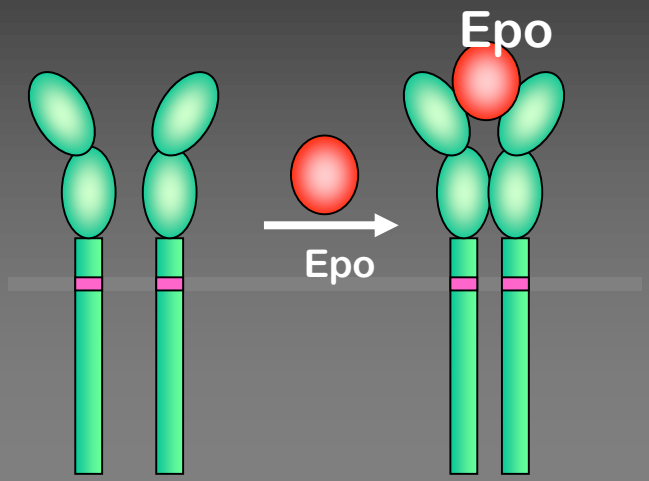
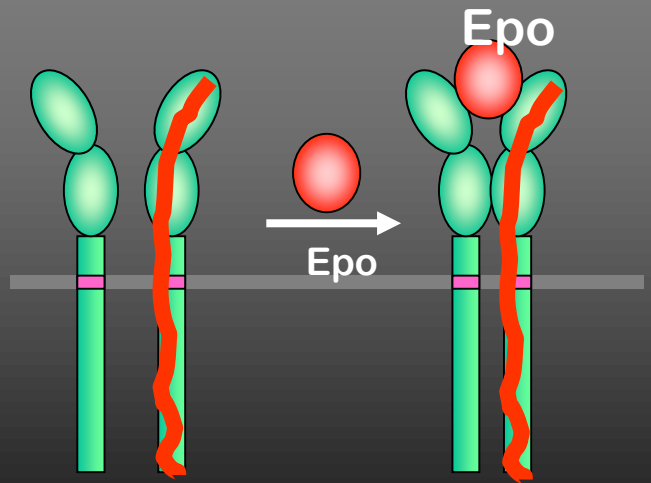
Un altro recettore dell'Epo!

Famiglia dei recettori delle citochine



EPOR-EPOR



EPOR-CD131

Legame del ligando

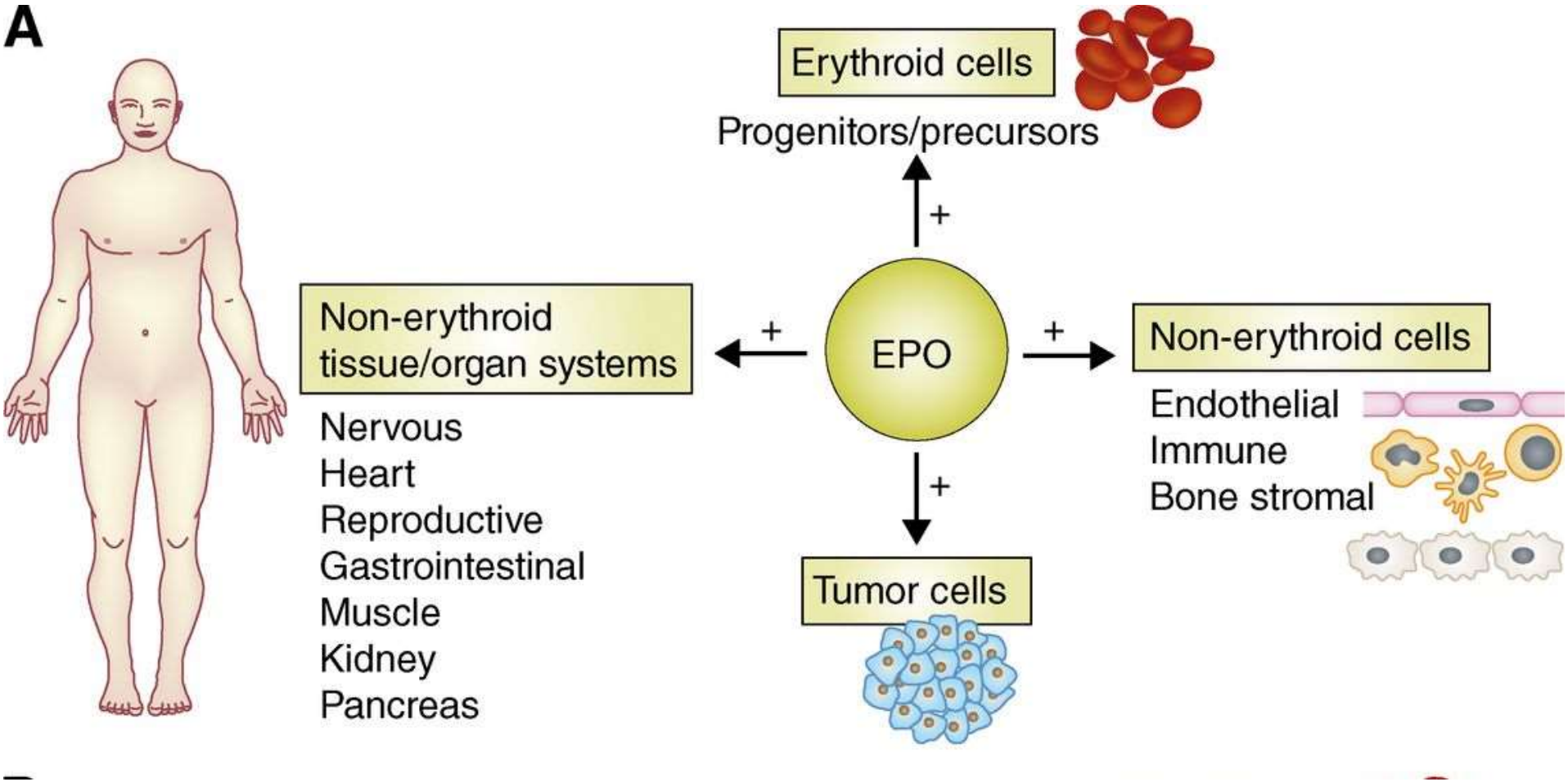
↓

Dimerizzazione

↓

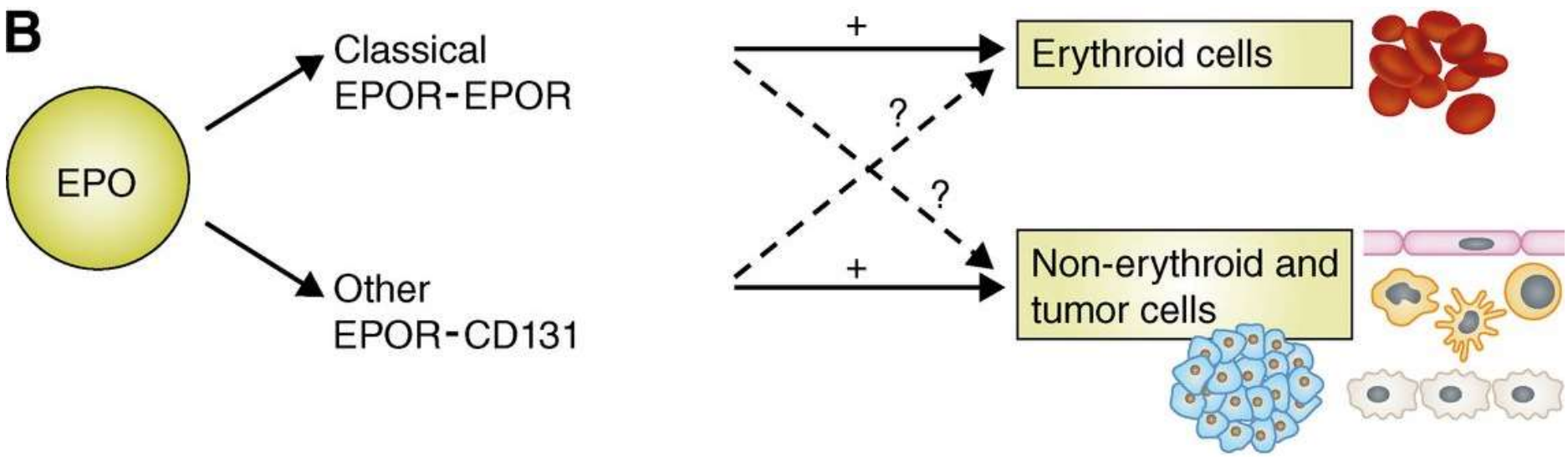
Attivazione del recettore

EPO targets many cell types and tissues



Broxmeyer H E J Exp Med 2013;210:205-208

Multifaceted effects and targets of EPO



EPO targets many cell types and tissues, including erythroid cells and their progenitors, tumor cells, and a variety of other nonerythroid cells and tissues

EPO signals in erythroid cells via EPOR-EPOR homodimers

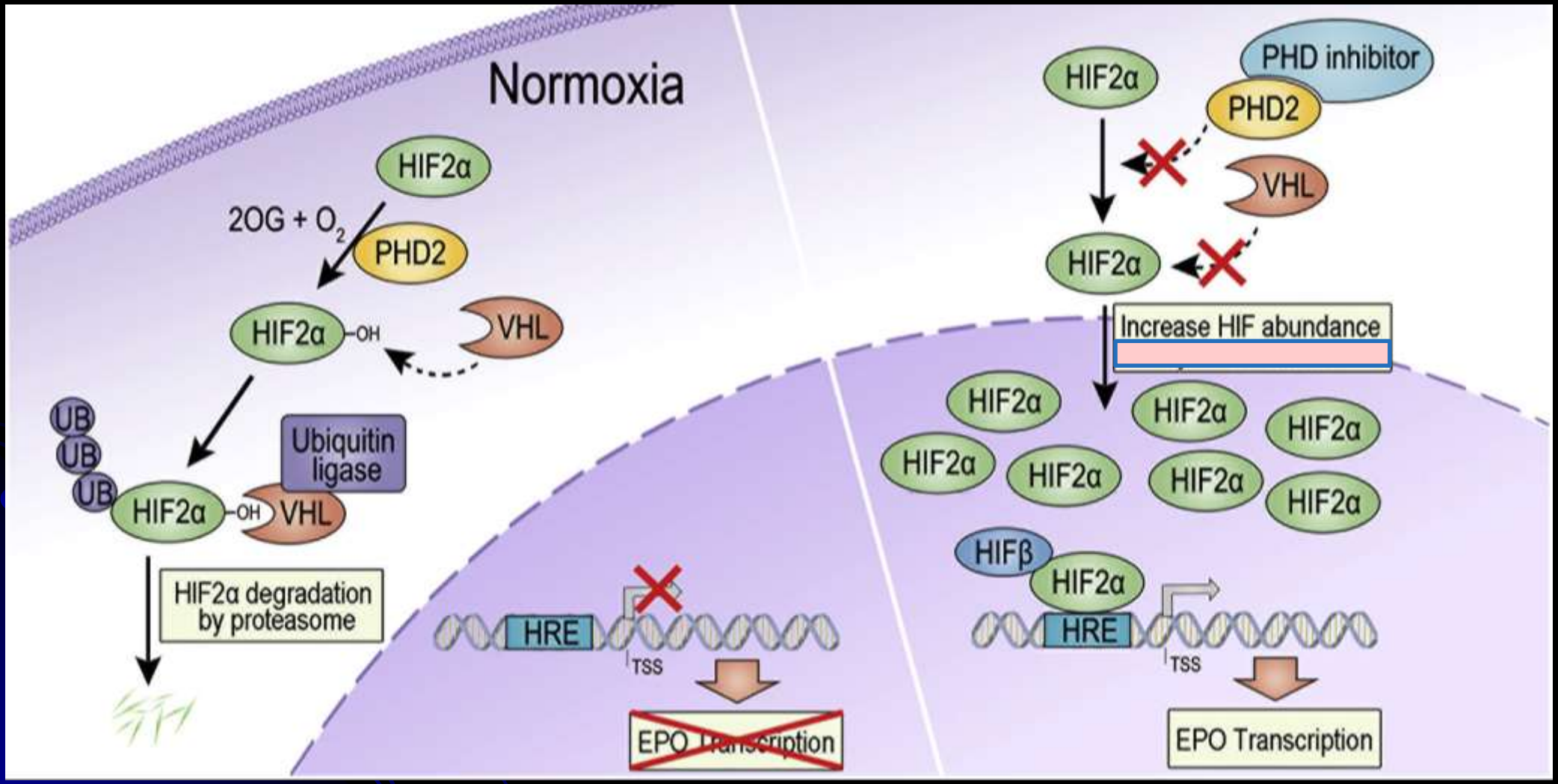
and in nonerythroid cells via EPOR-CD131 heterodimers

Broxmeyer H E J Exp Med 2013;210:205-208

JEM

Mutazioni nella Pathway oxygen sensing

Mutazioni nella Pathway oxygen sensing



Policitemia di Chuvash

Ang et al. Nature Genetics 2002

- Policitemia autosomica recessiva trovata in Russia

Table 1 • Biochemical parameters in Chuvash polycythemia

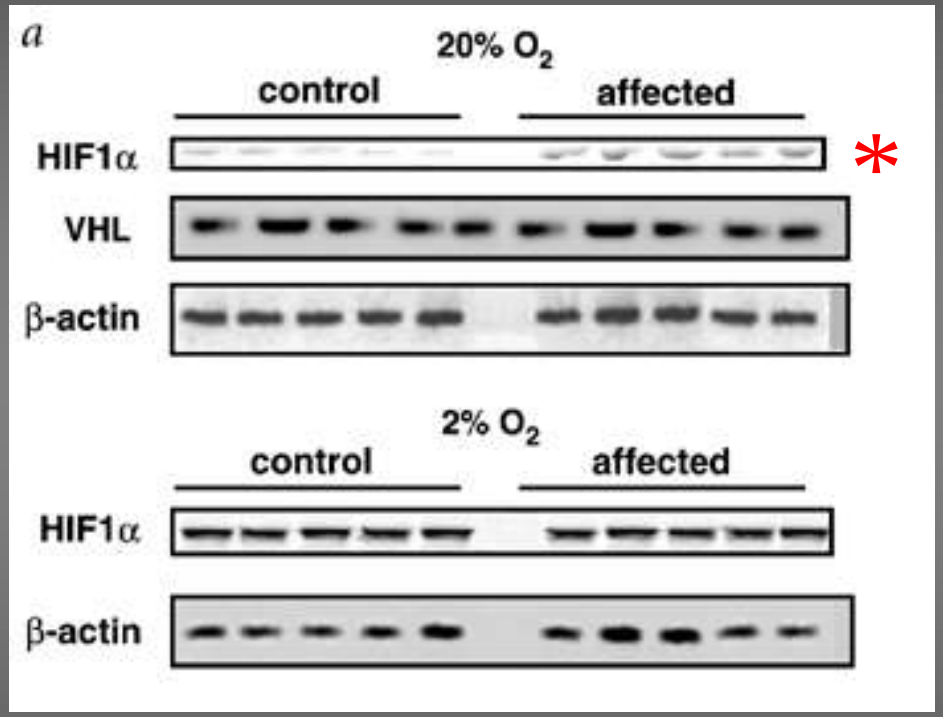
	Individuals with Chuvash polycythemia ($n = 20$)	Unaffected relatives ($n = 51$)	P
Erythropoietin (mIU ml^{-1})	61.9 ± 12.8	6.4 ± 6.9	0.001

Sequenziamento gene von Hippel Lindau (VHL) → **C/T transition, Arg/Trp200** (Pazienti omozigoti)

Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia

Sonny O. Ang

Nature genetics 2002, volume 32 no. 4 pp 614 - 621



Western blot, 5 pazienti + 5 controlli

20% O_2:

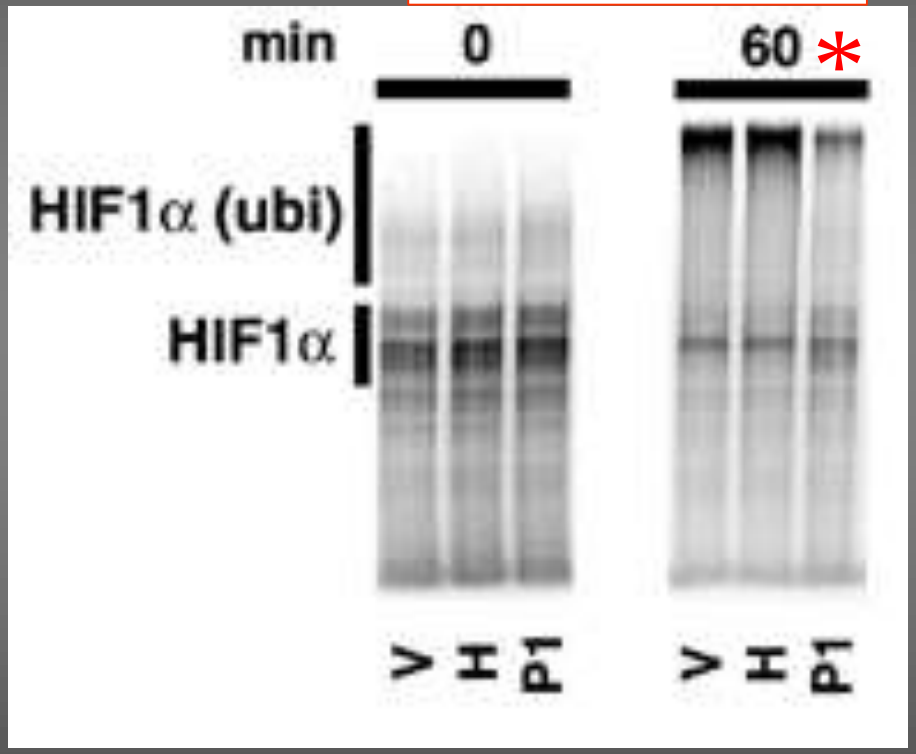
- Livelli di proteina VHL normali in mutato e Wt
- Livelli di HIF1α maggiori nei soggetti affetti

Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia

Sonny O. Ang

Nature genetics 2002, volume 32 no. 4 pp 614 - 621

NORMOSSIA



V= controllo (Wild type)

H= eterozigote

P1= paziente (omozigote)

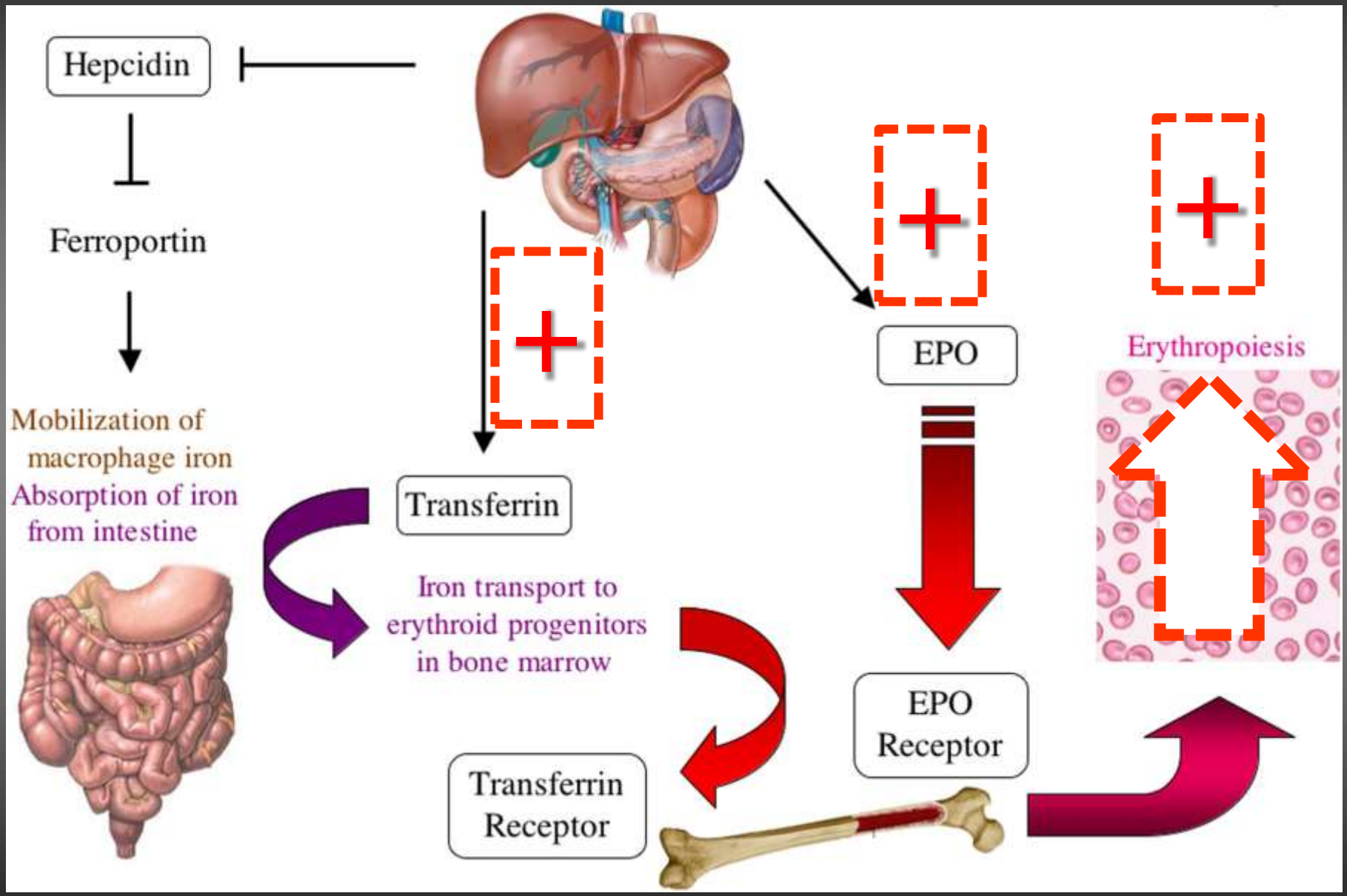
La forma ubiquitinizzata è meno presente nelle cellule del paziente

Mutazione Arg200Trp:

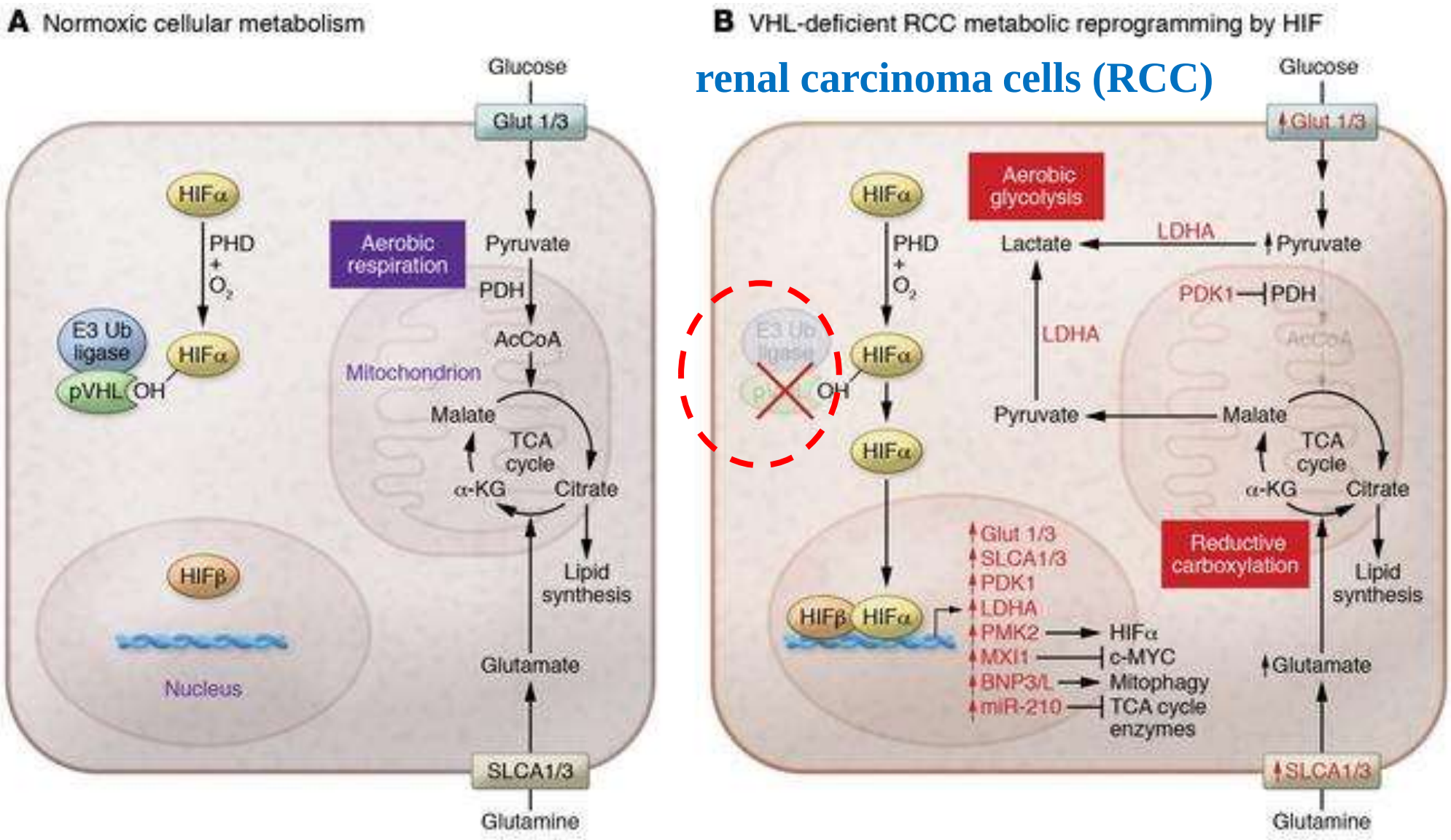
- Ridotta ubiquitinizzazione di HIF1α

- Aumentata espressione del gene Epo → policitemia

Response to hypoxia - chronic adaptation



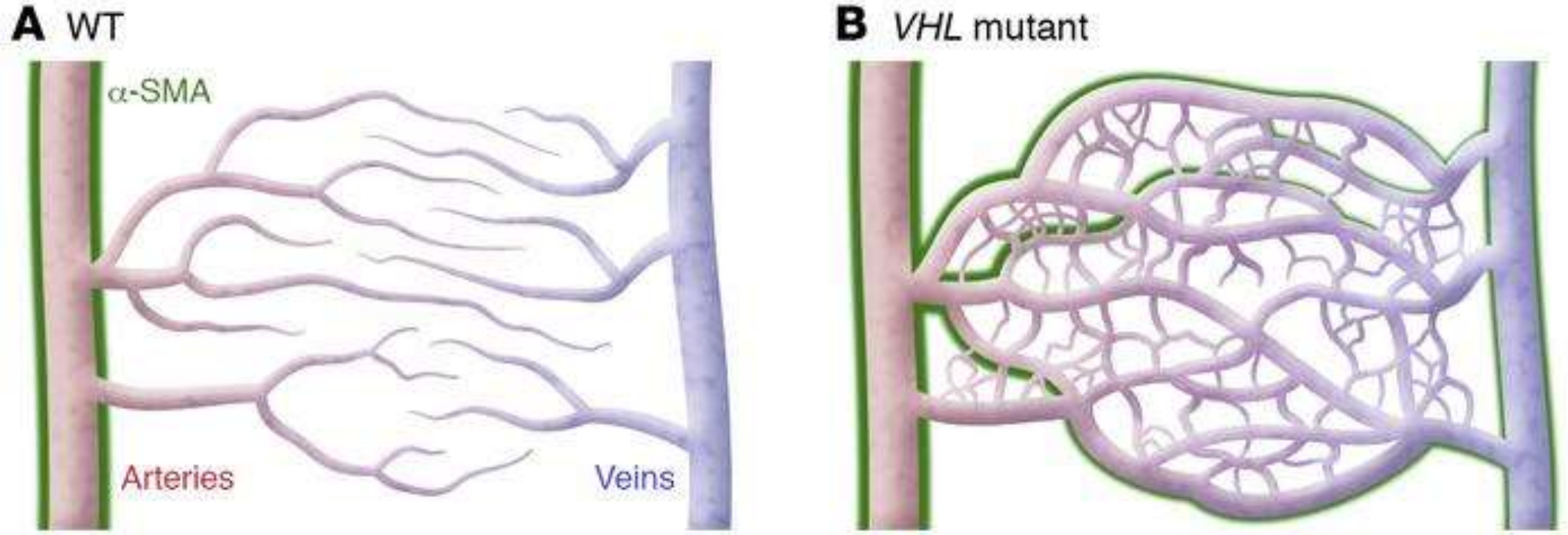
VHL mutation Hypoxia, angiogenesis, and metabolism in the hereditary kidney cancers



J Clin Invest DOI: 10.1172/JCI120855

Adattamento cronico all'ipossia…..

Figure 3: Hypoxia and angiogenesis,in the hereditary kidney cancers



vascular abnormalities

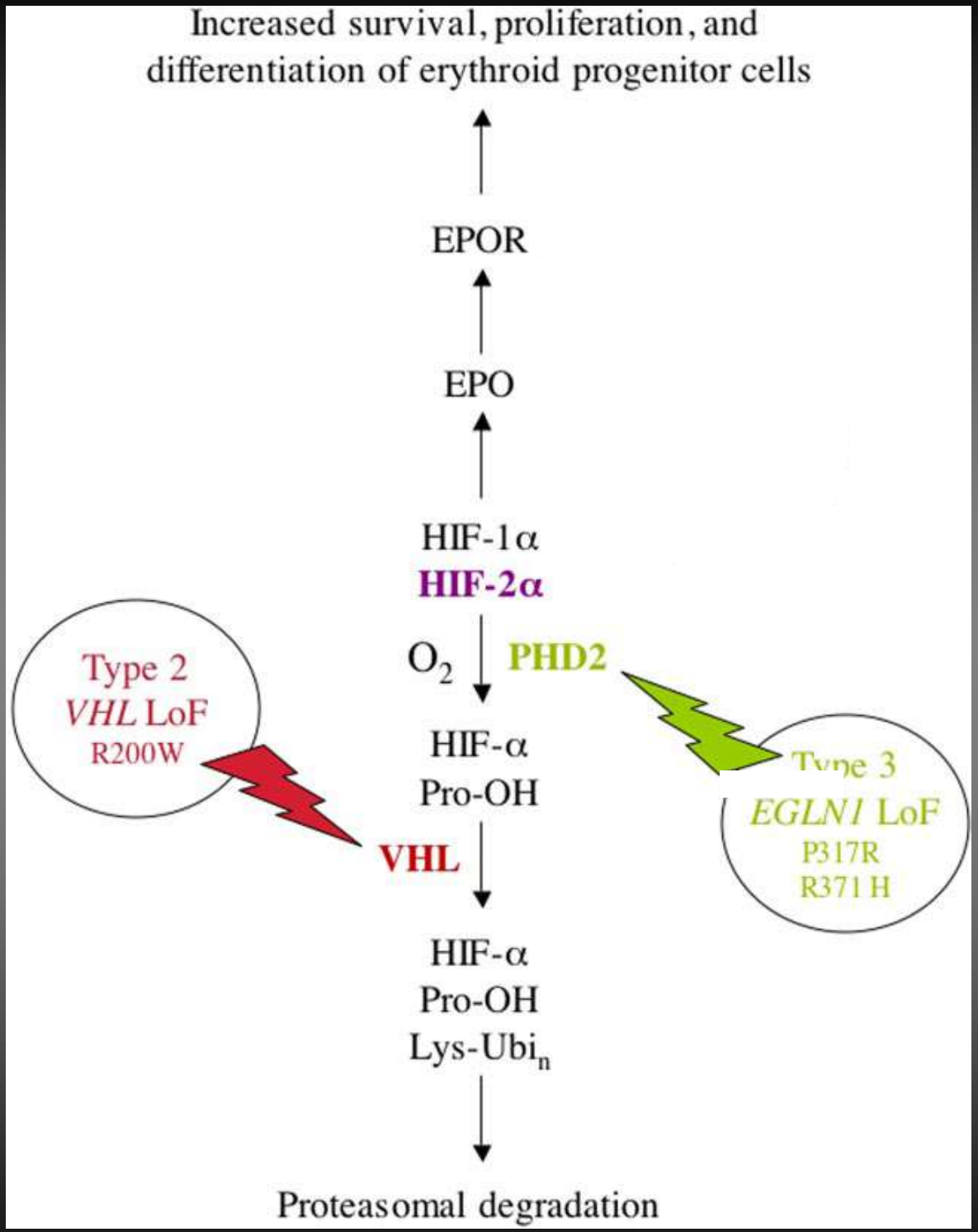
1 ectopic expression of smooth muscle α-actin (α-SMA; green) by vascular pericytes

2 elevated vessel density

3 development of arteriovenous shunts

J Clin Invest DOI: 10.1172/JCI120855

Mutazioni nella Pathway oxygen sensing: **PHD**


Increased survival, proliferation, and
differentiation of erythroid progenitor cells
EPOR
EPO
HIF-1α
HIF-2α
O2
PHD2
Type 2
VHL LoF
R200W
HIF-α
Pro-OH
VHL
Type 3
EGLN1 LoF
P317R
R371H
HIF-α
Pro-OH
Lys-Ubin
Proteasomal degradation

Mutazioni nella Pathway oxygen sensing: **HIF**

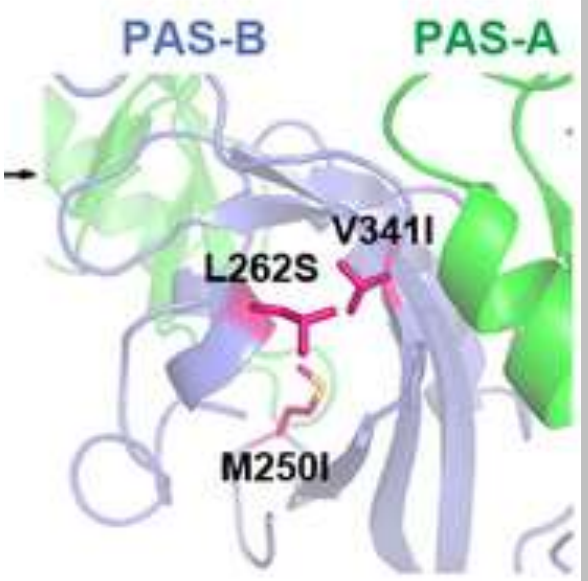
a

Location	Possible Role	Primary Tissue (Subtype)	Associated Histology
HIF-2α			
K18E		Stomach	Adenocarcinoma
A23V		Endometrium	Endometrioid carcinoma
V47M		Central nervous system (brain)	Glioma
F98L		Large intestine (colon)	Adenocarcinoma
R166L		Kidney	Clear cell renal cell carcinoma
I223M		Lung	Adenocarcinoma
H248N		Large intestine (colon)	Adenocarcinoma
R275H		Cervix	Squamous cell carcinoma
A277P		Lung	Squamous cell carcinoma
E279V		Liver	Hepatocellular carcinoma
HIF-1α			
K19Q		Endometrium	Endometrioid carcinoma
R30Q		Skin	Malignant melanoma
L54I		Kidney	Clear cell renal cell carcinoma
V116E		Kidney	Clear cell renal cell carcinoma
M120T		Large intestine (colon)	Adenocarcinoma
M171I		Kidney	Clear cell renal cell carcinoma
M250I		Lung	Adenocarcinoma
L262S		Skin	Malignant melanoma
V341I		Endometrium	Endometrioid carcinoma

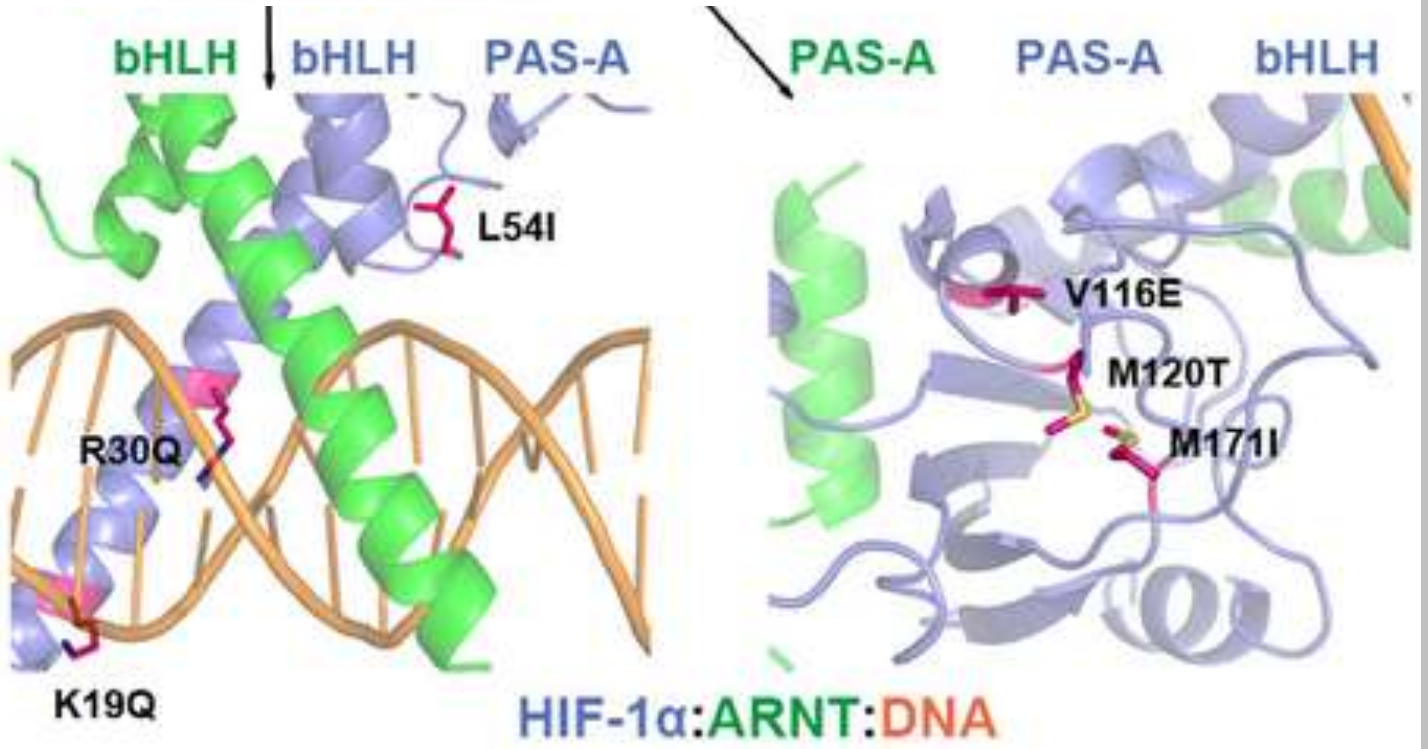
cancer-related mutations in HIF-2α and HIF-1α

Genere: carcinoma endometrio

HIF-1α		
K19Q	bHLH α1	DNA interaction
R30Q	bHLH α1	DNA interaction
L54I	bHLH α1-α2 loop	Interface 6 (bHLH/PAS-A)
V116E	PAS-A Cα	Internal stability
M120T	PAS-A Cα	Internal stability
M171I	PAS-A Gβ	Internal stability
M250I	PAS-B Aβ-Bβ loop	Internal stability
L262S	PAS-B Cα	Internal stability
V341I	PAS-B Iβ	Internal stability



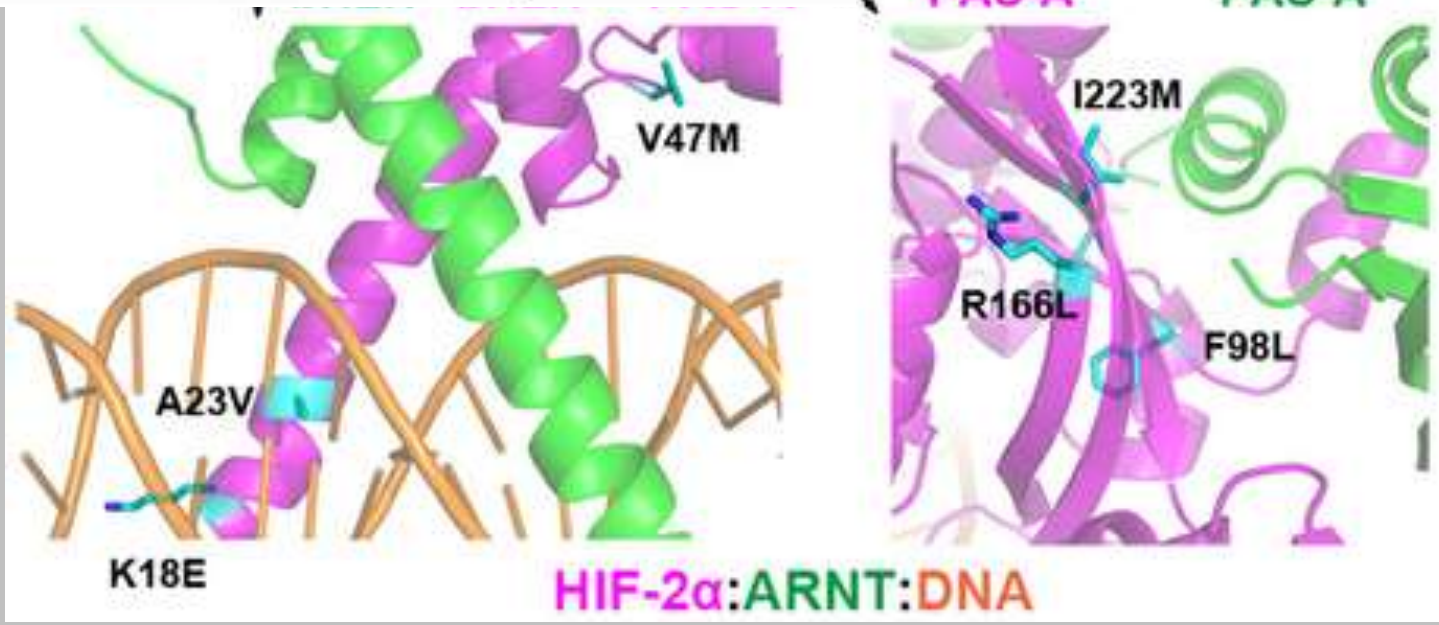
cancer-related mutations in HIF-1α



a

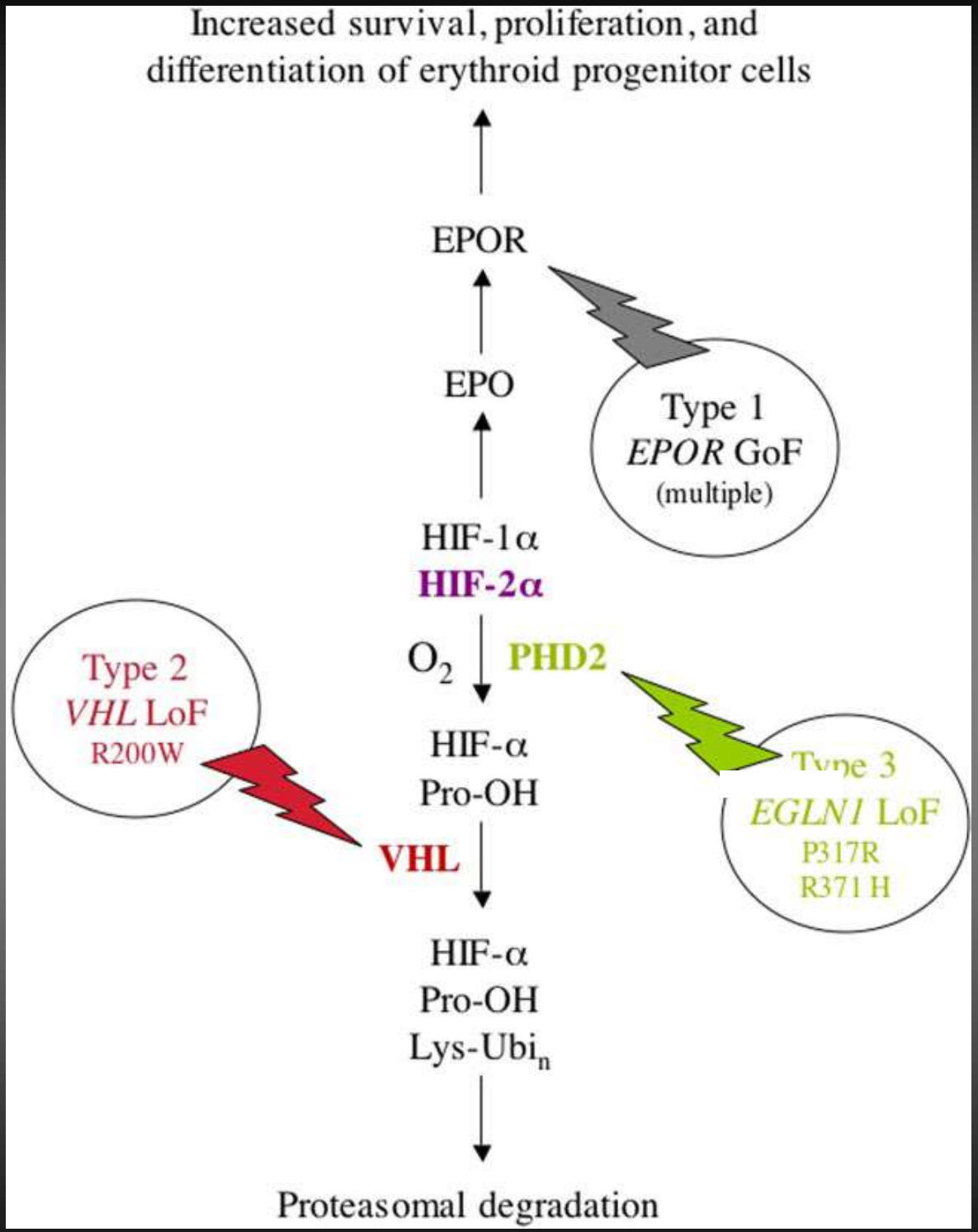
	Location	Possible Role
HIF-2α		
K18E	bHLH α1	DNA interaction
A23V	bHLH α1	DNA interaction
V47M	bHLH α1-α2 loop	Interface 6 (bHLH/PAS-A)
F98L	PAS-A Aβ	Internal stability
R166L	PAS-A Gβ	Internal stability
I223M	PAS-A Iβ	Interface 2 (PAS-A/PAS-A)
H248N	PAS-B Aβ	Internal stability
R275H	PAS-B Dα-Eα loop	Internal stability
A277P	PAS-B Eα	Internal stability
E279V	PAS-B Eα	Internal stability

cancer-related mutations in HIF-2α

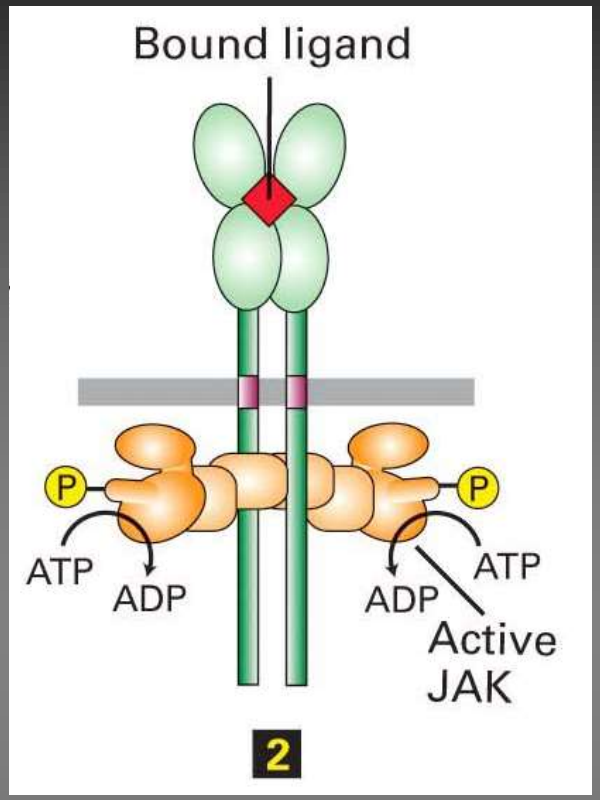


Mutazioni nella Pathway oxygen sensing:

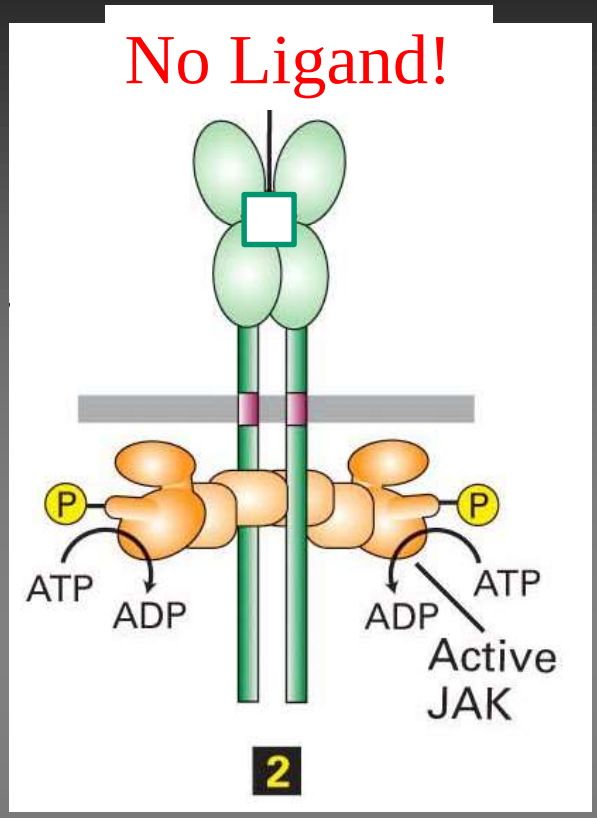
Gain of Function «GoF» EPOR


Increased survival, proliferation, and
differentiation of erythroid progenitor cells
EPOR
EPO
Type 1
EPOR GoF
(multiple)
HIF-1α
HIF-2α
O_2
PHD2
Type 2
VHL LoF
R200W
HIF-α
Pro-OH
VHL
Type 3
EGLN1 LoF
P317R
R371H
HIF-α
Pro-OH
Lys-Ubi_n
Proteasomal degradation

Trasduzione del segnale continua



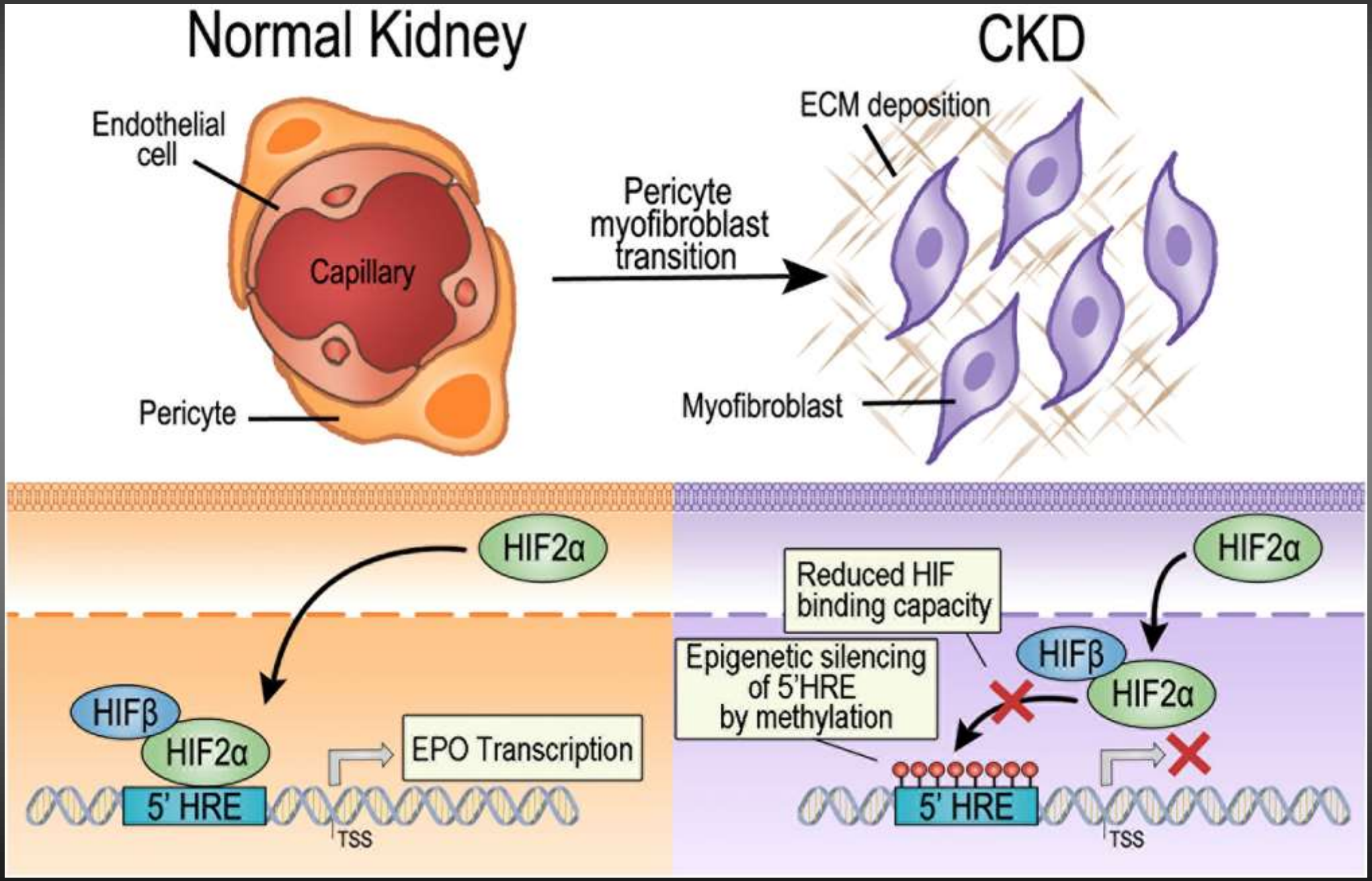
Dimerizzazione di EpoR
Fosforilazione di JAK e
attivazione di JAK chinasi



Dimerizzazione di EpoR
Fosforilazione di JAK e
attivazione di JAK chinasi

Mutated Receptor
Mutated Jak…

Chronic Kidney Disease



Anemia

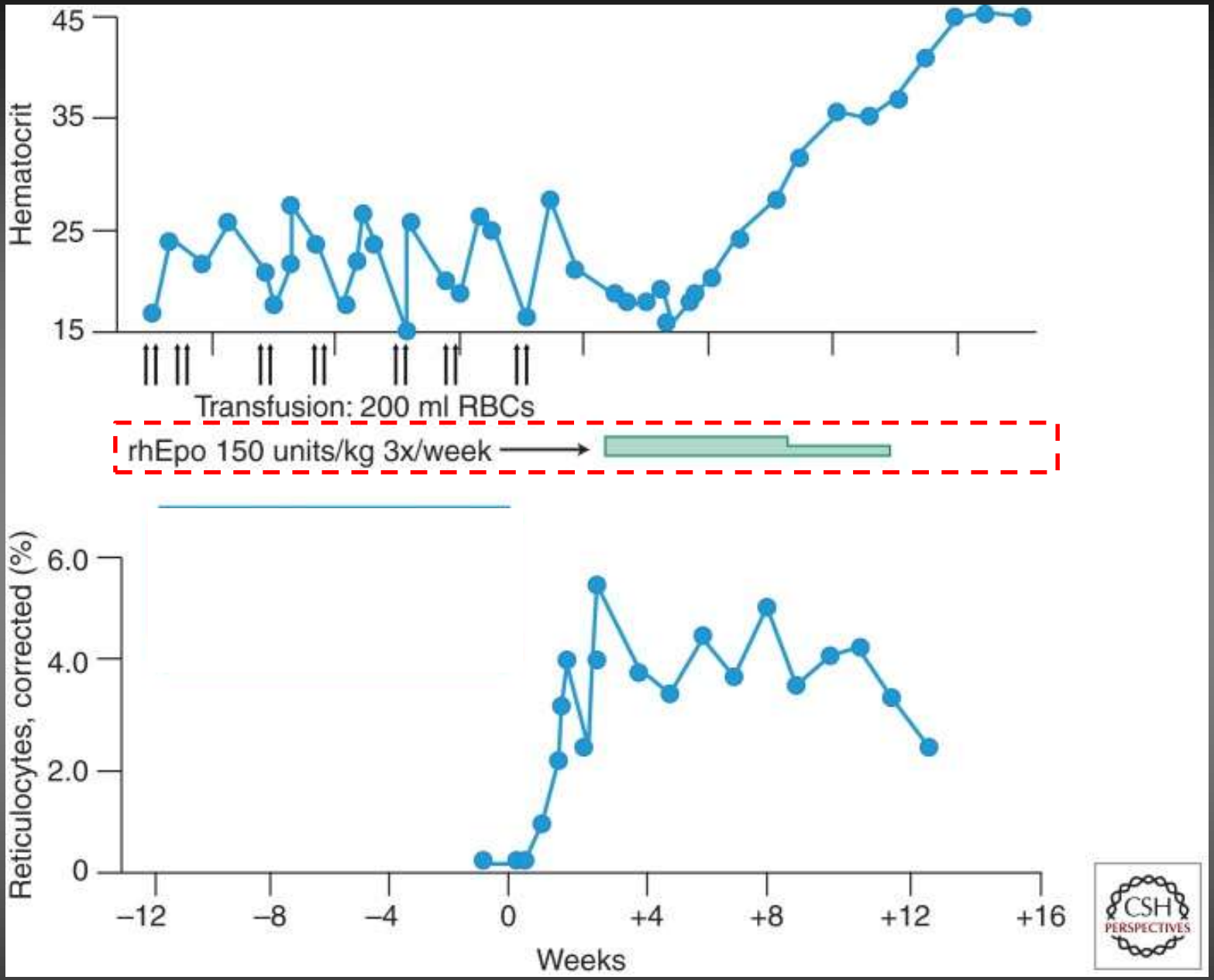
Inadeguata produzione endogena
(patologia renale)

↓

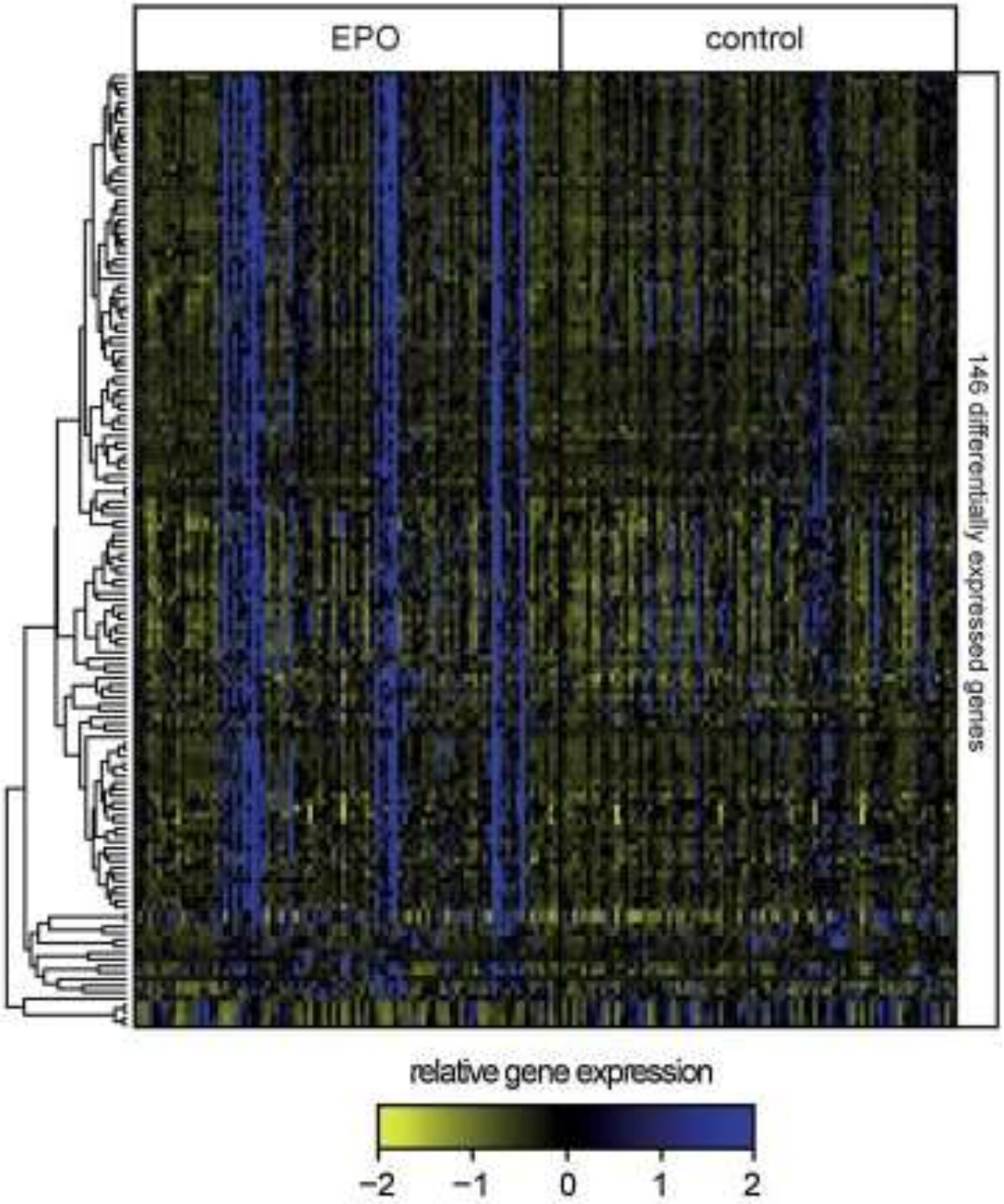
Carenza di globuli rossi

↓

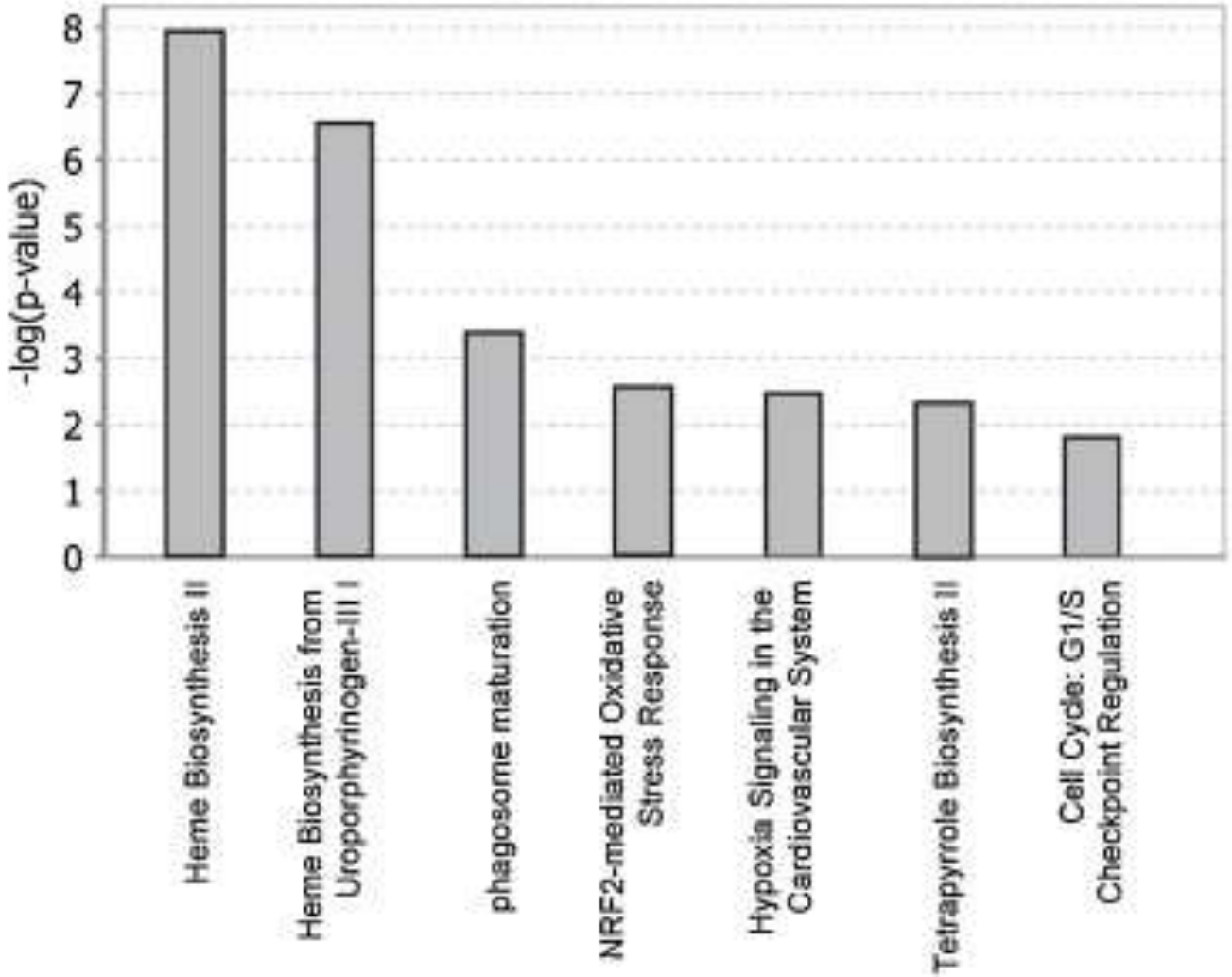
Anemia


Hematocrit
45
35
25
15
Transfusion: 200 ml RBCs
rhEpo 150 units/kg 3x/week
Reticulocytes, corrected (%)
6.0
4.0
2.0
0
−12
−8
−4
0
+4
+8
+12
+16
Weeks
CSH
PERSPECTIVES

Effect of erythropoietin (EPO) on the blood transcriptome after cardiac arrest



Effects of erythropoietin (EPO) on the transcriptome after cardiac arrest



Trattamento dell'anemia

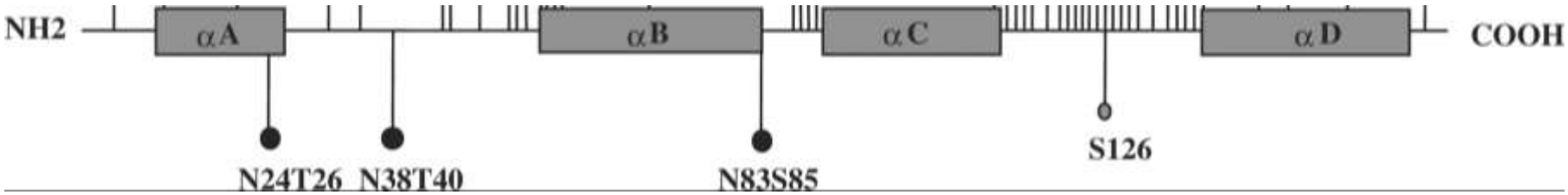
Epo ricombinante (rHuEPO)

Produzione su larga scala di Epo umana ricombinante

rHuEPO

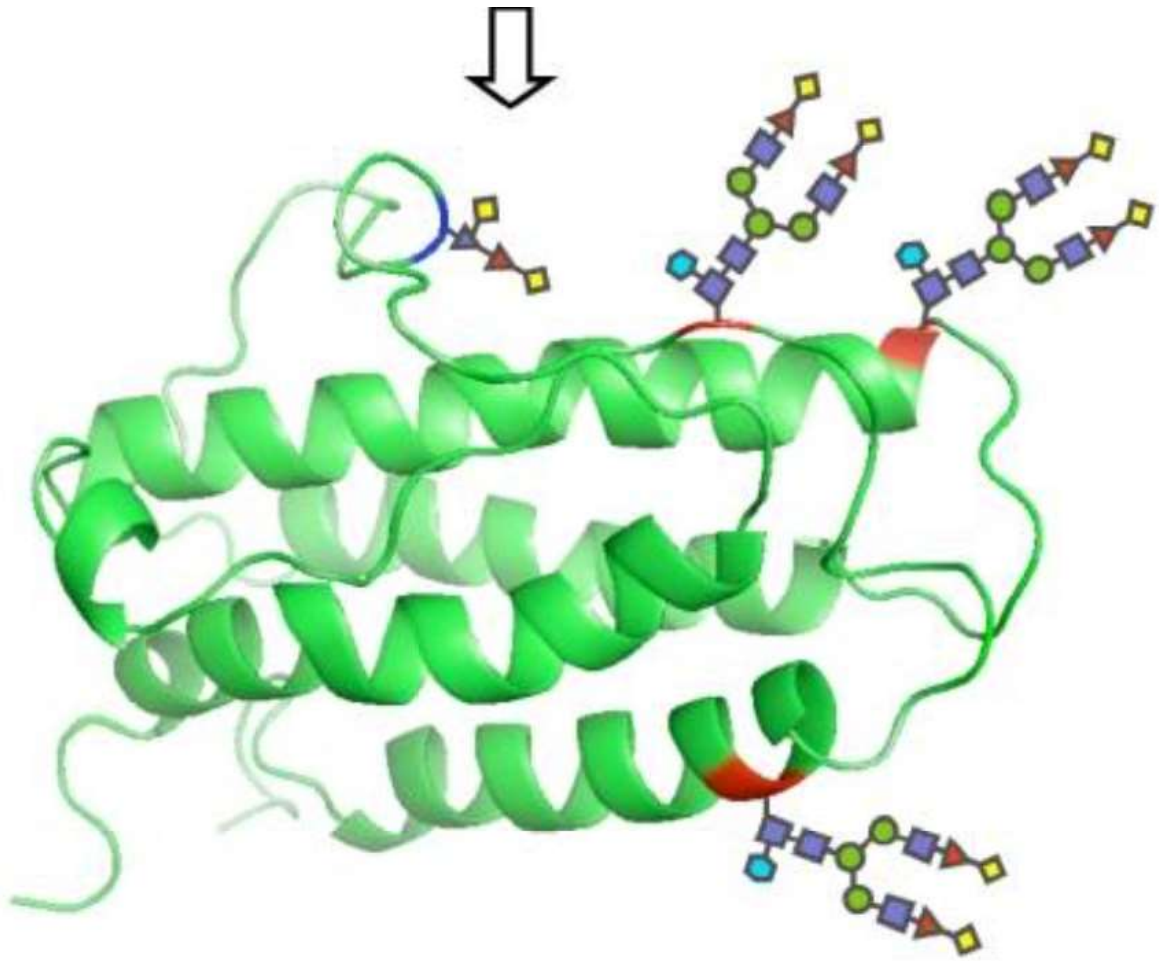
- 34000 Da
- prodotta in cellule mammarie in cui è stato introdotto il gene dell'Epo

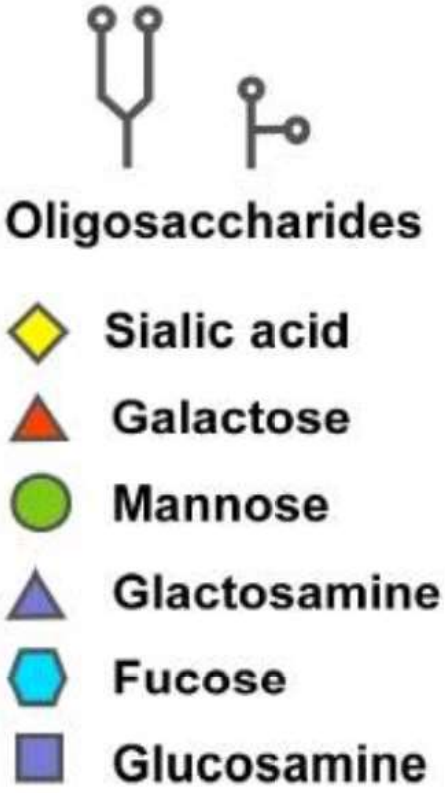
Epo contains **one** O-linked and **three** N-linked carbohydrate chains, each having 2–4 branches that often end in a negatively charged sialic acid.



Ser126 is the site for O-linked carbohydrate.

Steve Elliott et al. J. Biol. Chem. 2004;279:16854-16862





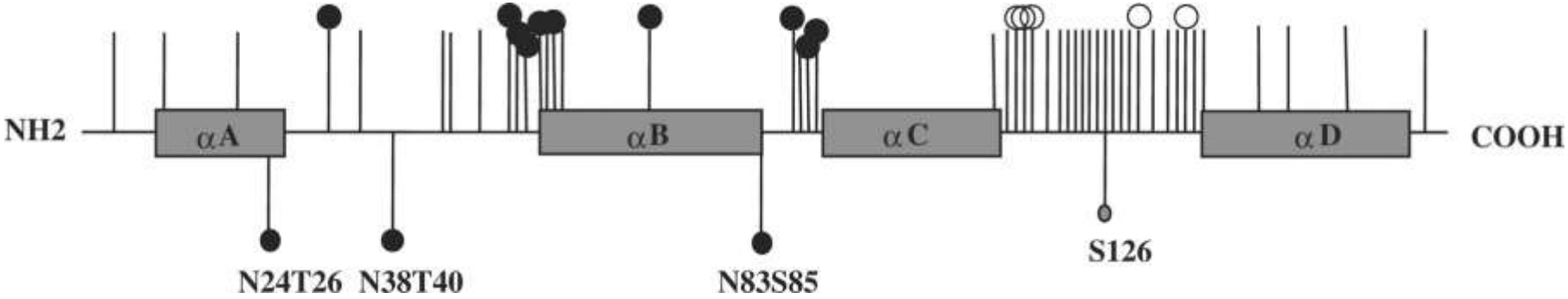
Carbohydrate chains are not required for receptor binding in vitro or stimulation of growth of EpoR-expressing cultured cells

required for the in vivo bioactivity

These carbohydrate chains are not required for receptor binding in vitro or stimulation of growth of EpoR-expressing cultured cells but are **required for the in vivo bioactivity**

Introduced (recombinant) consensus sequences in the rHuEPO amino acid backbone.

introduced N-linked consensus sequences



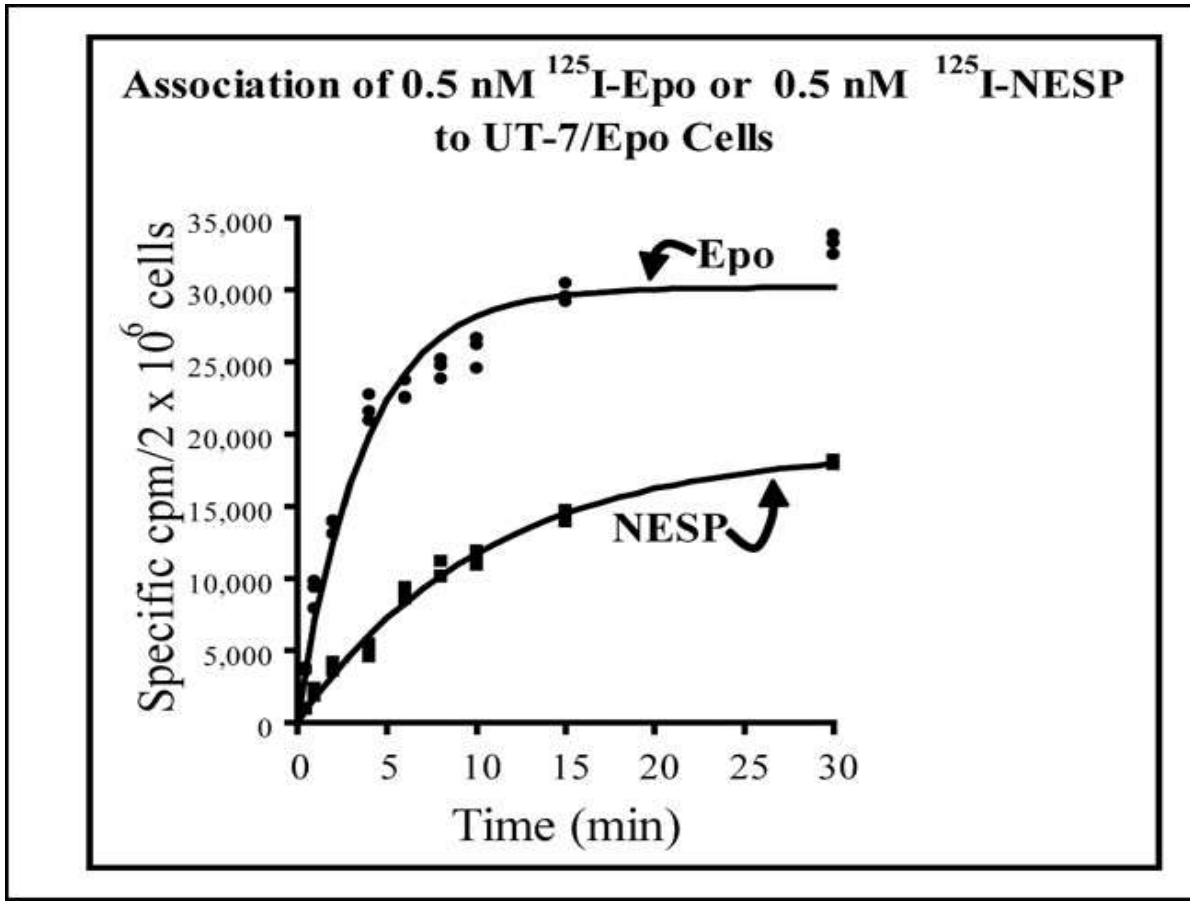
natural glycosylation sites

- more than half the molecules contained additional carbohydrate

Steve Elliott et al. J. Biol. Chem. 2004;279:16854-16862

- 2 additional N- linked glycosylation sites
- Novel Erythropoiesis Stimulating Protein (NESP)
  - Aumentato contenuto di carboidrati conferisce un aumento dell'emivita

Net binding of 125I-Epo or 125I-NESP with UT-7/Epo cells at 37 ℃.



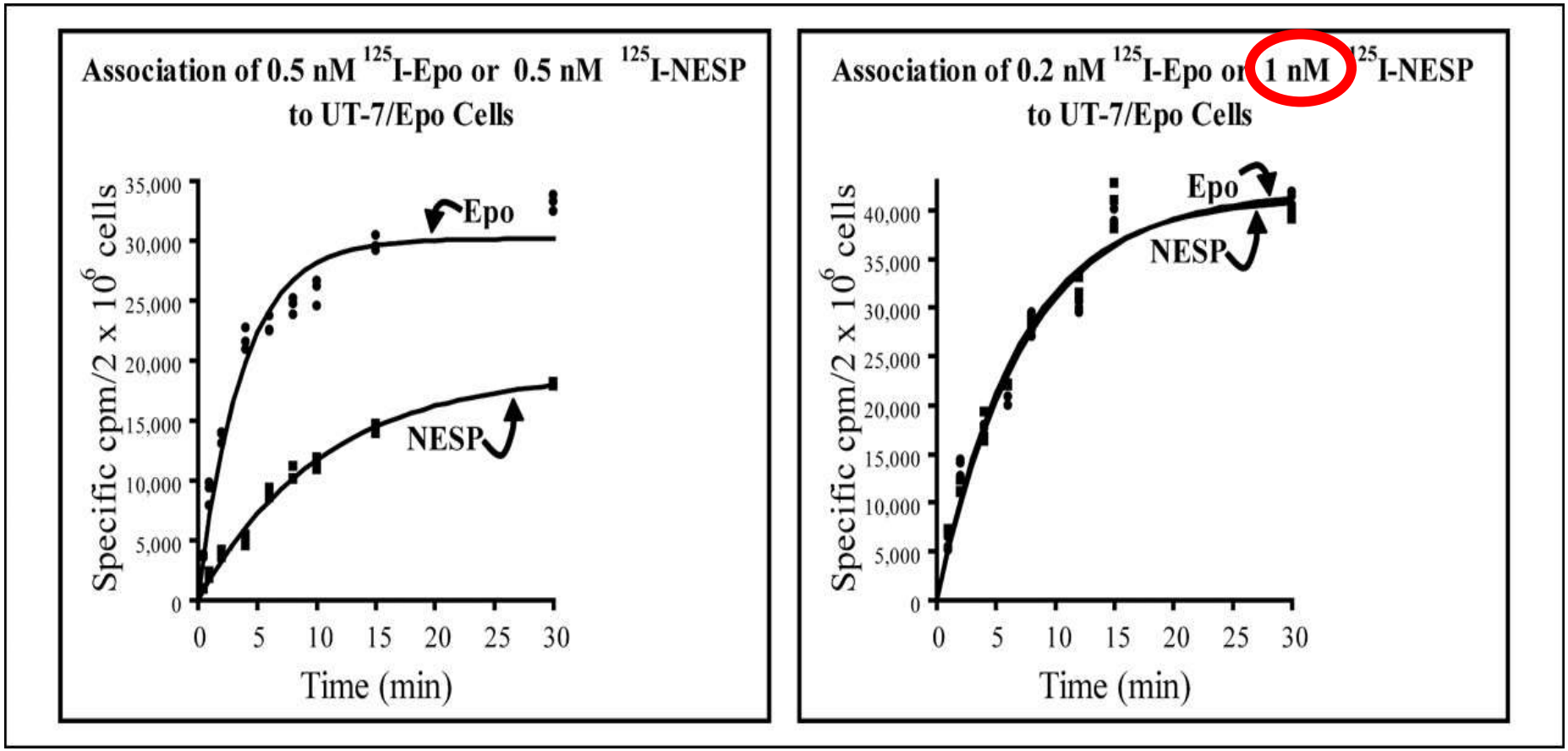
Cells were collected and rapidly separated from the medium after the indicated time

Cell-associated radioactivity was measured.

Gross A W , Lodish H F J. Biol. Chem. 2006;281:2024-2032

jbc

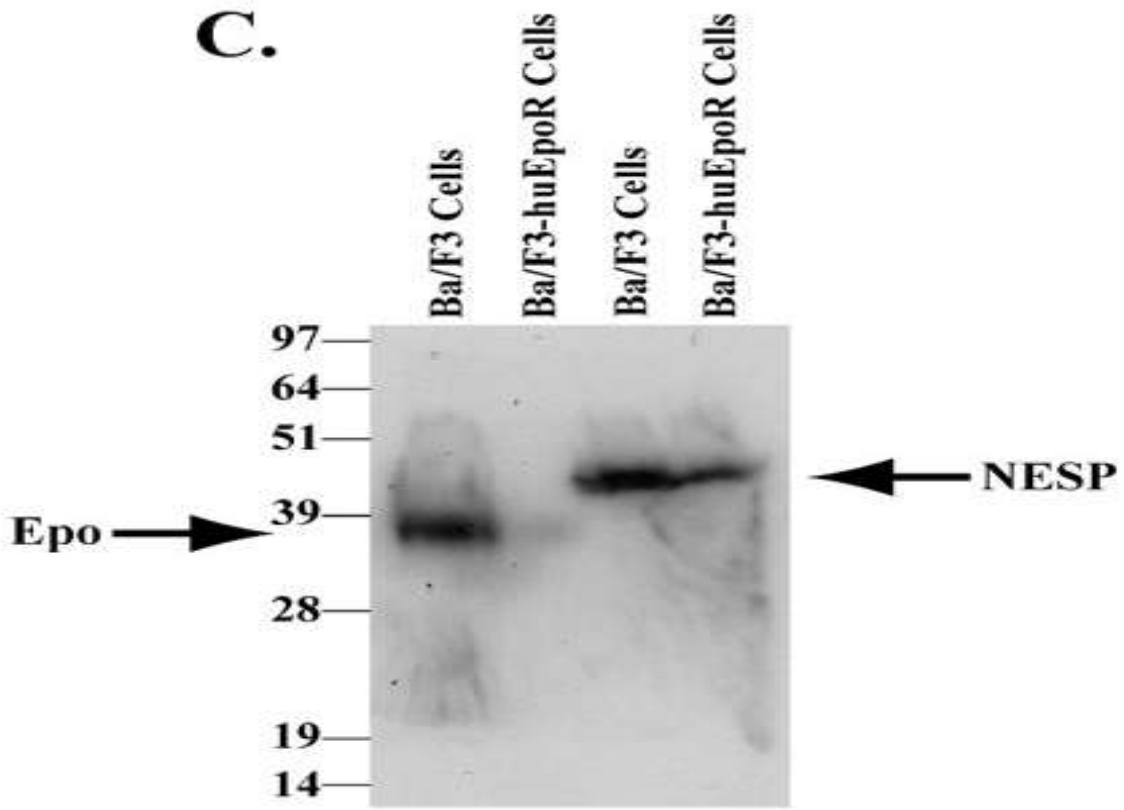
Net binding of 125I-Epo or 125I-NESP with UT-7/Epo cells at 37 ℃.



Novel Erythropoiesis Stimulating Protein (NESP)

- Epo isoforms with higher sialic acid content have
- a lower affinity for EpoR
- a longer serum half-life?

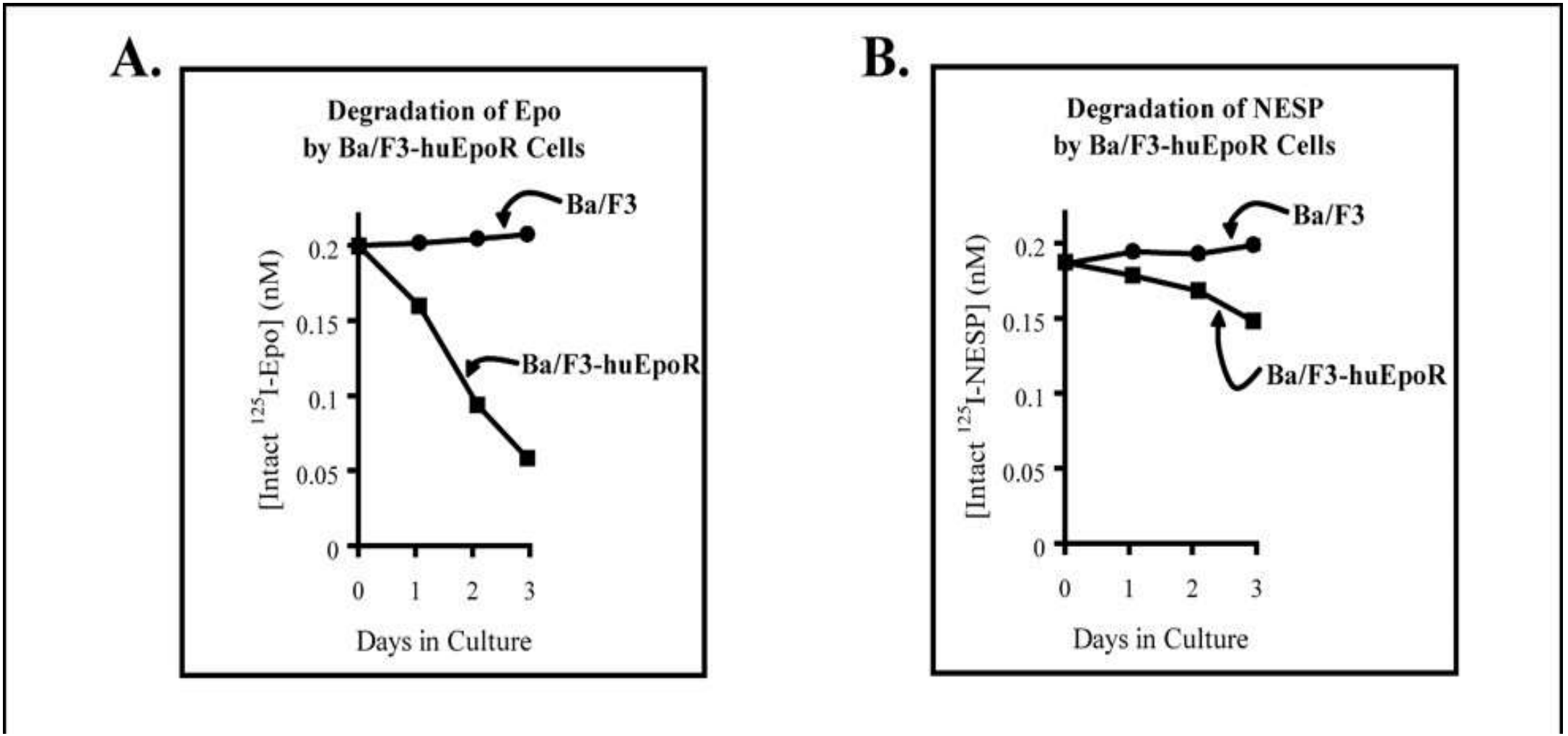
Degradation and endocytosis of Epo and NESP by Ba/F3-huEpoR cells.



proteins precipitated by trichloroacetic acid from the media of the cultures were separated by SDS-PAGE and analyzed by autoradiography

jbc

Degradation and endocytosis of Epo and NESP by Ba/F3-huEpoR cells.



Gross A W , Lodish H F J. Biol. Chem. 2006;281:2024-2032

Novel Erythropoiesis Stimulating Protein (NESP)

- Epo isoforms with higher sialic acid content have
- a lower affinity for EpoR
- a longer serum half-life

are more effective for stimulating the production of red blood cells in vivo.

Somministrazione meno frequente