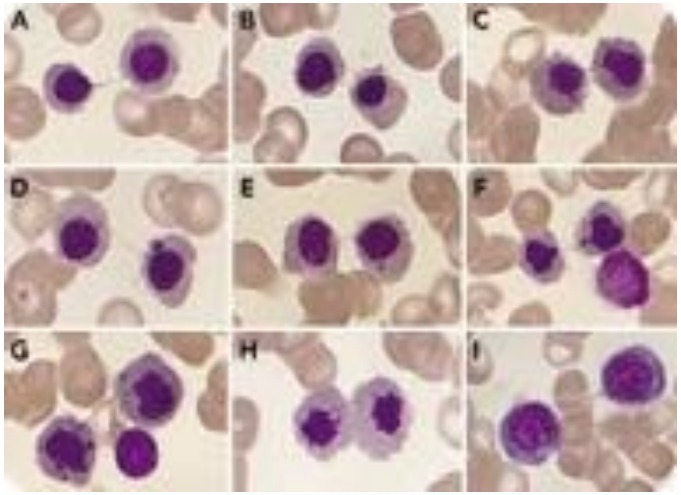
Overview of hematologic diseases characterized by dyserytropoiesis

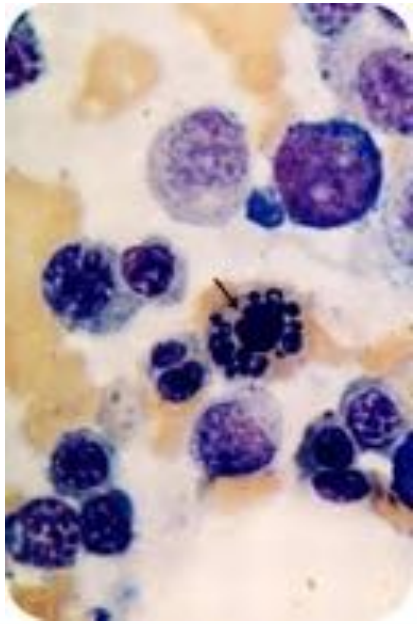
Ineffective erythropoiesis

Ineffective erythropoiesis (IE) refers to the inability to produce an adequate number of red cells in the presence of increased immature erythroid precursors leading to anemia and secondary marrow expansion

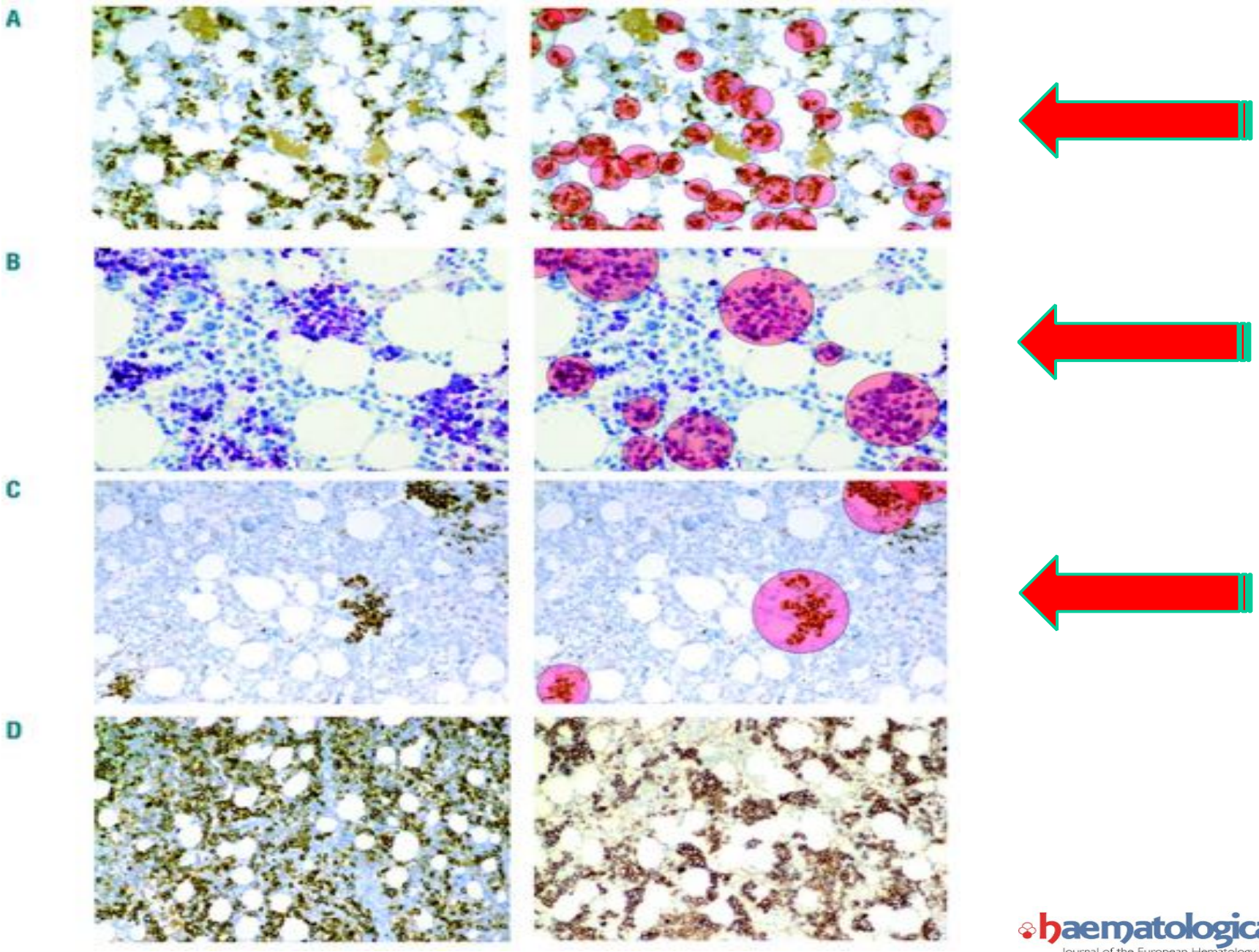
IE is also present in healthy individuals, where up to 25% of erythropoiesis is ineffective







Structure and size of erythroid islands in the bone marrow.



haematologica
Journal of the European Hematology Association
Published by the Ferrata Storti Foundation

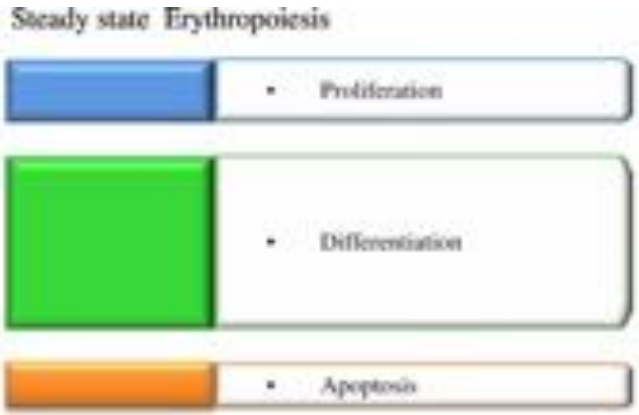
Peter Valent et al. Haematologica 2018;103:1593-1603

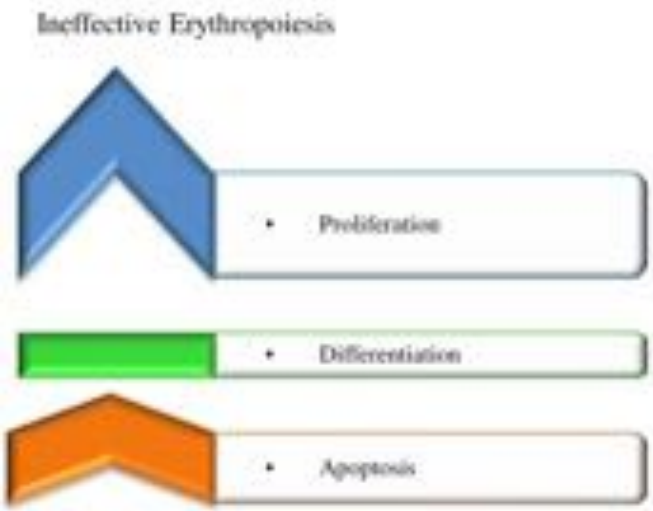
Causes of inefficacy erythropoiesis

Type of regulator	Major example
Growth factors for multipotent and early erythopoietic progenitor cells	SFC, G-CSF,Il-3
Later-acting erythropoietic differentiation factors	EPO, TGF-β,GDF11, Activin A
Essential transcription factors	GATA-1,STAT 5
Important survival factors for erythropoietic cells	MCL,BCL-XL,HSP70
Negative growth regulators of erythropoietic progenitor cells	TGF-beta,BID,FAS ligand,FAS, Caspase
Essential vitamins and trace elements	Vit B12, Folic Acid,Copper, Others
Iron and proteins involved in iron distribution and iron metabolism	Ferritin, transferrin, transferrin receptor, Ferroportin, Hepcidin

+ genes mutation

Markers of ineffective erythropoiesis





A single measure of IE does not exist

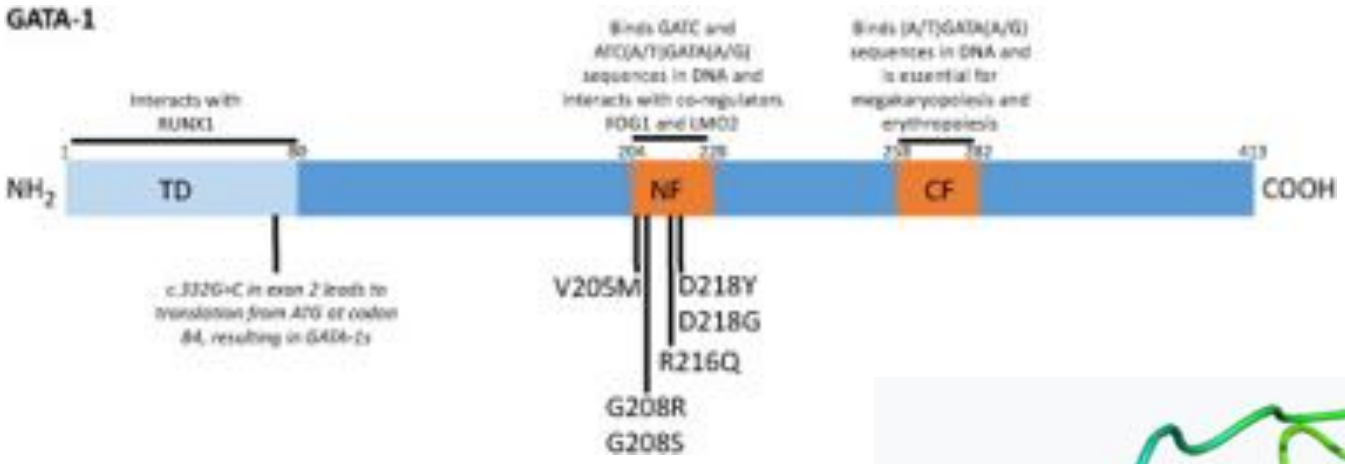
Reticulocyte
NRBC
EPO

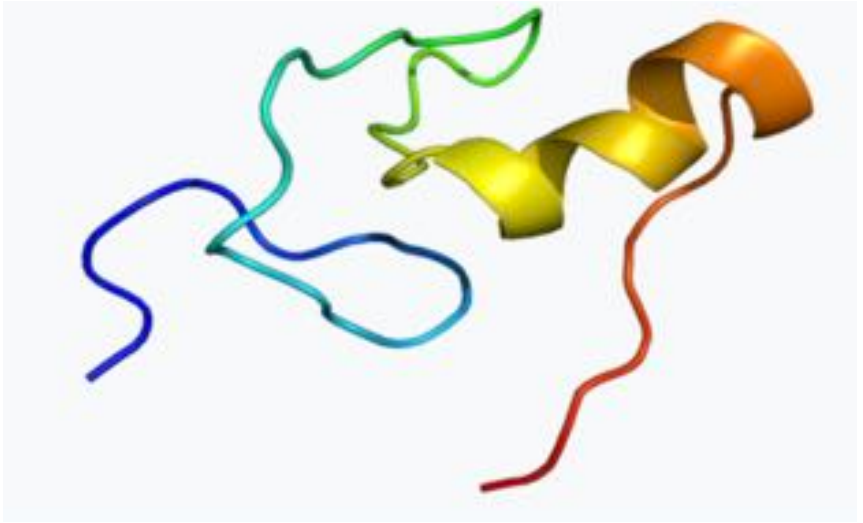
Common Laboratory tests

GDF
solubileTfR
ERFE
Hepcidine
Mutations

Experimental Markers of erythron activity

GATA-1 and hematological disease

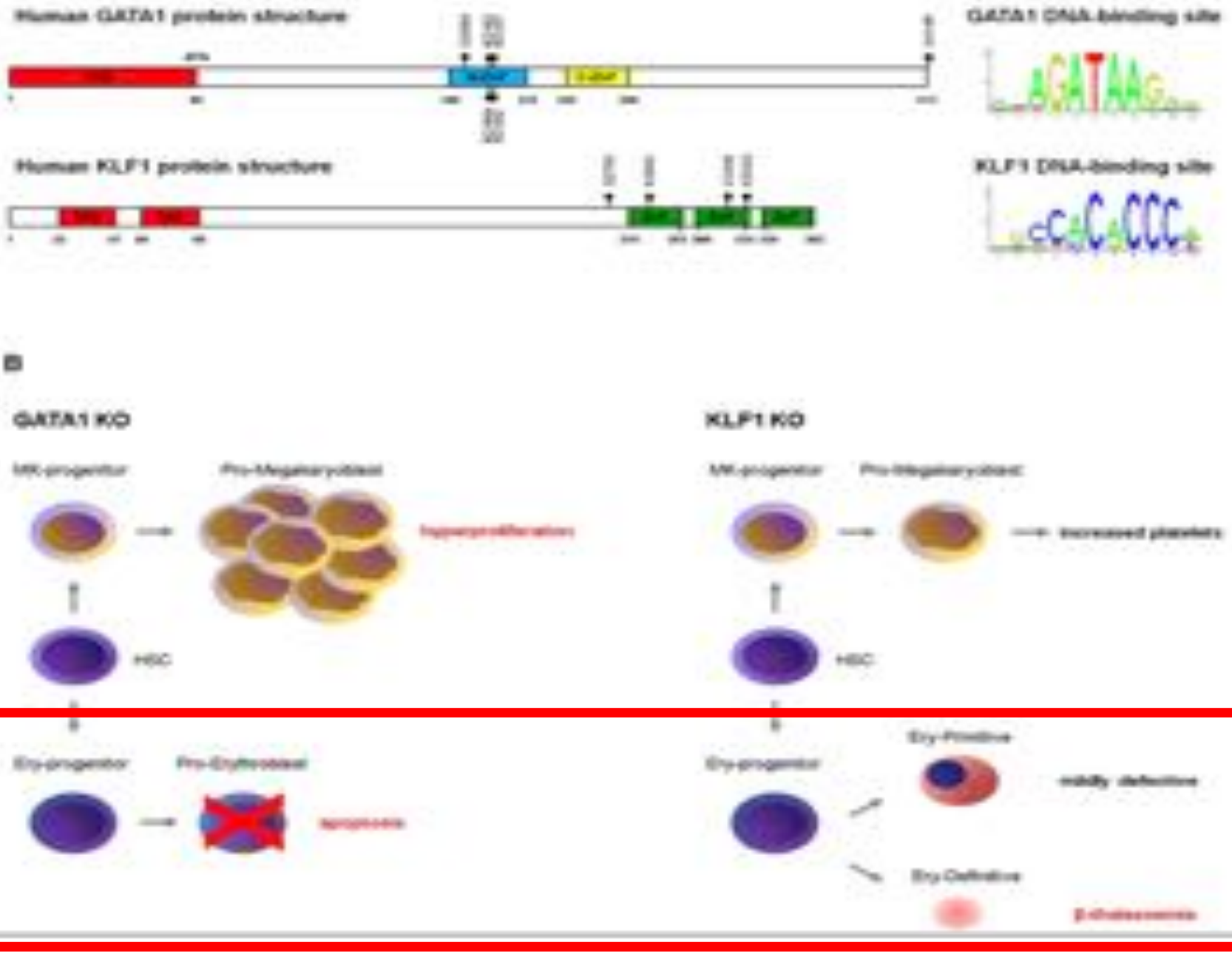




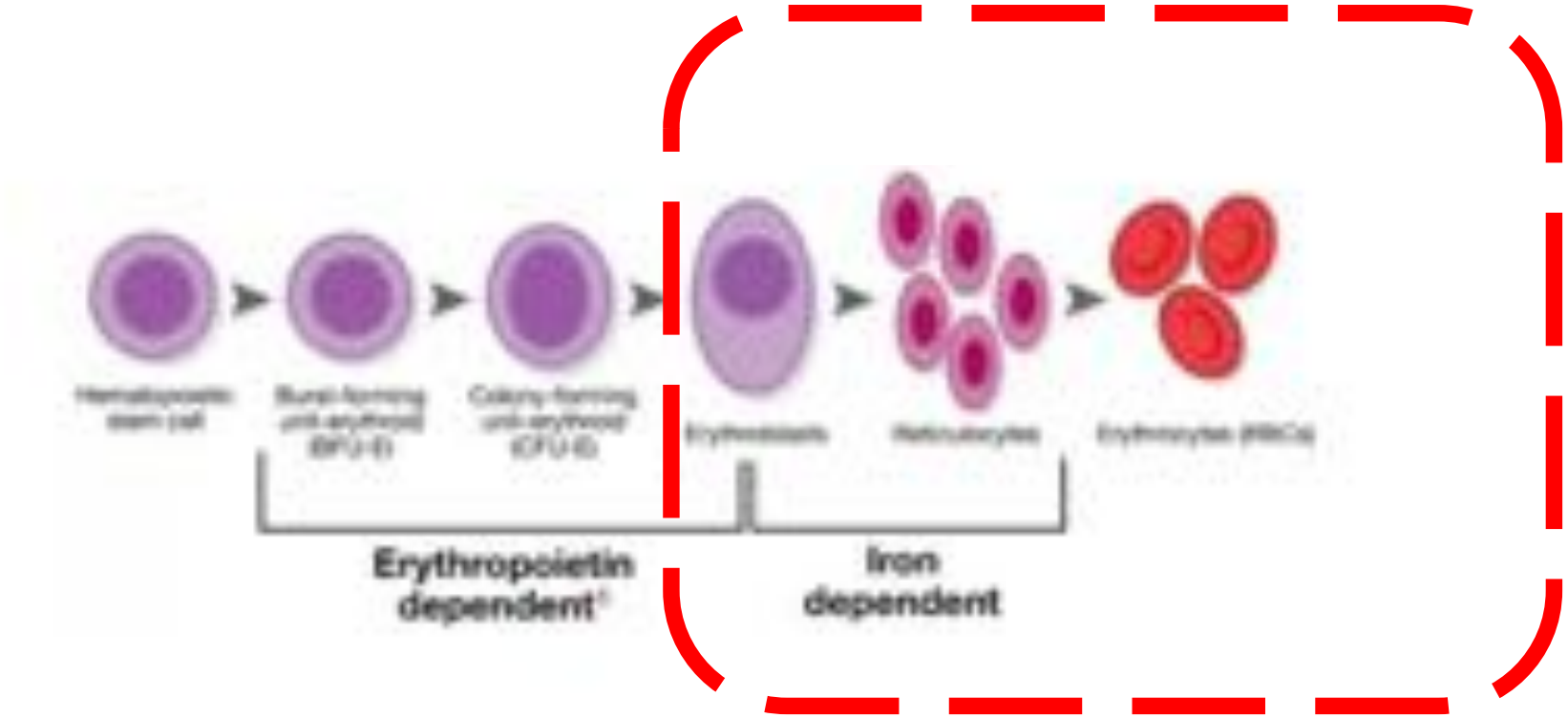
Reduced GATA-1 protein levels:

- β-thalassemia
- CDA variant
- Diamond Blackfan anemia (DBA)
- Myelodysplastic syndrome
- Myelofibrosis
- Aplastic anemia
- Leukemia

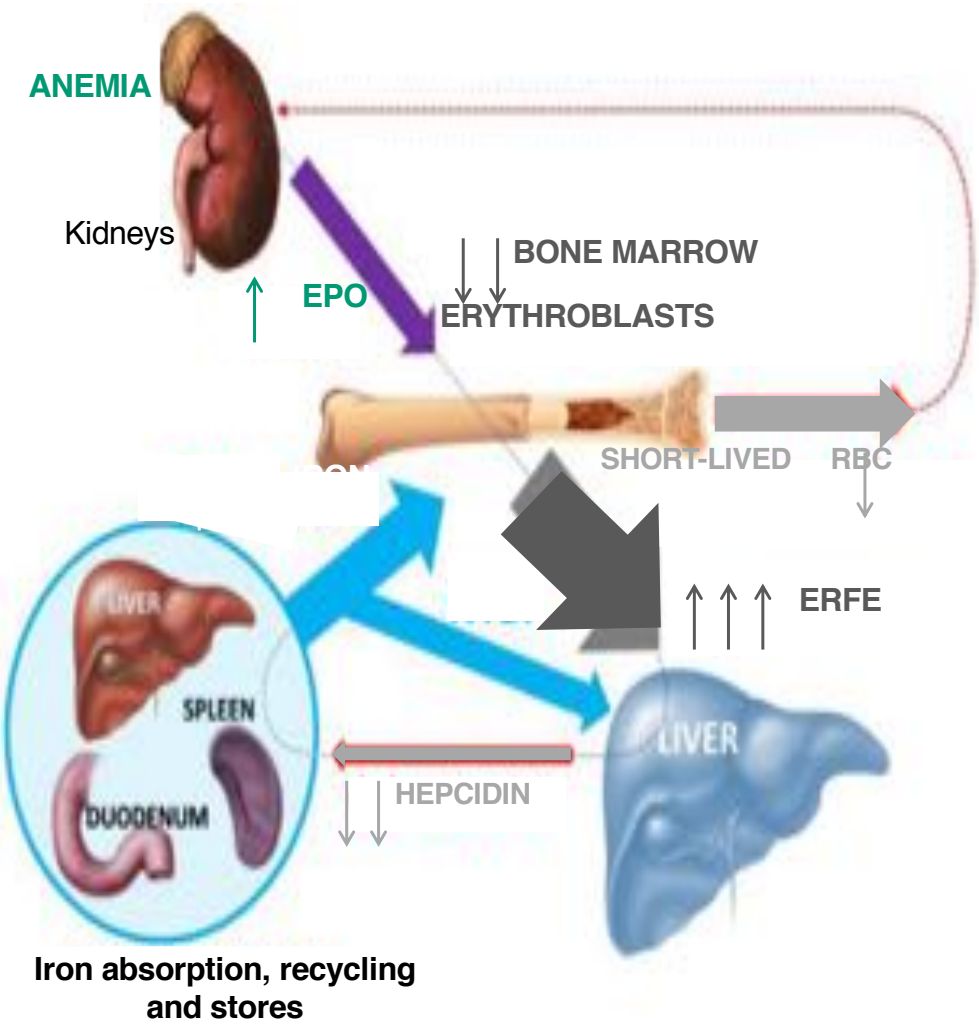
TRANSCRIPTION FACTOR'S DEFECT



Front. Physiol., 12 February 2019


Erythropoietin dependent
Iron dependent

Erythropoiesis in iron-loading anemias



In anemias with ineffective erythropoiesis, most erythroblasts do not successfully differentiate into mature erythrocytes, leading to anemia and increased EPO production by the kidneys. ERFE secretion is increased both because of EPO but also of the ERFE-secreting erythroblasts

Hepcidin is suppressed and iron is mobilized but erythrocyte production cannot increase and the additional iron is not utilized, generating NTBI and resulting in organ failure

Over the course of time, the combination of tissue hypoxia, increased erythropoietin and ineffective erythropoiesis creates a vicious cycle that may ultimately lead to a massive expansion of erythroblasts

Ganz T. Free Radic Biol Med. 2019;133:69-74

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Inherited disorders

Thalassaemia syndromes

NTDT β-thalassaemia intermedia

NTDT haemoglobin E/β-thalassaemia

NTDT haemoglobin H or α-thalassaemia

Congenital Dyserythropoietic Anaemias

Type I (*CDAN1* mutations)

Type II (*SEC23B* mutations)

Type III (*KIF23* mutations)

Variants (*KLF1* and *GATA1* mutations)

Inherited Sideroblastic Anaemias

X-linked sideroblastic anaemia (*ALAS2* mutations)

Recessive sideroblastic anaemia (*SLC25A38* mutations, *GLRX5* mutations)

Others

Haemolytic anaemias

Pyruvate kinase deficiency (selected cases)

Sickle cell anaemia (selected cases)

Acquired disorders

Myelodysplastic syndromes (MDS)

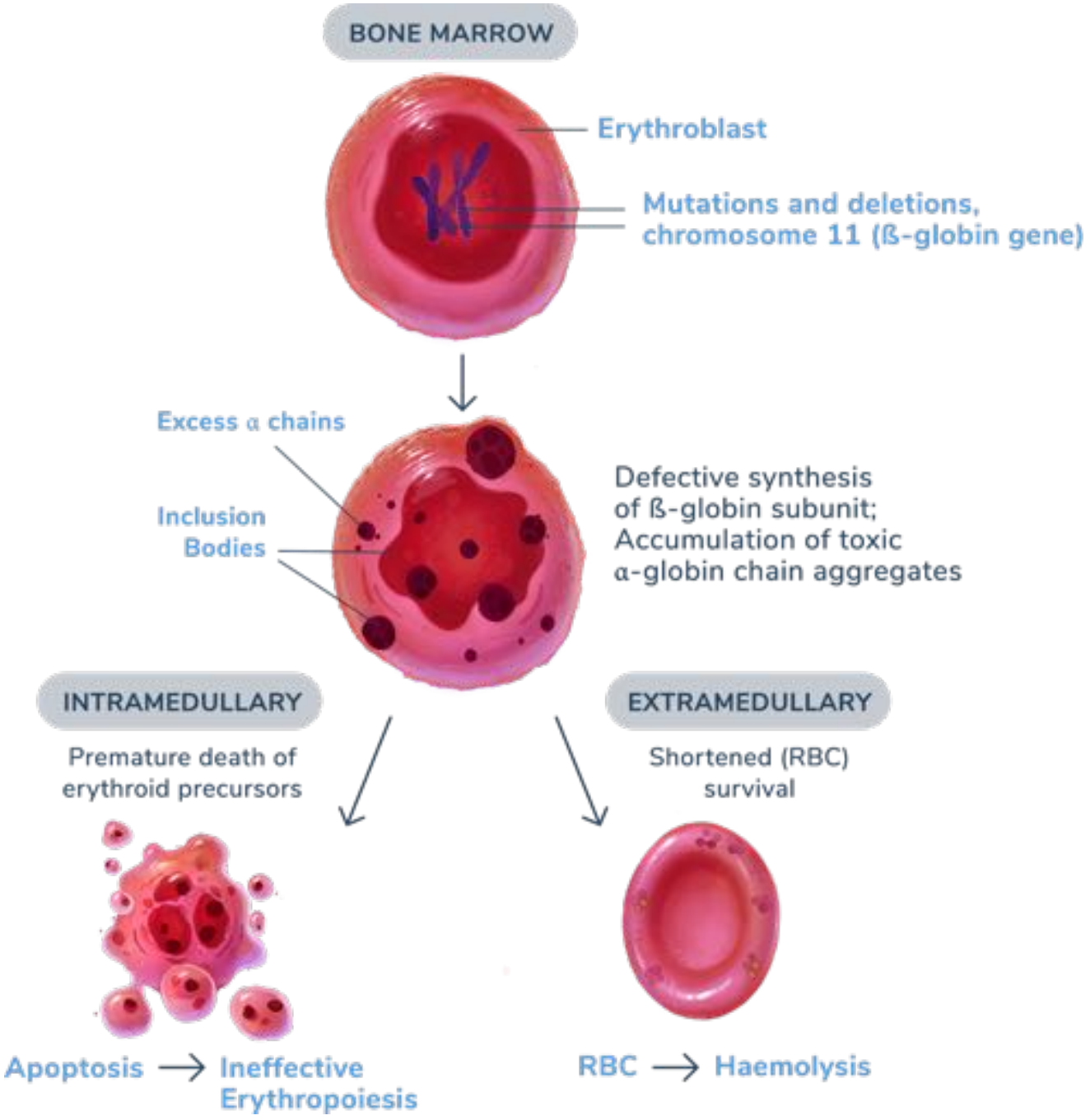
Refractory anaemia with ringed sideroblasts (RARS)

Refractory Cytopenia With Unilineage Dysplasia (RCUD), previously termed Refractory anaemias (RA)

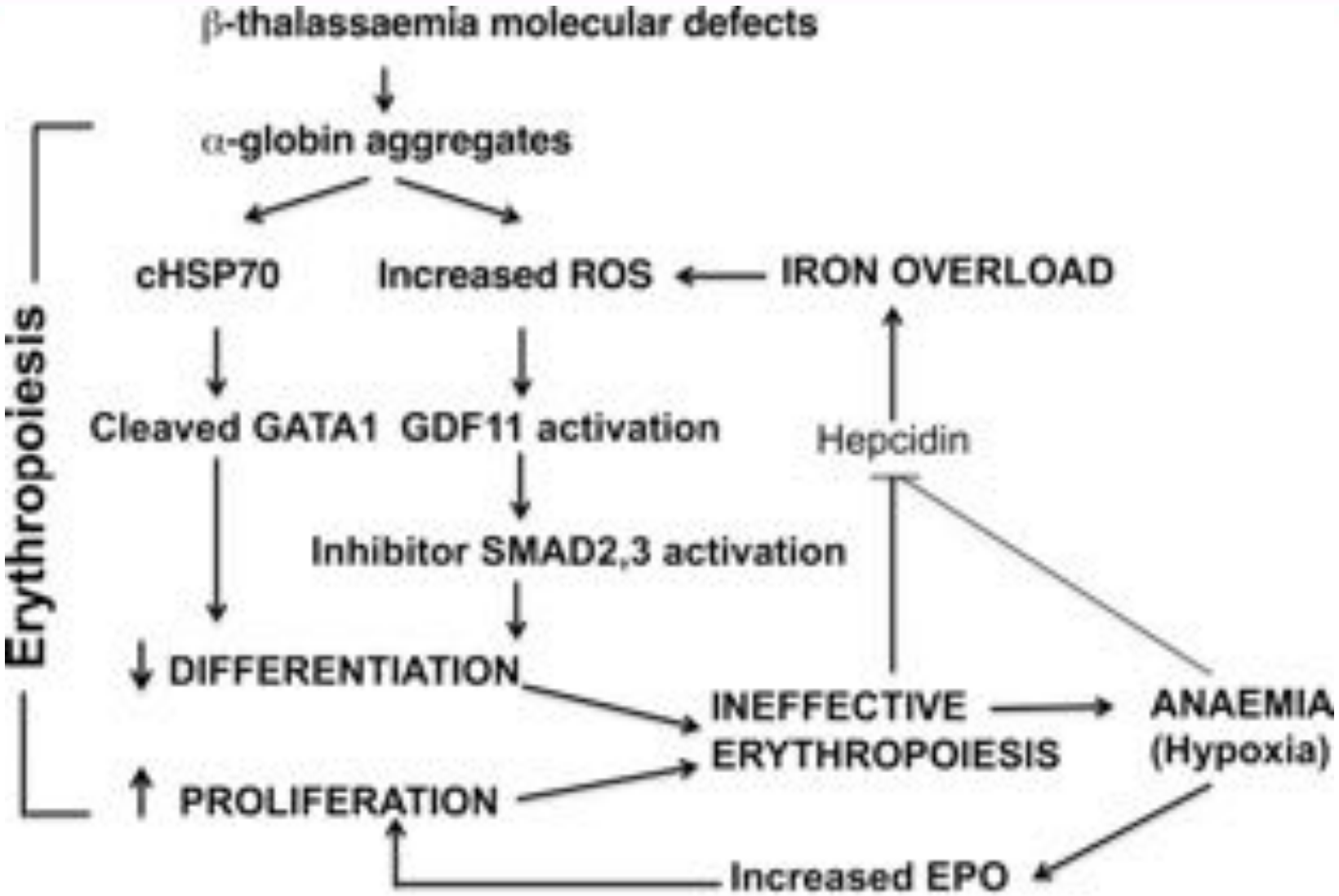
NTDT, non transfusion dependent thalassaemia.

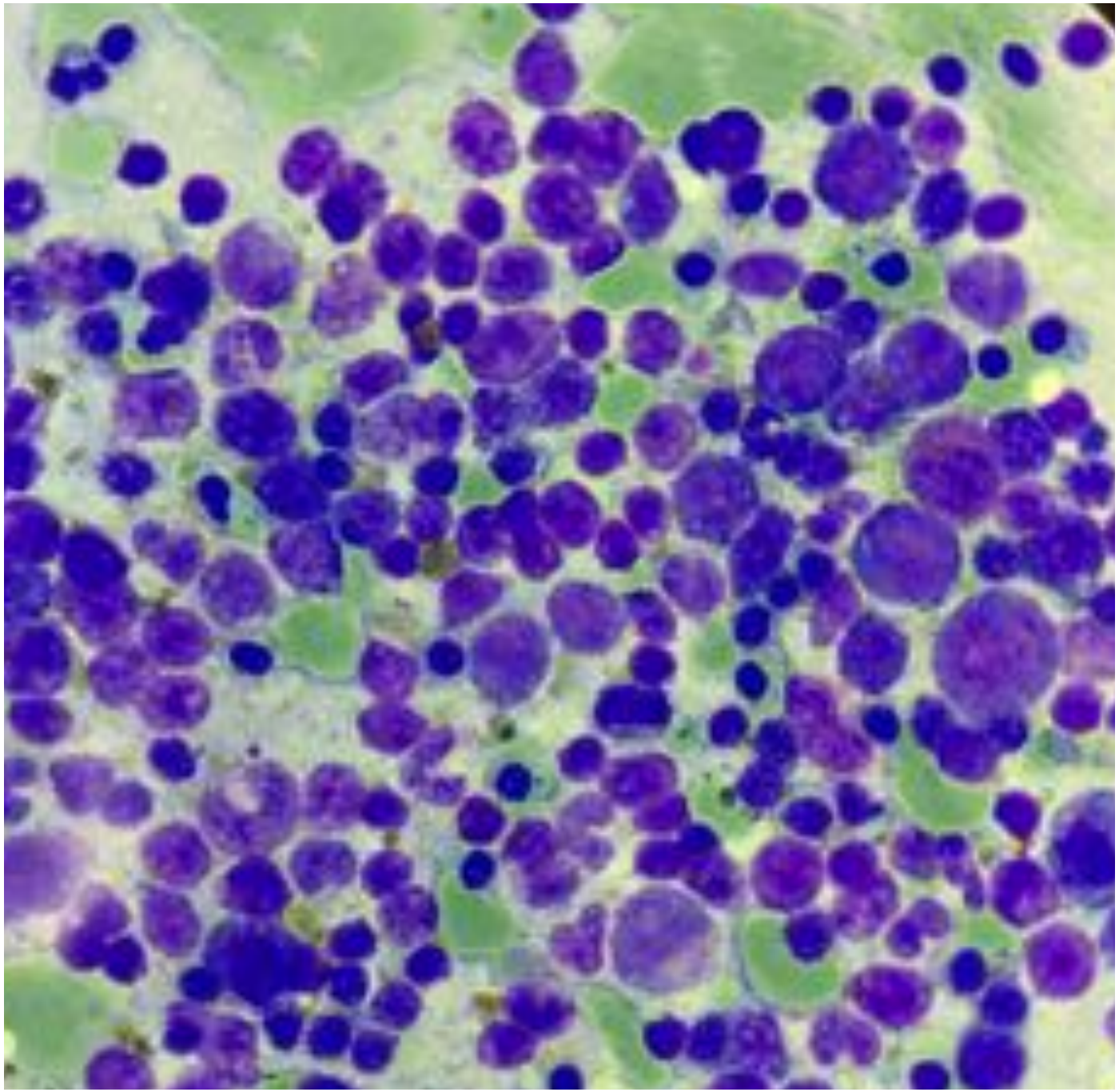
Anaemias characterized by ineffective erythropoiesis

Ineffective erythropoiesis in β-thalassemia

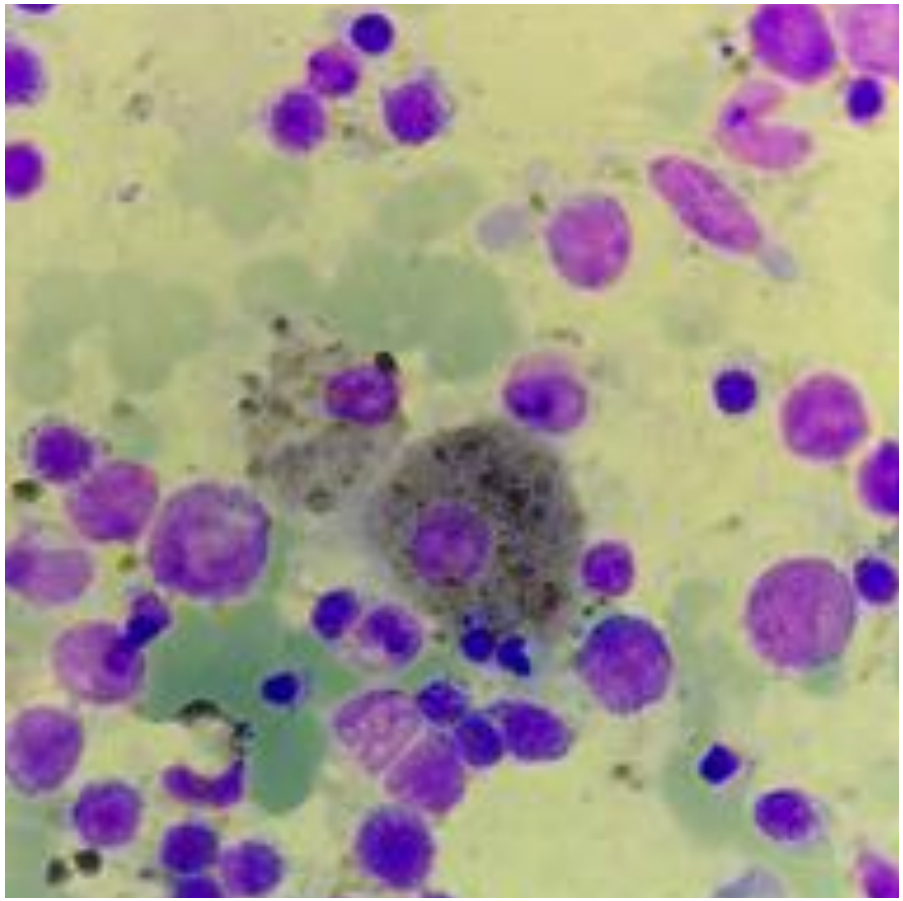


Pathophysiology of ineffective erythropoiesis in NTDT

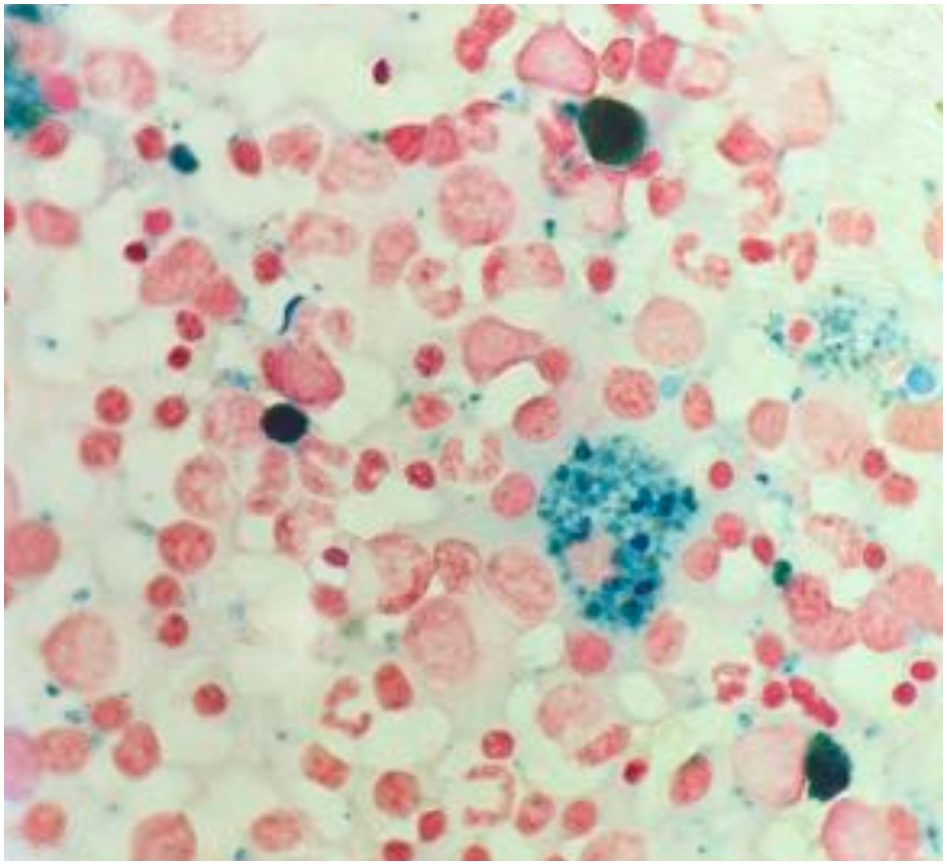




Personal imagines' library. Bone marrow aspirate from β-TM (hematoxylin-eosin staining)



hematoxylin-eosin staining



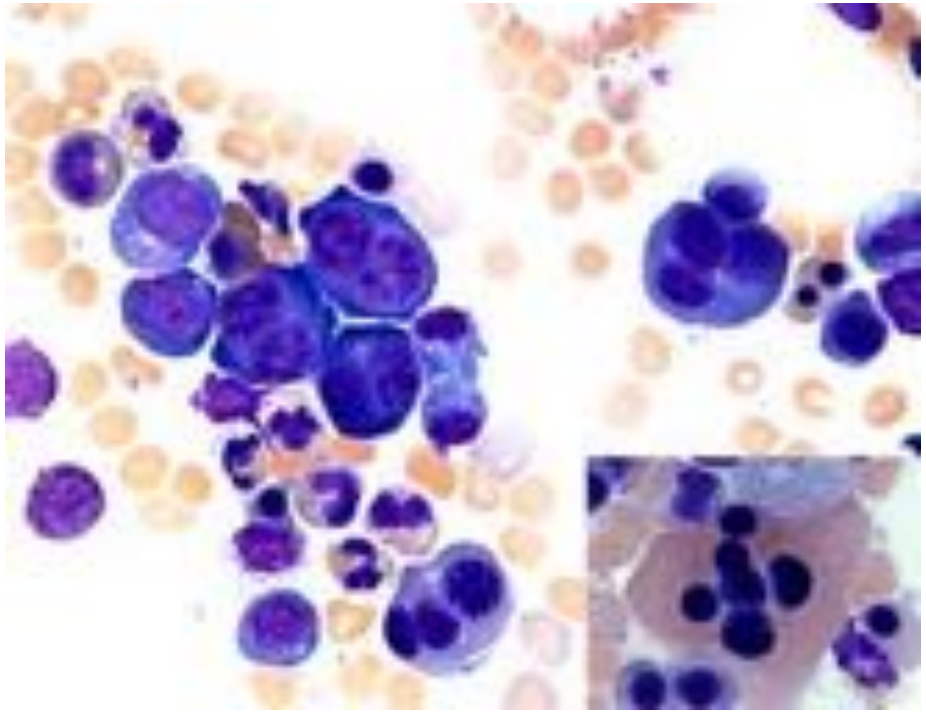
Perls’ Prussian Blue staining

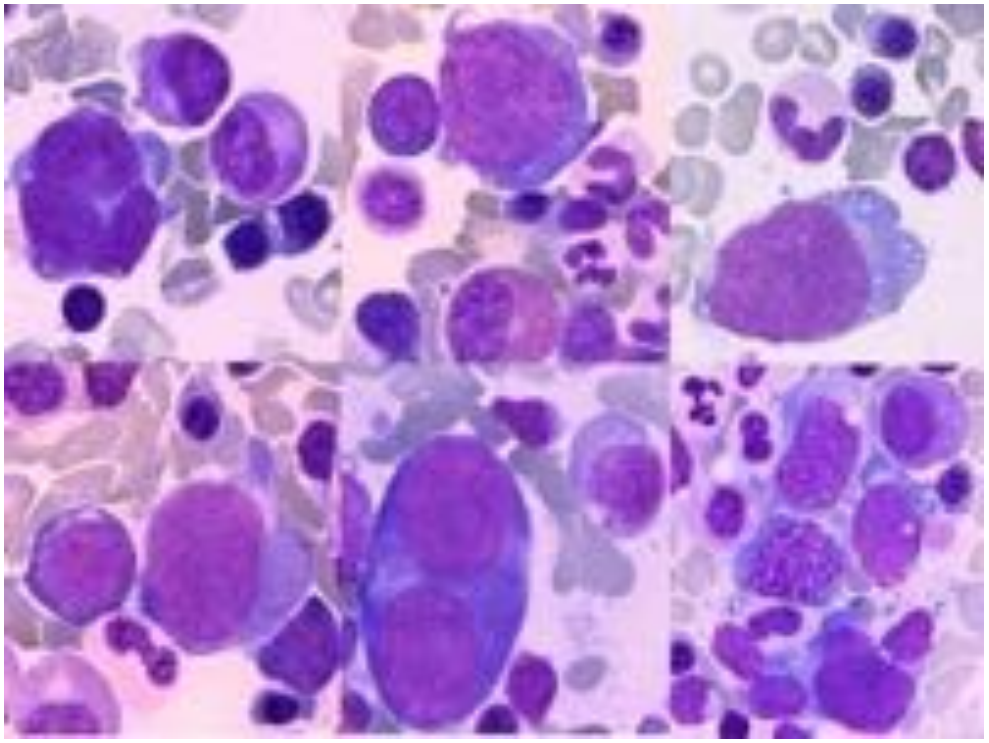
Personal imagines' library. Bone marrow aspirate from β-TM

Pathophysiology of ineffective erythropoiesis in MDS

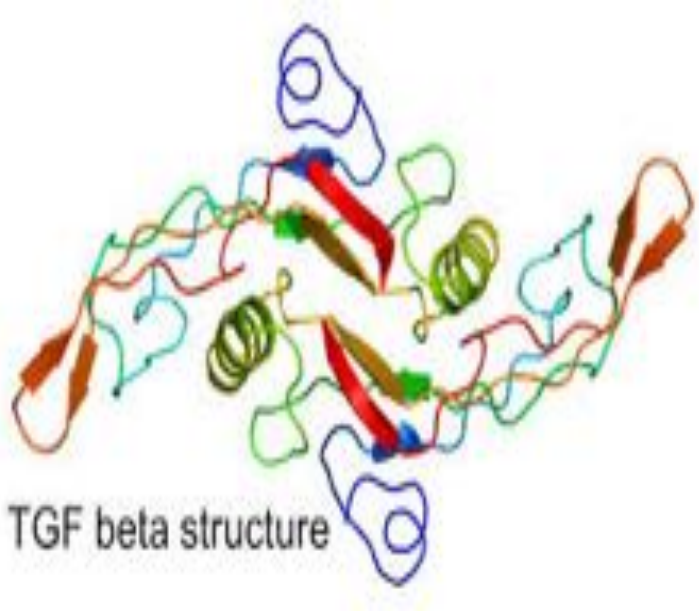
- paradoxical combination of erythroid bone marrow hyperplasia and peripheral anemia.
- extrinsic apoptotic pathway activation
- intrinsic apoptotic pathway activation (release of apoptogenic factors from mitochondria including cytochrome c)
- accumulation of iron in the mitochondrial matrix of ringed sideroblasts and the occurrence of mitochondrial DNA mutations.
- exacerbation of the physiologic mechanisms of Fas-mediated control of erythropoiesis and exhibit a Fas-dependent apoptosis, even in the presence of high Epo levels.

Pathophysiology of ineffective erythropoiesis MDS





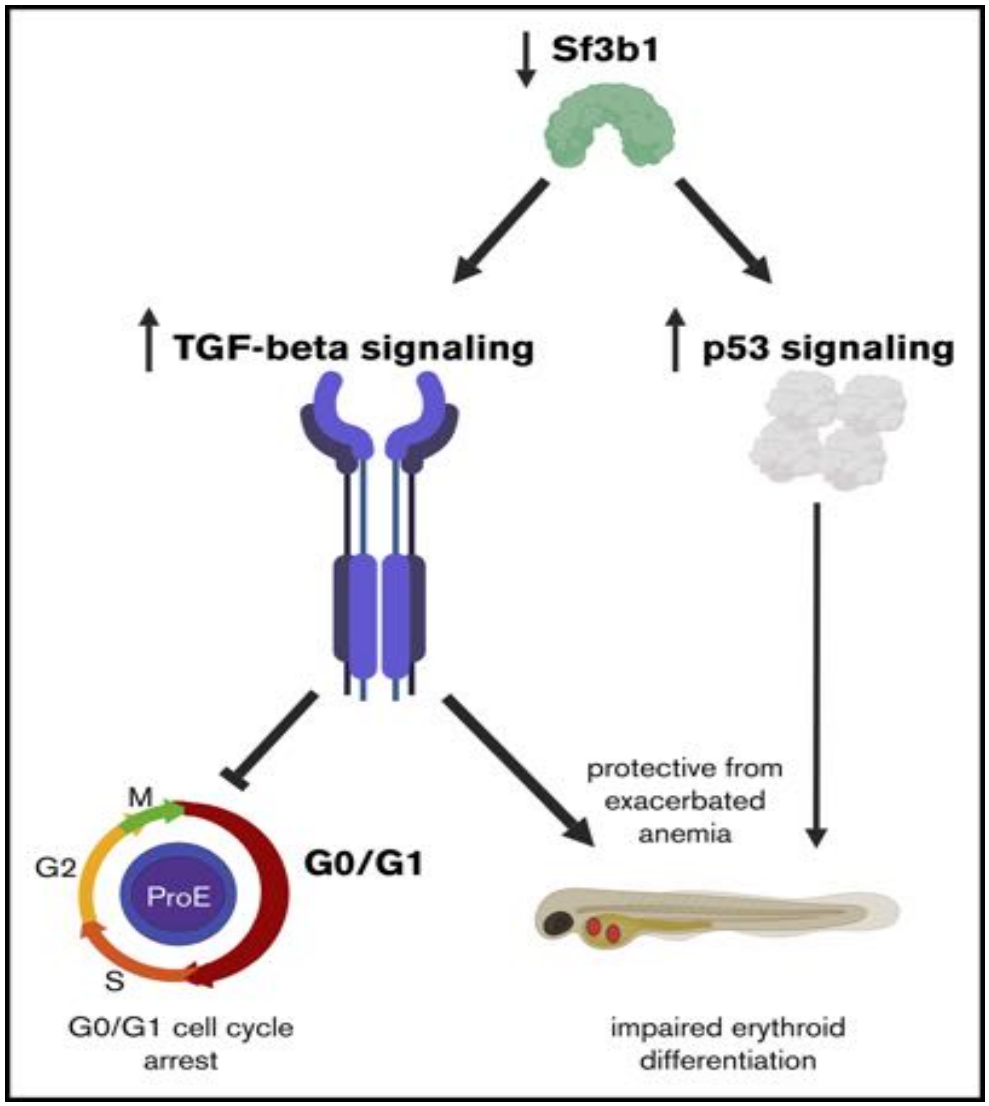
TGF-β pathtway upregulation in MDS



- Apoptosis of HSC mediated by Activin A has been detected In MDS, especially in case whit del 5q
- Elevated levels of GDF11 in plasma of MDS patient
- Increased transcription of TGF-β genes via SMAD 2/3 Pathway ,

Suragani RNVS Nat Med 2014, Bhagat TD Blood 2013, Zhou L Cancer Res 2011

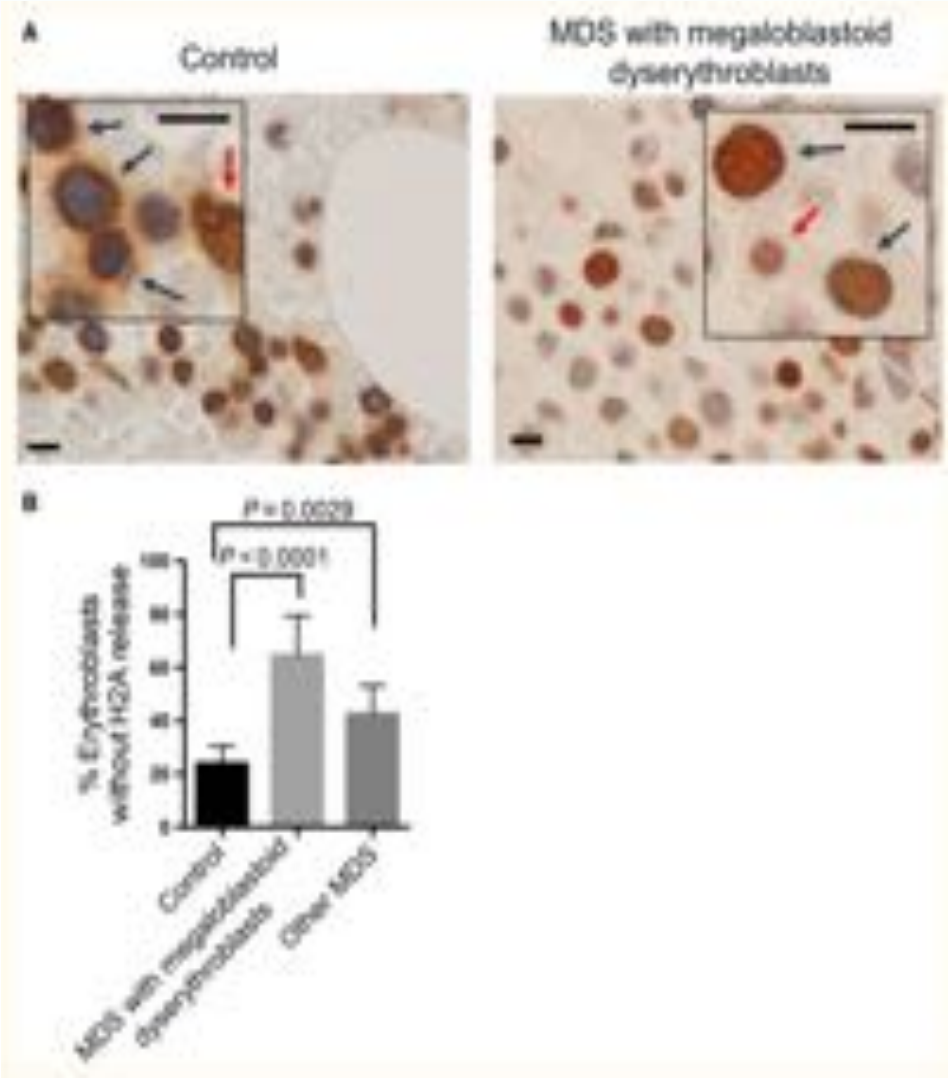
The splicing factor Sf3b1 regulates erythroid maturation and proliferation via TGFβ signaling in zebrafish



- ***sf3b1*** **deficiency led to the production of fewer erythrocytes that show maturation defects and dysplasia**
- **Genes in the TGFβ and the p53 pathways were significantly upregulated upon *sf3b1* loss.**
- **TGFβ pathway activity induced a G0/G1 cell-cycle arrest in *sf3b1*-mutant proerythroblasts.**

Adriana De La Garza,Rosannah C. Cameron,Varun Gupta,Ellen Fraint,Sara Nik,Teresa V. Bowman, The splicing factor Sf3b1 regulates erythroid maturation and proliferation via TGFβ signaling in zebrafish, Blood Adv, 2019,

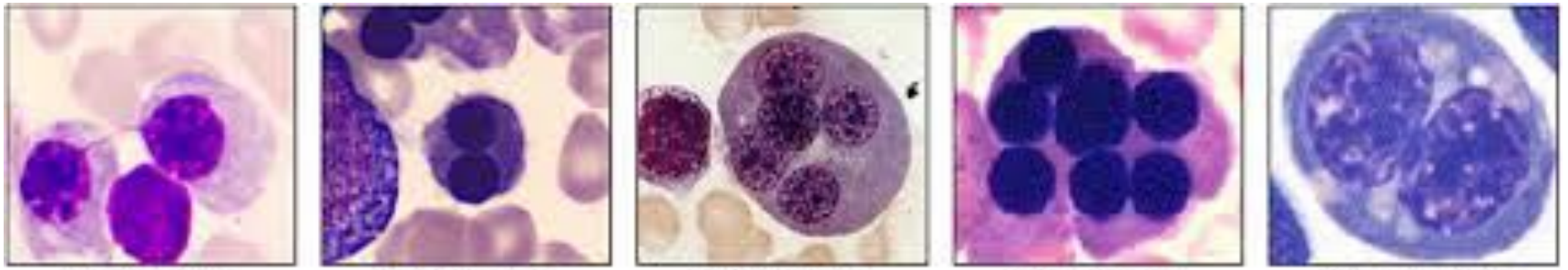
Disruption of erythroid nuclear opening and histone release in myelodysplastic syndromes



- Caspase-3 inhibition leads to the blockage of histones release and nuclear condensation, and differentiation in human terminal erythropoiesis
- Compared to healthy individuals, erythroblasts from MDS patients exhibit impaired histones release and chromatin condensation, which phenocopies the effect of caspase-3 inhibition.
- These findings indicate that defects of nuclear opening formation and histones release contribute to the pathogenesis of megaloblastoid changes in dyserythropoiesis in MDS.

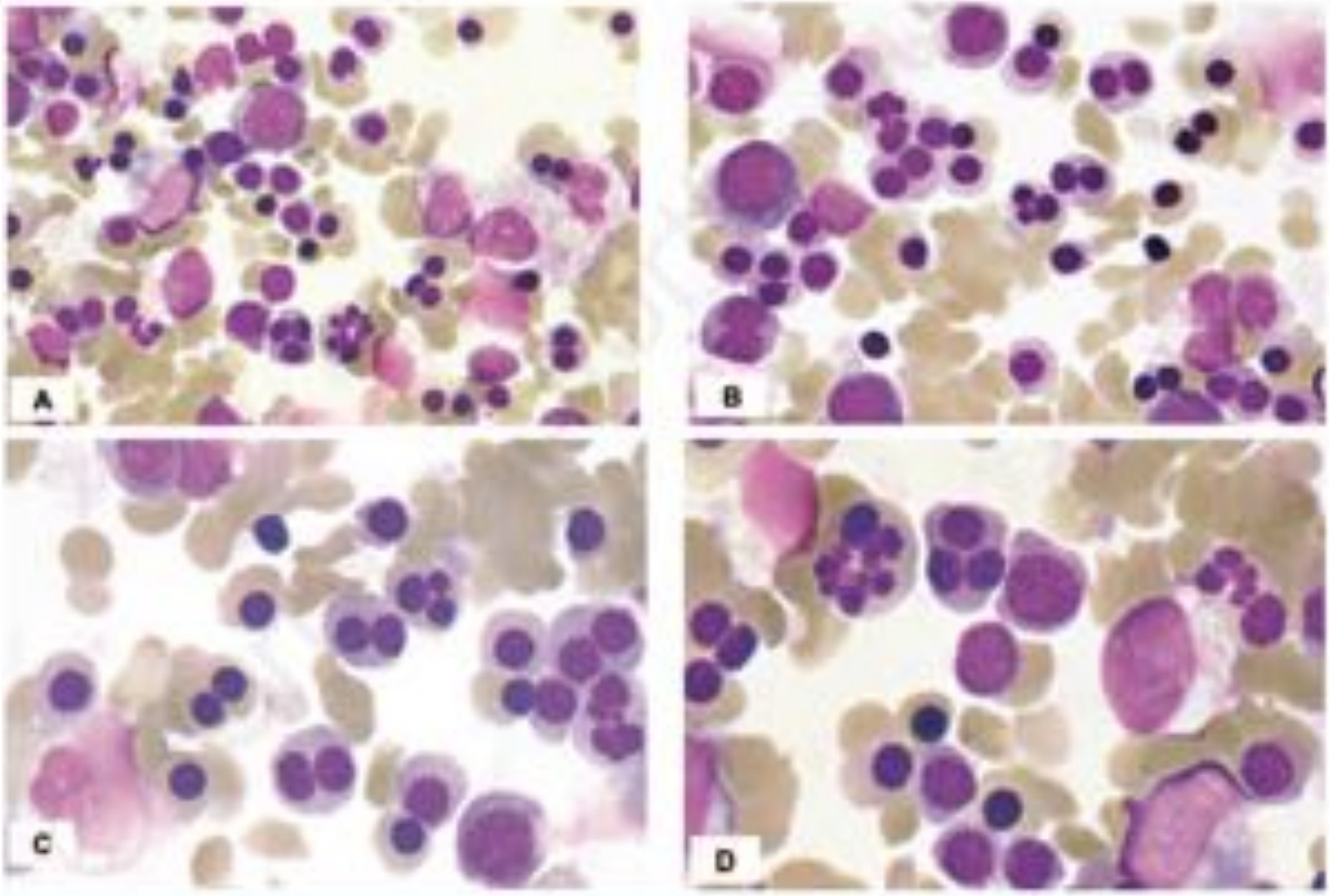
Cancer Med. 2019 Mar; 8(3): 1169–1174.

Congenital dyserythropoietic anemia

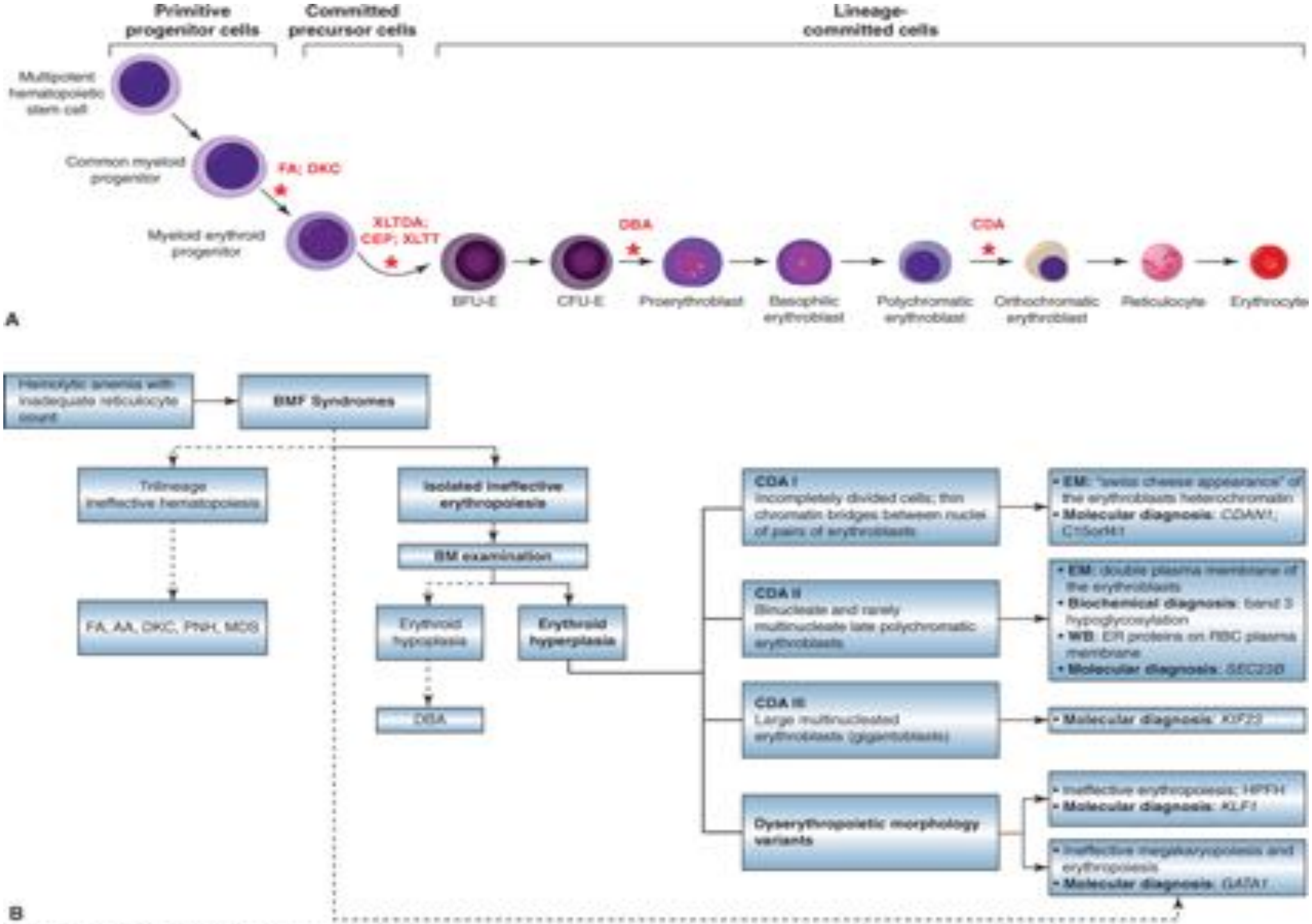


CDA type I | CDA type II | CDA type III familial | CDA type III sporadic | CDA variants

Congenital dyserythropoietic anemia (CDA)



haematologica | 2010; 95(5)



Source: K. Kaushansky, M.A. Lichtman, J.T. Prchal, M.M. Levi, O.W. Press, L.J. Burns, M. Caligiuri: Williams Hematology, 9th Edition
www.accessmedicine.com

CASO CLINICO

M 51 aa , Consulenza ematologica per pancitopenia e splenomegalia

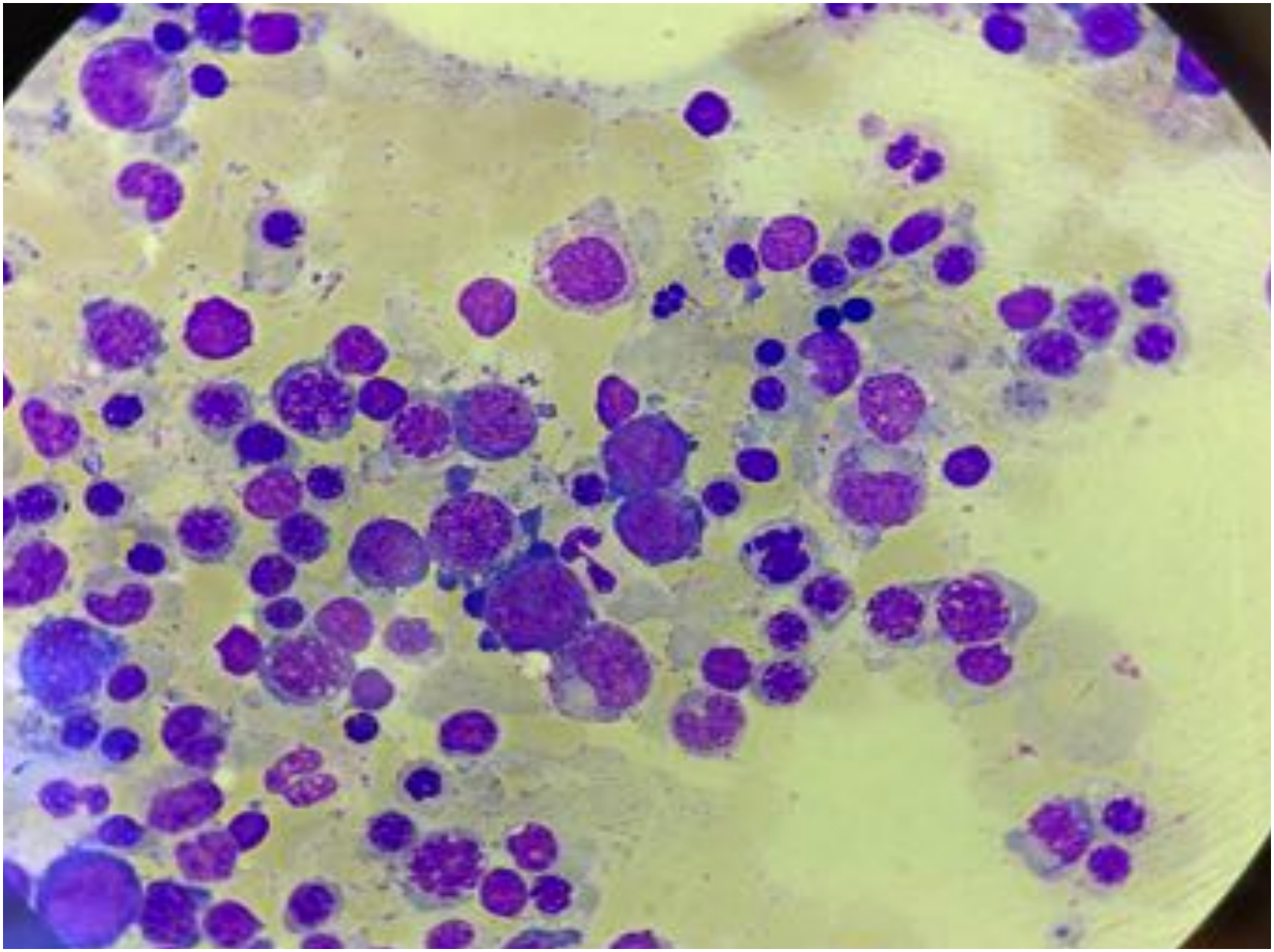
Anamnesi: β-talassemia minor, Lupus discoide non in trattamento epatopatia autoimmune in corso di definizione diagnostica al momento della consulenza. Trasfusione dipendente da circa 1 anno. Severo accumulo marziale epatico valutato con MRI

OB: Milza in FISx in ap, epatomegalia , non linfoadenomegalie. Non segni clinici di emolisi

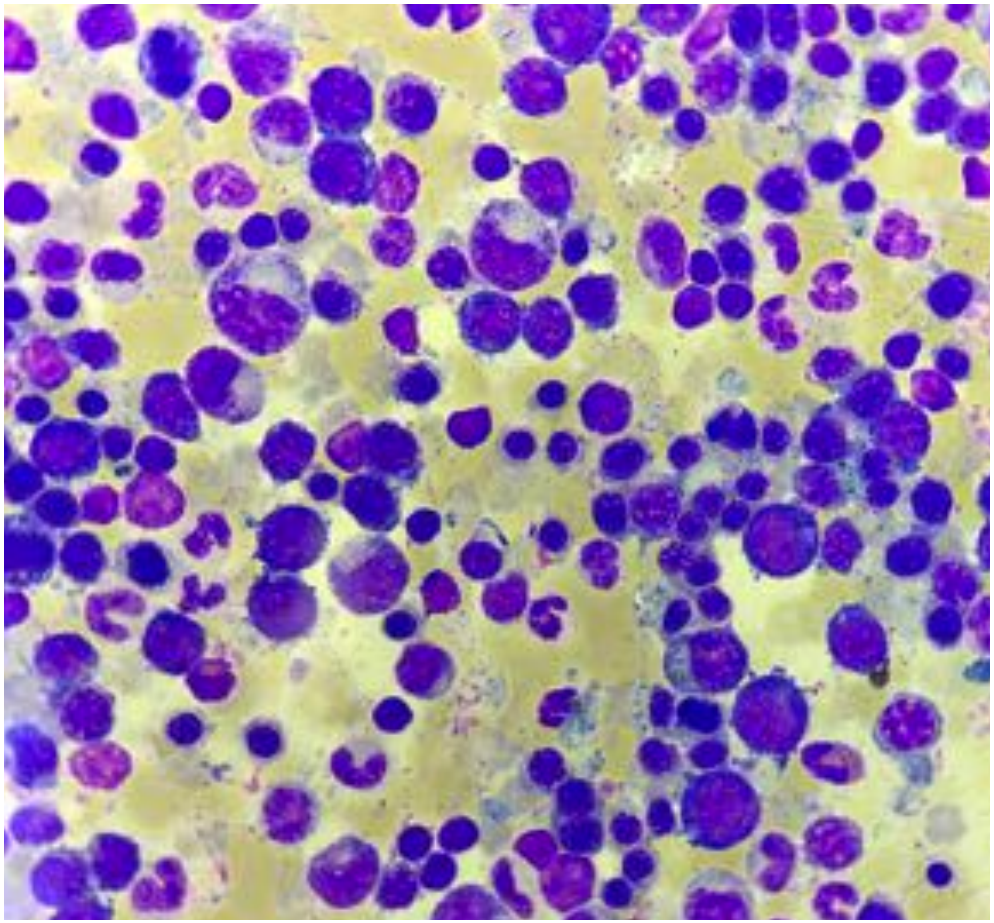
Emocromo: Hb 8,4gr/dL, MCV 69, GB 2380 (GN 1650), Plt 46.000. Reticolociti 1,8%

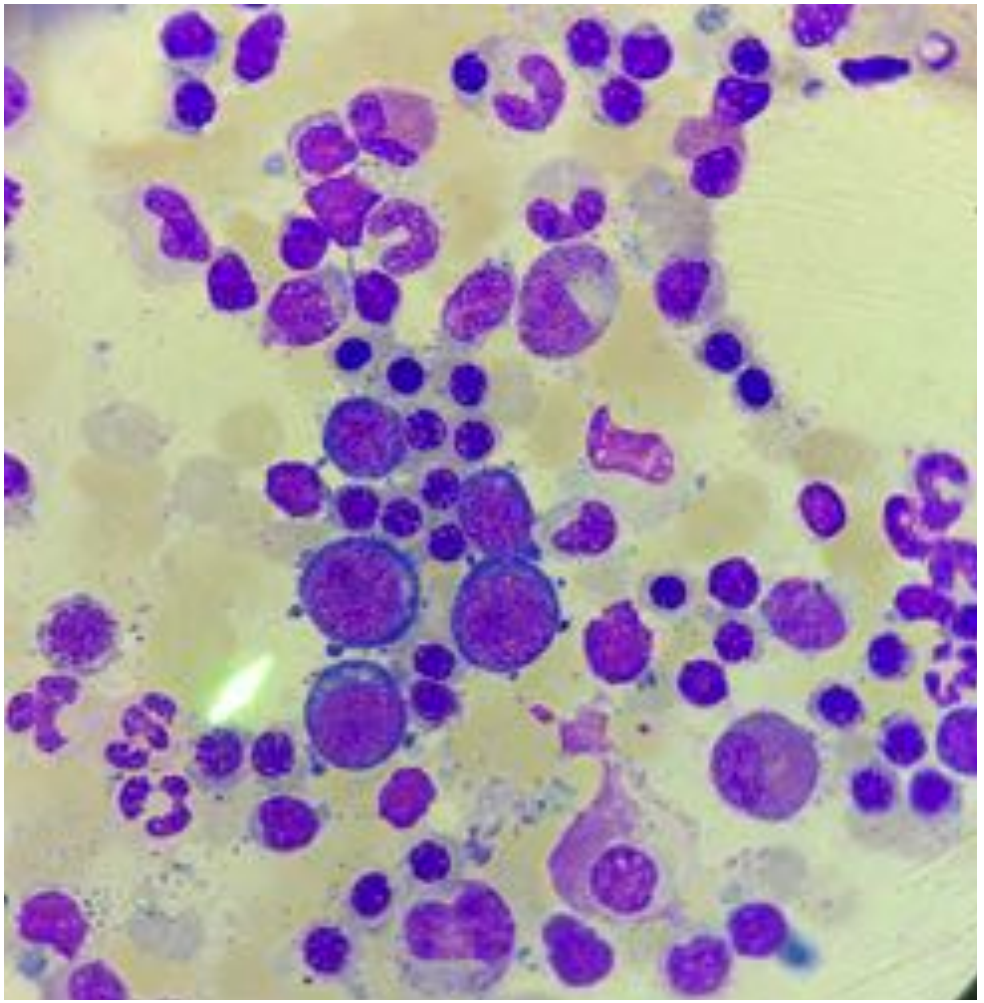
Chimica: Aptoglobina consumata, Iperbilirubinemia dir/ind, TDC diretto e ind neg , LDH normale

Pratica: Aspirato midollare, Biospia osteomidollare, citogenetica, indagini molecolari per malattie mielo/linfoproliferative



Personal imagines' library. Bone marrow aspirate . Hematoxylin-eosin staining





Personal imagines' library. Bone marrow aspirate . Hematoxylin-eosin staining

CASO CLINICO

Aspirato midollare: marcata iperplasia eritroide con note displastiche, Granulopoiesi e Megacariocitopoiesi nella norma
Non aumento forme immature

BOM : Cellularità 45%, prevalenza dell'eritropoiesi in assenza di precursori immaturi. Non fibrosi. Non infiltrato linfoide clonale. Non diagnostico per emolinfopatia

Biol Molecolare: BCR/ABL e JAK2 Negativi
Immunofenotipo: Negativo per EPN

Citogenetica: 46 XY

CONCLUSIONE: CASO CLINICO

Invio campioni per screening malattie con diseritropoiesi congenite rare

Stomatocitosi ereditaria con emazie disidratate (DHSt)

Prevalenza 1:50.000
Autosomica dominante
Mutazioni nel gene *PIEZO1*, che codifica per una porzione di un canale ionico meccano-sensibile.
Sintomi: pazienti adulti presenta lieve anemia o emolisi completamente compensata, con affaticamento, ittero, splenomegalia, complicanze da sovraccarico di ferro.
Diagnosi si basa sul riscontro degli stomatociti tipici nel sangue periferico, aumento del volume dei globuli rossi (macrocitosi), diminuzione della concentrazione cellulare media di emoglobina (MCHC) e aumento del numero dei reticolociti

β-trait !!!!!

HEMOLYTIC ANEMIAS' CLASSIFICATION

	INTRACORPUSCULAR DEFECTS	EXTRACORPUSCULAR FACTORS
HEREDITARY	•HEMOGLOBINOPATHIES •ENZYMOPATHIES •MEMBRANE-CYTOSKELETAL DEFECTS	•FAMILIAL HEMOLYTIC UREMIC SYNDROME
ACQUIRED	•PAROXYSMAL NOCTURNAL HEMOGLOBINURIA	•MECHANICAL DESTRUCTION [MICROANGIOPATHIC] •TOXIC AGENTS •DRUGS •INFECTIOUS •AUTOIMMUNE

Thanks for your very kind attention

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