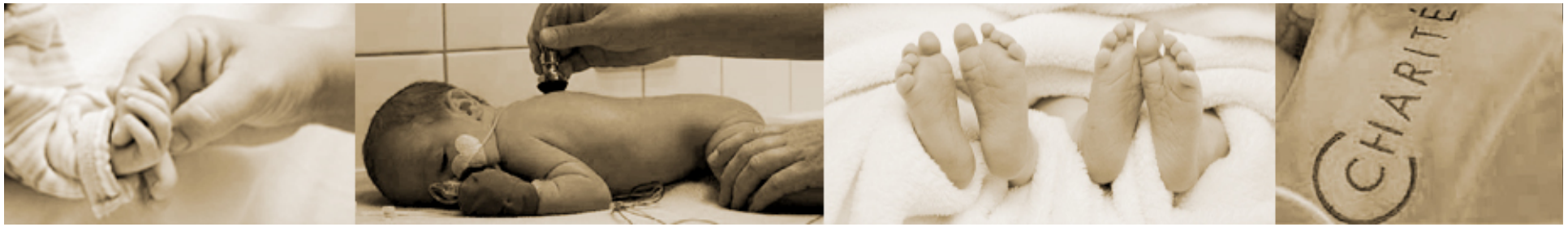


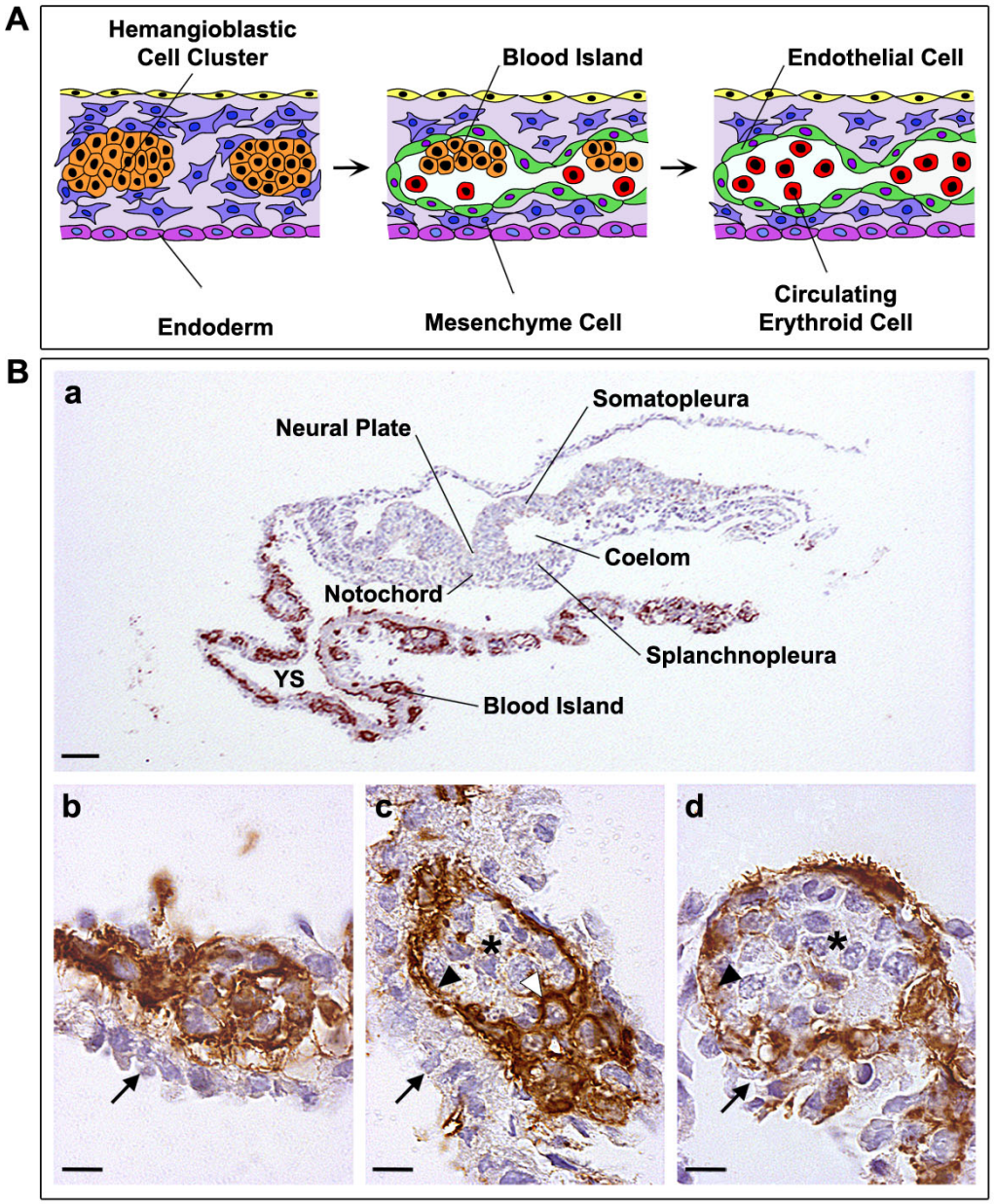
Grundlagen der Erythropoiese

Christof Dame

Klinik für Neonatologie
Charité - Universitätsmedizin Berlin

christof.dame@charite.de

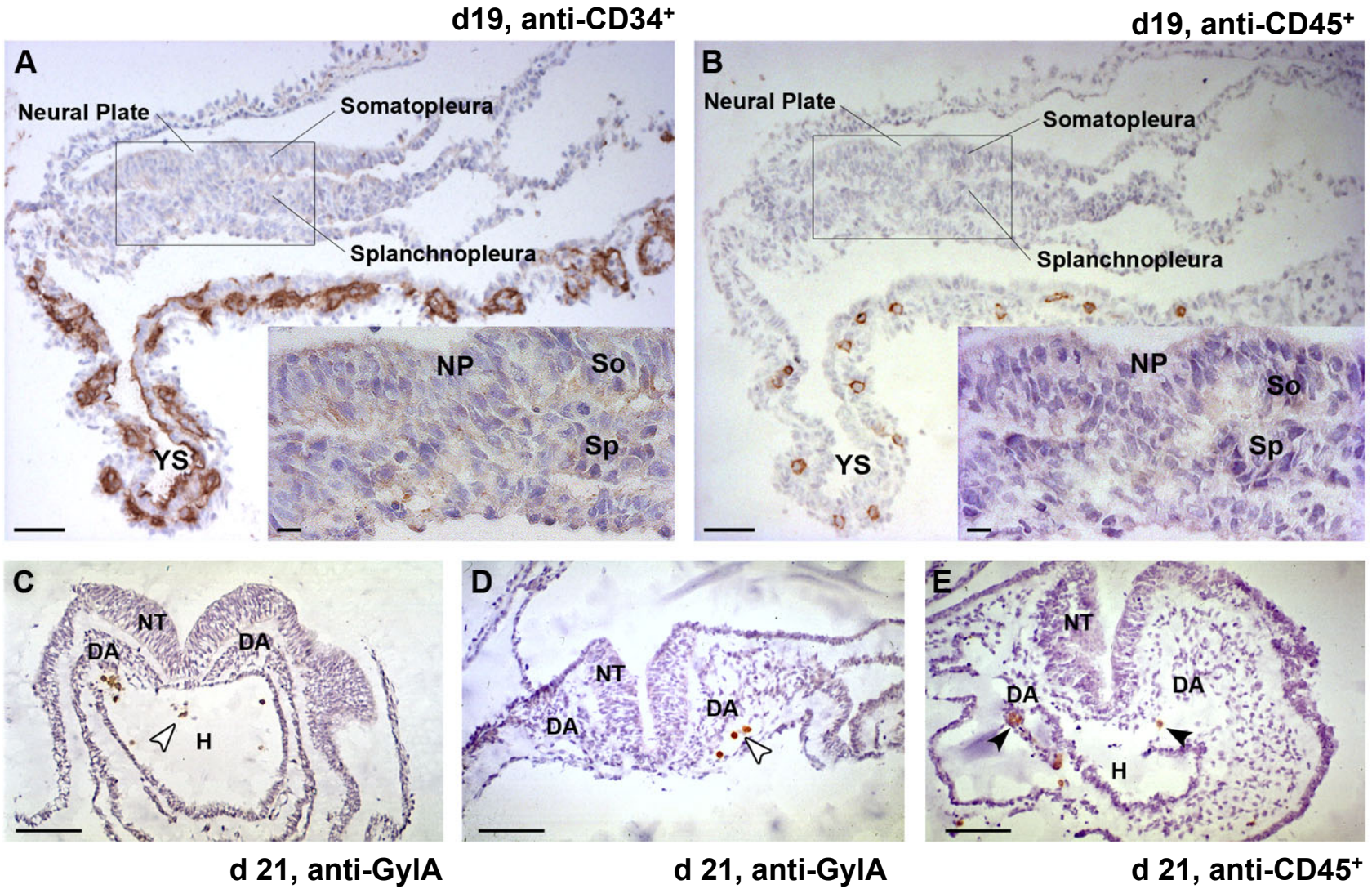
Yolk Sac Blood Islands



Human, d19
anti-CD34^{+}

Tavian M, Biasch K, Péault B. in Dame C. et al., Atlas of the Cellular and Molecular Development of Human Hematopoiesis, 2013

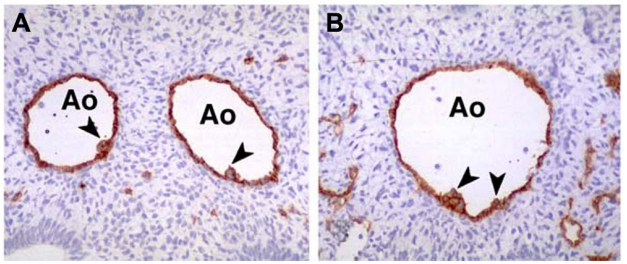
Onset of Blood Circulation

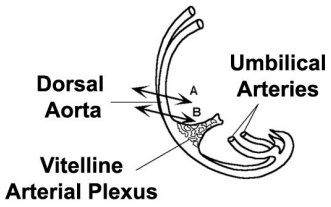


Tavian M, Biasch K, Péault B. in Dame C. et al., Atlas of the Cellular and Molecular Development of Human Hematopoiesis, 2013

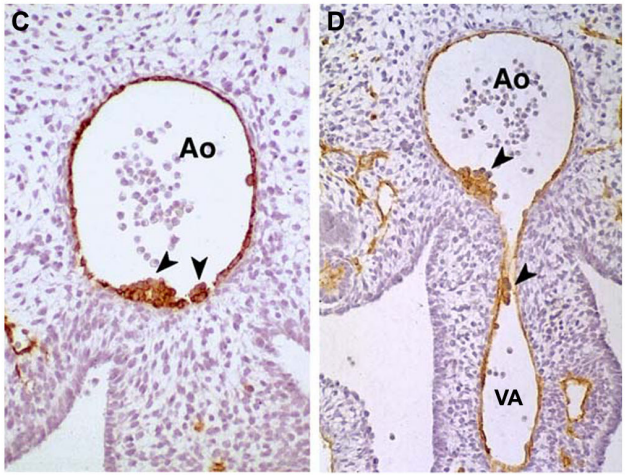
Hematopoietic cells clustered on arterial endothelium

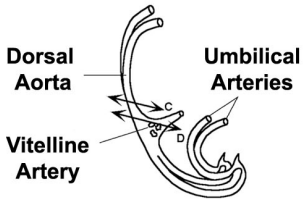
d27, anti-CD34+



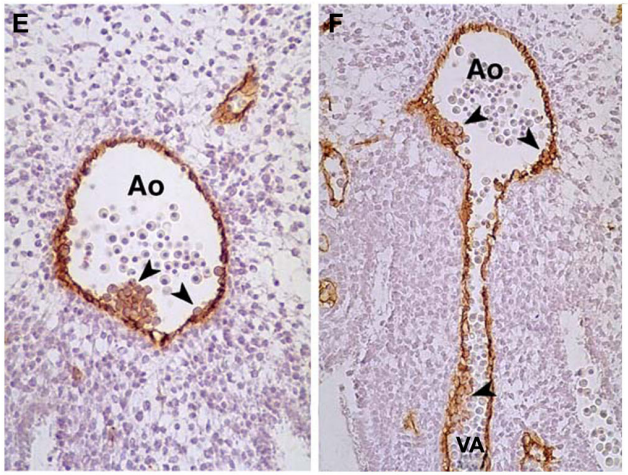


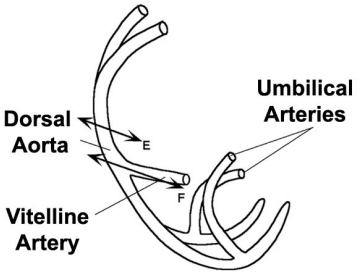
d32, anti-CD34+





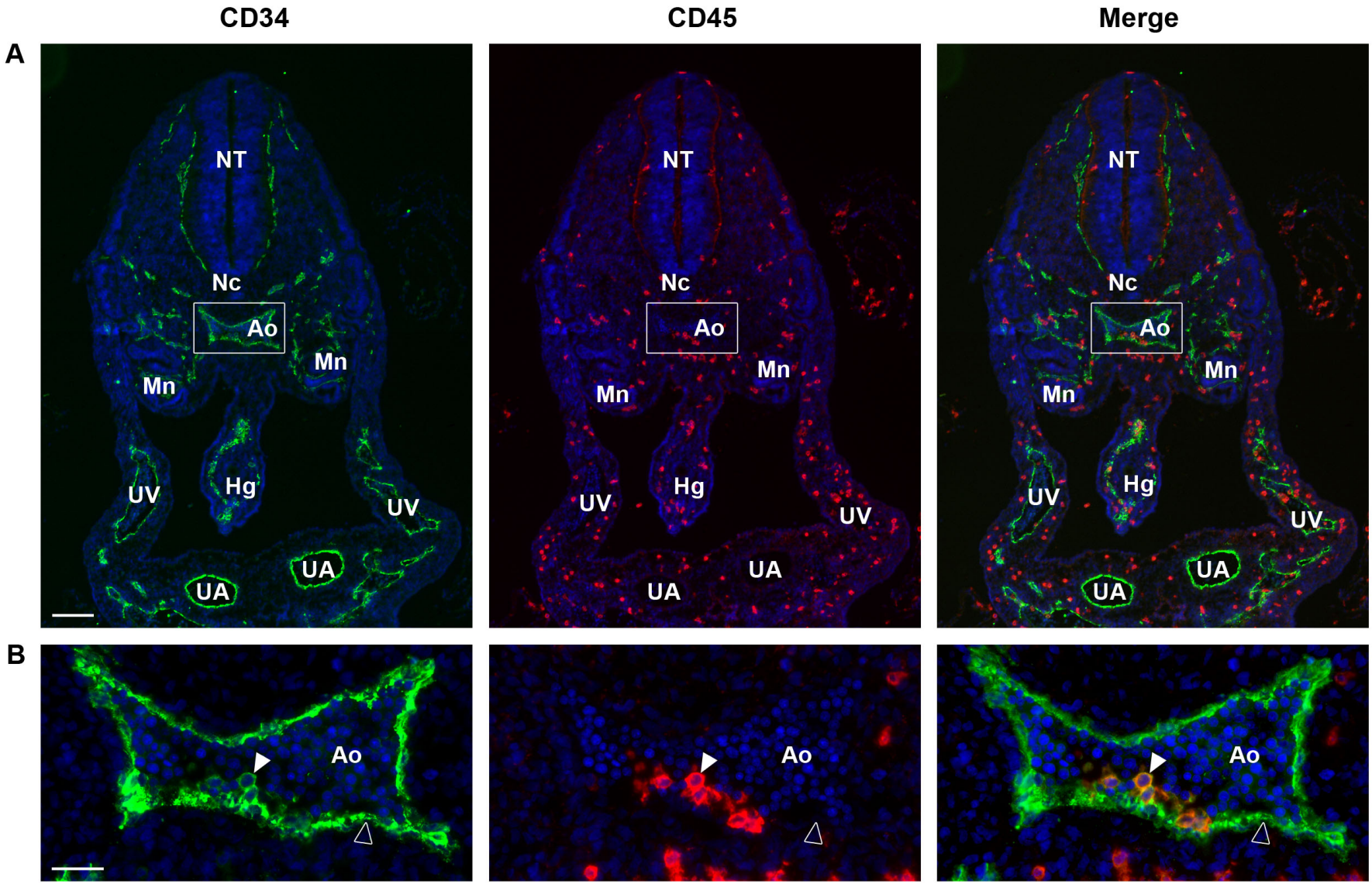
d36, anti-CD34+





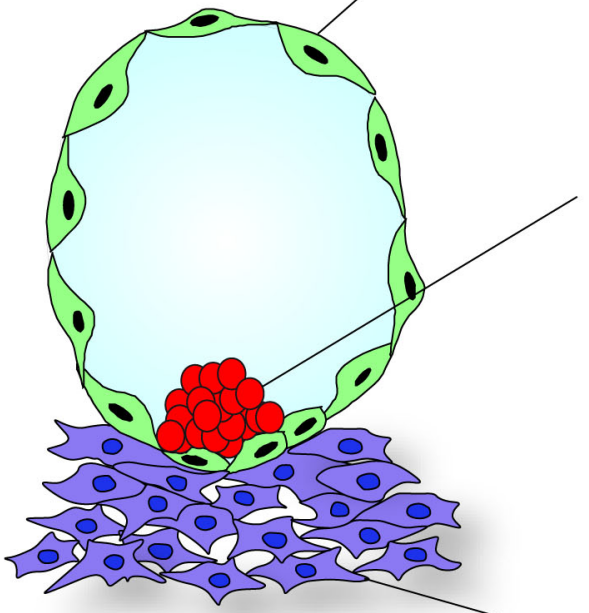
Tavian M et al., Development 1996; 793-803

Hematopoietic stem cell clusters inside the human embryonic aorta



Tavian M, Biasch K, Péault B. in Dame C. et al., Atlas of the Cellular and Molecular Development of Human Hematopoiesis, 2013

Cellular Environment of hematopoietic stem cells within the human AGM



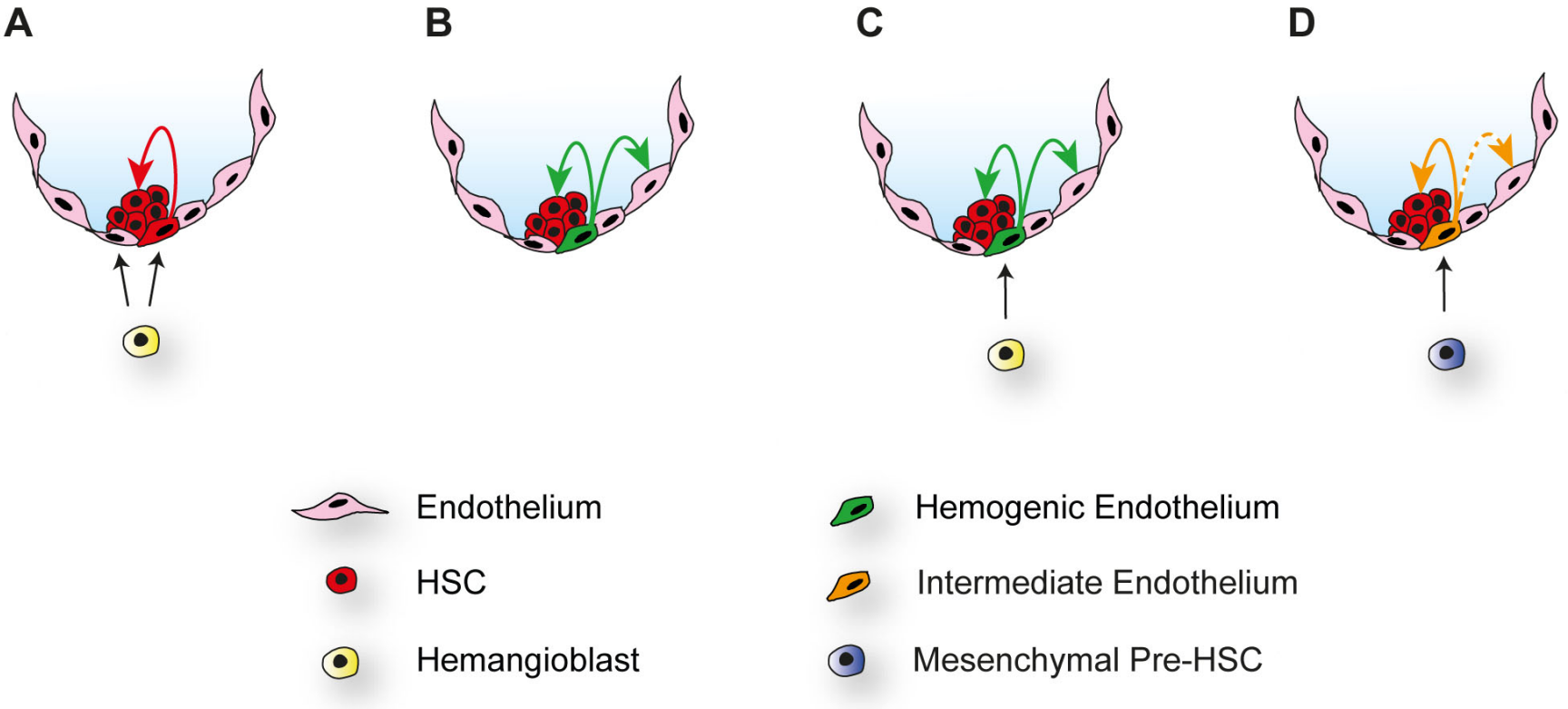
Endothelial Cells
CD31, CD34, CD143/ACE, CD93,
VE-Cadherin, V-CAM, CD146,
c-Kit, Flk-1/KDR, Flt3, Flt3-Ligand,
VEGF, GATA-2, Tal-1/SCL, KG-1 kinase

HSC Clusters
CD31, CD34, CD143/ACE, CD93,
VE-Cadherin, V-CAM, CD43, CD44,
CD45, CD164, CD146, c-Kit, Flk-1/KDR,
Flt3, Flt3-Ligand, GATA-2, GATA-3,
c-Myb, RUNX1/AML-1, Tal-1/SCL

Mesenchyme Cells
Tenascin C, ALCAM/CD166,
c-Kit, BMP-4, SDF-1,
GATA-3, SMA

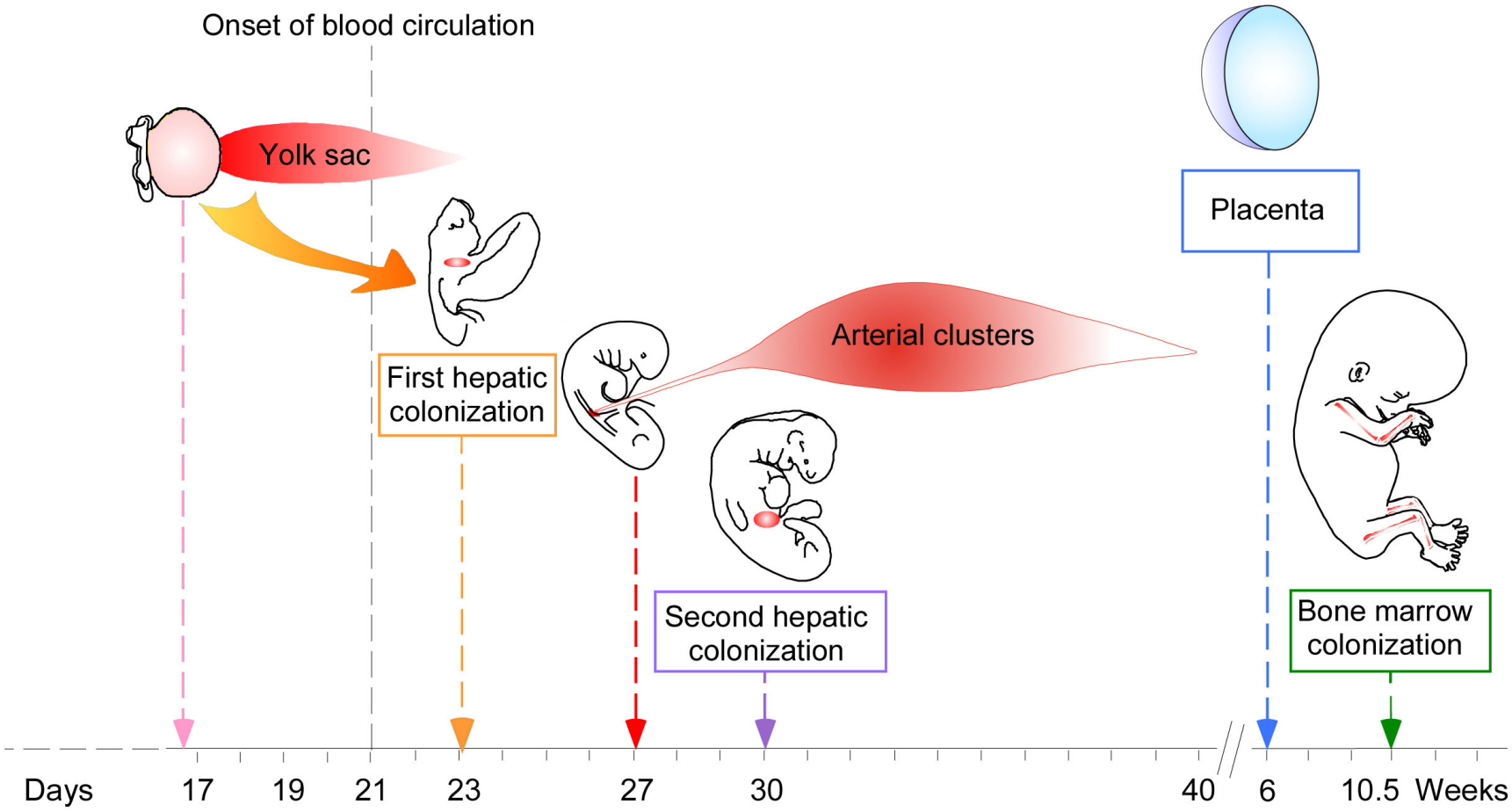
Tavian M, Biasch K, Péault B. in Dame C. et al., Atlas of the Cellular and Molecular Development of Human Hematopoiesis, 2013

Possible Cellular Origins of Intraaortic HSC



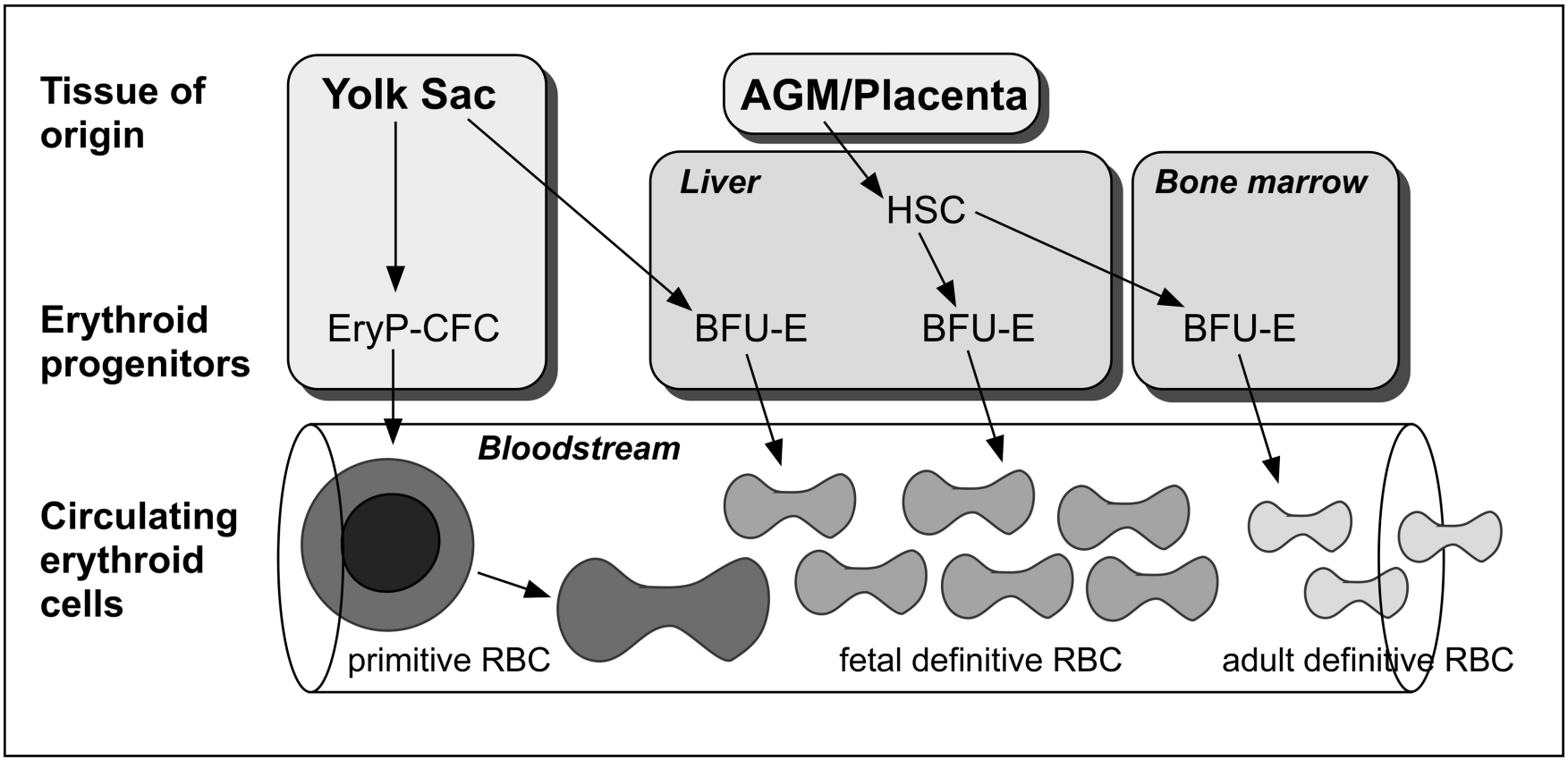
Tavian M, Biasch K, Péault B. in Dame C. et al., Atlas of the Cellular and Molecular Development of Human Hematopoiesis, 2013

Appearance of hematopoietic stem cells in the developing embryo



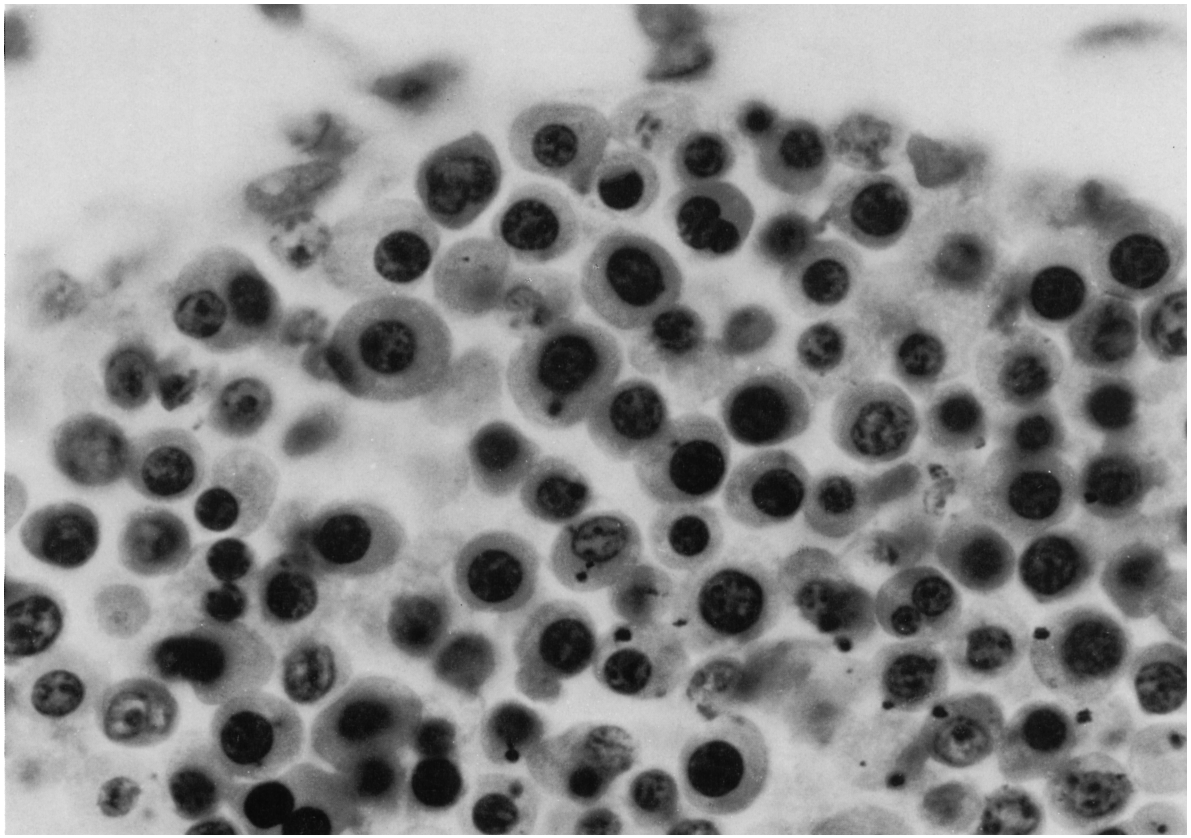
Tavian M, Biasch K, Péault B. *In:* Dame C. et al., Atlas of the Cellular and Molecular Development of Human Hematopoiesis, 2013

Transition of Primitive to Definitive Erythropoiesis in Humans



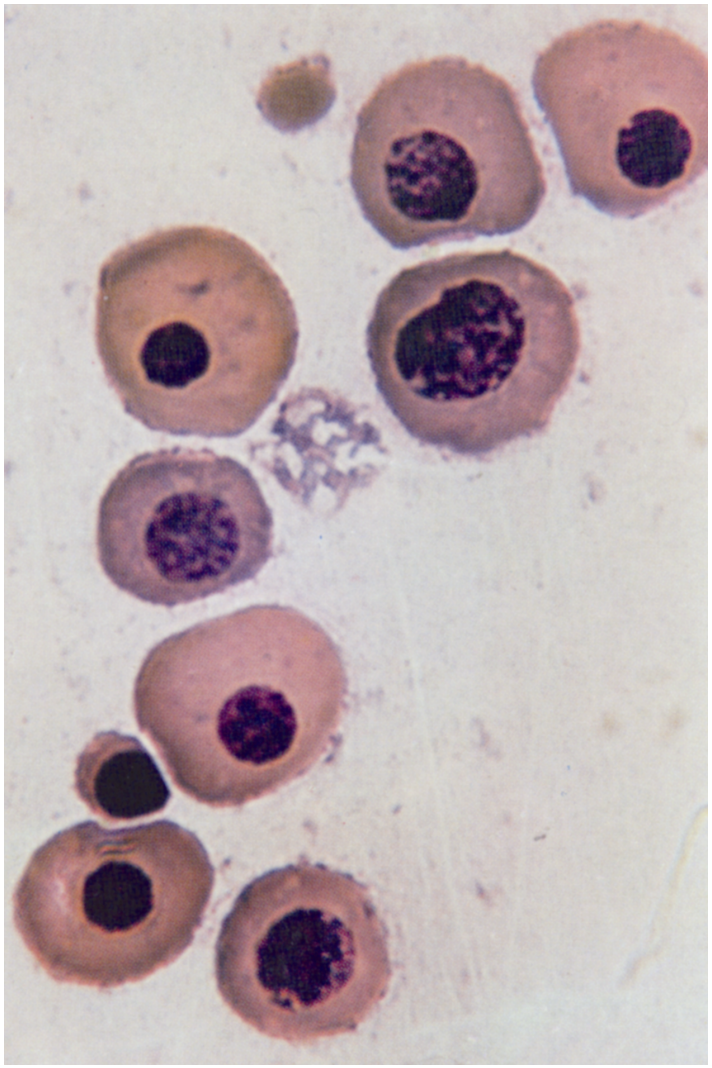
Palis J. in Dame C. et al., Atlas of the Cellular and Molecular Development of Human Hematopoiesis, 2013

Circulating primitive erythroblasts, human, 4-5 weeks pc



Original from: Kelemen E, Calvo W, Fliedner TM. Atlas of human hemopoietic development. Springer, 1979

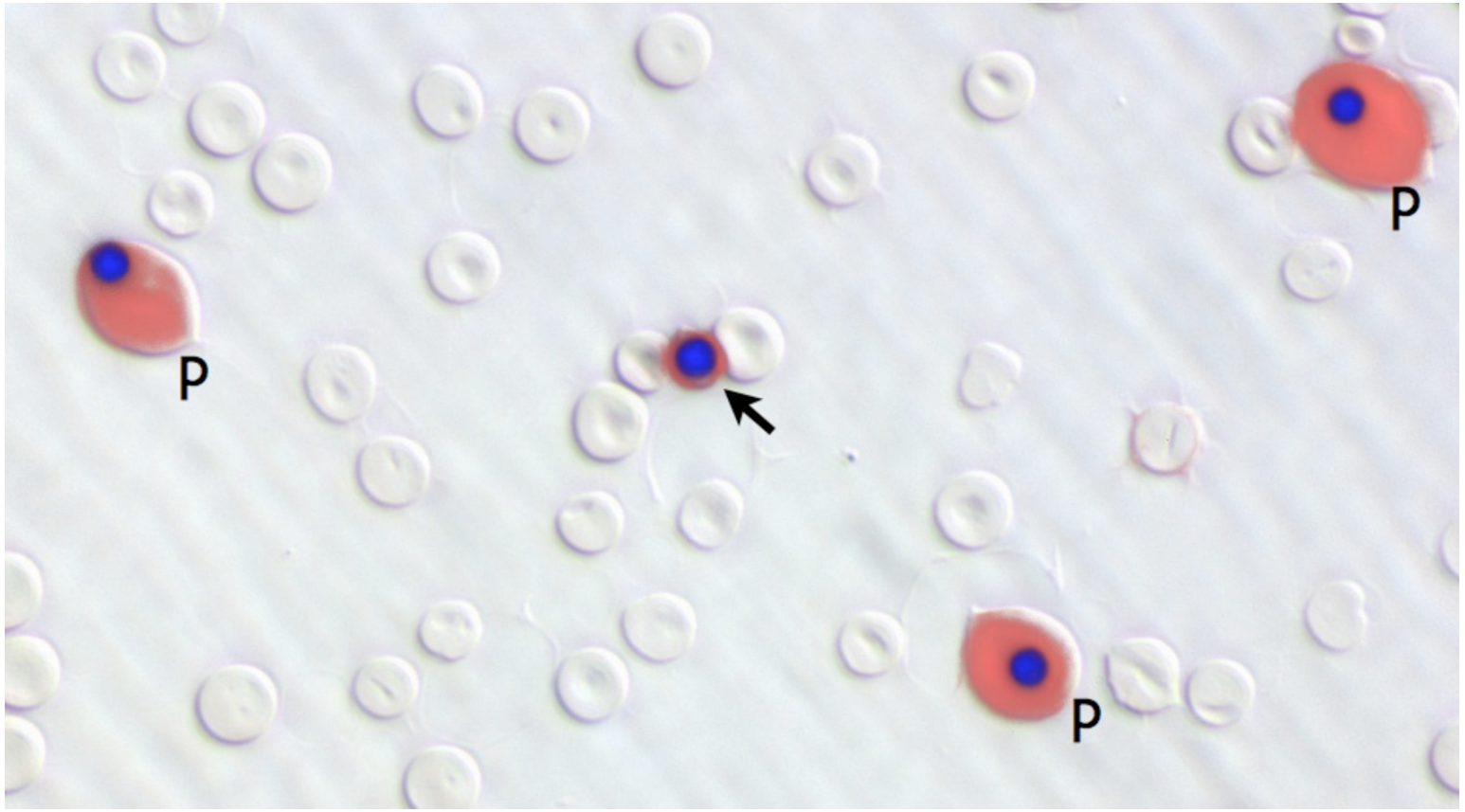
Circulating primitive erythroblasts, human, 7-8 weeks pc



Original from: Kelemen E, Calvo W, Fliedner TM. Atlas of human hemopoietic development. Springer, 1979

Late-stage Primitive Erythroblasts vs. Definitive Erythrocytes

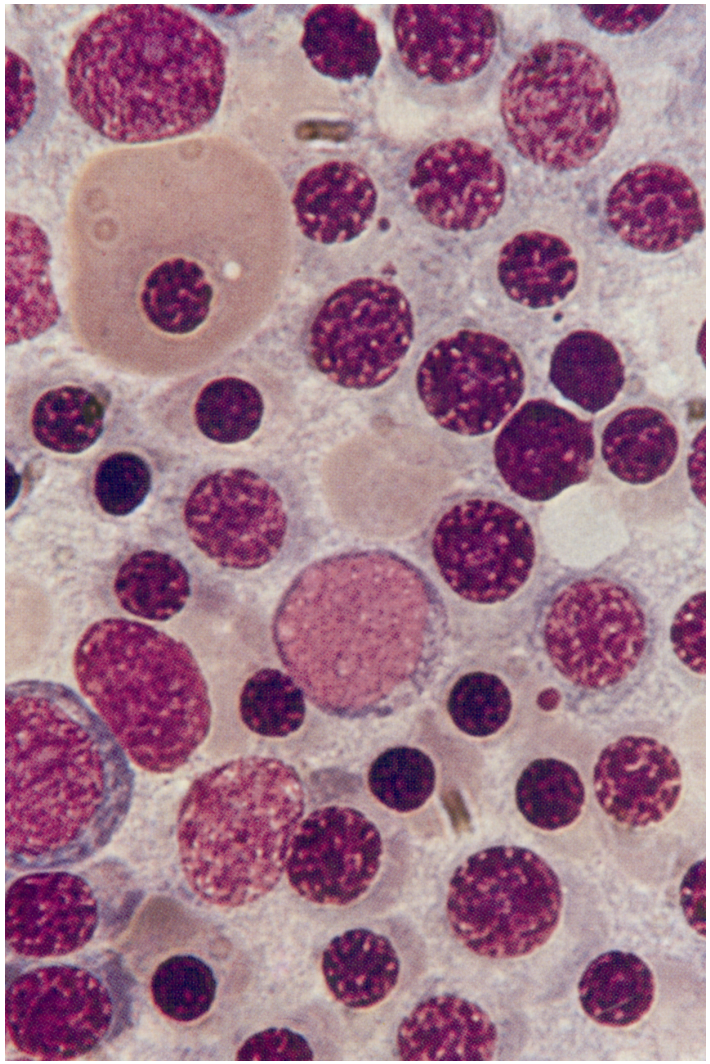
Mouse, e15.5



Palis J. in Dame C. et al., Atlas of the Cellular and Molecular Development of Human Hematopoiesis, 2013

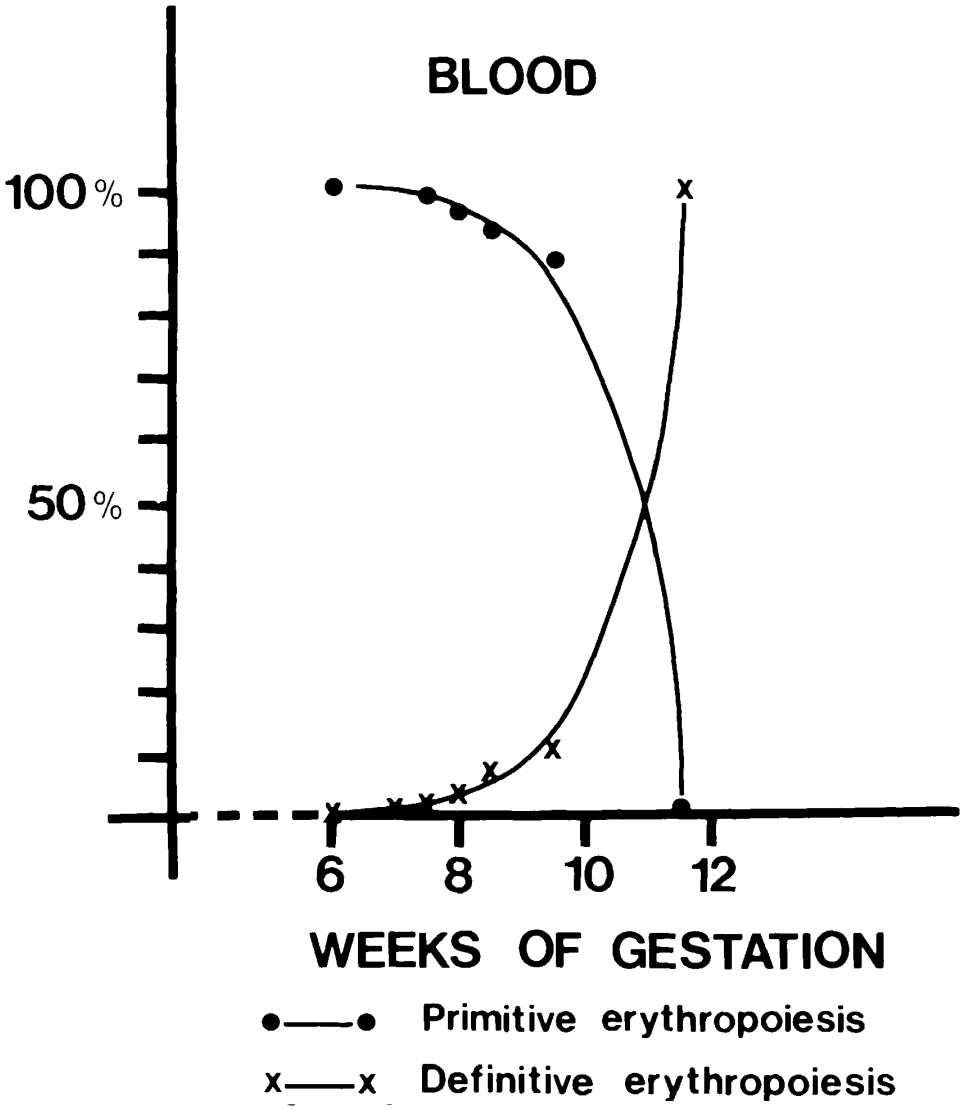
Definitive Erythroid Precursors

Liver, 7-8 weeks pc



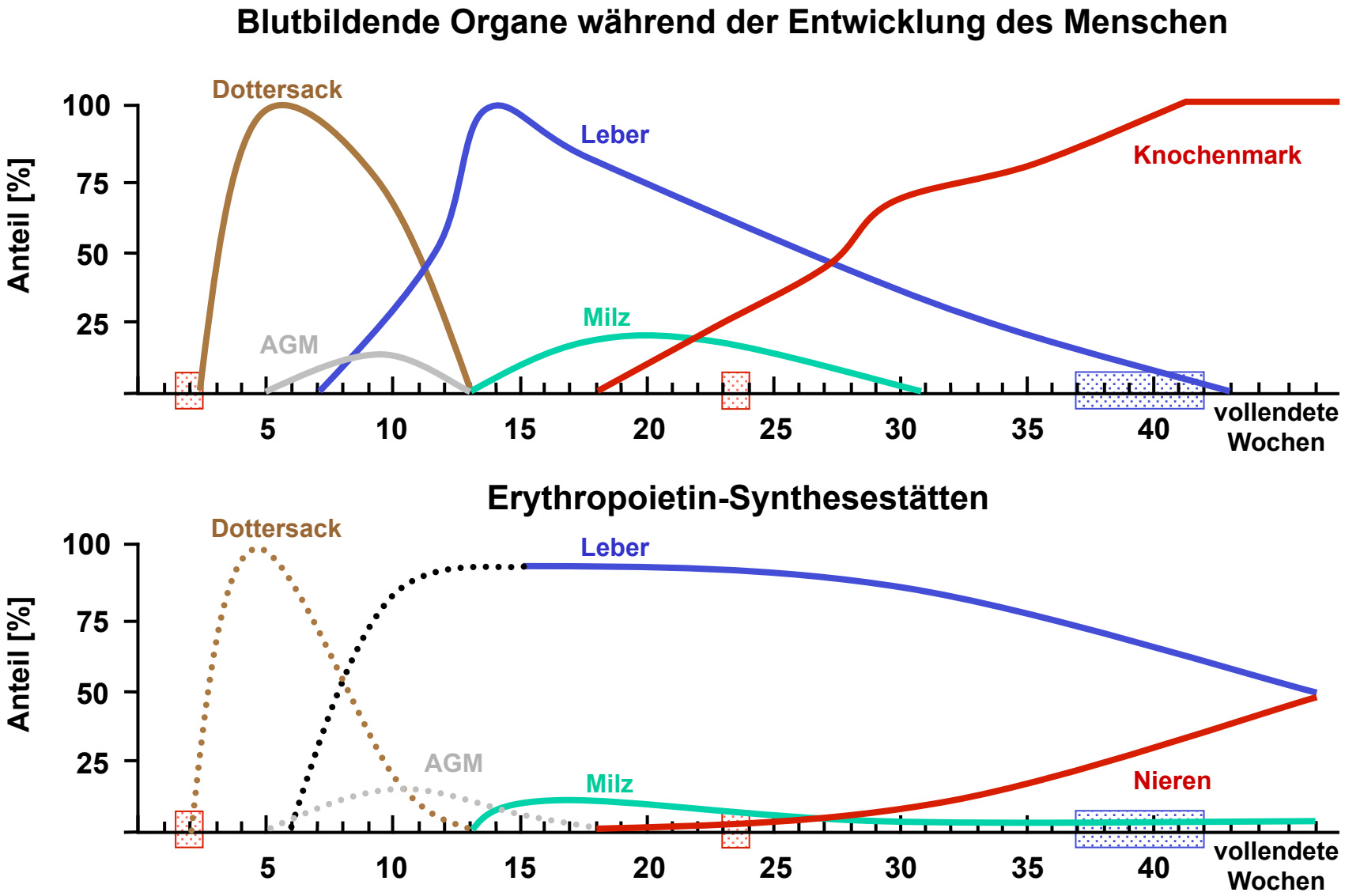
Original from: Kelemen E, Calvo W, Fliedner TM. Atlas of human hemopoietic development. Springer, 1979

Transition of Primitive to Definitive Erythropoiesis in Humans

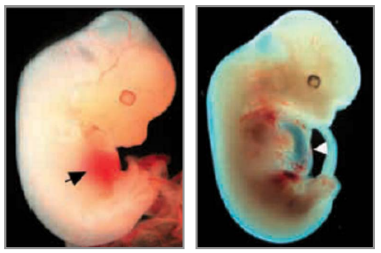


Original from: Kelemen E, Calvo W, Fliedner TM. Atlas of human hemopoietic development. Springer, 1979

Switch der Blutbildung



Erythropoietin (Epo) und sein Rezeptor (EpoR) in der Hämatopoiese

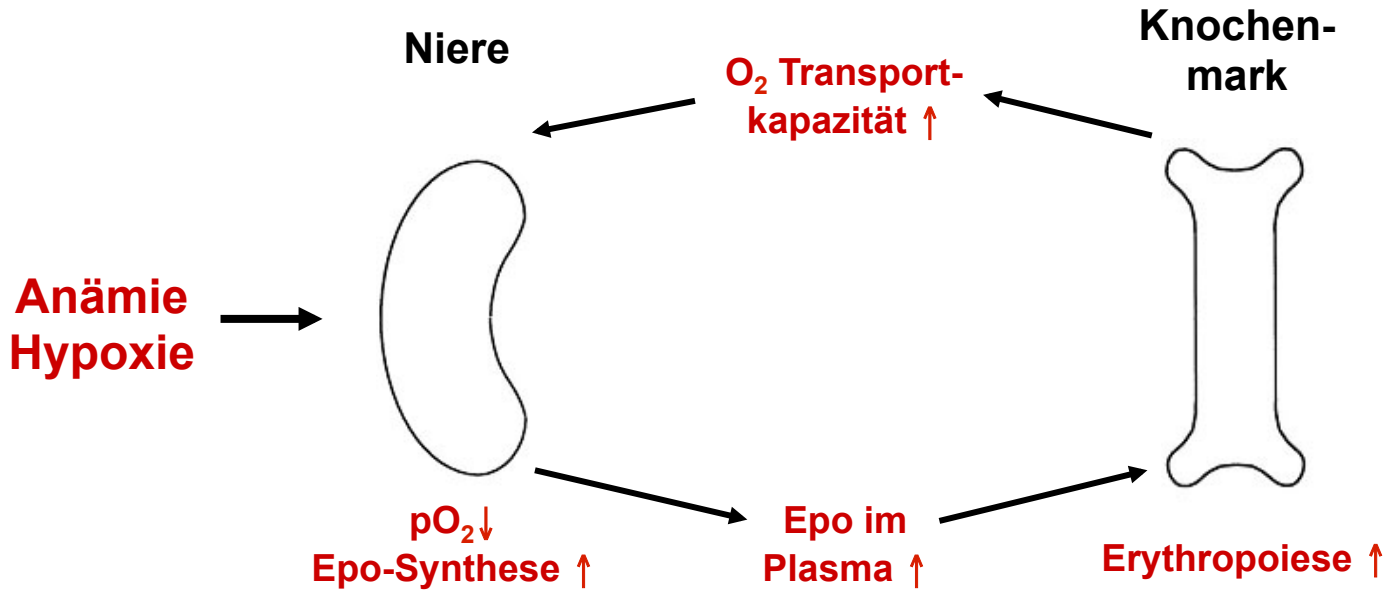


Wu H. *et al*., Cell 1995

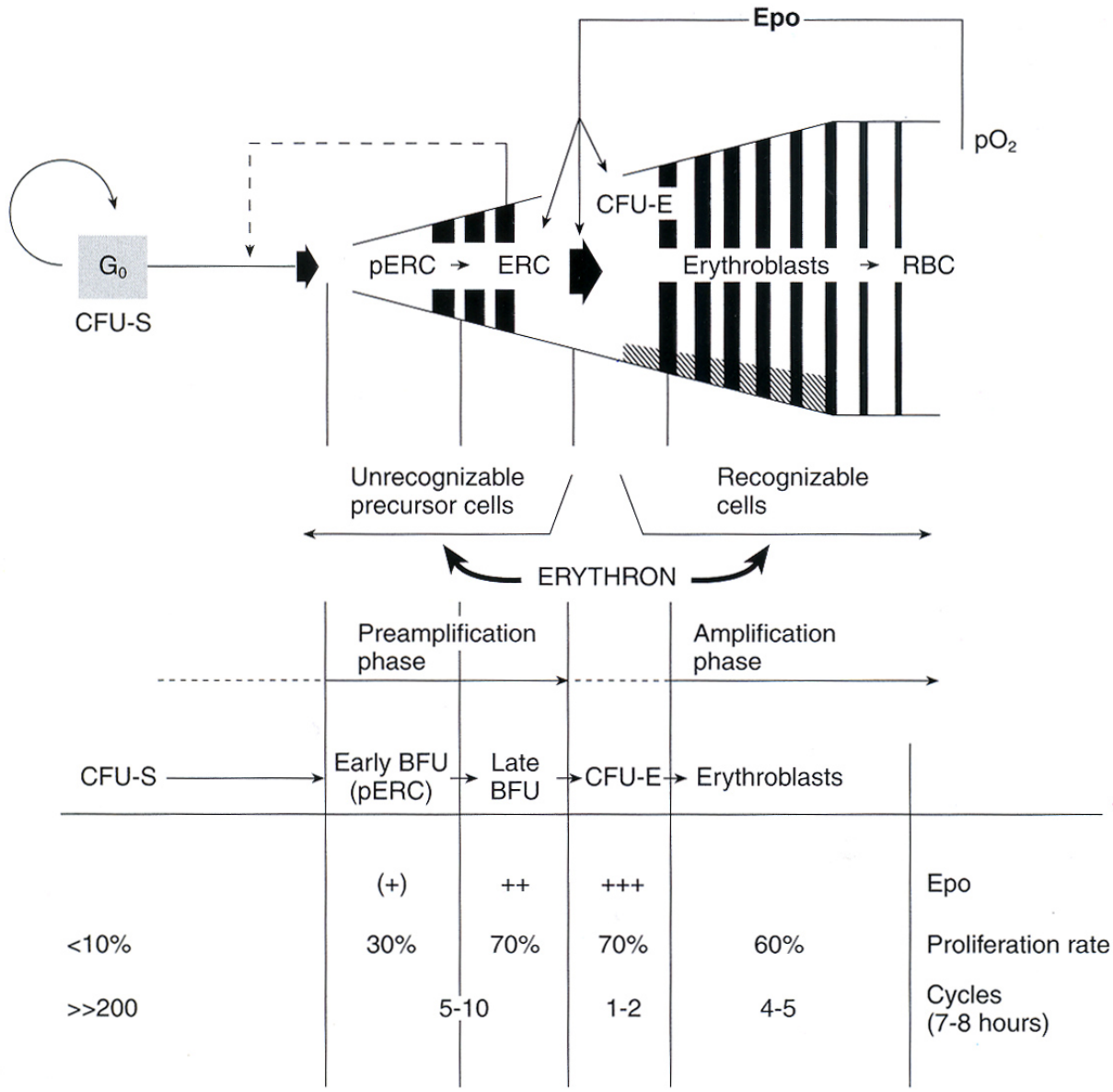
Epo

▼

Hämatopoiese

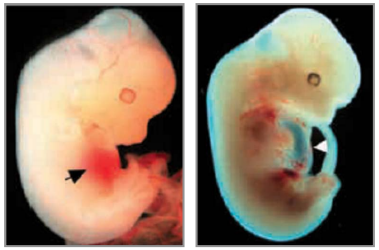


Epo – the primary regulator of red blood cell production



Erythropoietin (Epo) und sein Rezeptor (EpoR) in der Hämatopoiese

Wildtyp ***EpoR*$^{-/-}$**

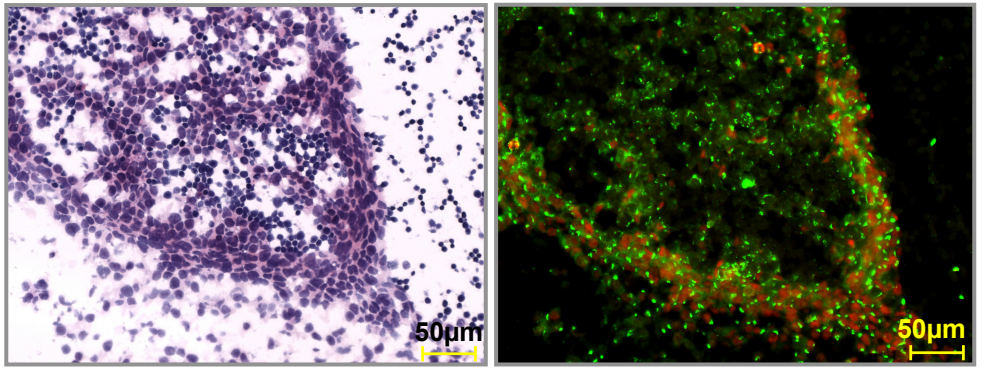


e11.5

Wu H. *et al*., Cell 1995

Leber (m) e11; H&E **Epo [Cy2]; Wt1 [Cy3]**

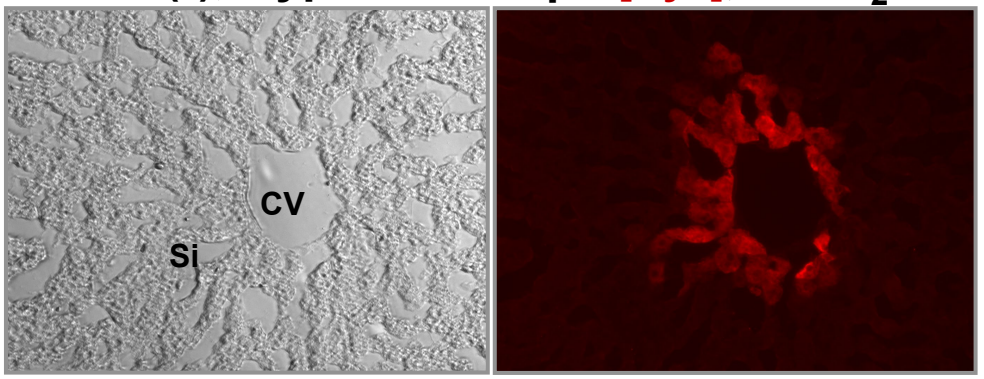
embryonal



Dame C. *et al*., Blood 2006

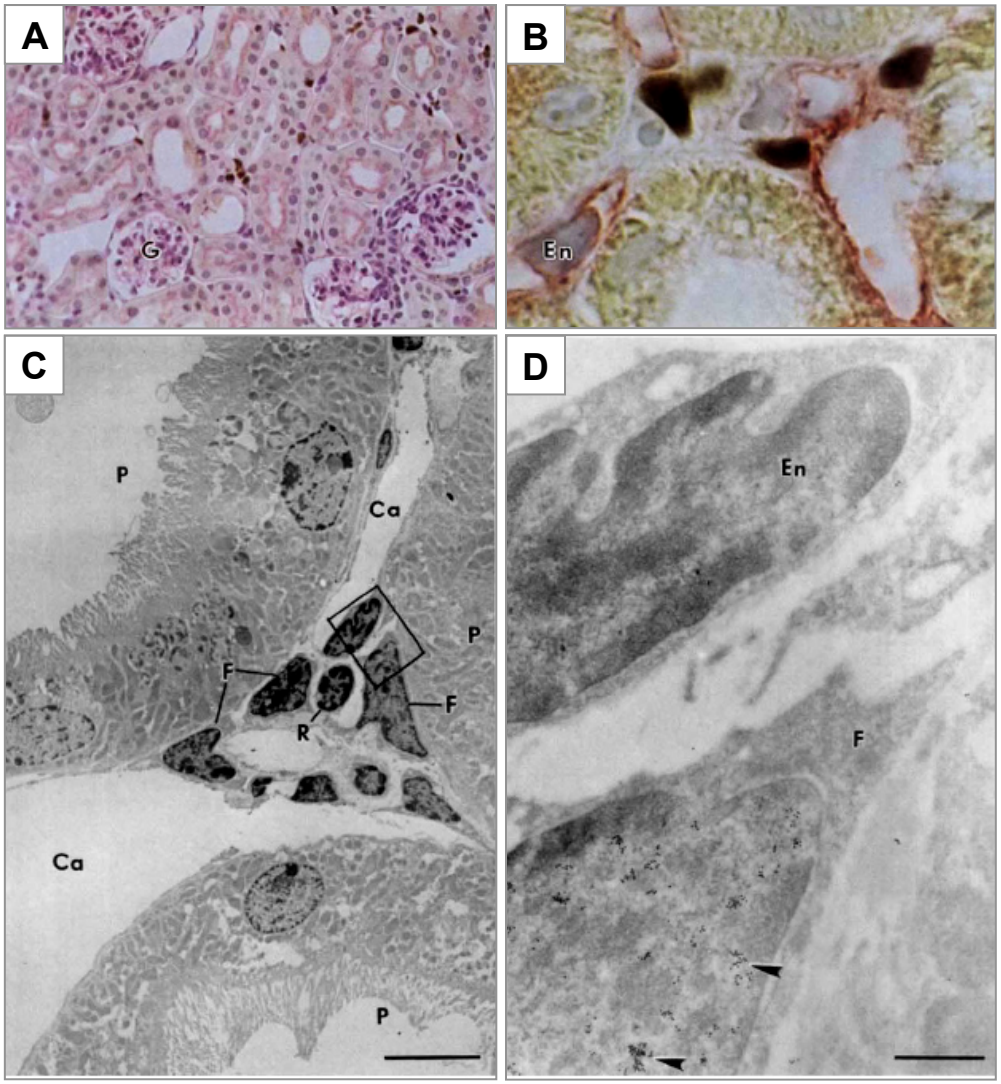
Leber (r), Hypoxie **Epo [Cy3]; 8% O_2**

adult



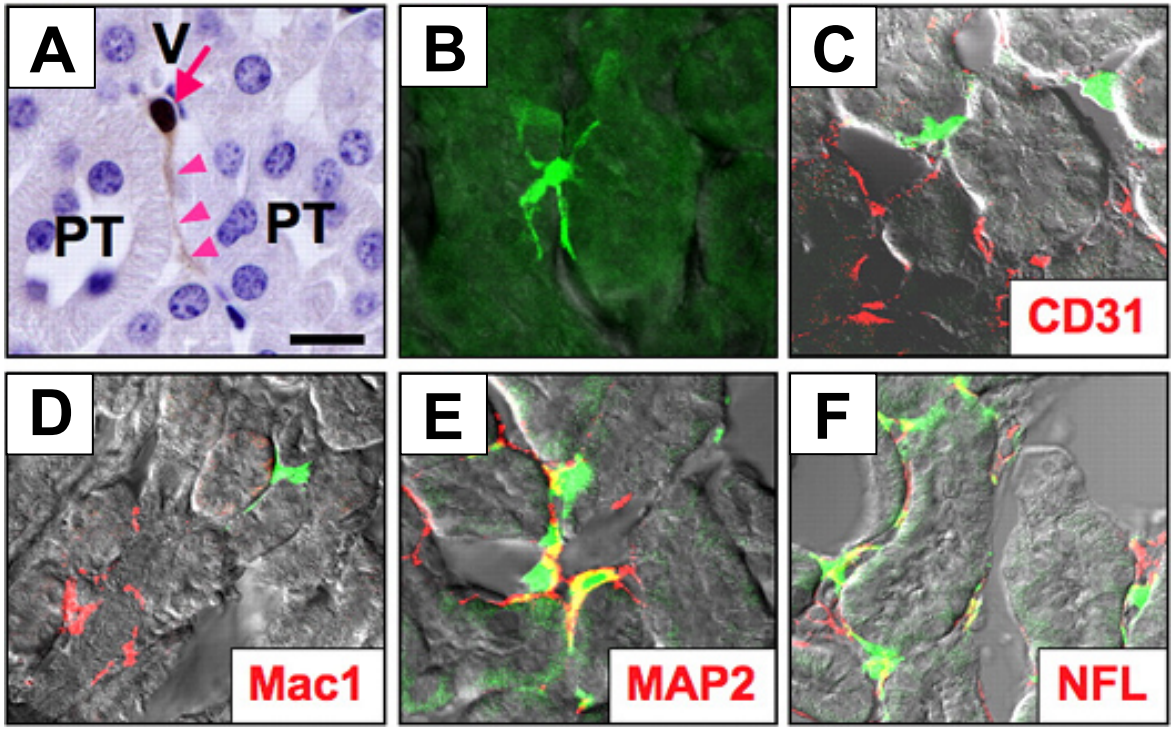
Dame C. Atlas of the Cellular and Molecular Development of Human Hematopoiesis, Springer, 2013

Erythropoietin (Epo) Synthese in der Niere



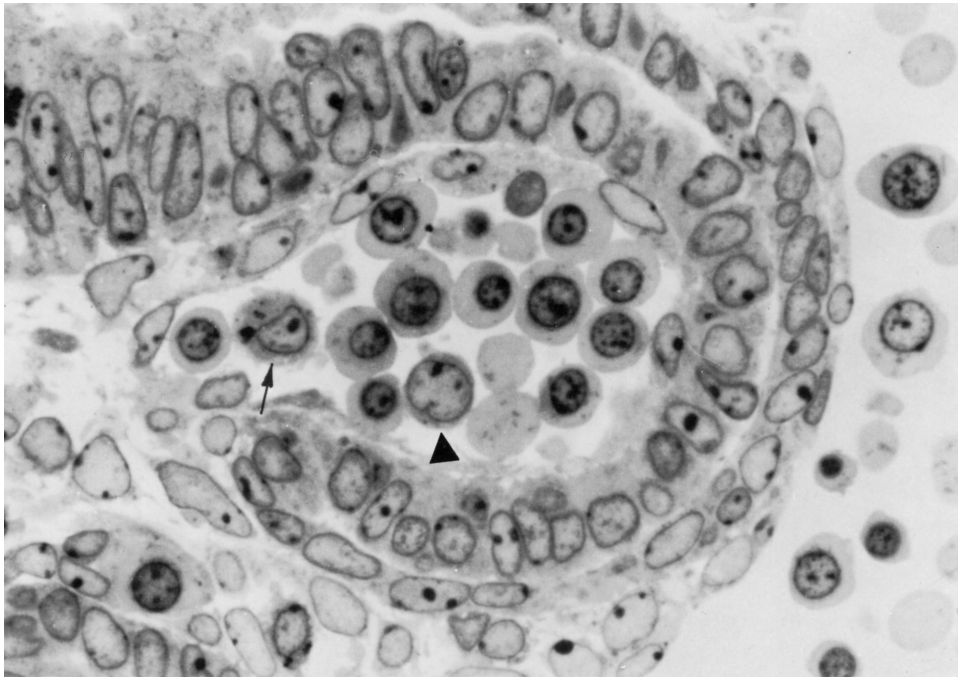
Maxwell PH *et al*., Kidney International 1993; 44:1149-1162

Erythropoietin (Epo) Synthese in der Niere



Obara N. *et al*., Blood 2008; 111:5223-32

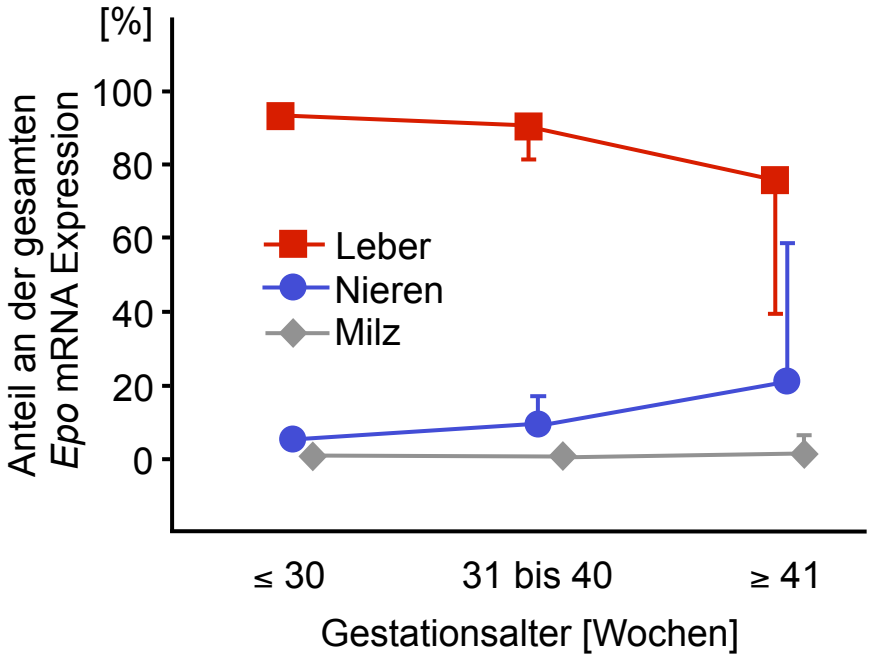
Island of primitive erythroblasts in the human mesonephros at 4-5 weeks of gestation



Original from: Kelemen E, Calvo W, Fliedner TM. Atlas of human hemopoietic development. Springer, 1979

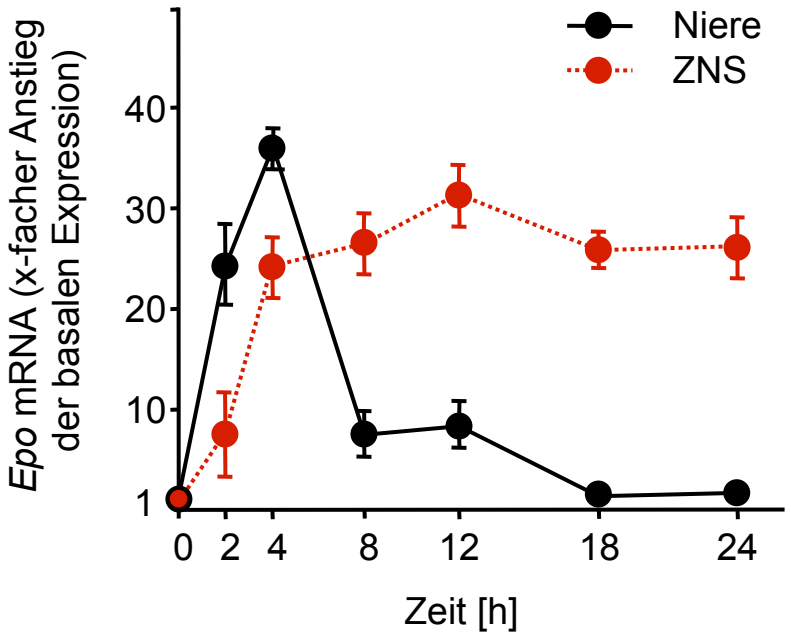
Gewebespezifische Regulation der *Epo* mRNA Expression

→ **Wechsel der Epo-Synthese von der Leber zur Niere**



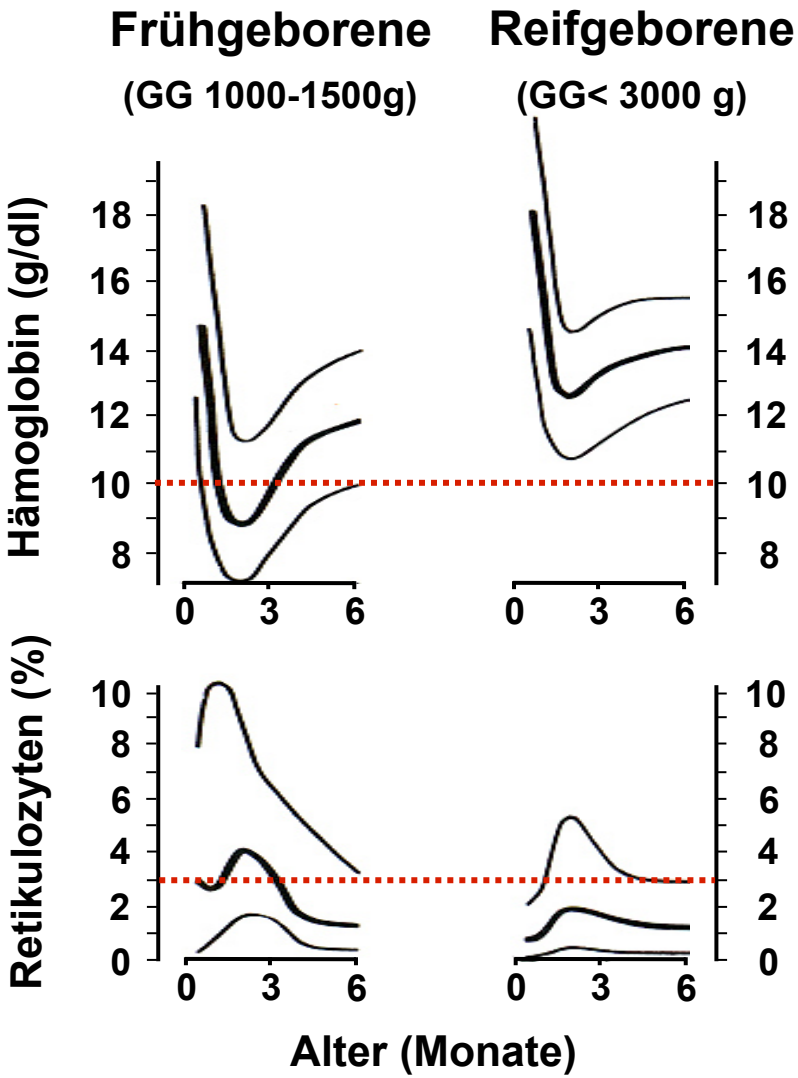
Dame C. *et al*., Blood 1998

→ **Organspezifische Antwort unter fortgesetzter Hypoxie**



Chikuma M. *et al*., Am J Physiol Endocrinol Metab 2000

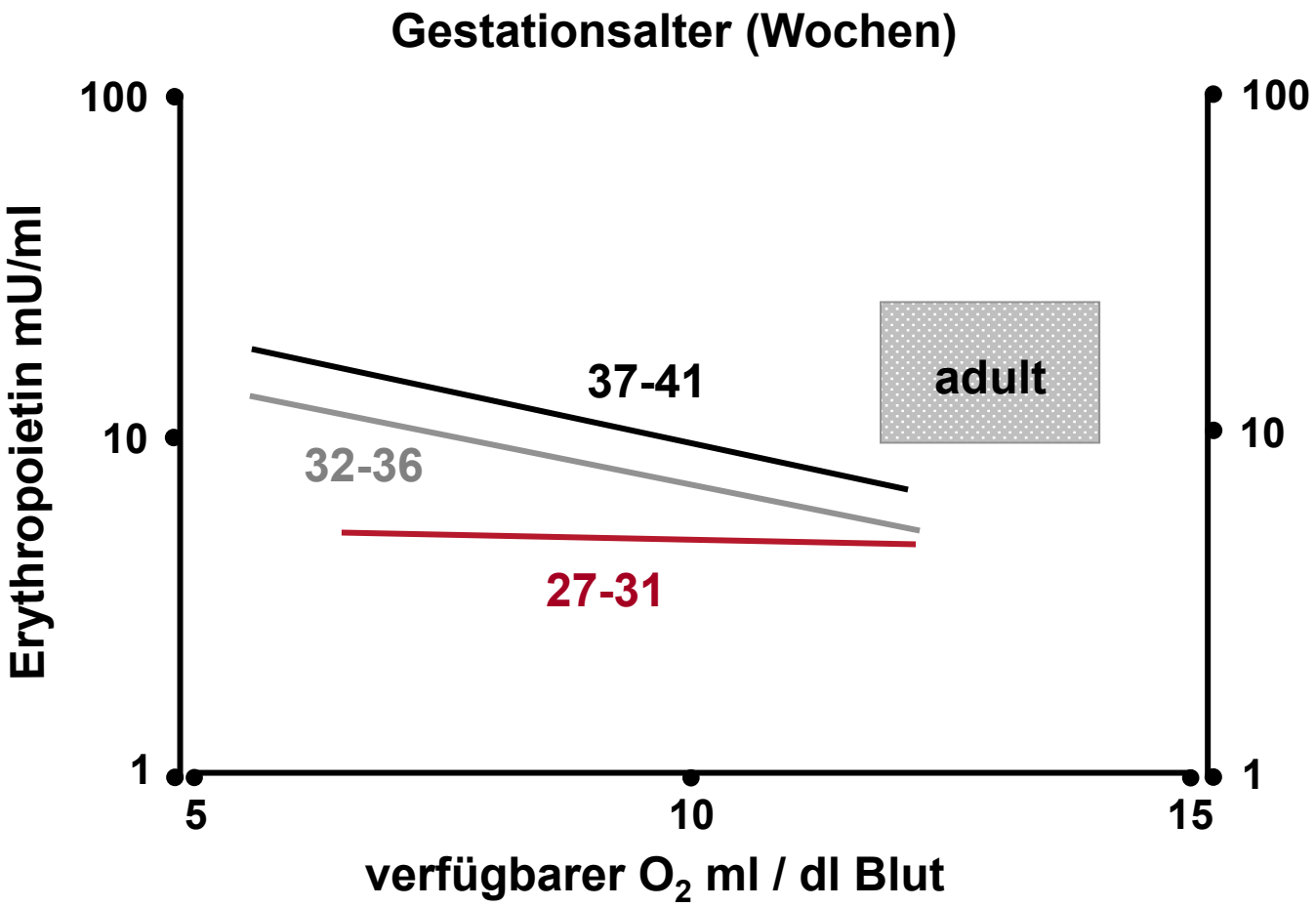
Frühgeborenen-Anämie



Dallman PR, Annu Rev Med, 1981

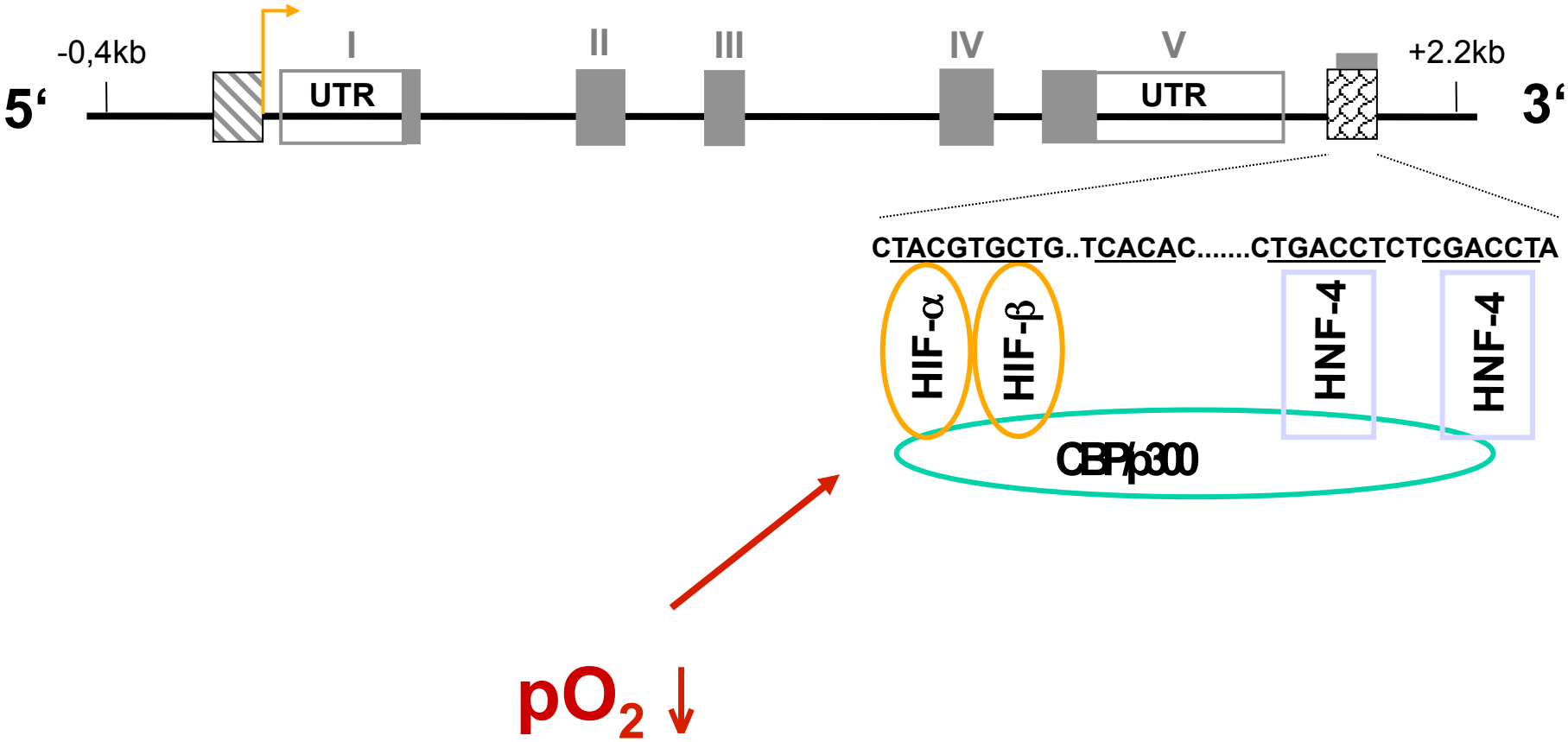
Epo in Relation zum verfügbaren O_2

Die besondere Situation beim Frühgeborenen



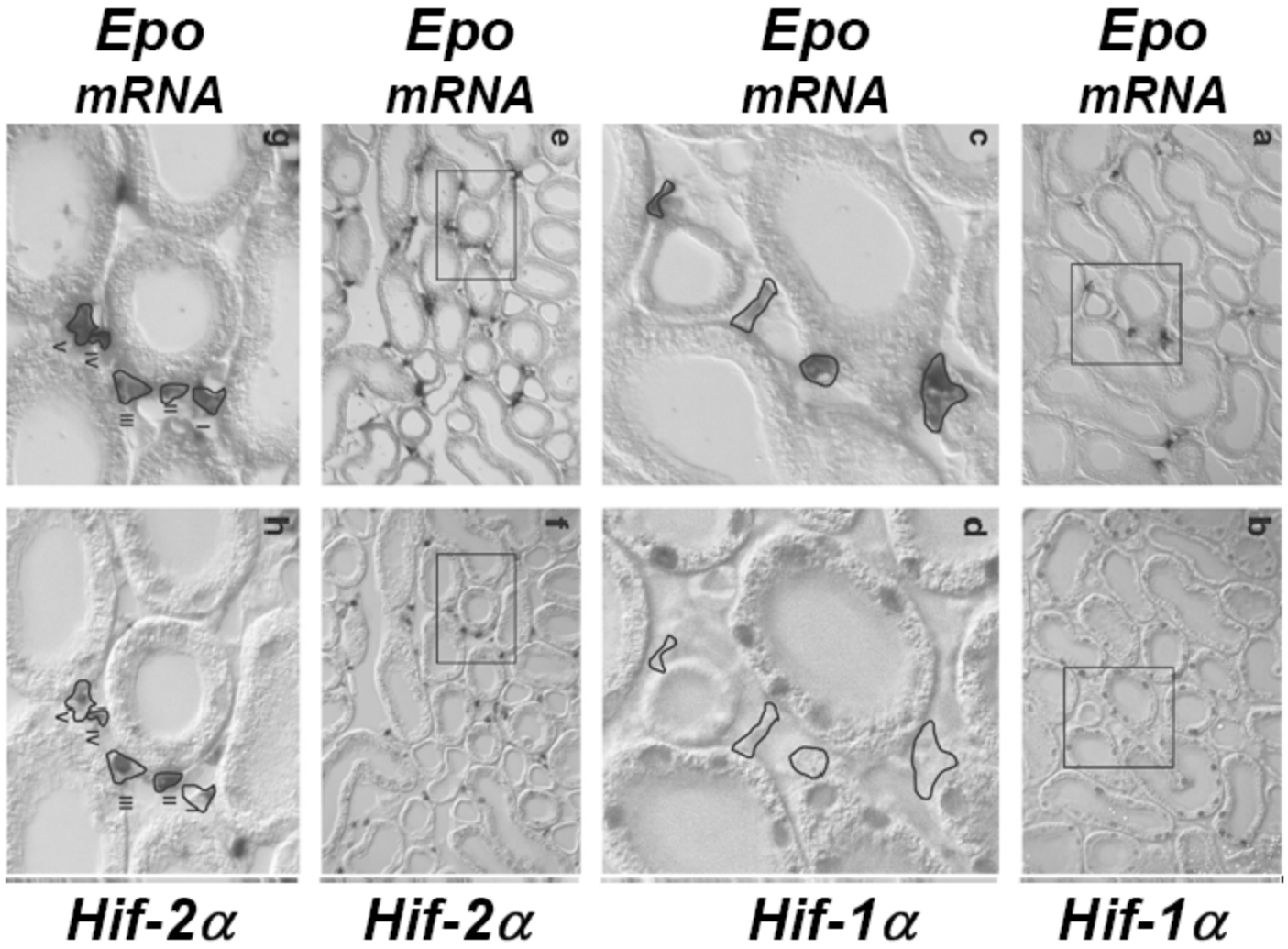
Dallman PR, J Pediatr 1984; 105:756-7

Transcriptional regulation at the 3‘ *Epo* enhancer



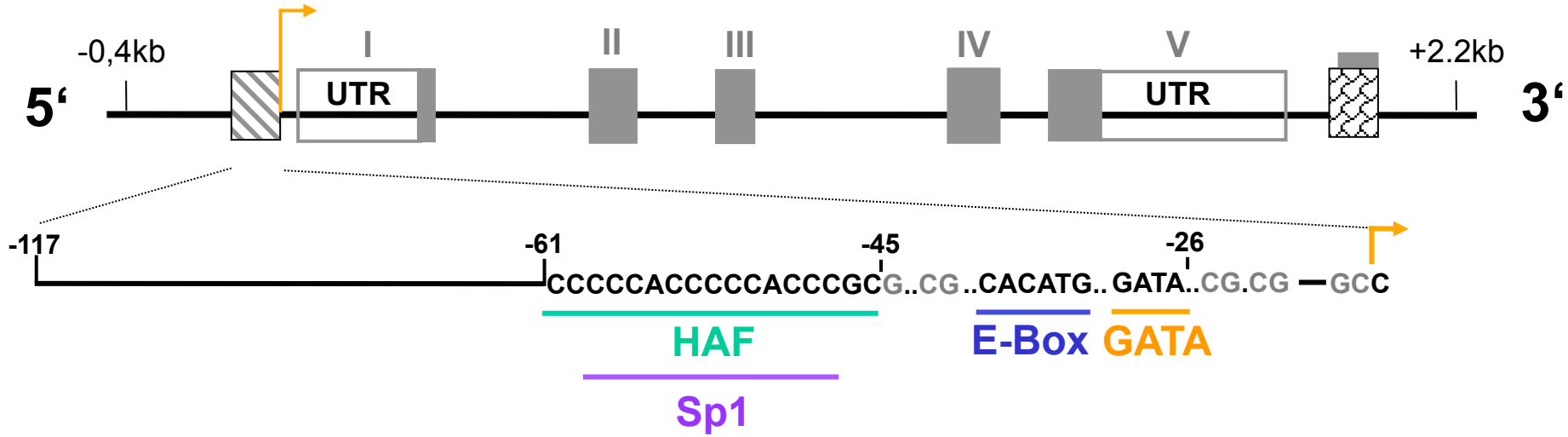
Review: Fandrey, J. Am J Physiol 2004; 286:R977-88

Epo and *Hif* Expression in the Murine Kidney



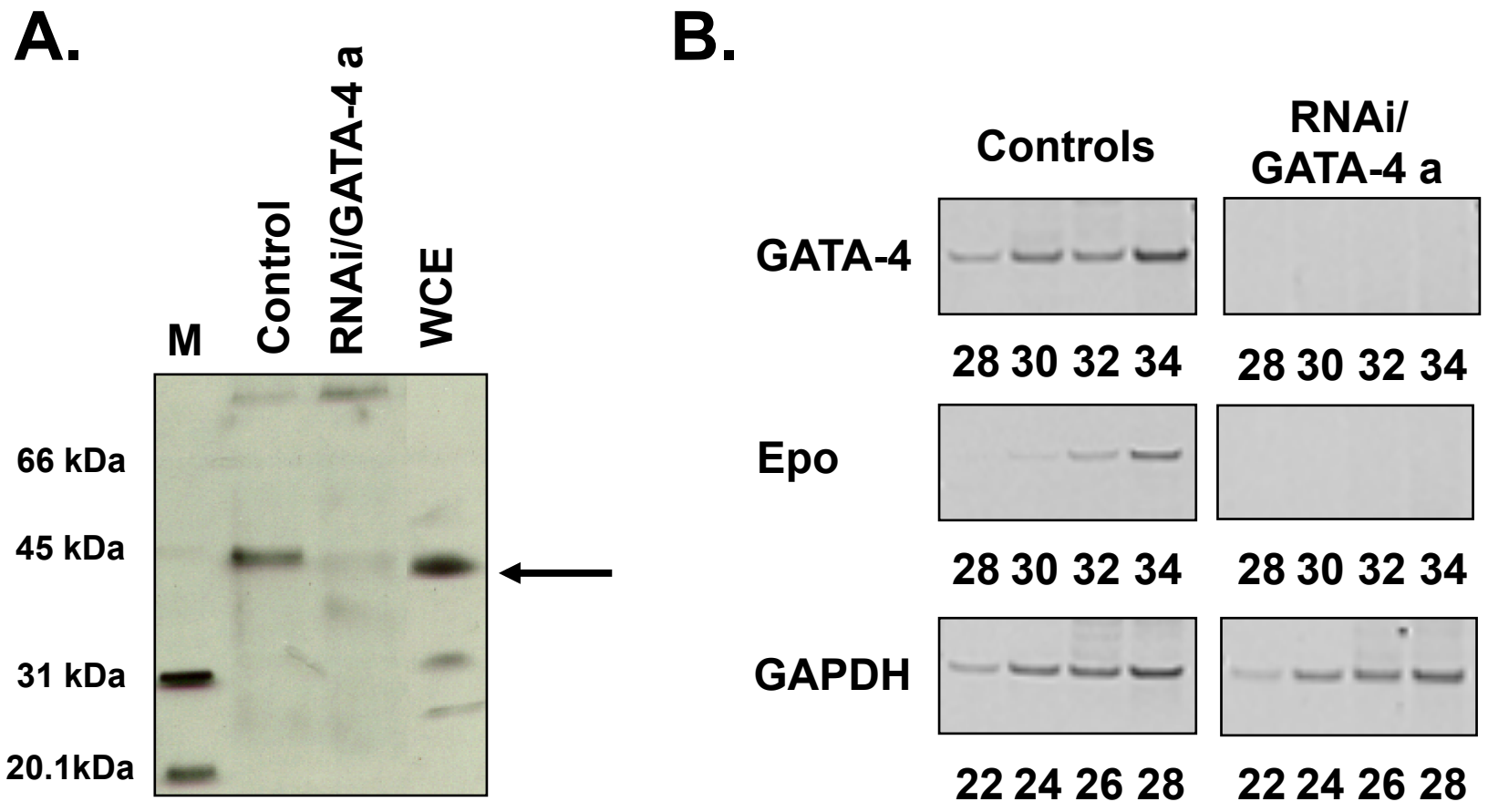
Paliege A *et al*., Kidney International 2010; 77:312-318

Transcriptional regulation at the 5‘ *Epo* promoter



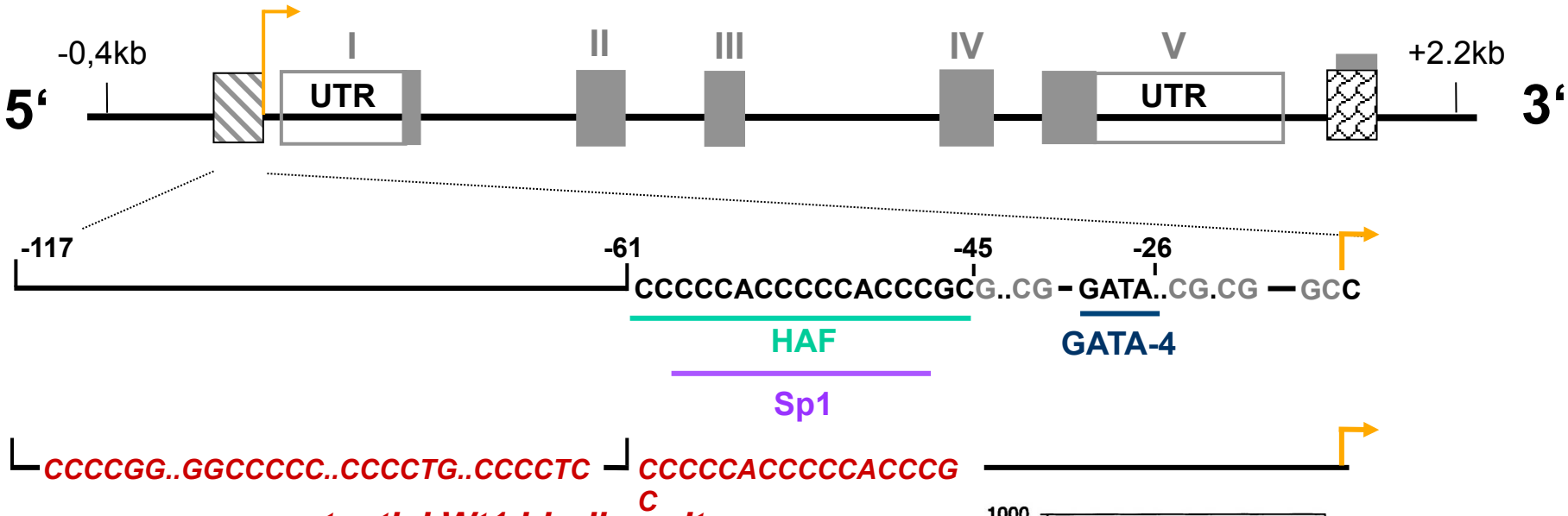
Gupta M. *et al.*, Blood. 2000; 96:491-7; Sanchez-Elsner T. *et al.*, J Mol Biol. 2004; 336:9-24

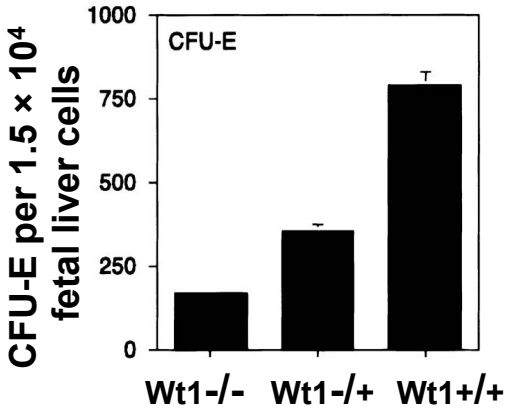
GATA-4 induces *Epo* mRNA expression in Hep3B cells



Dame *et al*., J Biol Chem, 2004; 279: 2955-61

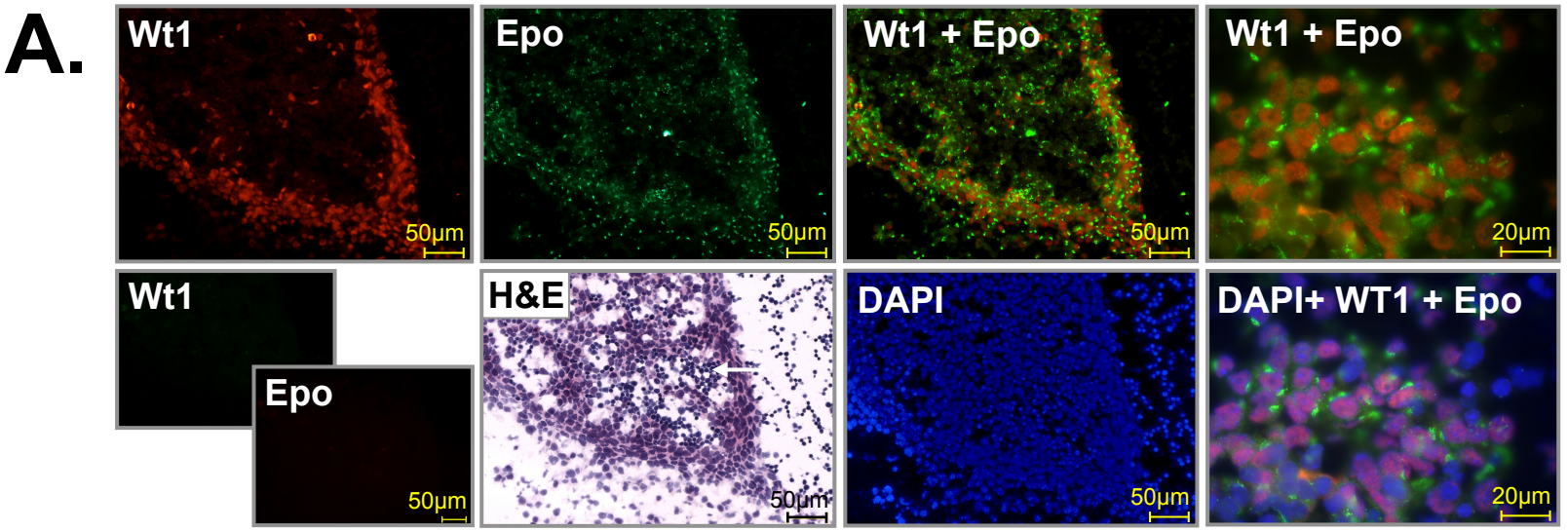
Transcriptional regulation at the 5‘ *Epo* promoter

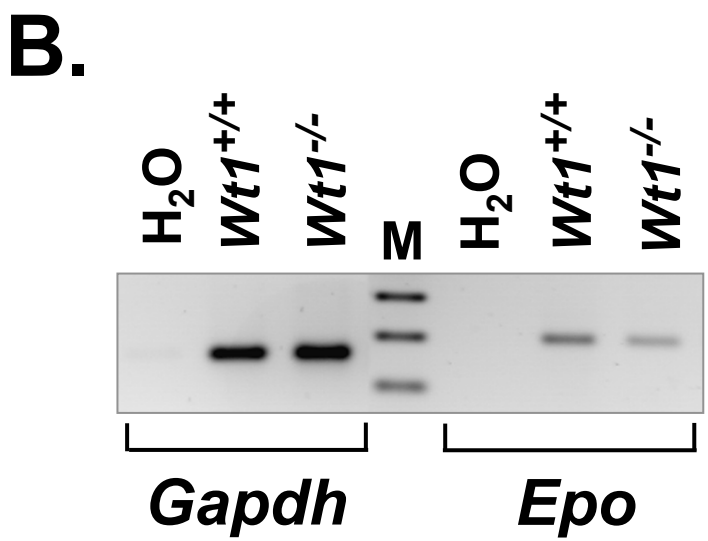


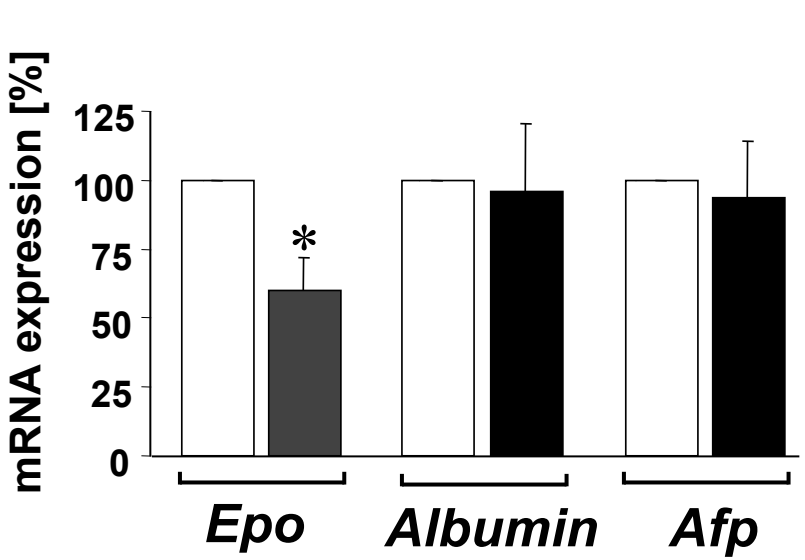


Alberta JA. *et al*., Blood. 2003; 101: 2570-4

In vivo relevance of Wt1 on *Epo* mRNA expression in the fetal liver



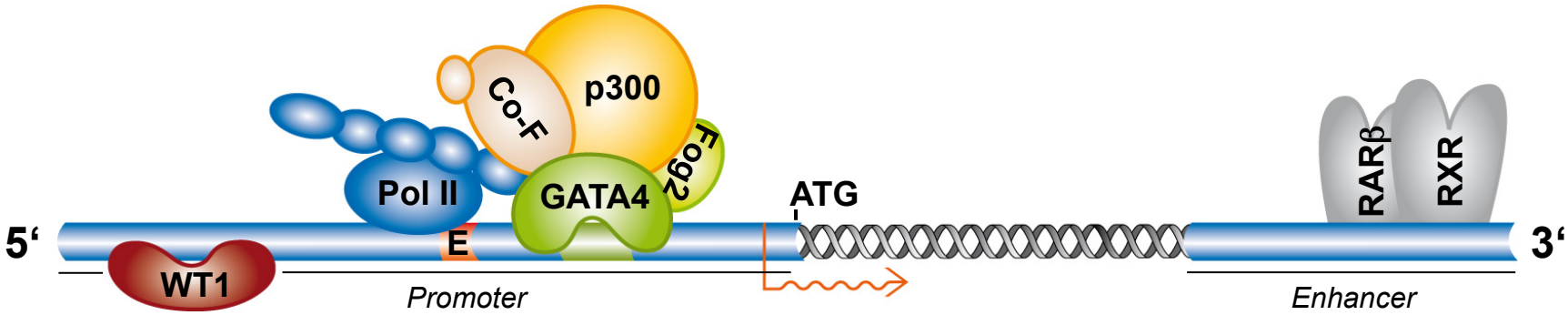




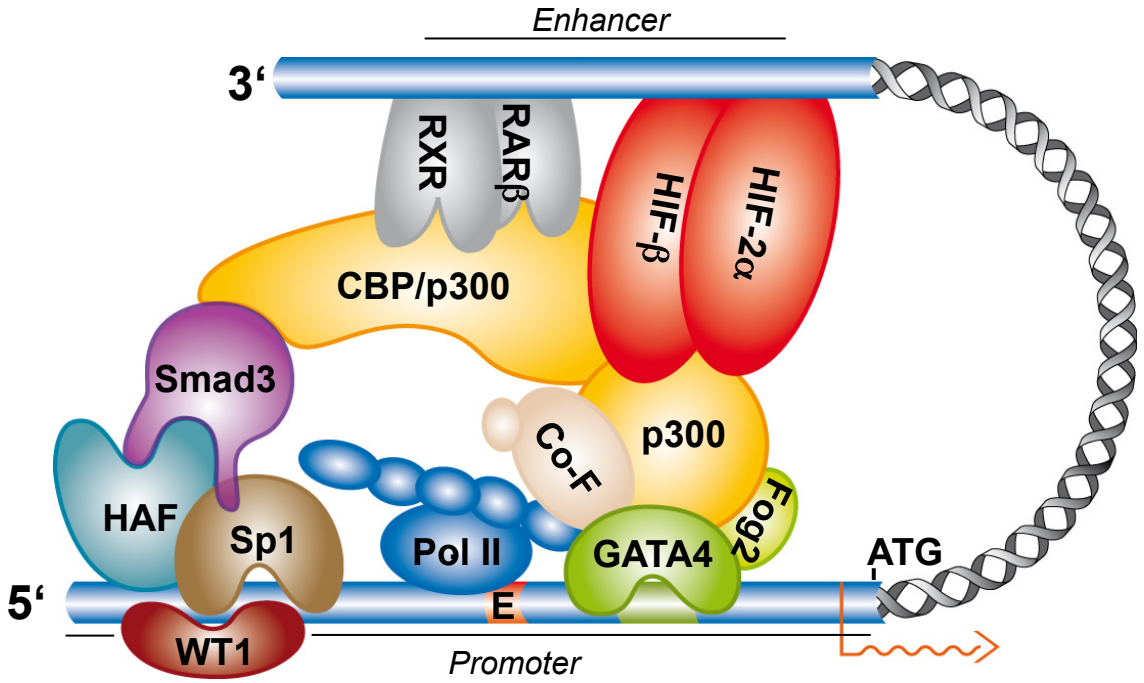
Dame C, *et al*. Blood, 2006, 107:4285-90

Molekulare Mechanismen der *Epo* Regulation

Normoxie

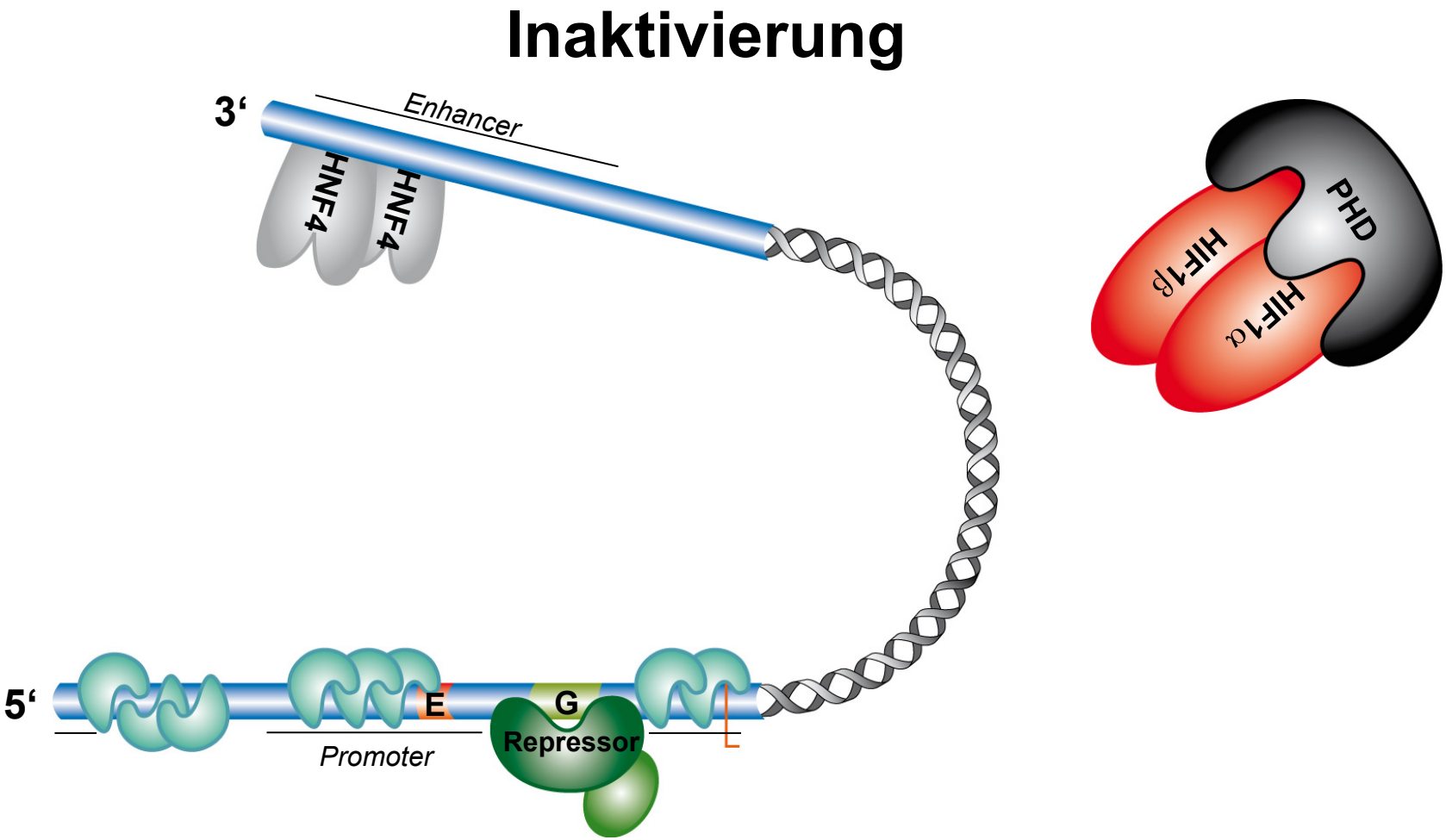


Hypoxie

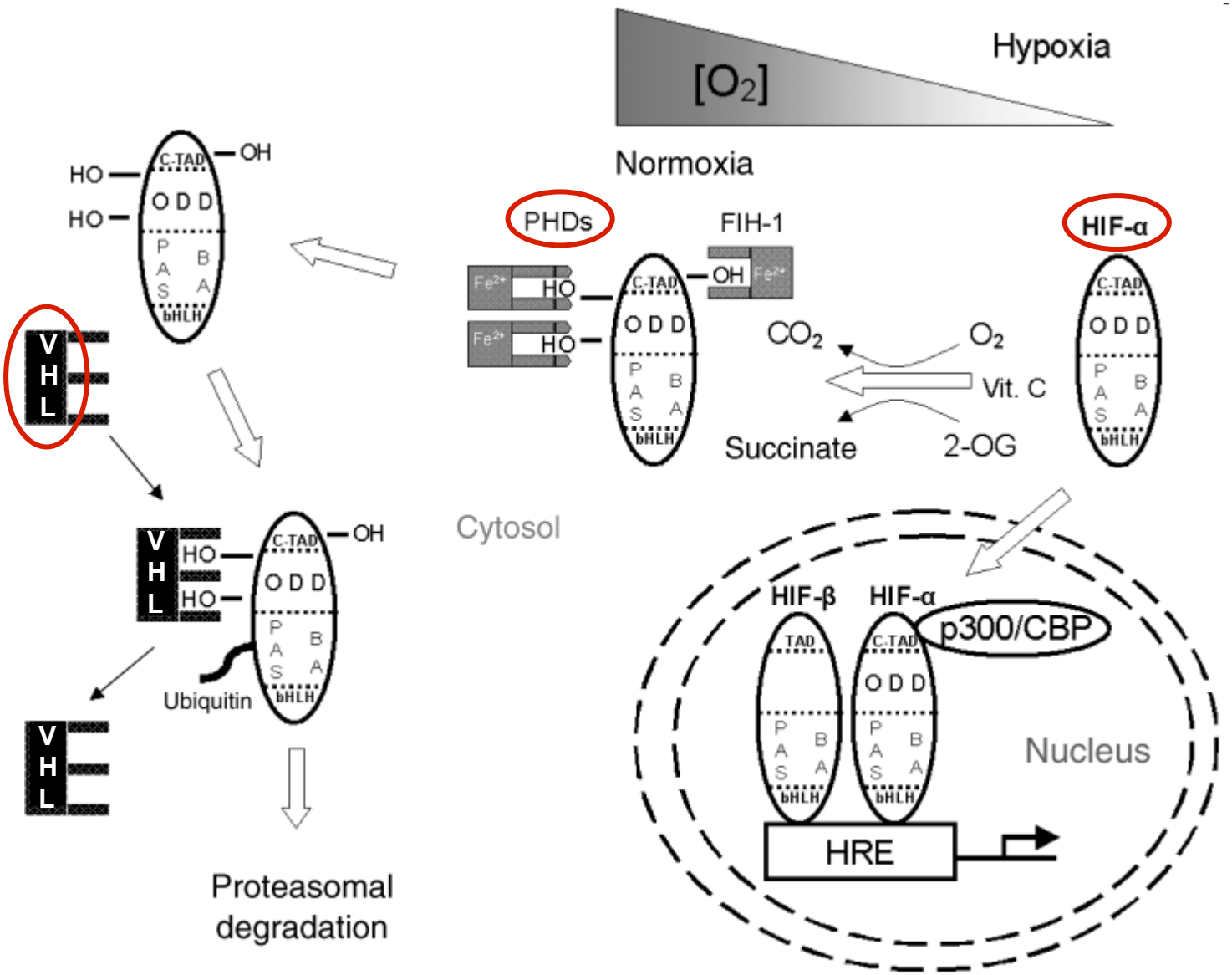


Dame C. *et al*., J Biol Chem, 2004; Dame C, *et al*. Blood, 2006;
Sanchez-Elsner T. *et al*., J Mol Biol. 2004; Obara N. *et al*. Blood 2008.

Molekulare Mechanismen der *Epo* Regulation

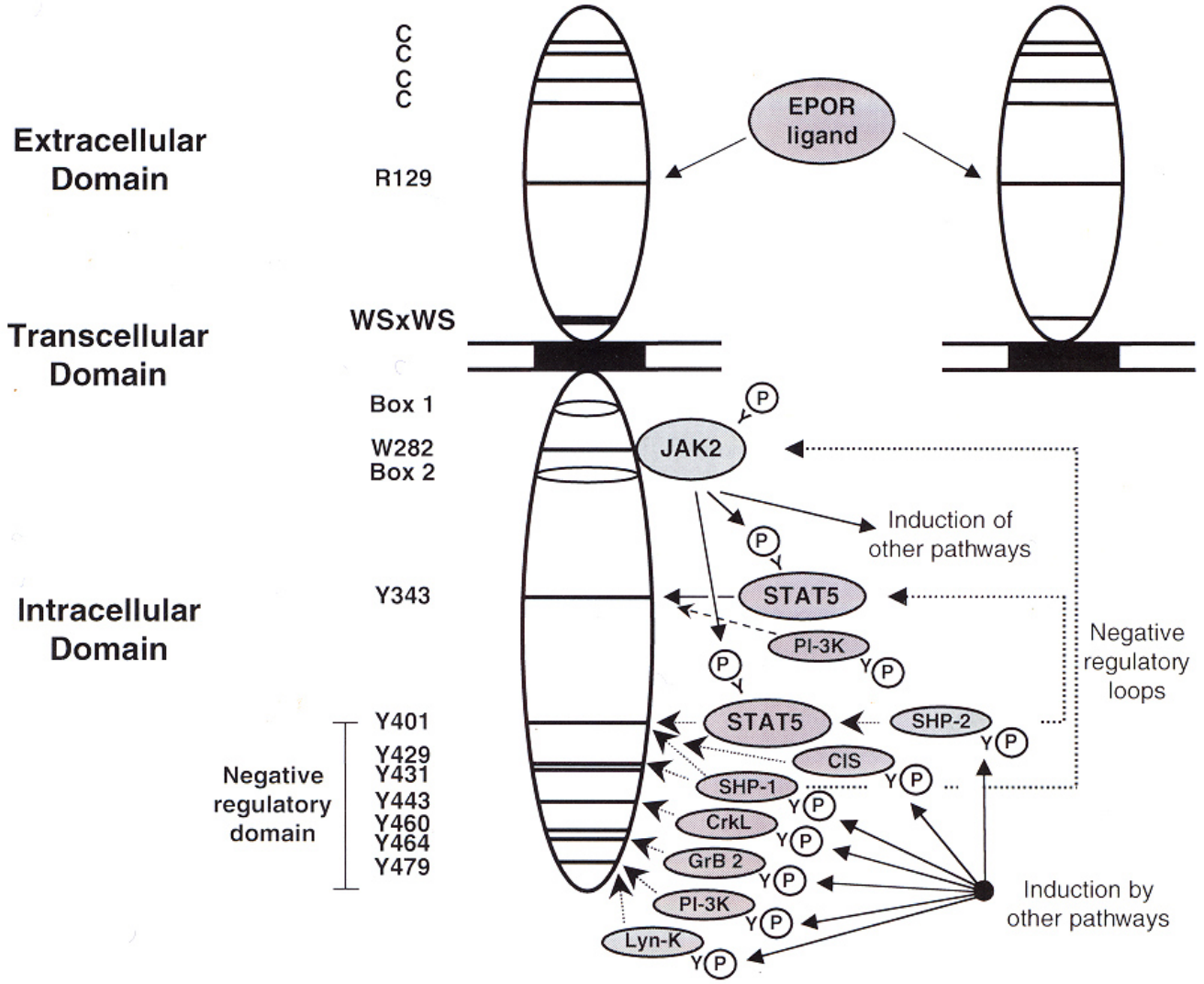


Epo Gene Regulation

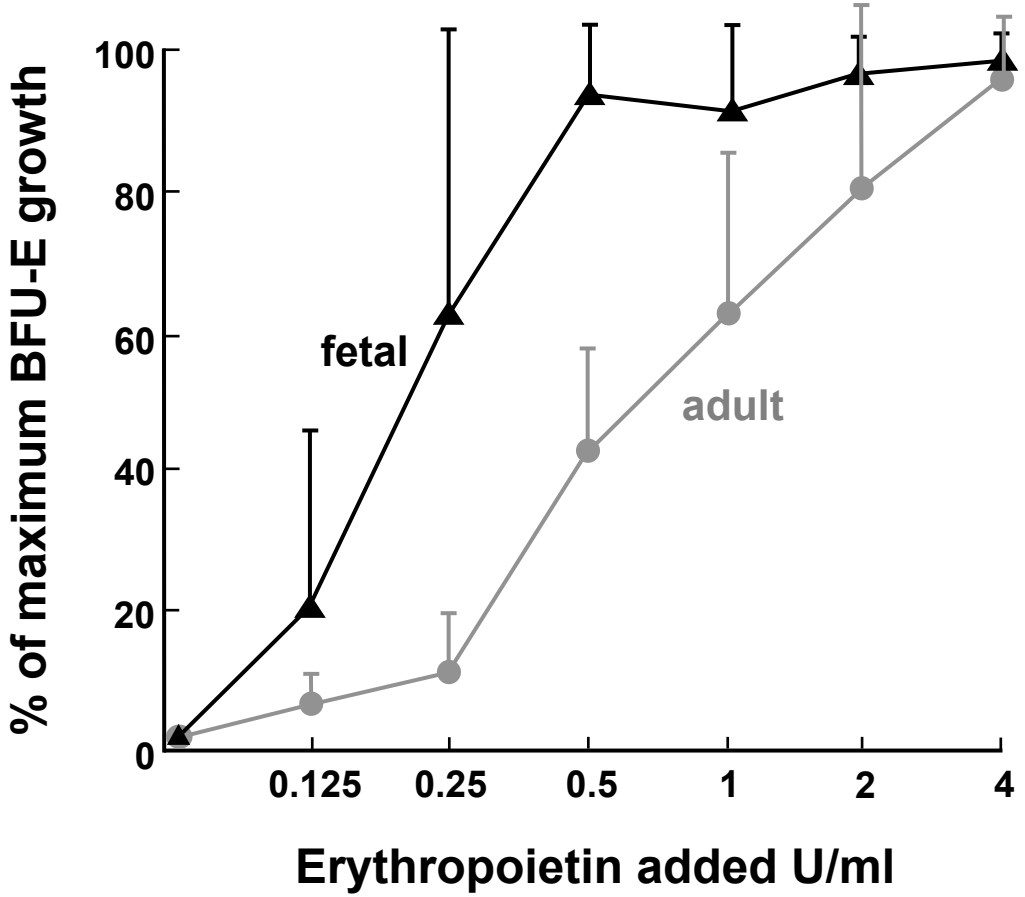


Stockmann C and Fandrey J. Clin Exp Pharmacol Physiol 2006

EpoR Signalling



Epo Sensitivity



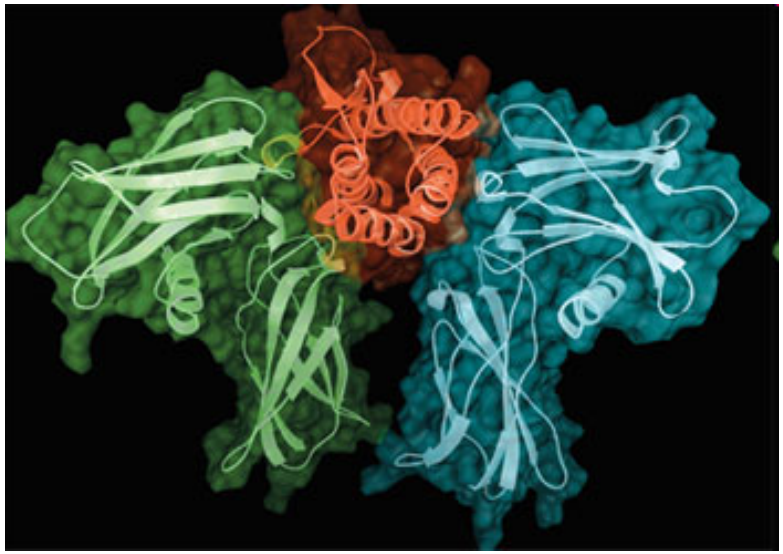
Linch DC *et al*., Blood 1982; 59:976-979

Behandlung der Frühgeborenen-Anämie

- Infektionsrisiko
- Erhöhtes ROP Risiko

Hesse L *et al*., Eur J Pediatr 1997
Brown MS *et al*., J AAPOS 2006

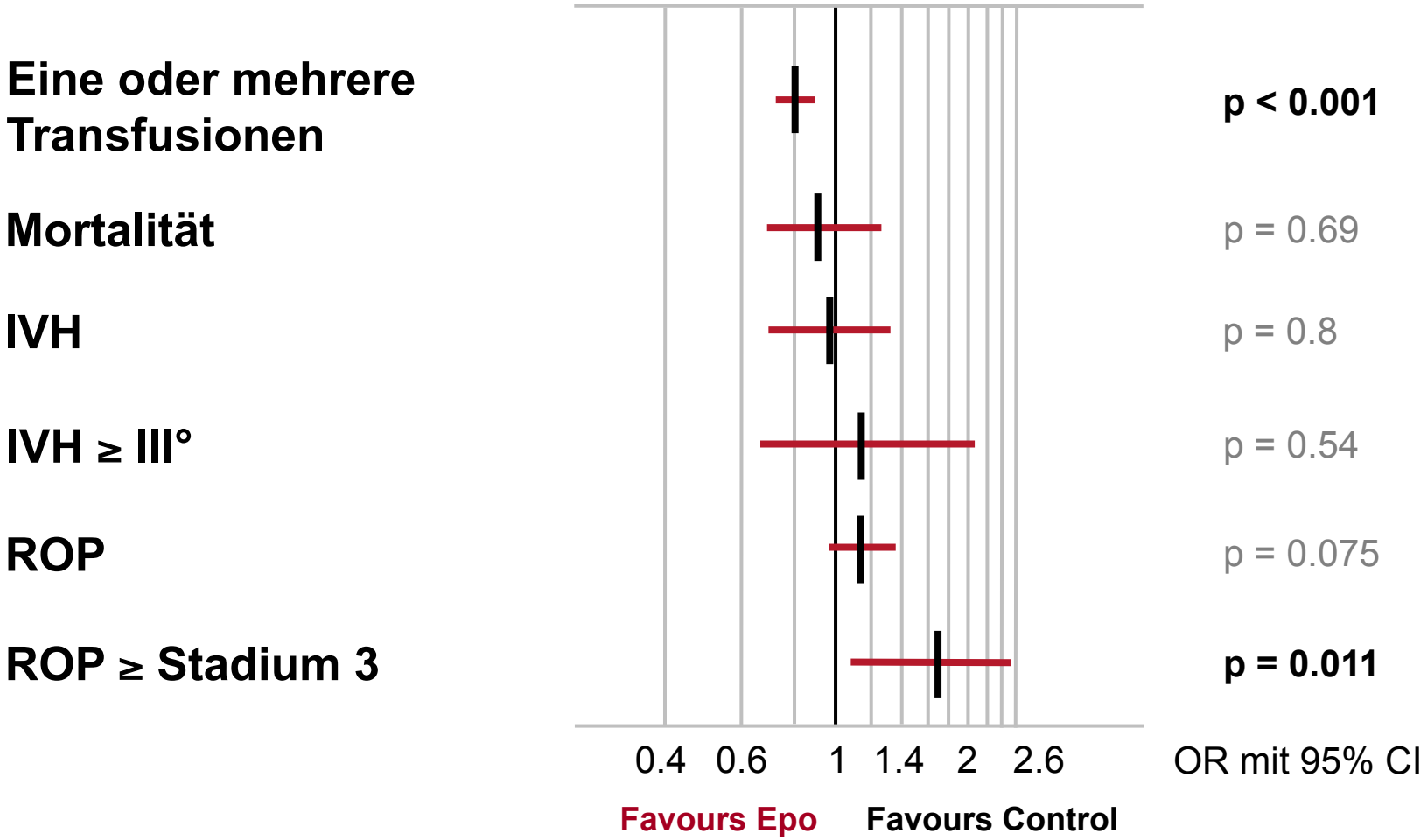
Sind rekombinantes Epo oder Epo-Derivate eine gute Alternative?



rEpo Behandlung der Frühgeborenenanämie

early rEpo Dosierung 70 bis 2.100 U/kg/Woche

16 Studien; rEpo n = 944; Placebo n = 881



Ohlsson A, Aher SM, Cochrane Database Syst Rev 2010

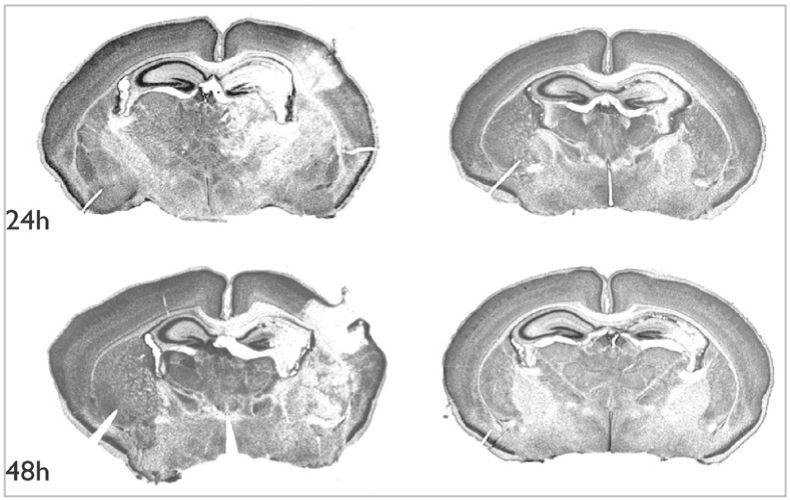
Erythropoietin jenseits der Hämatopoiese

Epo

Hämatopoiese

Neuroprotektion

Hypoxisch-ischämischer Insult

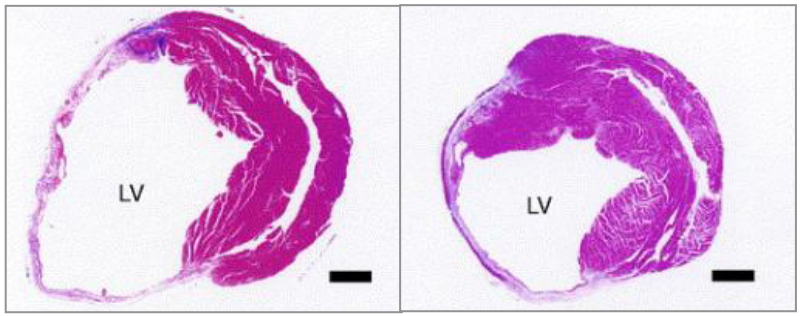


Kontrolle **rEpo 5.000 iE/kg**

Matsushita H. *et al*., NeuroReport 2003; 14:1757-61

Kardioprotektion

10 Wochen nach LV Myokardinfarkt

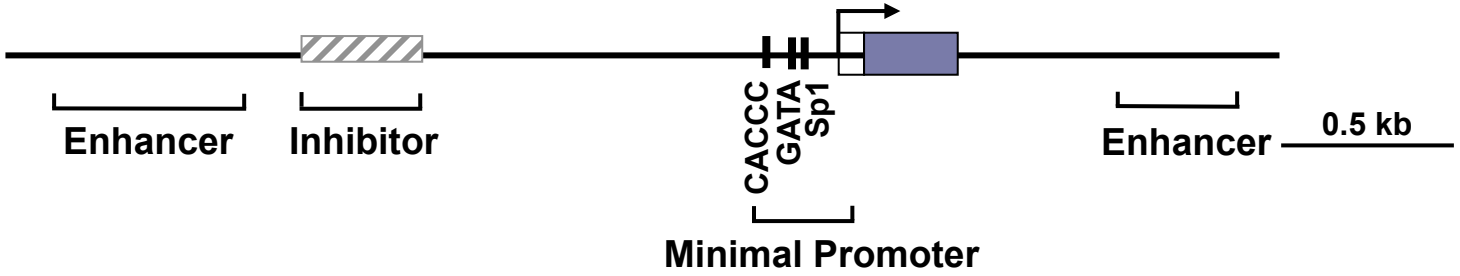


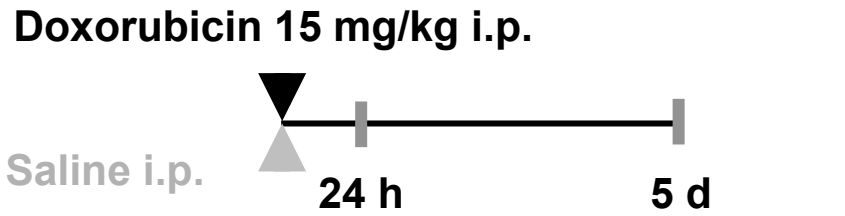
Kontrolle **rEpo s.c. 3.000 iE/kg/Woche**

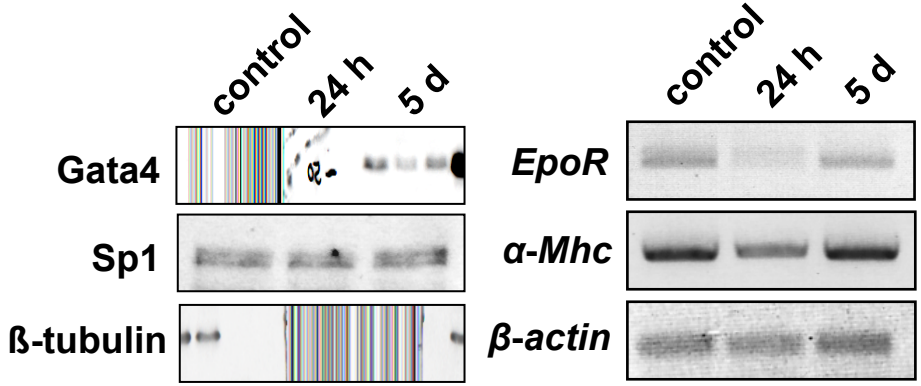
Li Y. *et al*., Cardiovasc Res 2006; 71:684-94

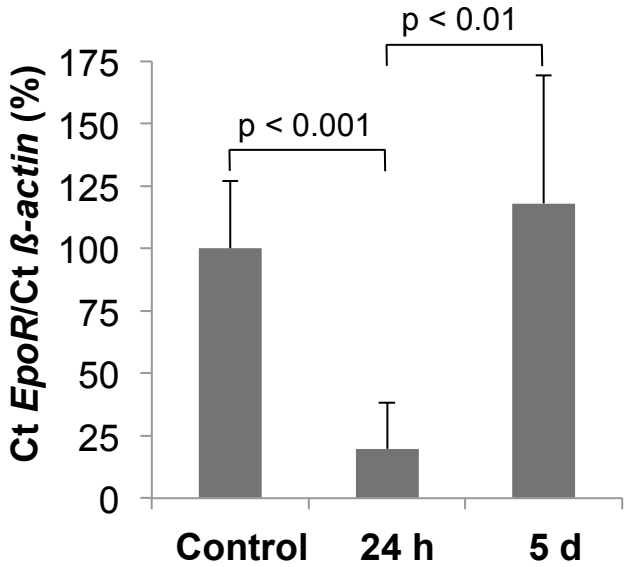
Transkriptionelle Regulation des *EpoR*

in Kardiomyozyten





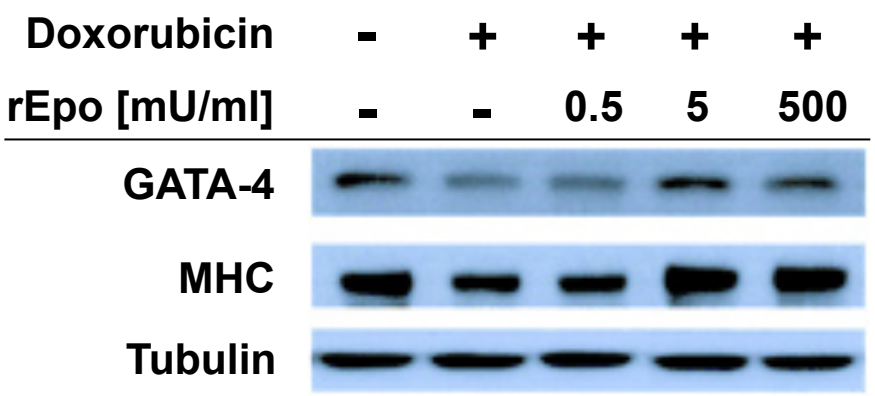


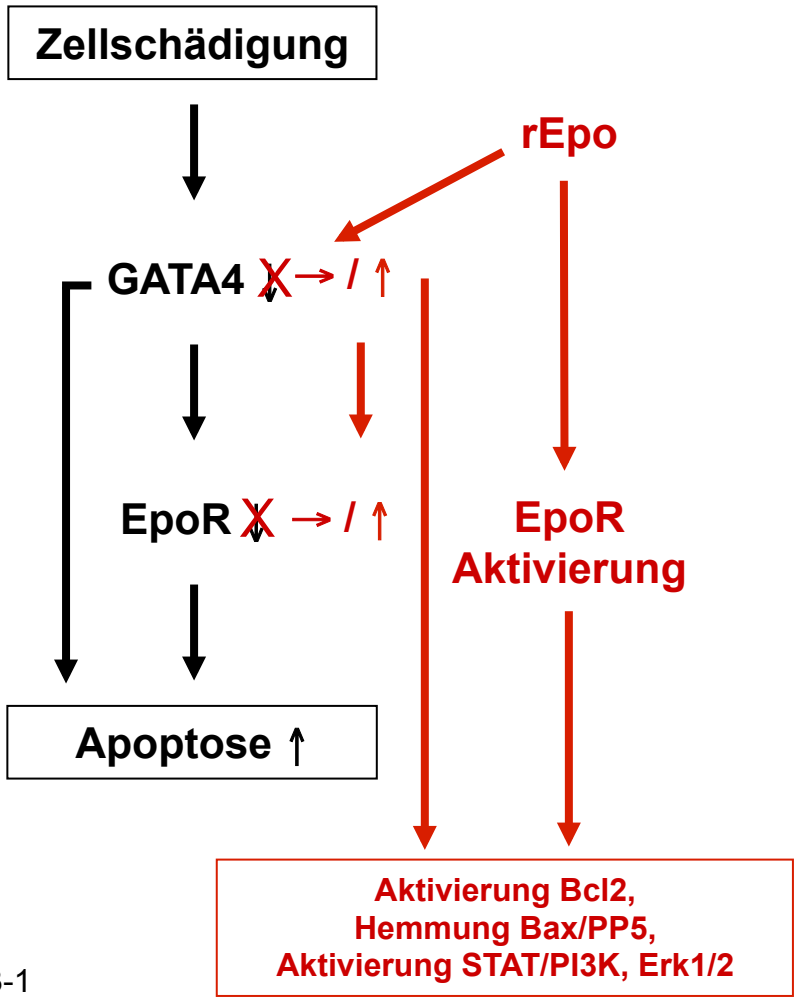


Von Salisch S. *et al*, J Cell Mol Med 2011

Modell:

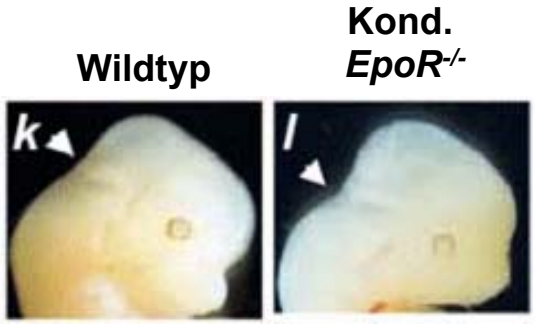
Epo induziert die Expression des EpoR via GATA4





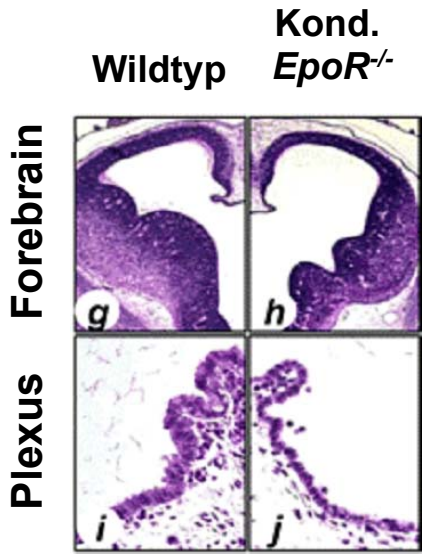
Expression und Funktion des Epo-R im Gehirn

Entwicklungsbiologische Funktion des EpoR im Gehirn

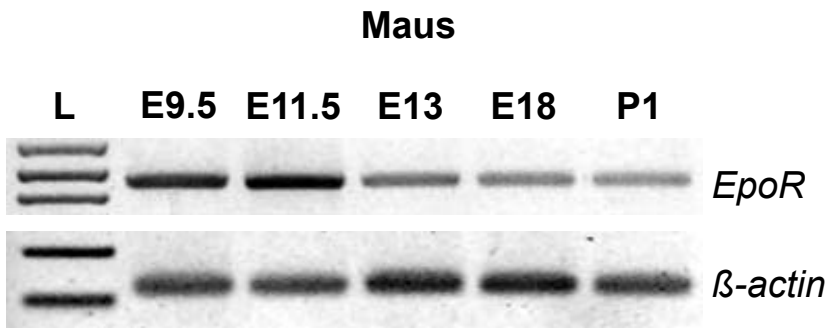


E13.5

Tsai PT *et al*. J Neurosci 2006

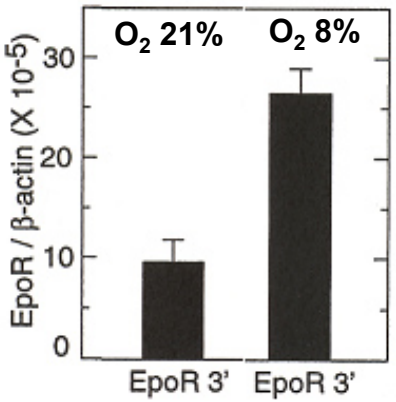


Entwicklungsabhängige Regulation



Wallach I *et al*., Pediatr Res 2009

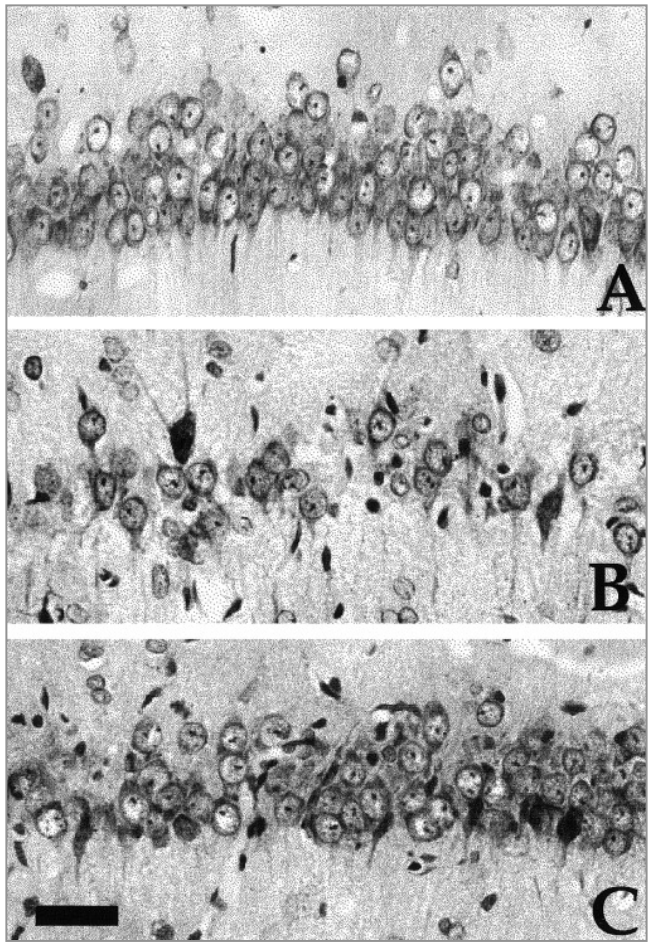
Induktion durch Hypoxie



Yu *et al*., Development 2002

Pharmakodynamische Aspekte bei der Neuroprotektion durch rekombinantes Epo

Hippocampus (CA1)



Wen et al., J Neurosci Res 2002

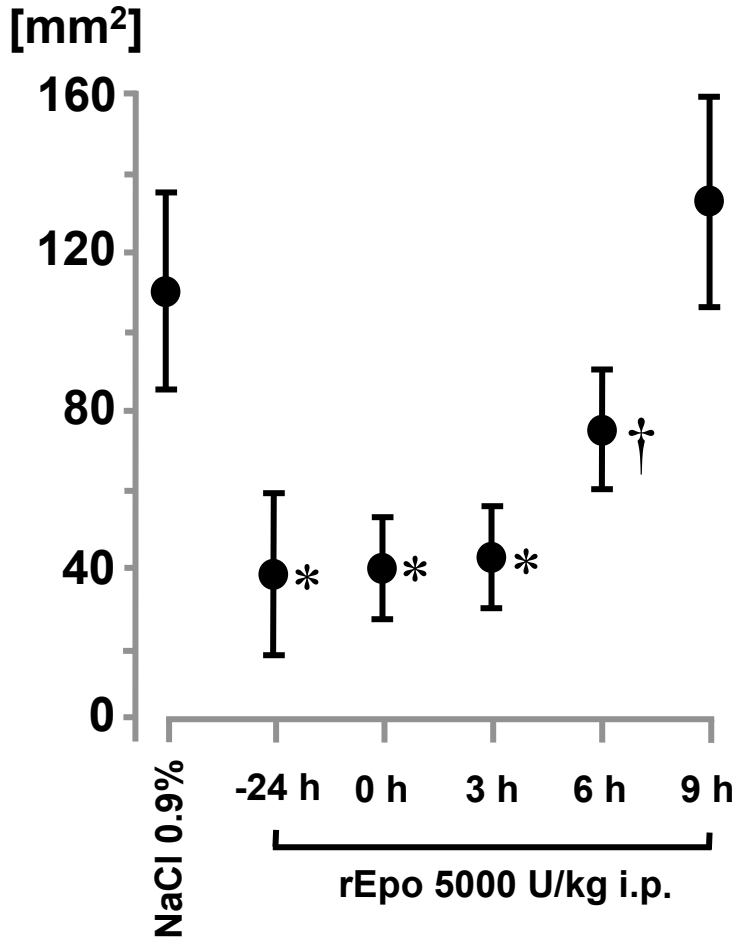
Kontrolle

Ischämie

rEpo, 3 min nach dem Insult

Nekrose-Volumen

(nach unilateraler Carotis-Ligation)



Brines ML, et al., Proc Natl Acad Sci USA, 2000

Interventionsstudien zur Neuroprotektion mit rEpo

***Reifgeborene*:**

Neurological Outcome after Erythropoietin Treatment for Neonatal Encephalopathy

Studiendesign: Phase I, bi-zentrisch, randomisiert, Plazebo-kontrolliert, n = 167

>37+SSW, >2500 g, moderate oder schwere HIE
rEpo 300 oder 500 iU/kg 1. Gabe s.c., alle 48 h über 2 Wochen

Outcome nach 18 Monaten: weniger Behinderung nach moderater HIE

Zhu C *et al*., Pediatrics 2009

Erythropoietin in Infants with Hypoxic Ischemic Encephalopathy

Studiendesign: Phase I, monozentrisch, Fall-kontrolliert, n = 30

38+0 – 42+0 SSW, milde oder moderate HIE
rEpo 2 500 iU/kg s.c., 1xtgl über 2 Wochen

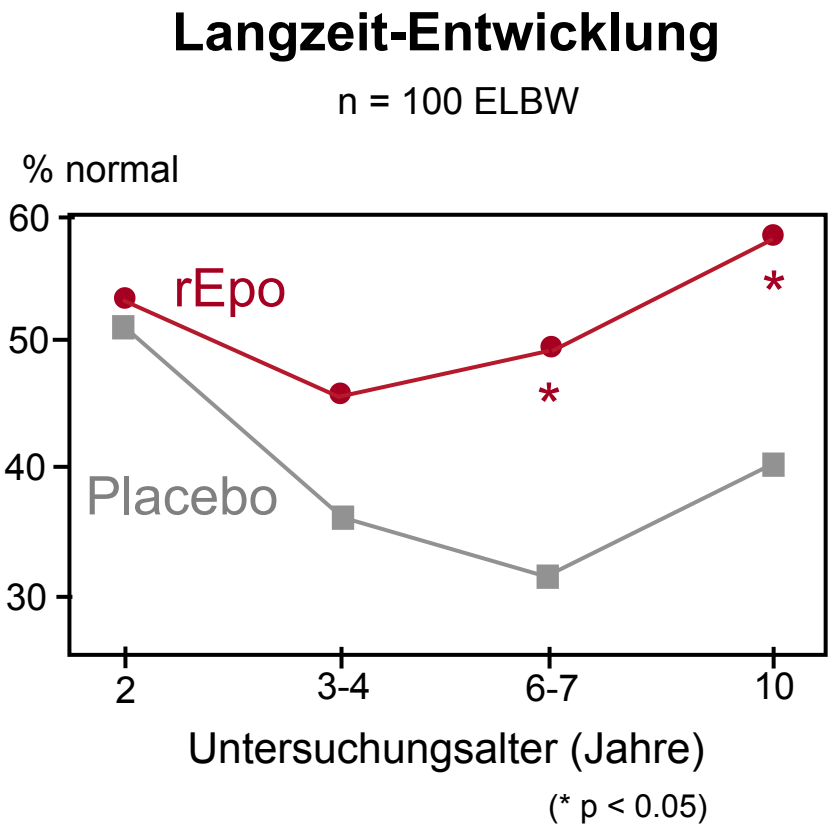
Outcome nach 6 Monaten: weniger Behinderung (DDST II)

Elmahdy *et al*., Pediatrics 2010

Darbe Administration in Newborns Undergoing Cooling for Encephalopathy (DANCE)

NCT001471015, Beginn 03/2012; Abschluß: 03/2014

Epo und Neuroprotektion: Retrospektive Daten



	IVH		
	rEpo treated (n = 29)	Untreated (n = 17)	
Composite IQ	90.3	67.0	p<0.05
Verbal IQ	94.9	74.9	p<0.05
Nonverbal IQ	85.4	64.3	p<0.05

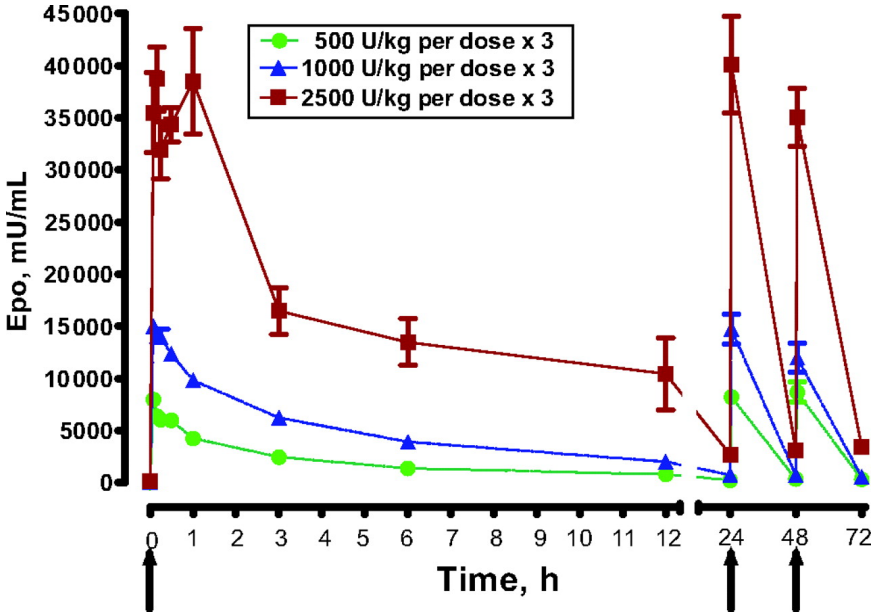
Neubauer AP *et al.*, Ann Neurol 2010

Randomisierte kontrollierte Studien
zur Neuroprotektion bei ELBW / VLBW Frühgeborenen

US Epo Trial

Phase I, single center,
dose escalation,open label trial

Einschlußkriterien:
Gestationsalter 24+0 - 27+6
Geburtsgewicht <1000 g
n = 30



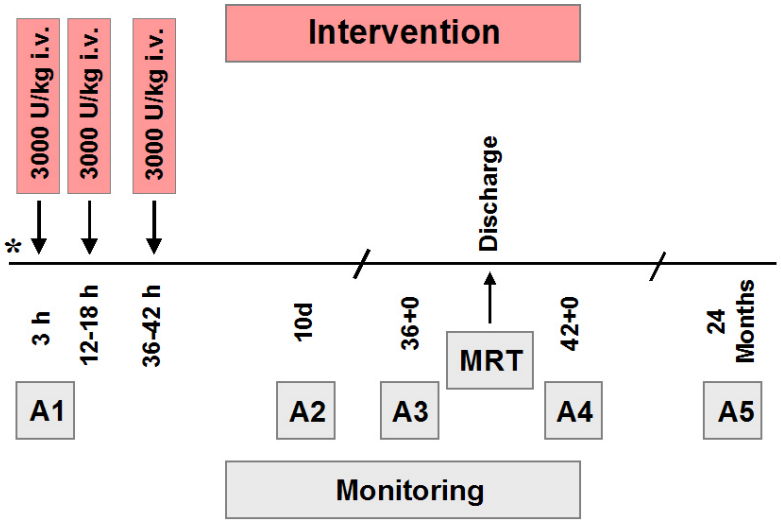
Juul SE *et al.,* Pediatrics, 2008

Swiss Epo Trial

Phase I/II, single center,
double-blind, placebo controlled trial

Einschlußkriterien:
Gestationsalter 24+0 - 31+6

n = 30 (rEpo:Kontrolle = 2:1)



Fauchère JC *et al.*, *Pediatrics* 2008

Interventionsstudien zur Neuroprotektion mit rEpo

***Frühgeborene*:**

Does Erythropoietin Improve Outcome in Very Preterm Infants?

NCT004139946, Beginn 01/2006; Abschluß: 12/2016

Studiendesign: multizentrisch, randomisiert, doppel-blind, Plazebo-kontrolliert, n = 420
VLBW 26+0 – 31+6 SSW
3 x rEpo 3000 iU/kg i.v. , 3 h, 12-18 h, 36-42 h nach Geburt

Outcome Kriterien:
MRT 40 SSW
Bayley II (MDI, PDI), Zerebralparese, Seh- und Hörfunktion mit korrigiert 24 Monaten

Trial of Erythropoietin Neuroprotection in Extremely Preterm Infants (PENUT)

NCT01378273, Beginn 07/2012; Abschluß: 12/2017

Studiendesign: multizentrisch, randomisiert, doppel-blind, Plazebo-kontrolliert, n = 475
<28+6 SSW
6 x rEpo 500 oder 1000 iU/kg i.v., erste Gabe innerhalb 48 h nach Geburt, dann alle 2 Tage anschließend 3 x 400 iU/kg/Woche s.c. bis 32+6 SSW.

Outcome Kriterien:
MRT 40 SSW
Bayley III (MDI, PDI), Zerebralparese mit korrigiert 24 Monaten

Vielen Dank !