Cliniques universitaires SAINT-LUC UCL BRUXELLES

Corata

Session Hematologie

Jeudi 20 septembre 2018

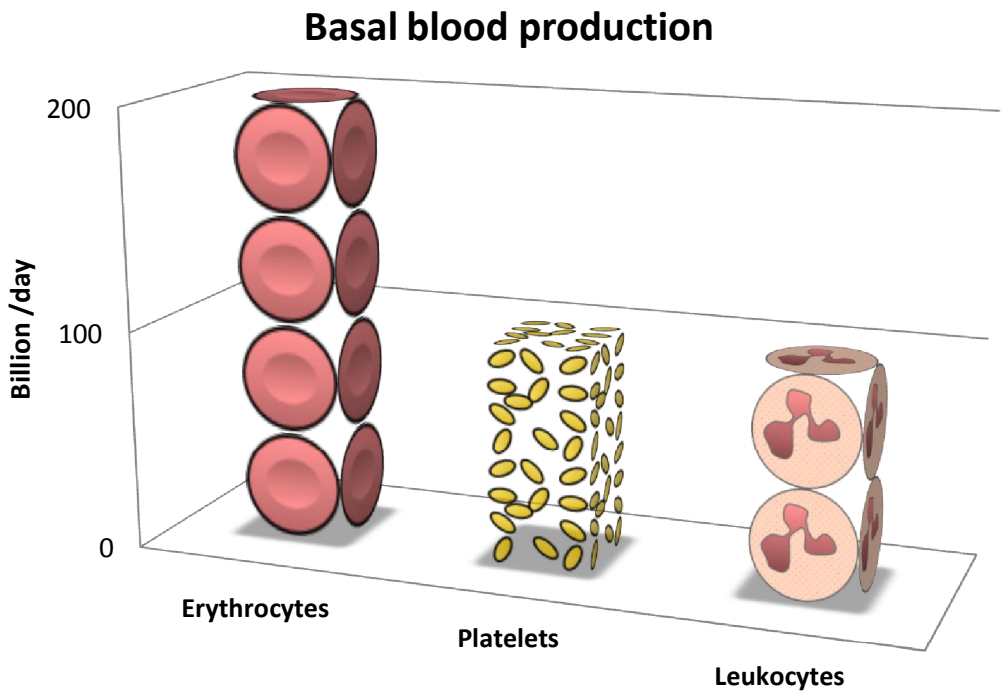
Hôtel Mercure Champ de Mars 12 Avenue Aristide Briand 76000 ROUEN (France)

LUDWIG CANCER RESEARCH

Thrombopoïétine (TPO) et érythropoïétine (EPO)

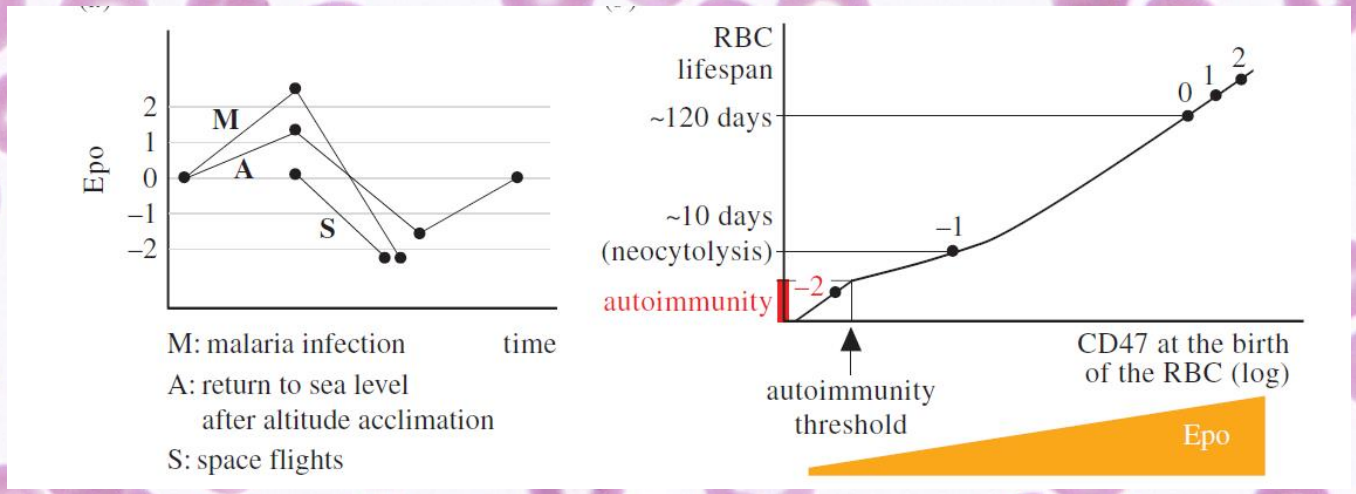
Professeur Jean-Philippe DEFOUR - Cliniques Universitaires Saint-Luc / Bruxelles

Blood is a highly regenerative tissue,
with balanced production of mature blood cells.



Lifespan in adult 120days

antagonist effects of CD47 “don’t eat me” signal on the phagocytic activity of macrophages



Fernandez A. R. Soc. open sci. **2017**

Magnification: 100 x

10 µm

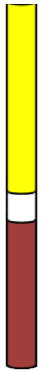
Epo Discovery

	Globules
A Lima, le 4 Octobre 1889 (veille de mon départ pour la Cordillère), mon sang contient pas millimètre cube	5.000.000
A Morococha, le 19 Octobre (depuis quinze jours dans la Cordillère)	7.100.000
Dr. Mayorga (id.)	7.300.000
Mayorca, arriero (depuis trois ans à la mine)	7.840.000
R. Prieto, garçon de cuisine, métis	6.770.000
Dittman, allemand, administrateur de la mine	7.920.000
Atchachay, Indien	7.960.000
Margarita, Indienne	7.080.000
Charpentier, fils de Français, majordome	6.000.000
Rossi, Italien, àla Oroya	6.320.000
Mon sang, le 27 Octobre	8.000.000
Dr. Mayorga, (id.)	7.440.000
Jeune chienne vigoureuse	9.000.000
Coq d'un an vigoureux	6.000.000
Lama mâle	16.000.000

Prof. Francois Gilbert Viault: 1890

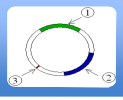
Paul Carnot

Clotilde-Camille Deflandre
enseignante à Rouen avt son PhD



Hemopoetin 1906

A l'origine extraction à partir d'urines humaines



Clonage en 1985: EPO recombinante

Disponible en 1988

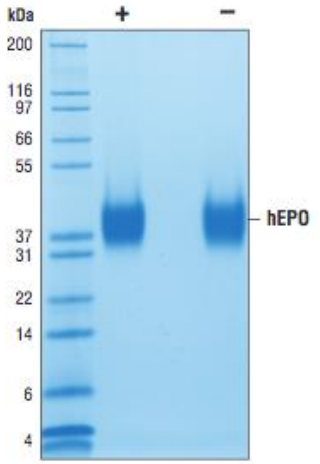
Prohibé par le comité olympique en 1990

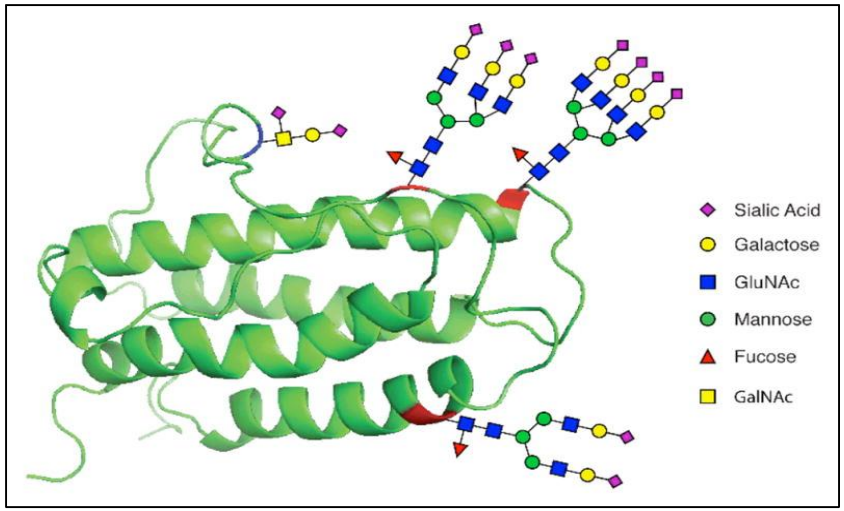
DCI	Spécialité	Lignée cellulaire	Structure	Demi-vie	Fréquence d'administration
Epoétine α	Eprex Binocrit	Ovaire d'hamster chinois = OHC	Très proche de l'EPO endogène Diffère seulement de quelques acides aminés et par la longueur des chaînes glycosylées	6-9 h	3 fois/semaine
Epoétine β	Neorecormon	OHC		6-9 h	
Epoétine δ	Dynepo	Cellules humaines (HT-1080)		5-13 h	
NESP = Darbepoétine α	Aranesp	OHC	5 chaînes glycosylées au lieu de 3	25 h	1 fois/semaine
CERA	Mircera	OHC	Forme pégylée d'époétine β	130-140 h	1 fois/mois

DCI : dénomination commune internationale

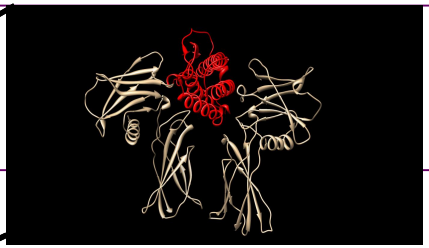
Epo structure

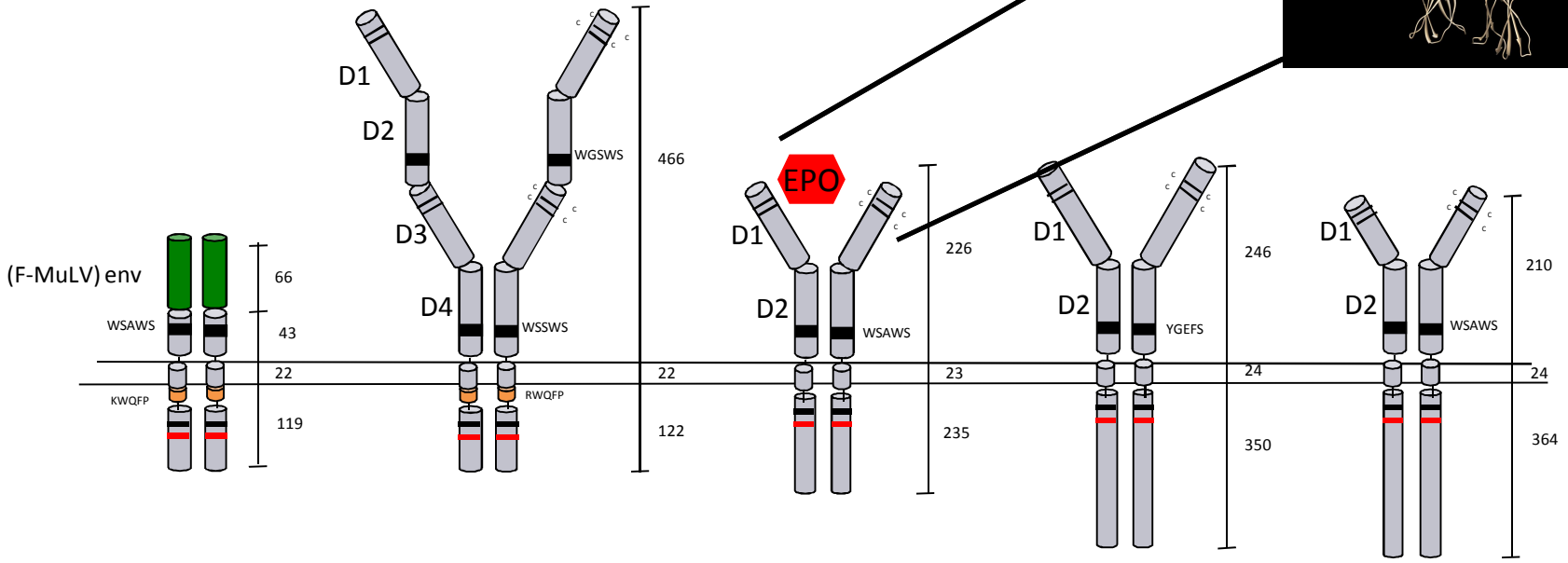
- chr7q
- 165 aa
- 37,1Kd
- fortement glycosylée
  - (40% of its weight)
- 3 sites de N glycosylation
  - N24, N38, N83
- 1 site de O glycosylation
  - S126





EpoR





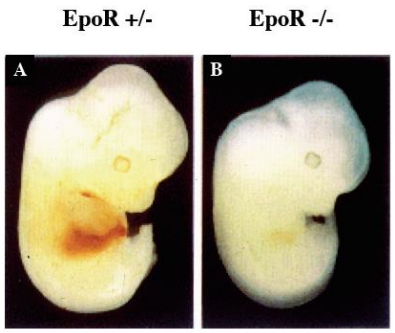
v-Mpl
(284aa)

TpoR/c-Mpl
(635 aa)

EpoR
(508aa)

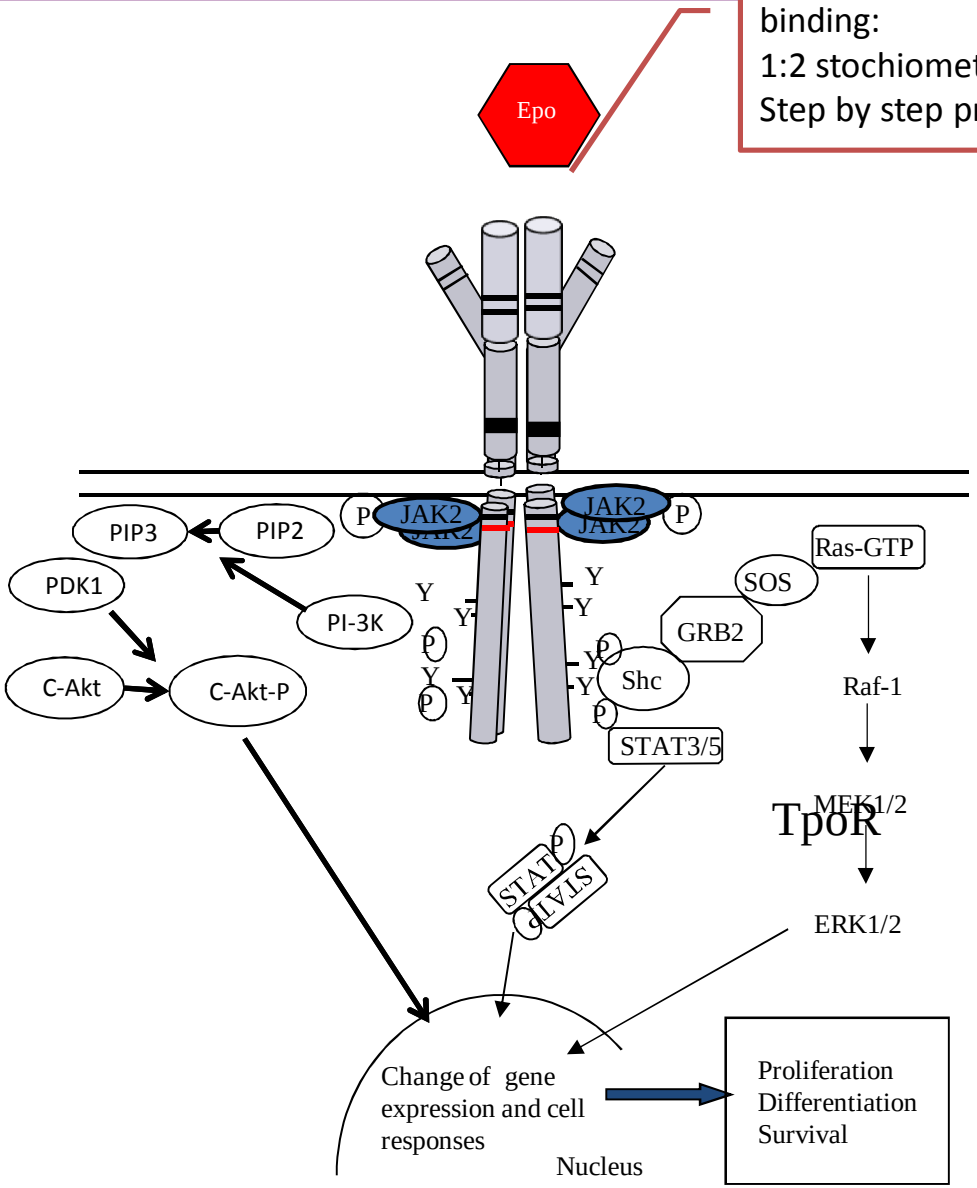
GHR
(638aa)

PrlR
(598aa)

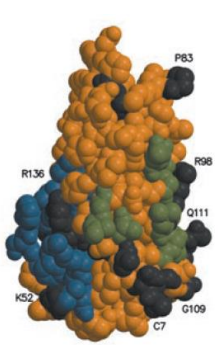


Transduction du signal

binding:
1:2 stochiometry
Step by step process



High affinity site
Kd 1 nM

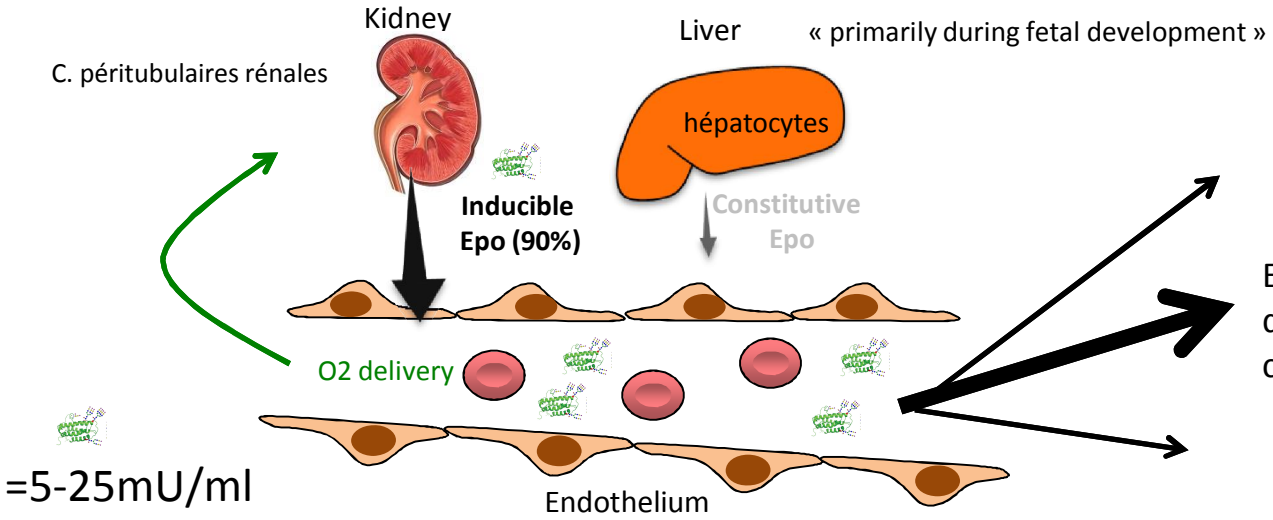


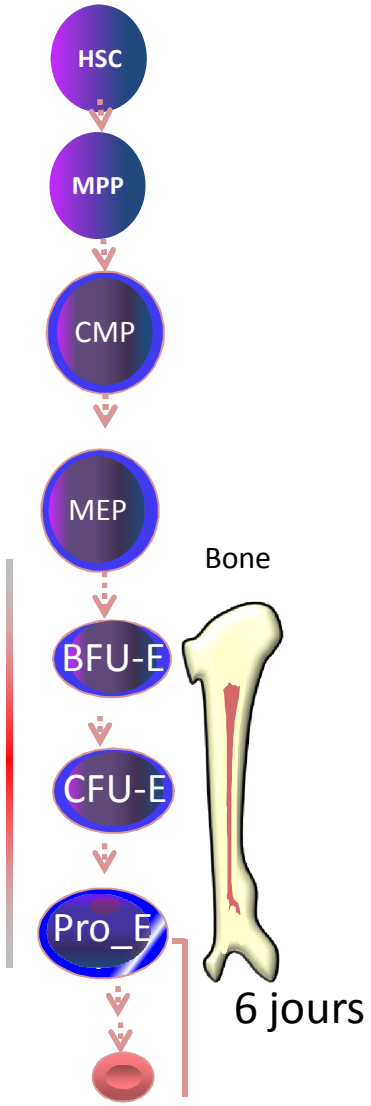
Epo/ Tpo common residues

Low affinity site
Kd 2 µM

receptor binding domain of Tpo

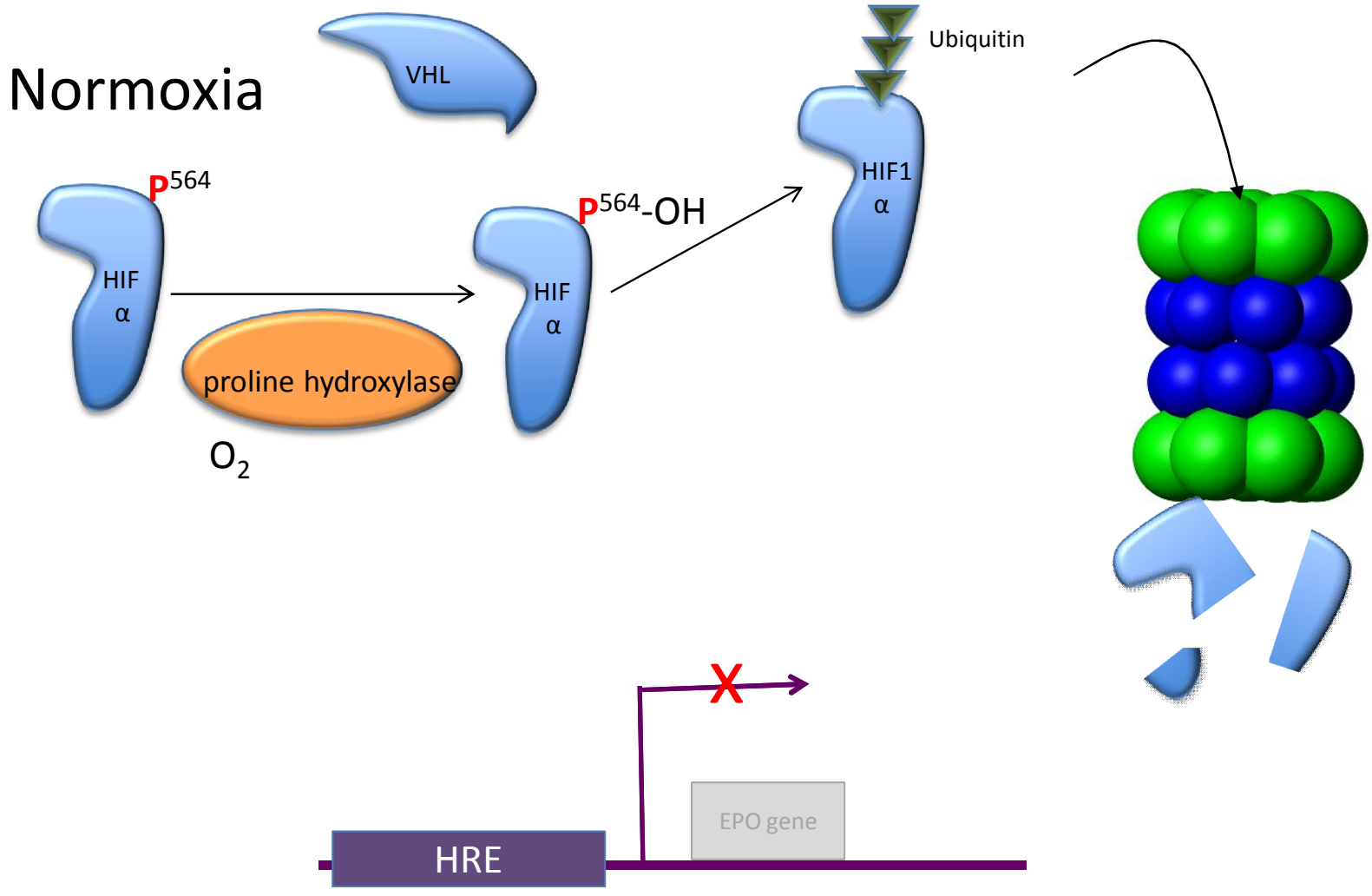
Epo function, production and regulation





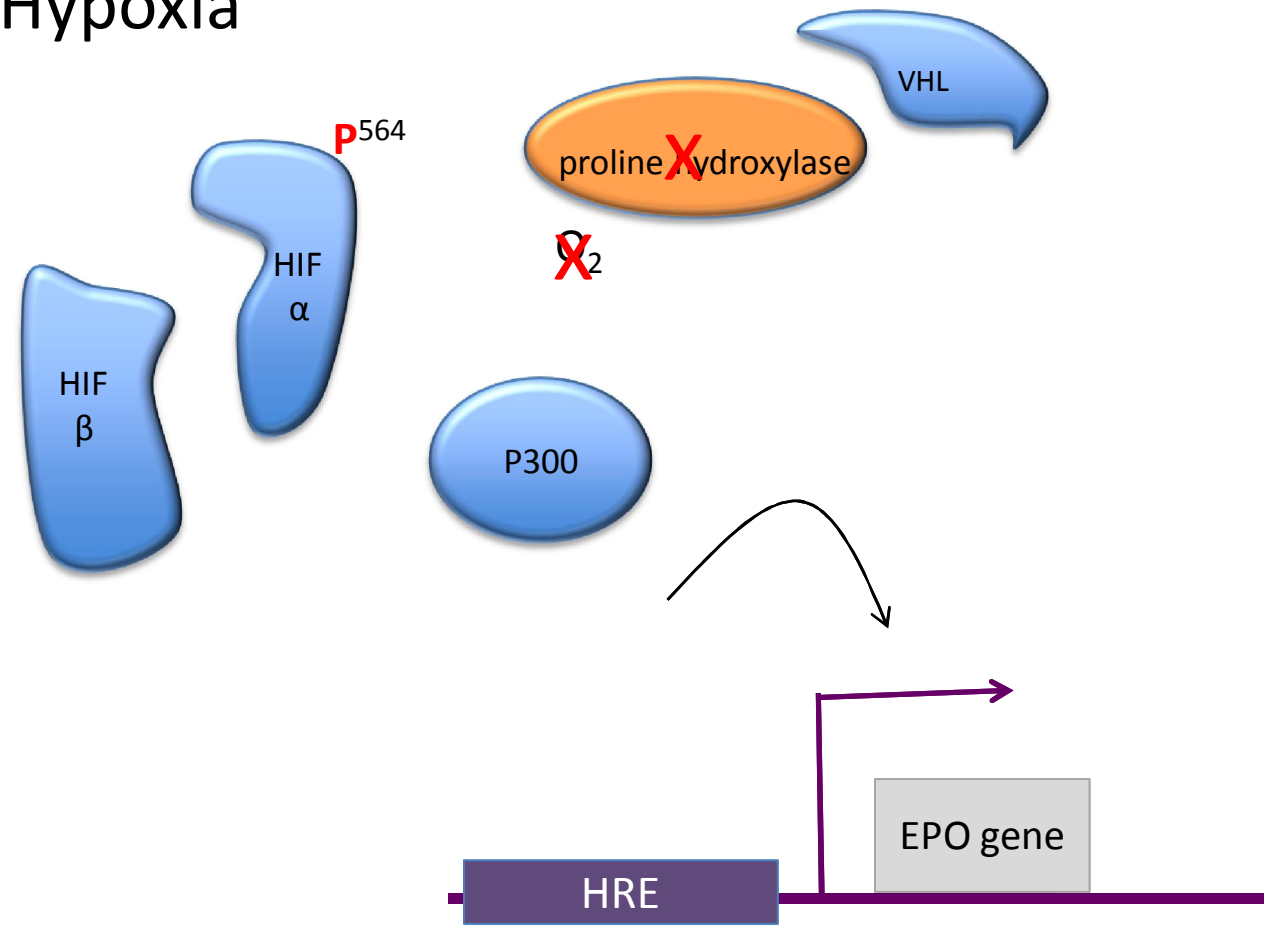
TOGETHER WITH:
G-CSF, IL-6, SCF, IL-l,
IL-3, IL-4, IL-9,
IL-11, GM-CSF, IGF-1

Erythropoiétine: Regulation



Erythropoiétine: Regulation

Hypoxia



N=2X24
non professional

Effects of erythropoietin on real life cycling performance

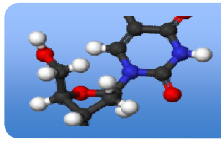
VENTOUX

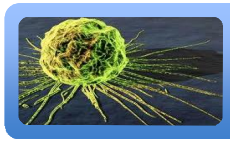
Jules A A C Heuberger et al, Lancet 2017

Indications de l'érythropoïétine

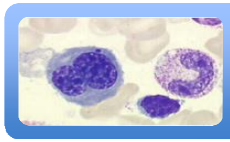
Darbepoetin Aranesp® Epoetin Eprex®

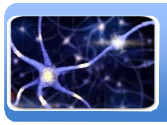

Anémie rénale hb : 8,0 et 11,0 g/dl.


Utilisation de Zidovudine chez les HIV (remboursement ?)

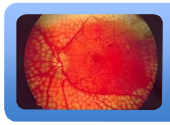

Patient onco hemato sous chimiothérapie **Hb<12g/dl**


chirurgie orthopédique


MDS IPSS faible , >18a, Epo<500mU/ml (compassionnel)


Neuroprotecteur*


Parkinson*

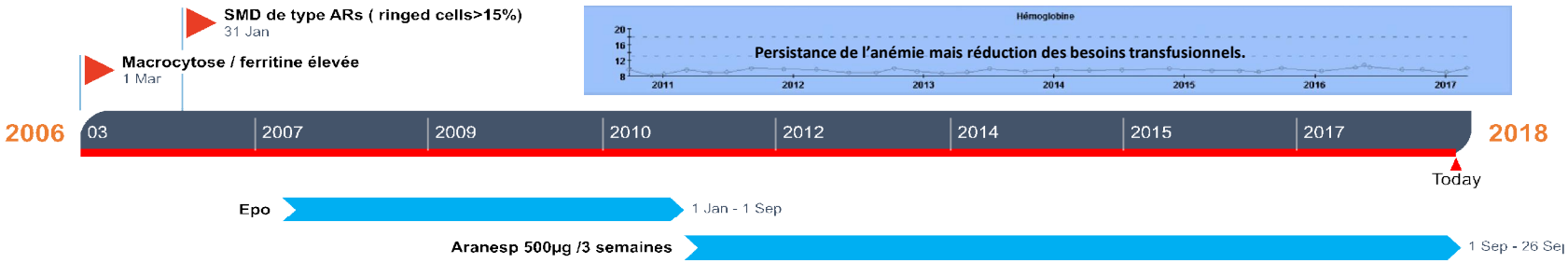

retinopathie diabétique*


AVC*


infarctus

Because EPO is present in other tissues in the body (e.g. endothelial,myocardial and neural cells.

* New Asialo forms cross the blood brain barrrier



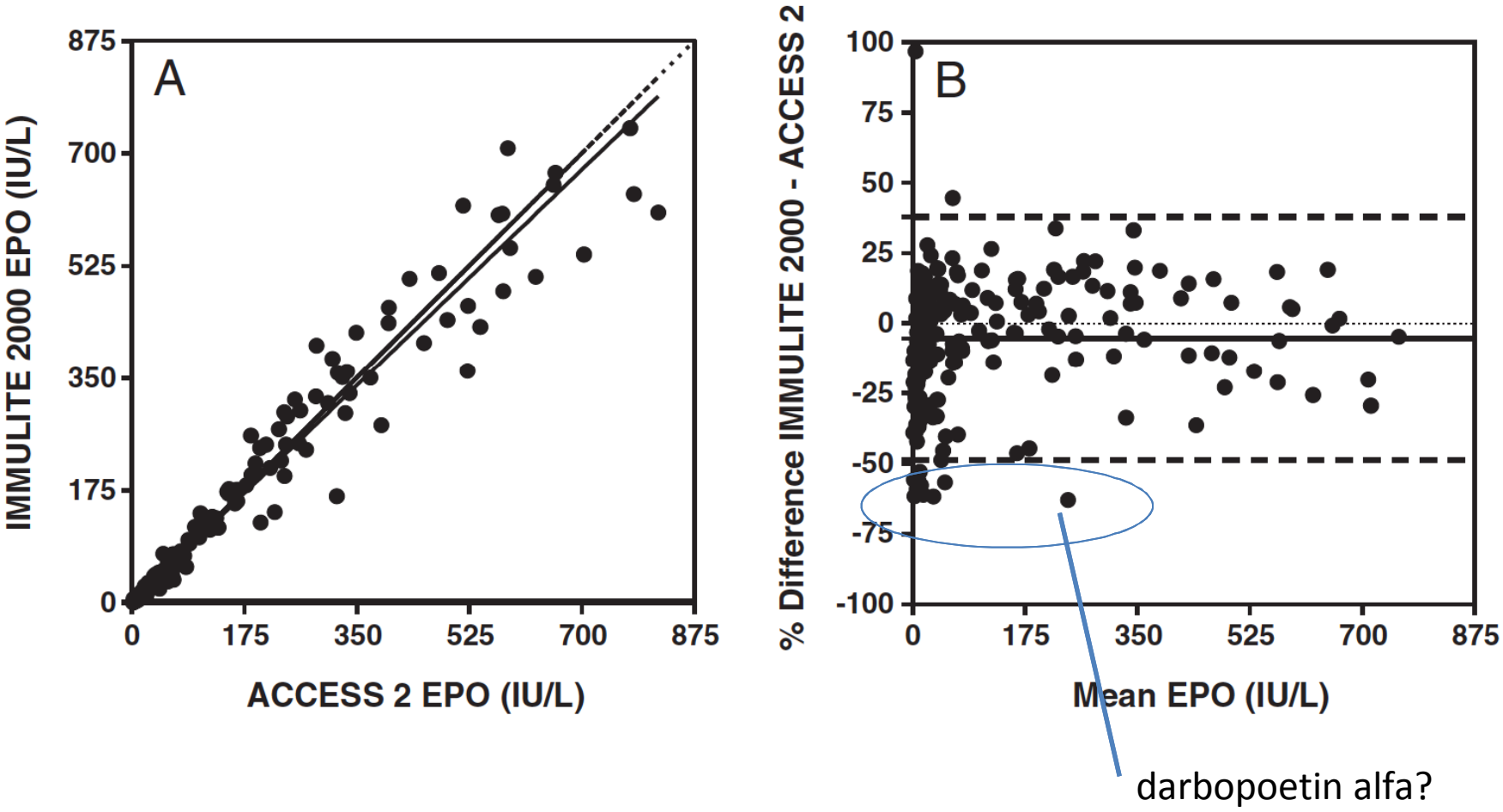
Epo: comment la doser ? ELISA /CLIA/RIA

Trousse/Distributeur	Méthode	Limite de détection (UI/L)	Gamme de calibration (UI/L)	Valeurs normales (UI/L) ou moyennes (SD)	Cut-off Polyglobulie de Vaquez	Cut-off Poyglobulie secondaire
Biomerica EPO/Cambridge Life Sciences	Elisa	1,2	7,5 à 494	4,3-32,9	Non publié	Non publié
Quantikine EPO/R et D Systems	Elisa	0,6	2,5 à 200	3,3-16,6	1,4 Se 65 % Sp 100 %	13,7 Se 19,7 % Sp 100 %
Immulite EPO/Diagnostic Products Corporation	Chimiluminescence	0,2	2 à 178	4,1-20,1	2,8 Se 78 % Sp 100 %	13,8 Se 34 % Sp 100 %
Acces EPO, DXI 800/Beckman Coulter	Chimiluminescence	< 0,6	5 à 750	2,6-18,5	Non publié	Non publié
Nichols Advantage/Nichols Institute Diagnostics	Chimiluminescence	5	5 à 700	5,0-25,0	Non publié	Non publié
EPO-Trac/Diasorin	RIA	4,4	6 à 280	9,1-30,8	Non publié	Non publié
EPORIA/Ramco Laboratories	RIA	3,3	6,25 à 200	16,2 (7,6)	Non publié	Non publié

Se : sensibilité, Sp : spécificité. La trousse Nichols Advantage/Nichols Institute Diagnostics n'est plus commercialisée.

Ann Biol Clin, vol. 67, no 5, septembre-octobre 2009

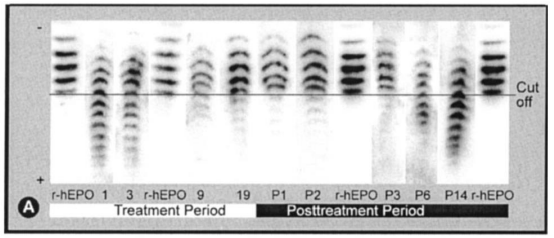
CLIA method comparison



Owen et al Clinica Chimica Acta (2014)

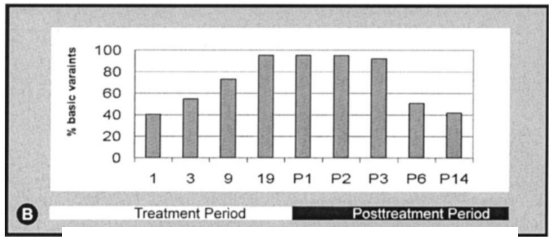
World antidoping agency (WADA)

- endogenous vs RhEpo ? difficult…
- various extent of glycosylation.
- focalisation isoélectrique (IEF)



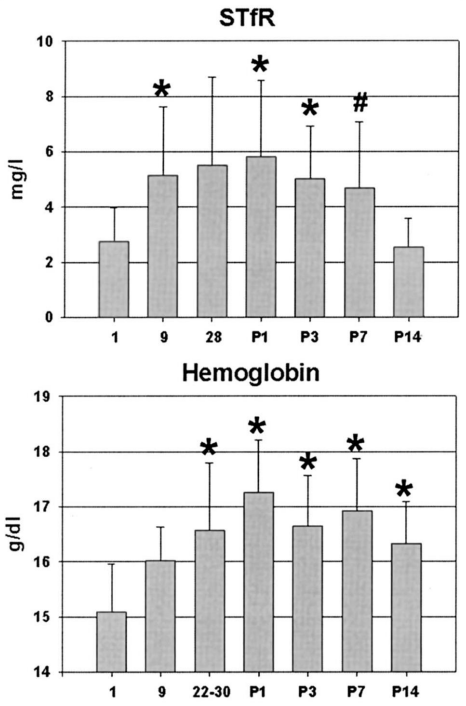
Cathode - basic pH

acid pH Anode +



sTfR (erythropoiesis) may be useful as screening tool.

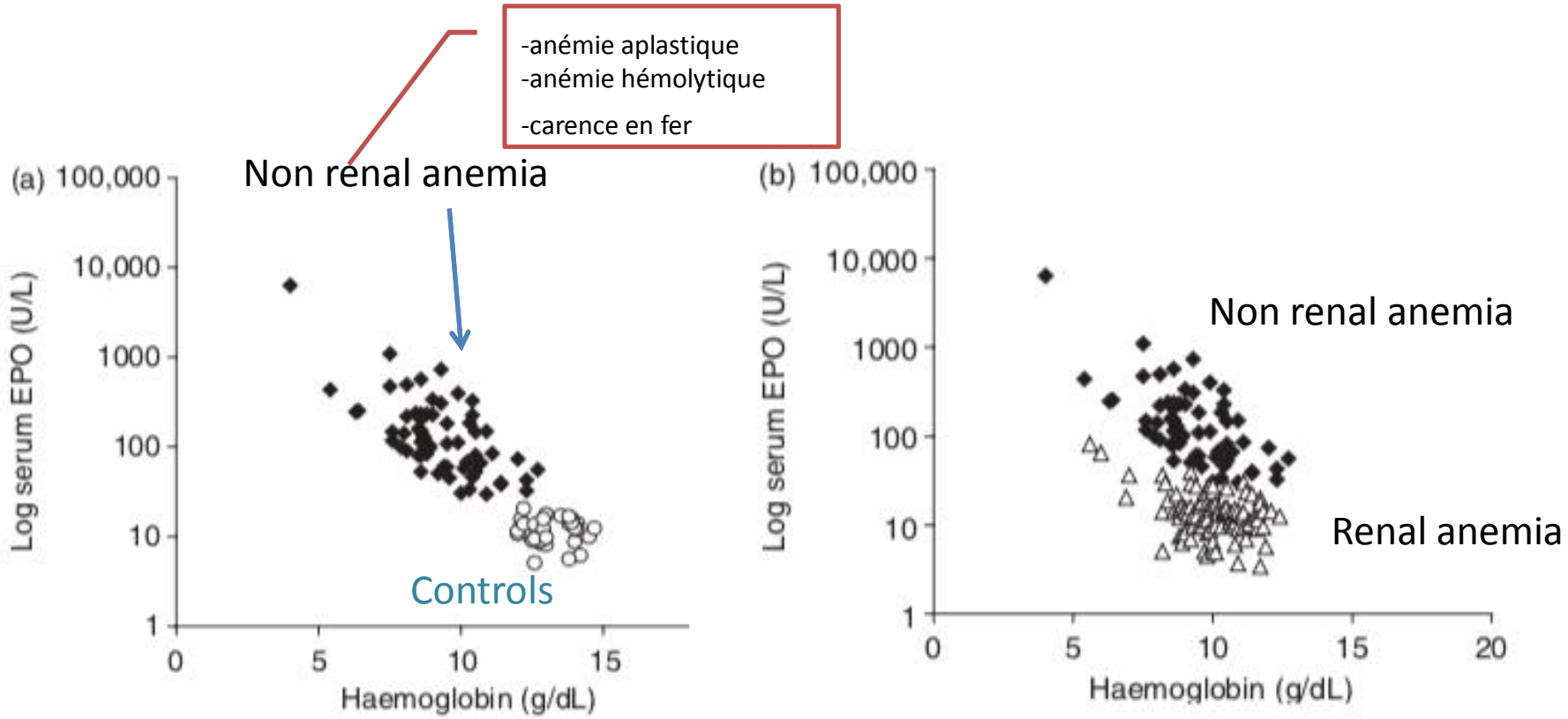
GRO NISSEN-LIE 2004



Epo: quand la doser?

1) Anemies

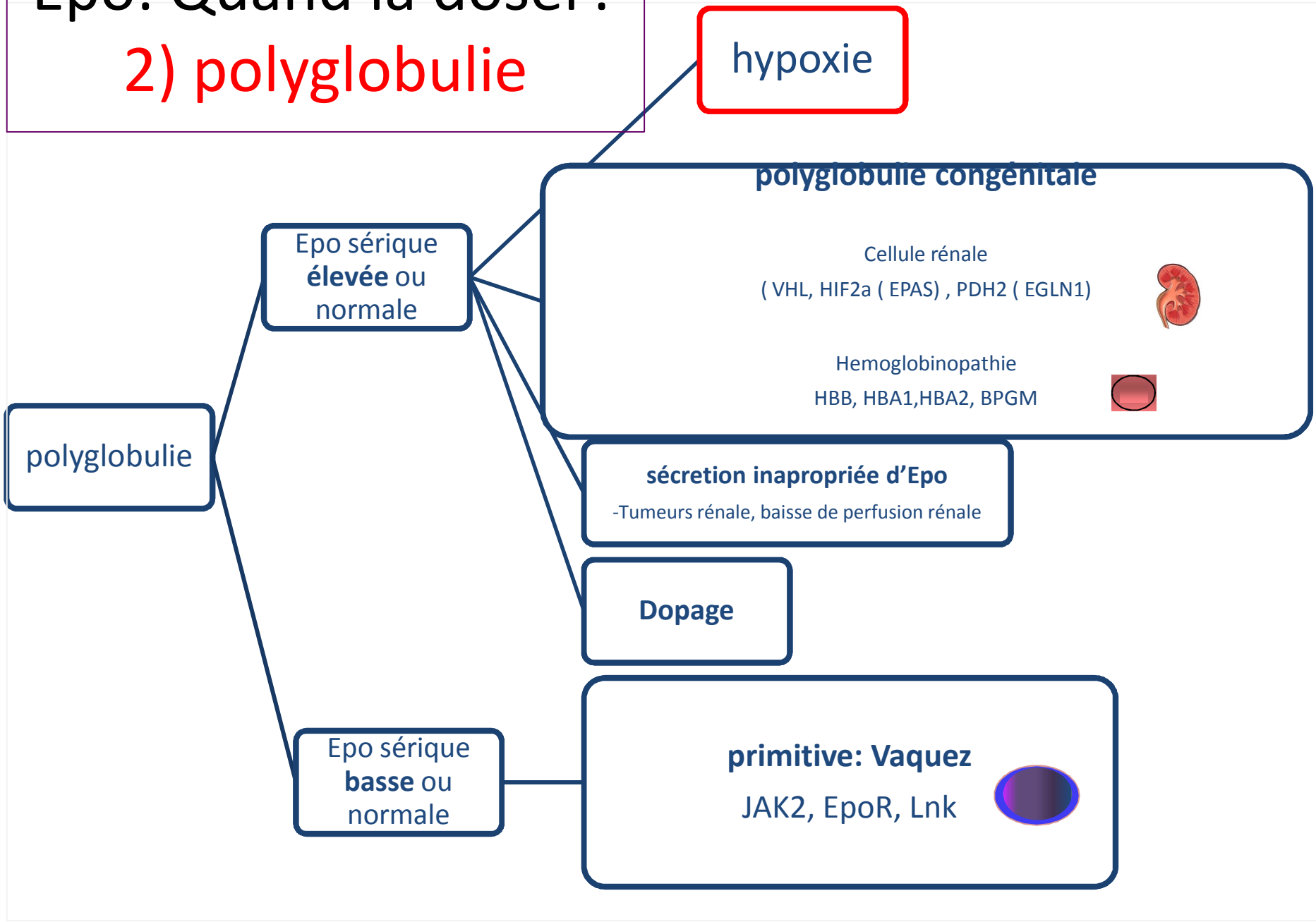
-anémie aplastique
-anémie hémolytique
-carence en fer



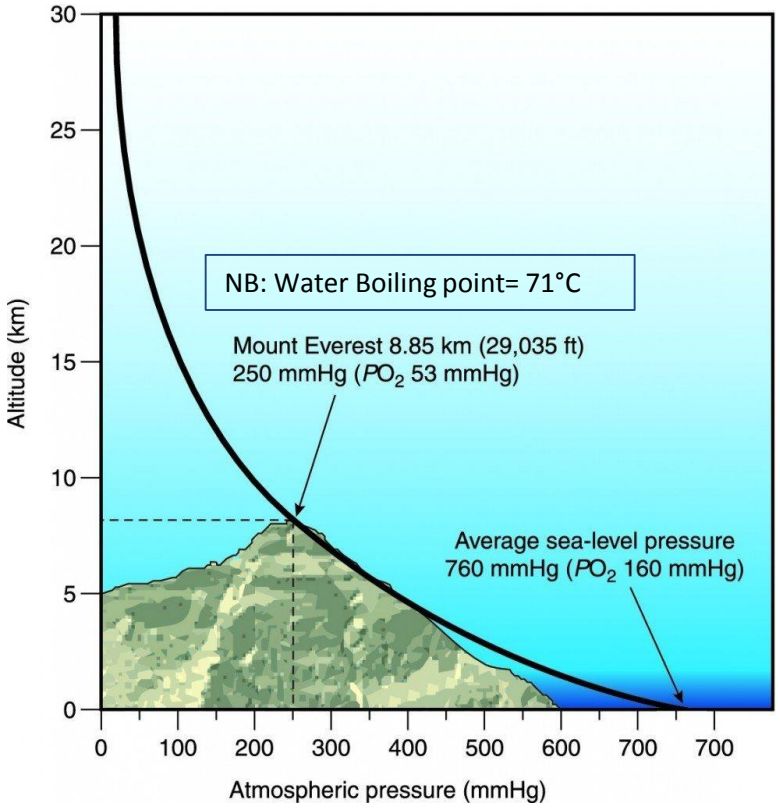
Marsden et al.

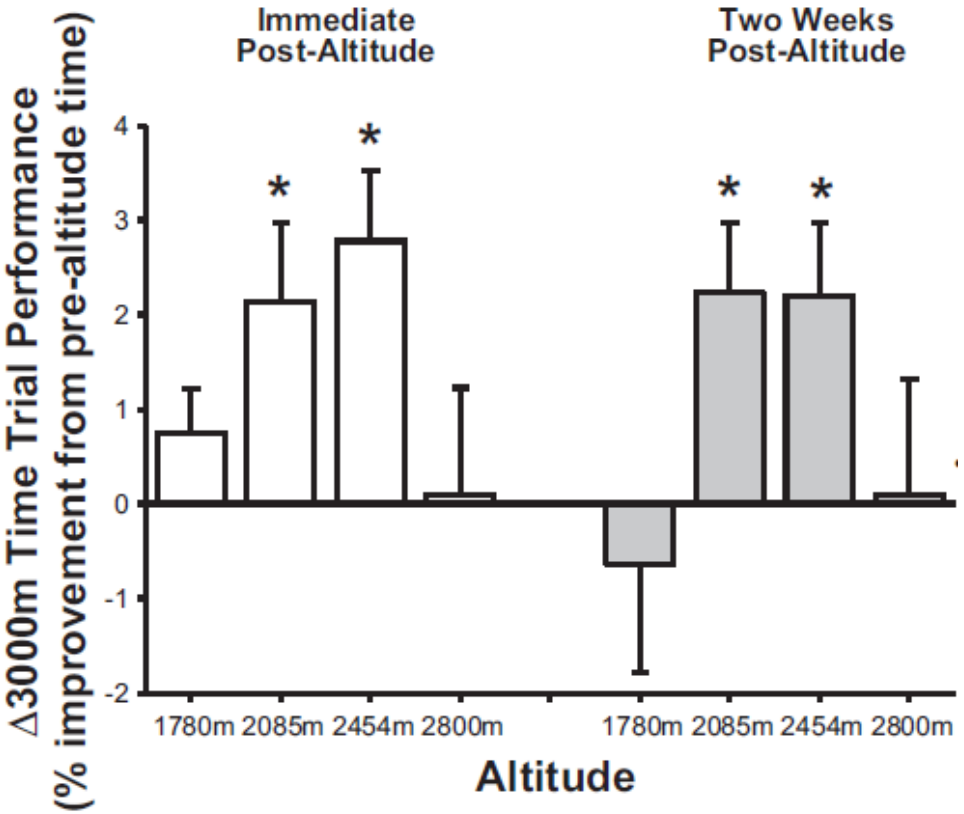
Epo: Quand la doser?

2) polyglobulie



Hypoxia: how high to live for optimal sea level perfomance?



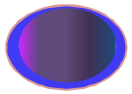


Chapman et al , J Appl Physiol 2013

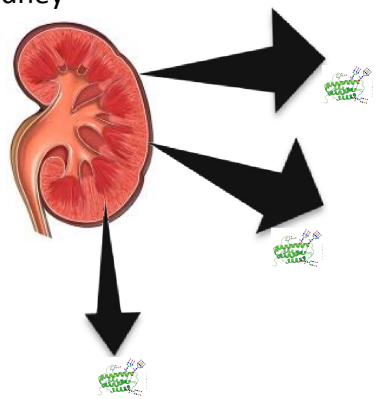
Epo: Quand la doser?

2) polyglobulie

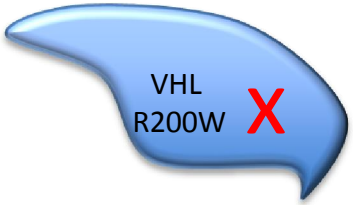
polyglobulie

- Epo sérique **élevée** ou normale
  - hypoxie
  - **polyglobulie congénitale**
    - Cellules rénales
      (VHL, HIF2a (EPAS) , PDH2 (EGLN1)
      
    - Hemoglobinopathies
      HBB, HBA1,HBA2, BPGM
      
  - **sécretion inappropriée d'Epo**
    -Tumeurs rénales, baisse de perfusion rénale
  - **Dopage**
- Epo sérique **basse** ou normale
  - **primitive: Vaquez**
    JAK2, EpoR, Lnk
    

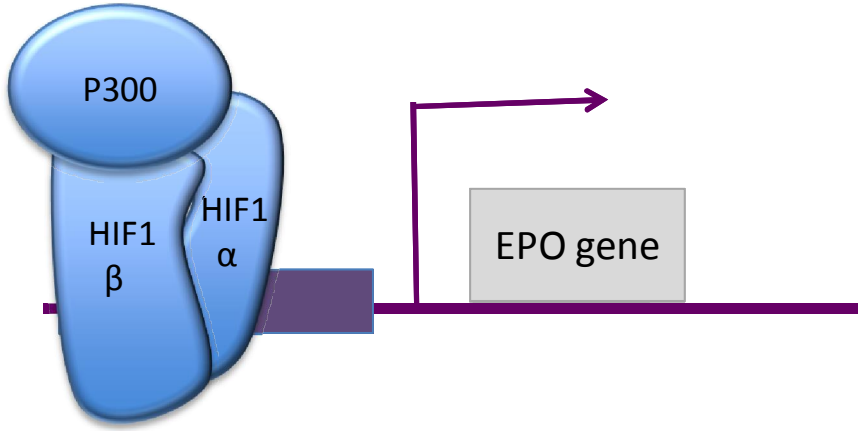
Kidney



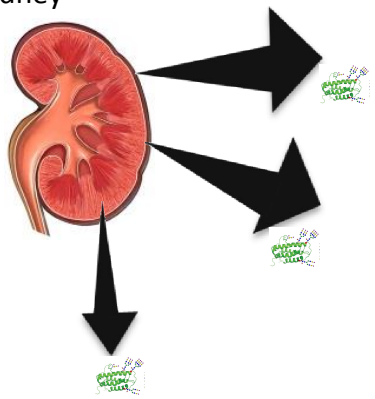
VHL mutation
(chuvash polycythemia)



Mutation in “von Hippel-Lindau” protein



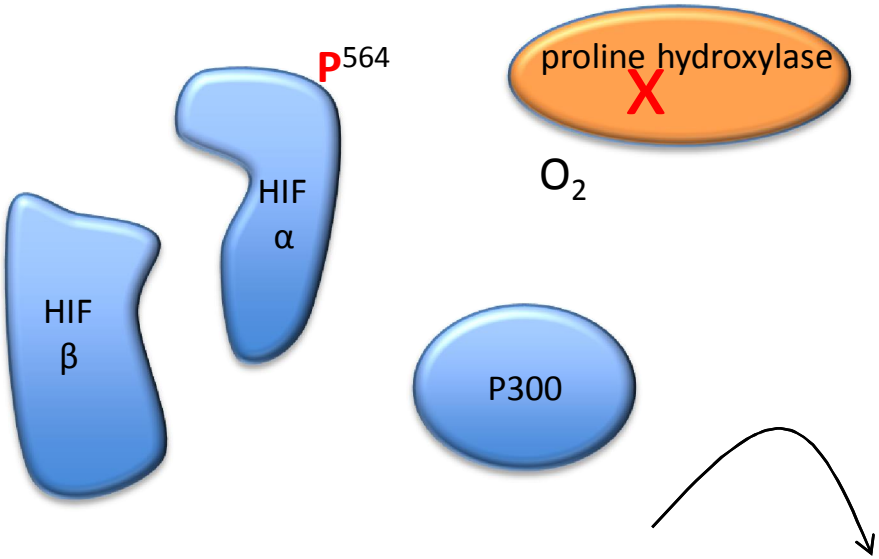
Kidney

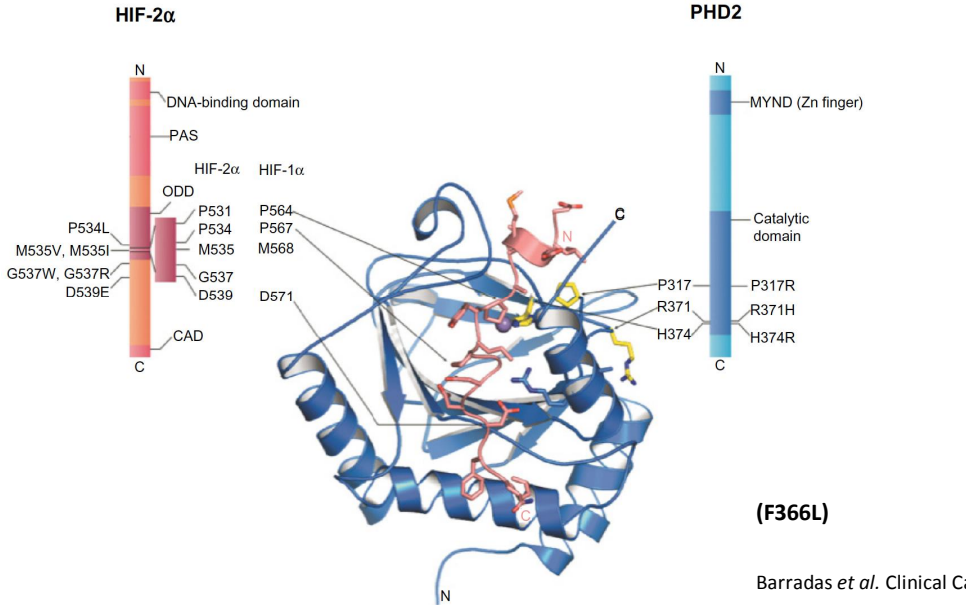


HIF2α (EPAS gene) and PHD2 (EGLN1 gene) mutations

gain of function

loss of function

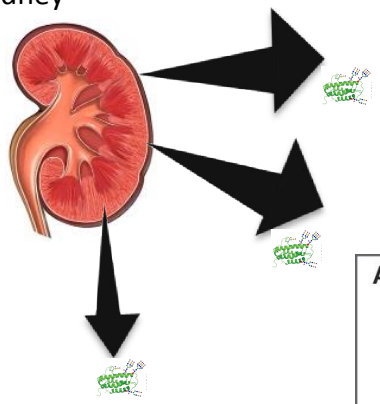




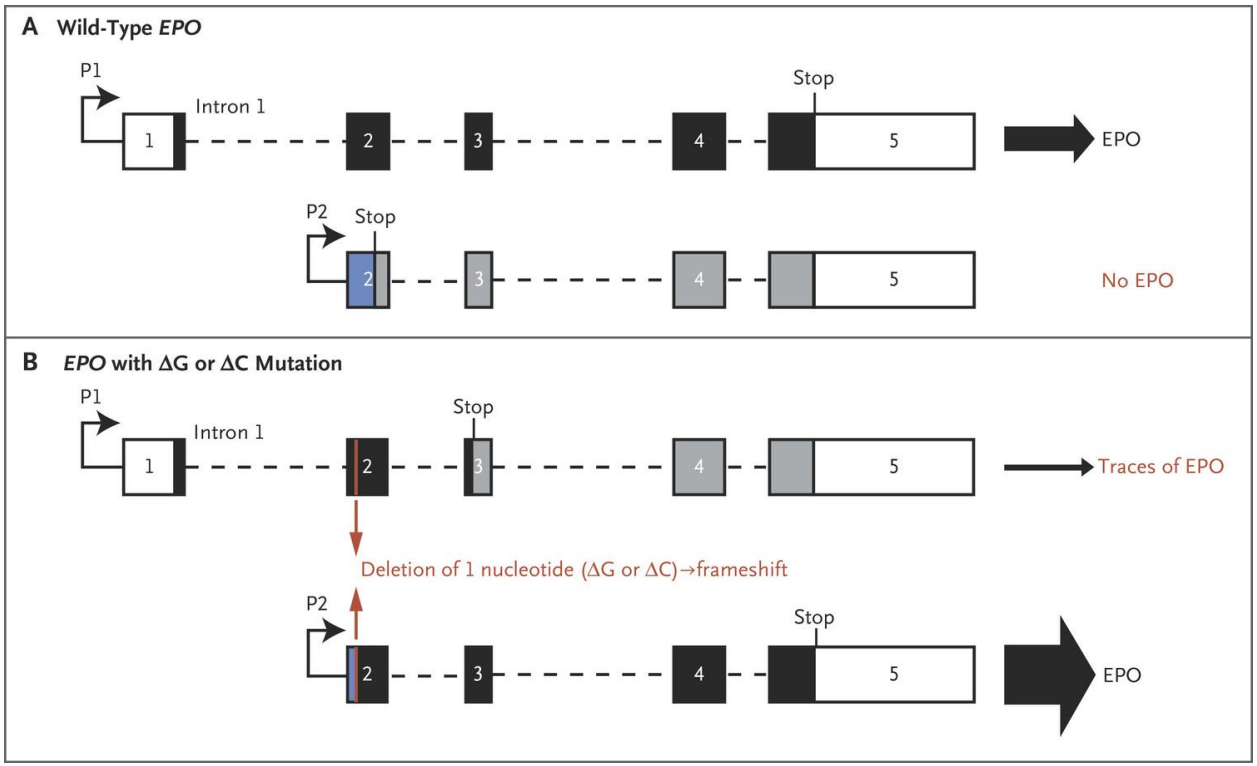
Barradas *et al.* Clinical C

Gardie, *et al*. hypoxia 2014

Kidney



EPO ΔG mutant in Exon2 : frameshift



Alternative promoter in intron1

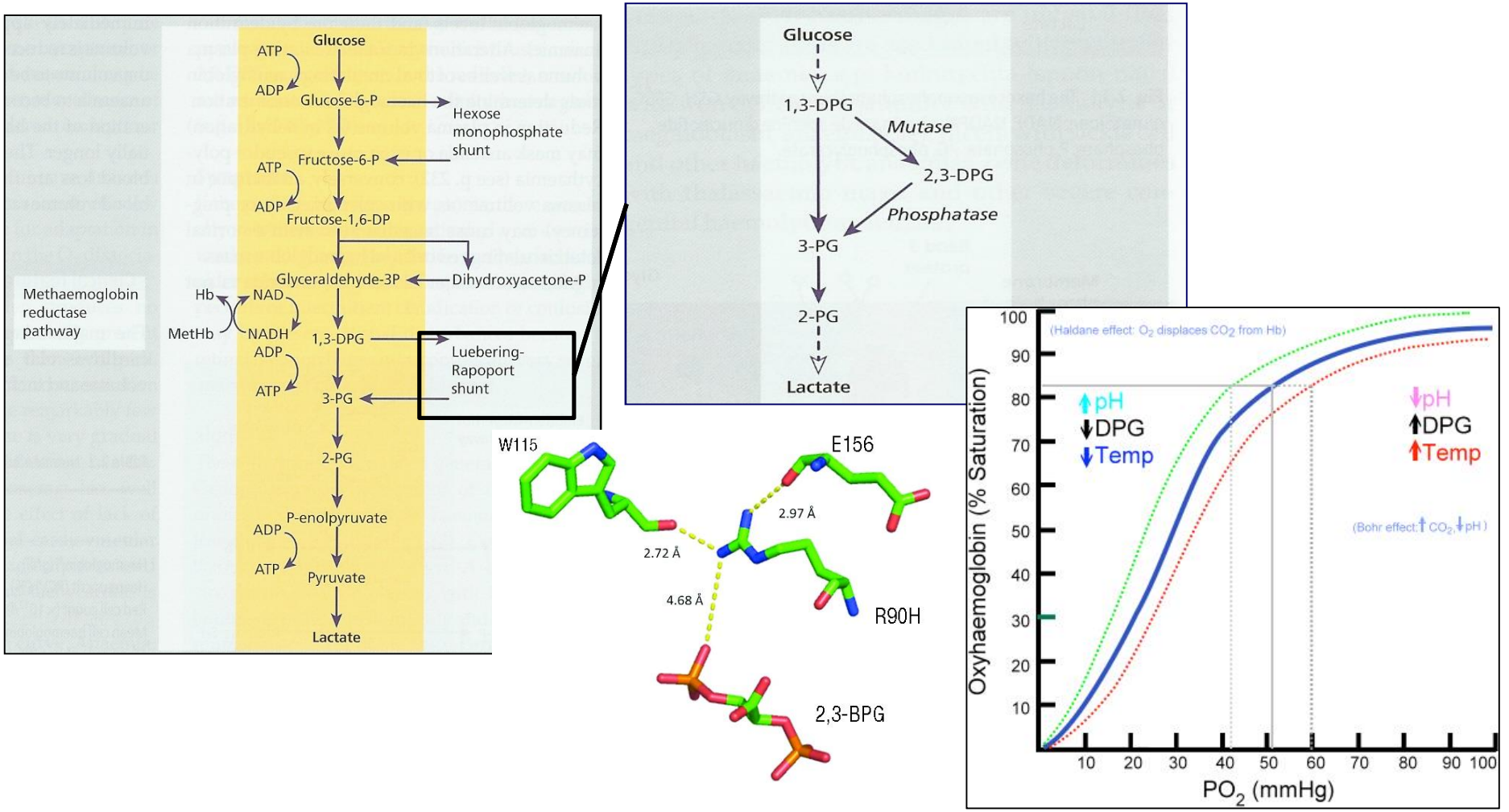
NB: L'effet d'une mutation (perte de fonction) doit être envisagé sur différents transcrits!

J Zmajkovic et al. N Engl J Med 2018

Secondary erythrocytosis with high Epo

BPGM heterozygous R90H mutation

(Bisphosphoglycerate mutase)



Nayia Petousi *et al* Hematologica 2014

Epo: Quand la doser?

2) polyglobulie

polyglobulie

Epo sérique **élevée** ou normale

- hypoxie
- **polyglobulie congénitale**
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    HBB, HBA1,HBA2, BPGM
- **sécretion inappropriée d'Epo**
  -Tumeurs rénales, baisse de perfusion rénale
- **Dopage**

Epo sérique **basse** ou normale

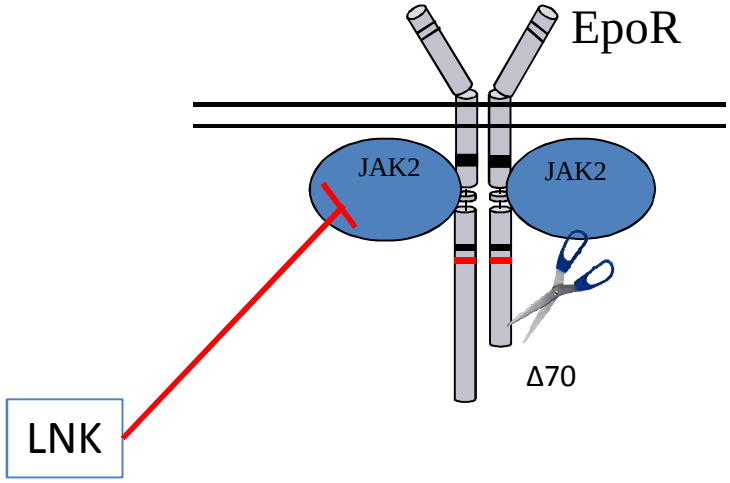
- **primitive**
  (germinale ou somatique)
  JAK2, EpoR, Lnk

Primary erythrocytosis with low serum Epo

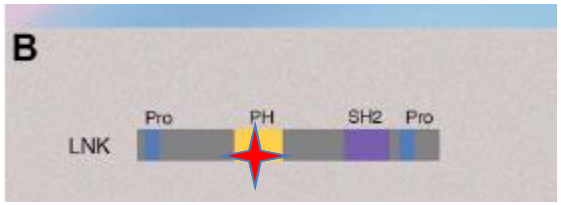
- EpoR
- JAK2
- Lnk/SH2B3

Ex:

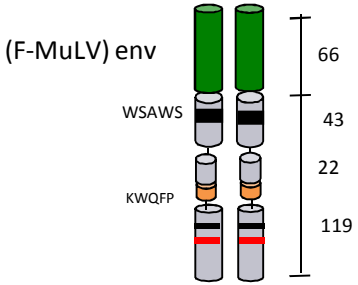
1. *EpoR* Δ70 A de la Chapelle, PNAS 1993
2. stop codon at amino acid 393 : Perrotta *et al* PLoS One. 2010



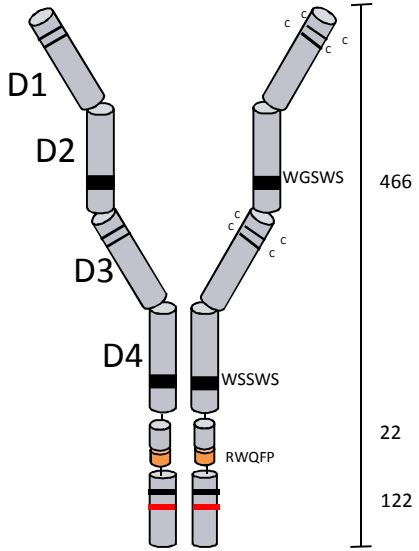
Eero Mantyranta won 3 olympic gold medals and 2 world championships.



Tpo Discovery



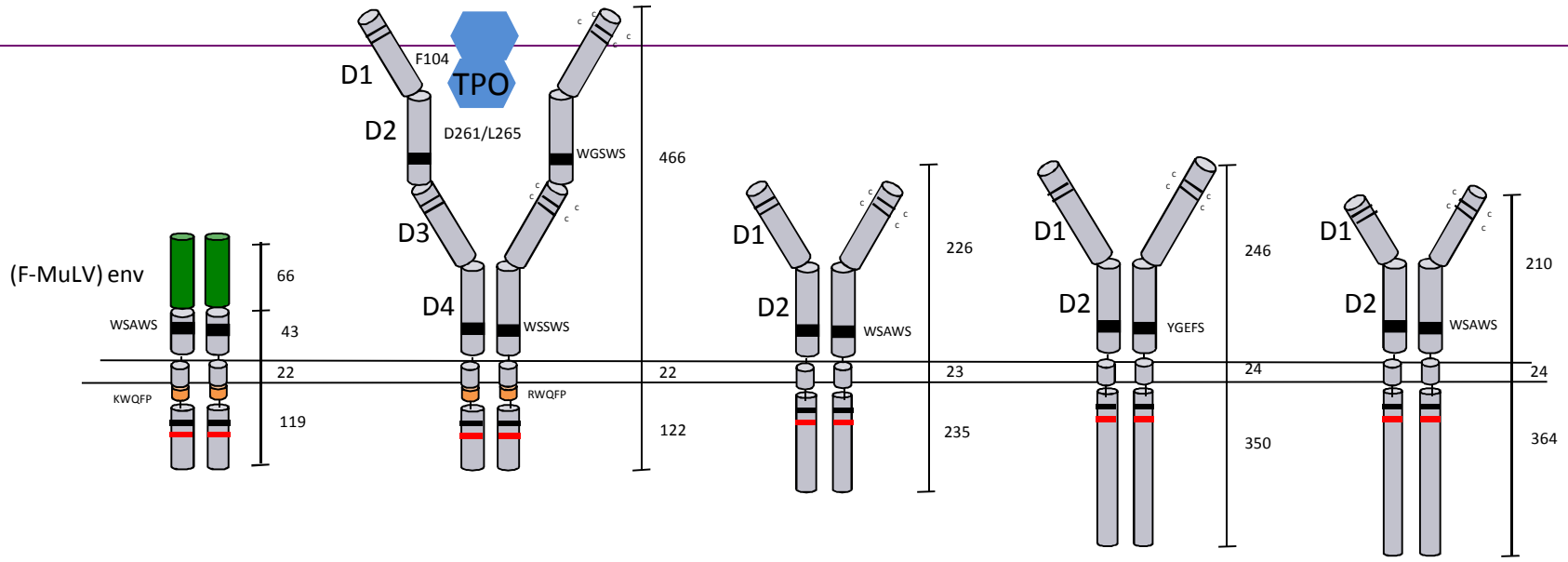
v-Mpl
(284aa)



TpoR/c-Mpl
(635 aa)

TpoR : Vigon *et al* PNAS 1992
Tpo : Bartley *et al* Cell **1994**

Homodimeric cytokine receptor



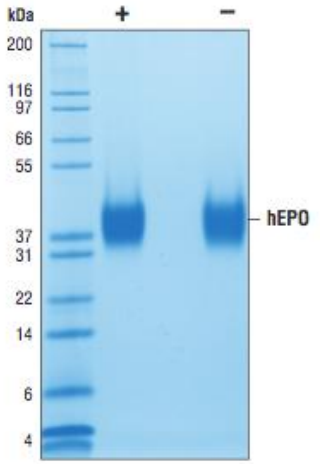
v-Mpl (284aa) | TpoR/c-Mpl (635 aa) | EpoR (508aa) | GHR (638aa) | PrlR (598aa)

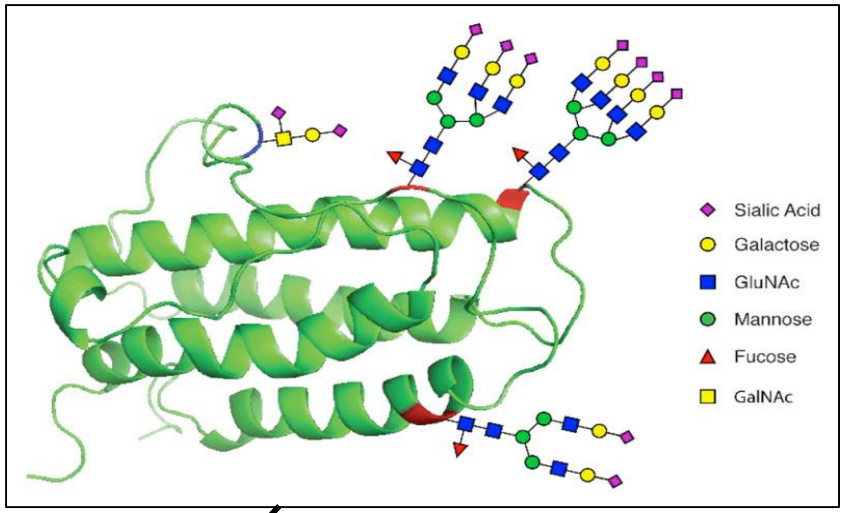
Mice	**TpoR+/+**	**TpoR-/-**
Platelets (X10^3 /ul) (PB)	938 ± 304	59 ± 13
Megacaryocytes (/10 hpf) (BM)	58 ± 8	5.5 ± 2
HSC	nle	Less
CFU-GM	nle	Less
CFU GEMM	nle	less
CFU-MK	nle	less

Epo/Tpo structure

Epo

- chr7q
- 165 aa
- 37,1Kd
- fortement glycosylée
  - (40% of its weight)
- 3 sites de N glycosylation
  - N24, N38, N83
- 1 site de O glycosylation
  - S126



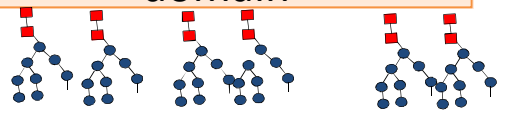


≅

receptor binding domain

Not present in Epo

C terminal Glycan domain



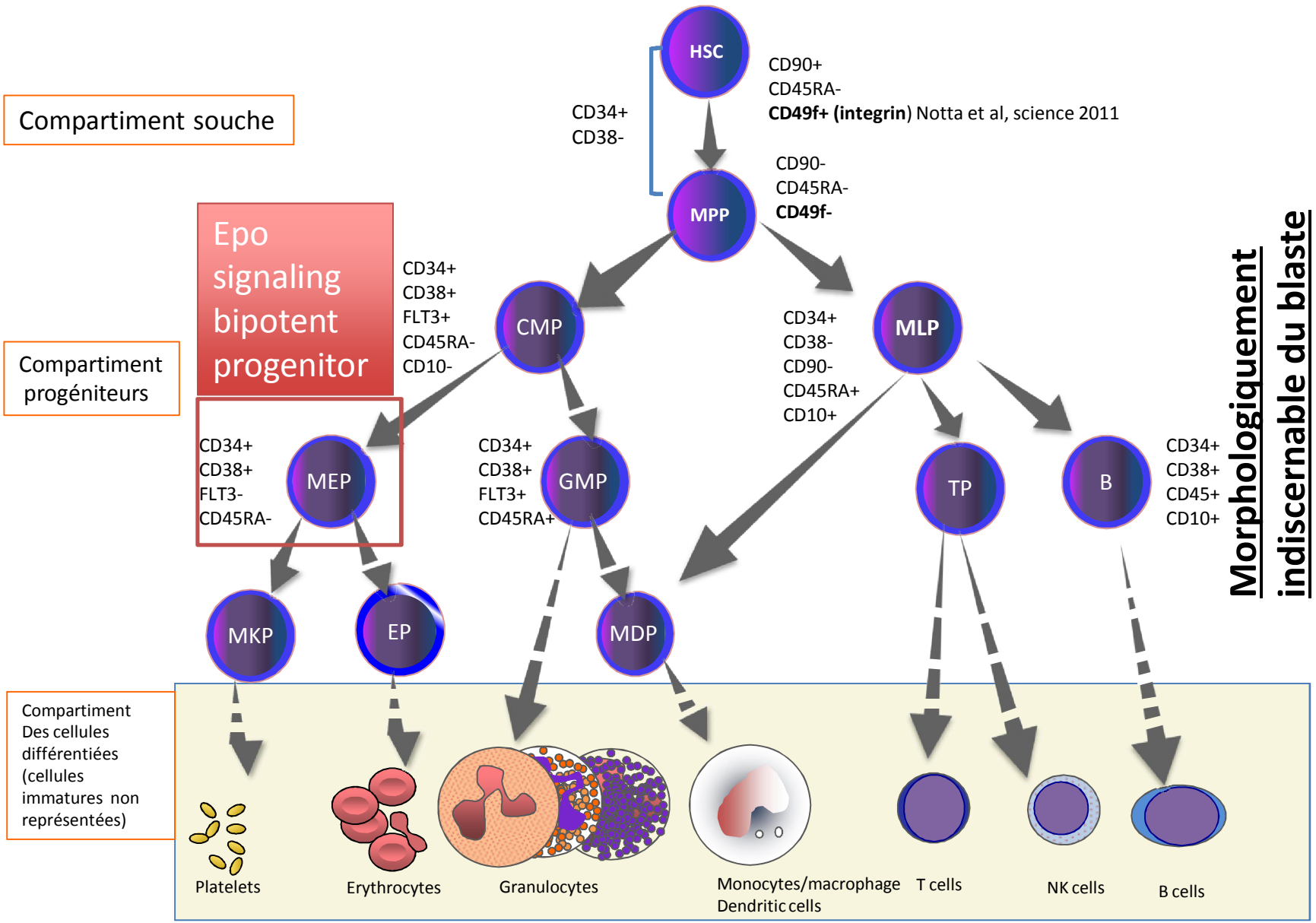
20% identity +
25% similarity with Epo

Tpo

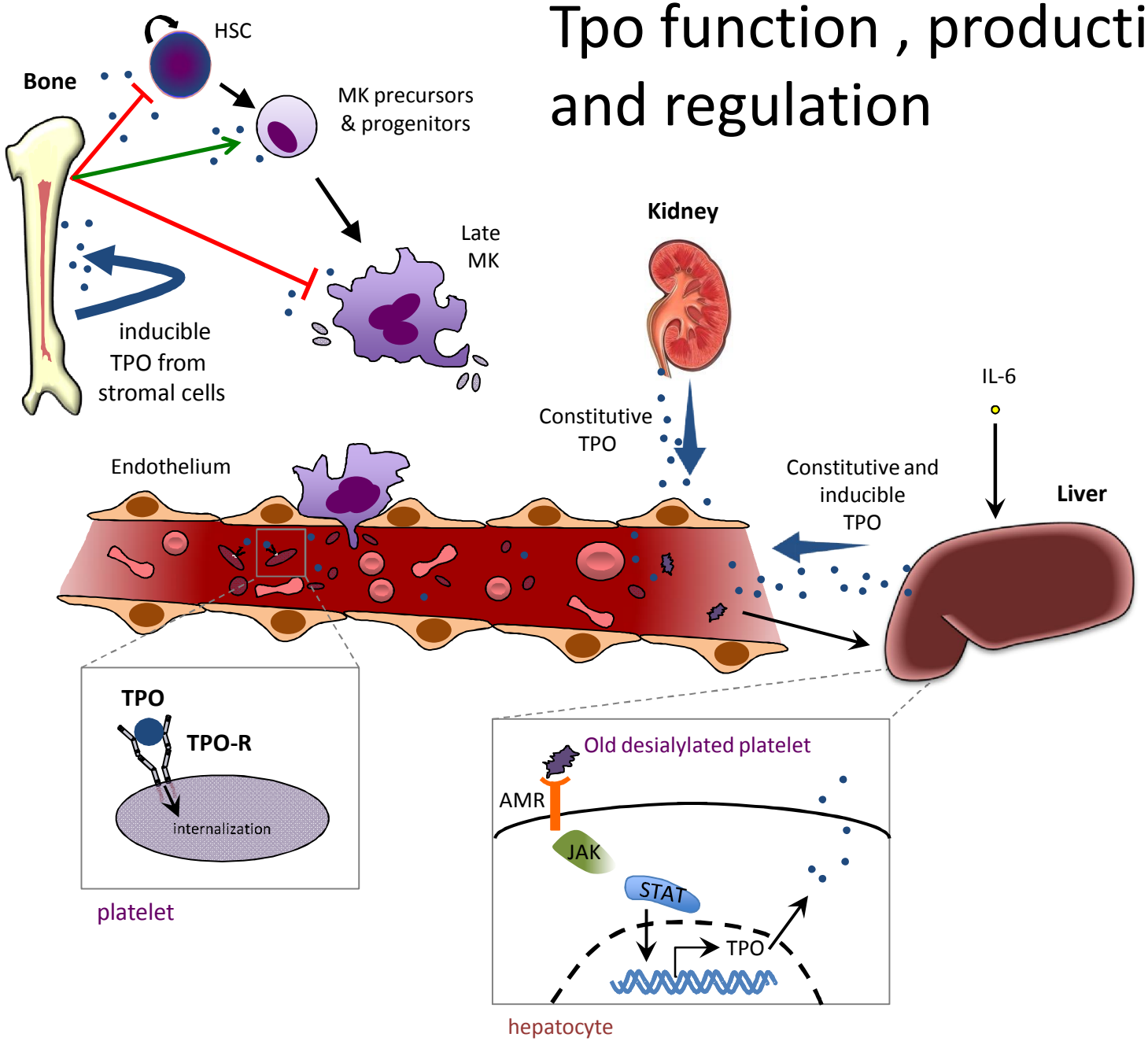
- chr3
- 332 aa
- 70Kd
- Fortement glycosylée en C-terminale

According to Kaushansky, Journal of Pediatric Hematology/Oncology: November 2003

Cross-Reactivity Between Erythropoietin and Thrombopoietin ?



Tpo function , production and regulation



Pas d'indication pour la thrombopoiétine

PLT

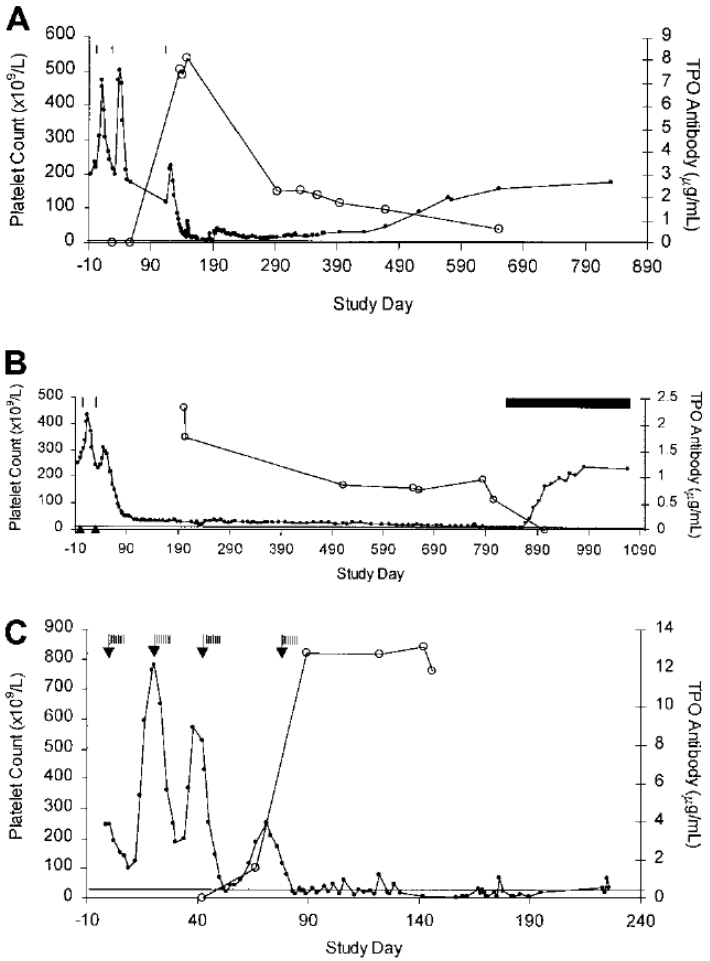
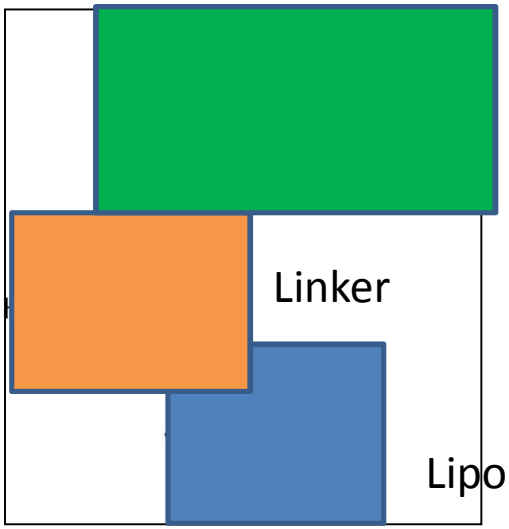


Figure 1. Platelet counts and anti-TPO antibody concentration. The platelet count (closed circles) is inversely related to anti-TPO antibody concentration (open circles) in thrombocytopenic subjects no. 1 (A), 2 (B), and 3 (C). PEG-rHuMGDF treatment is indicated by vertical lines and chemotherapy by the arrows. Horizontal line denotes normal cutoff of assay for anti-TPO IgG. In panel B, the solid bar indicates cyclosporine administration and solid triangles denote negative anti-TPO antibody screening tests. The anti-TPO antibody titer on day 56 for subject no. 1 (A) is slightly above the cutoff value.

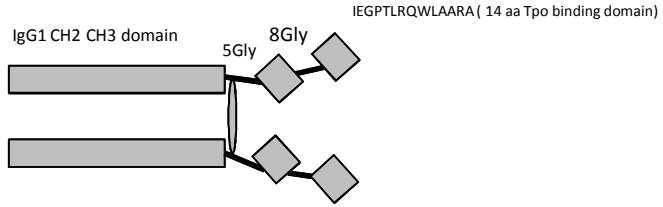
Anti thrombopoietin antibody

Junzhi Li *et al* Blood 2001

Agonistes non peptidiques de la thrombopoietine



Eltrombopag
SB-497115
442 Da small molecule
Oral

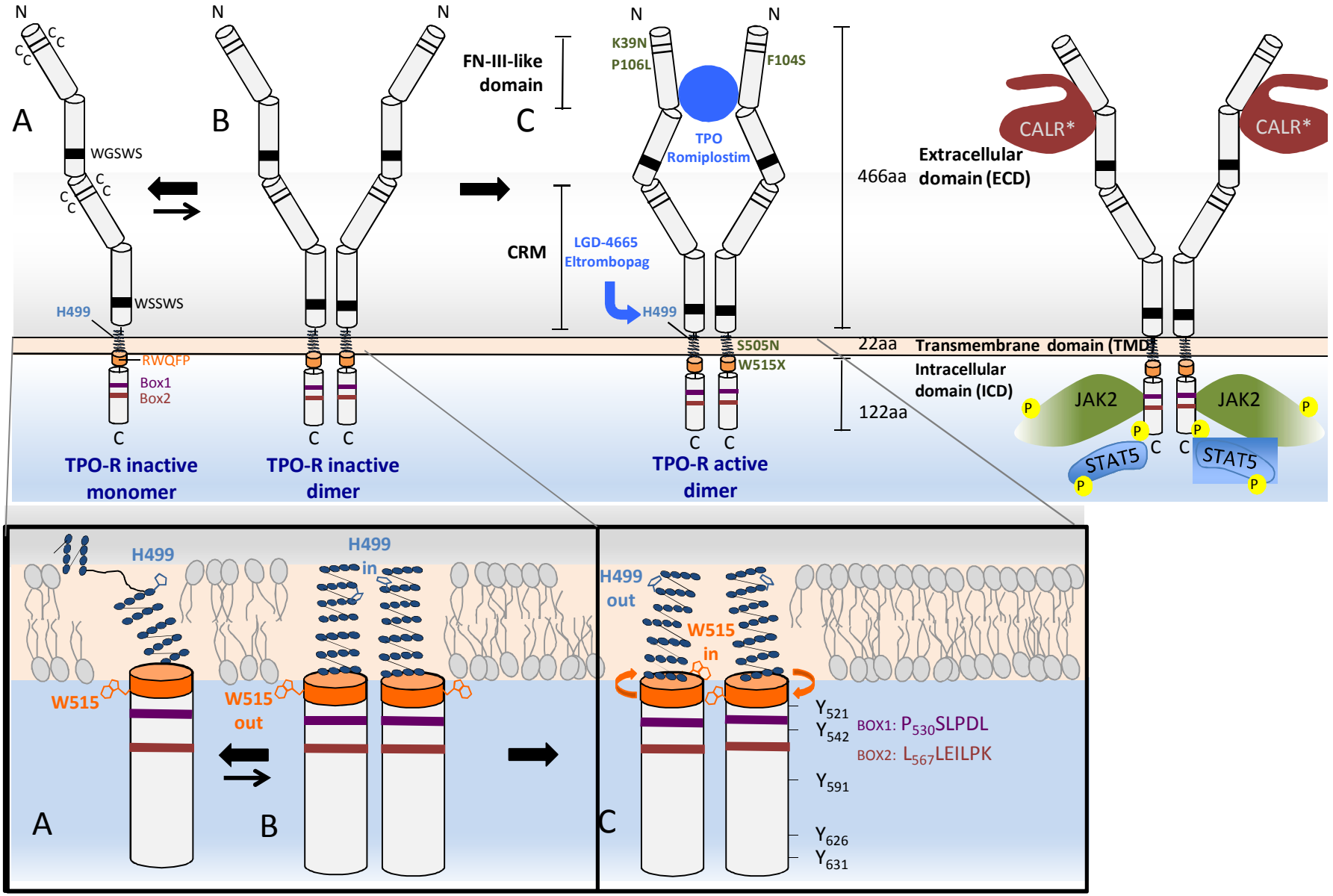


No sequence

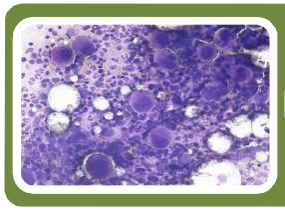
Fc-peptide fusion protein
(60kDa peptibody=peptide +antibody)

Romiplostim

subcutaneous

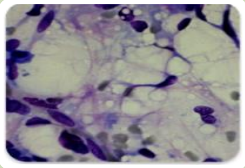

N
C
C
C
C
A
WGSWS
C
C
C
C
WSSWS
H499
RWQFP
Box1
Box2
C
TPO-R inactive monomer
B
N
N
C C
TPO-R inactive dimer
FN-III-like domain
C
CRM
LGD-4665
Eltrombopag
N
N
K39N
P106L
F104S
TPO
Romiplostim
H499
S505N
W515X
C C
TPO-R active dimer
466aa
Extracellular domain (ECD)
CALR*
CALR*
22aa
Transmembrane domain (TMD)
Intracellular domain (ICD)
122aa
JAK2
JAK2
P
P
P
P
C
C
STAT5
STAT5
P
P
H499
W515
A
H499 in
W515 out
B
H499 out
W515 in
C
Y521
Y542
Y591
Y626
Y631
BOX1: P530SLPDL
BOX2: L567LEILPK

Indication® des agonistes



PTI (PLT <30,000/µL splenectomisé ou CI)

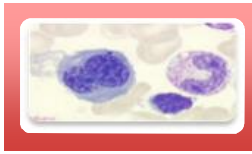
Revolade eltrombopag
Nplate Romiplostim



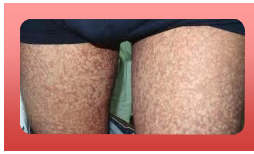
Anémie aplasique sévère (SAA) PLT <30,000/µL , >18a !

revolade eltrombopag only

Chronic liver disease (hepatitis C)



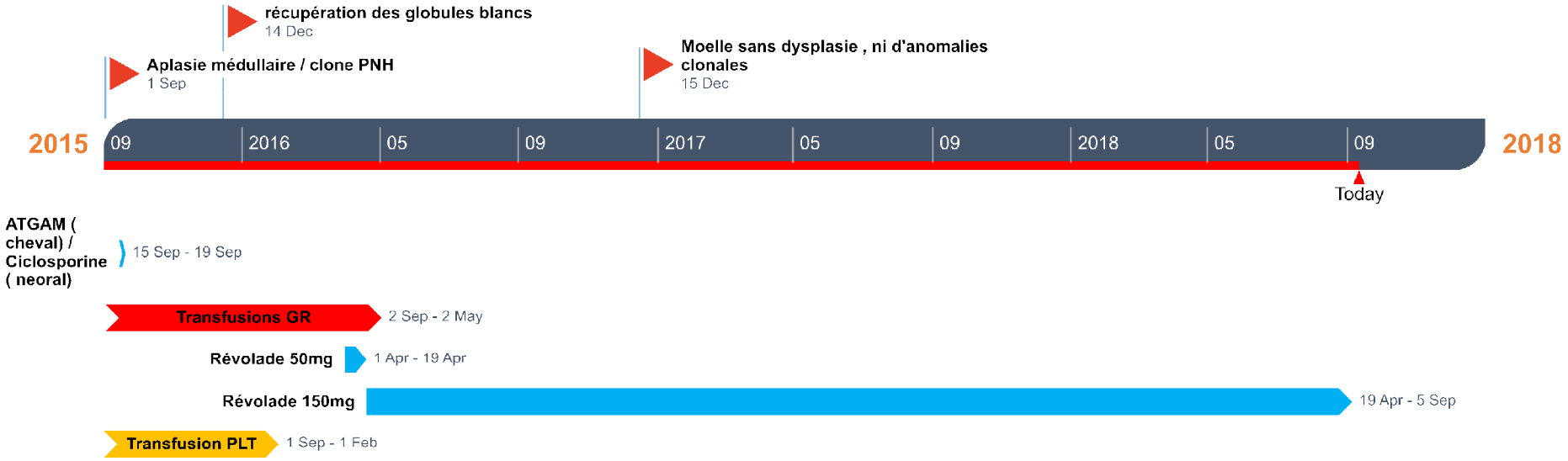
Myelodysplastic syndromes



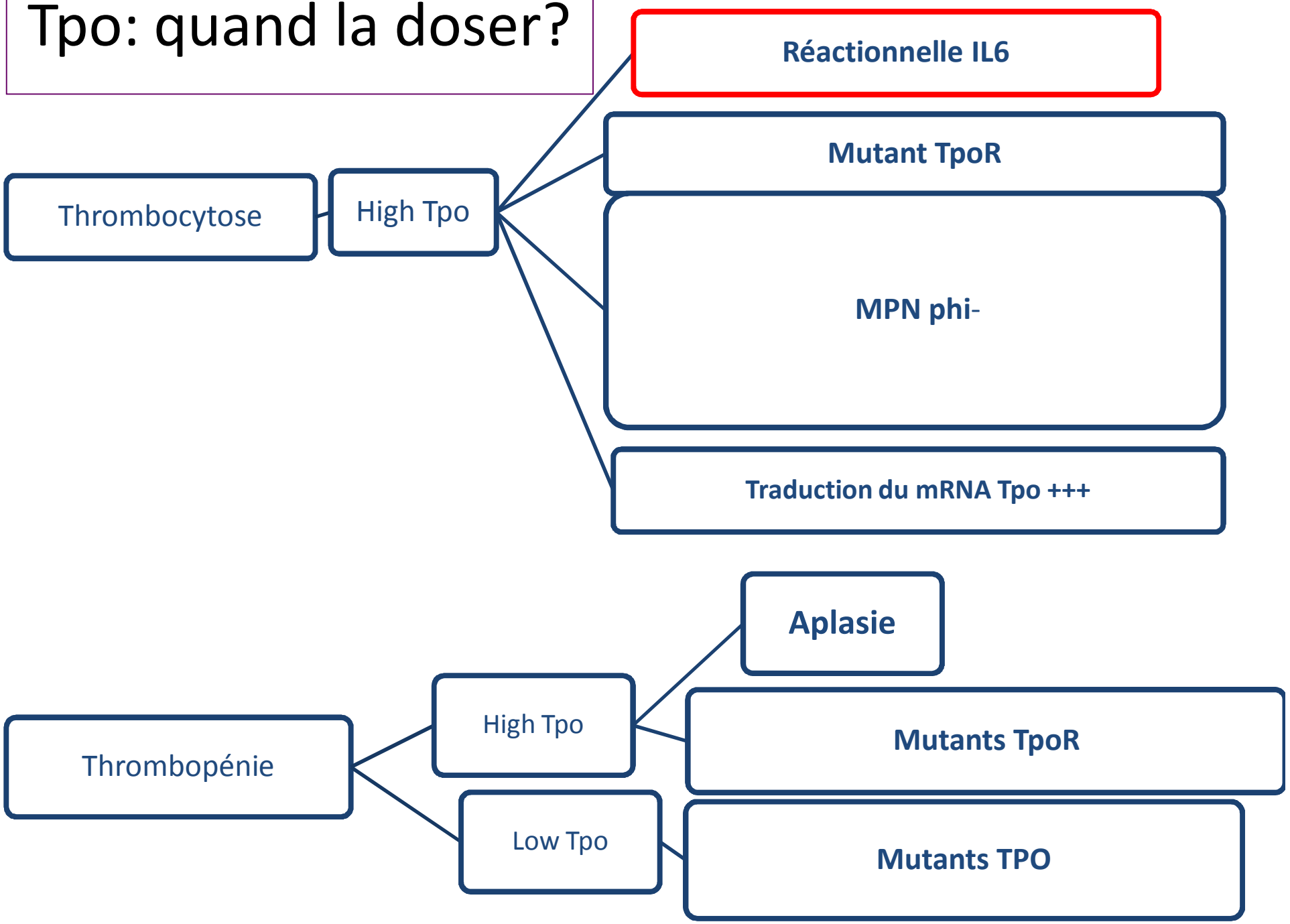
Congenital bone marrow failure (dyskeratosis, blackfan,…)

Therapy induced thrombocytopenia

High Tpo aplastic anemia responds to Eltrombopag (revolade)

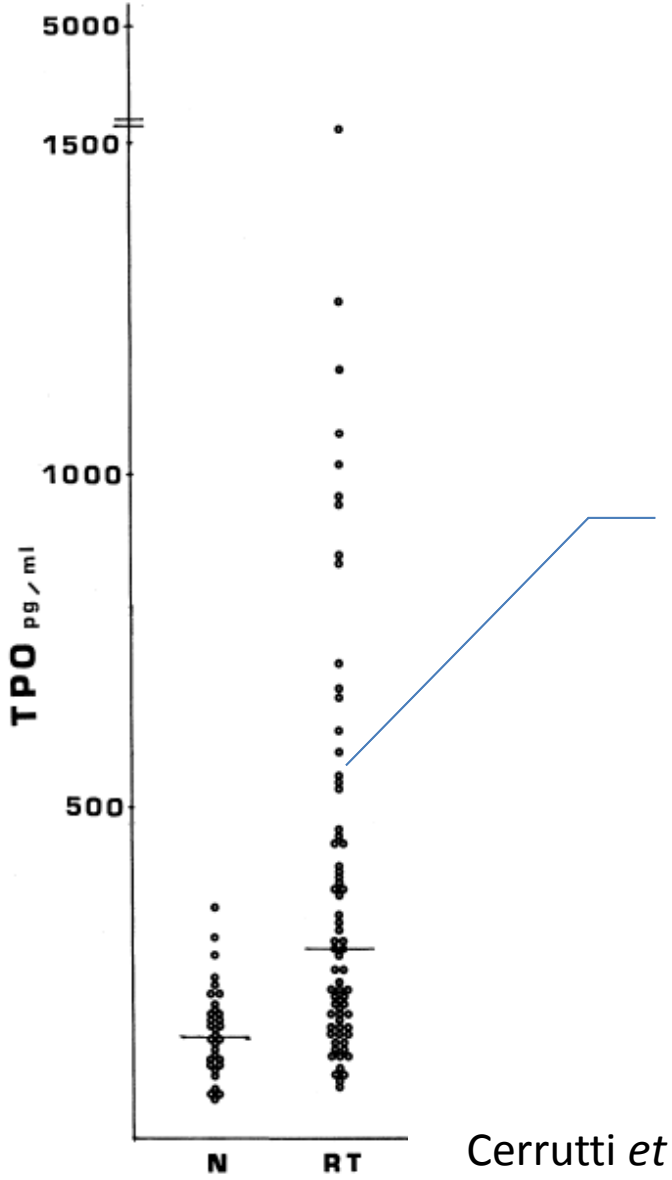


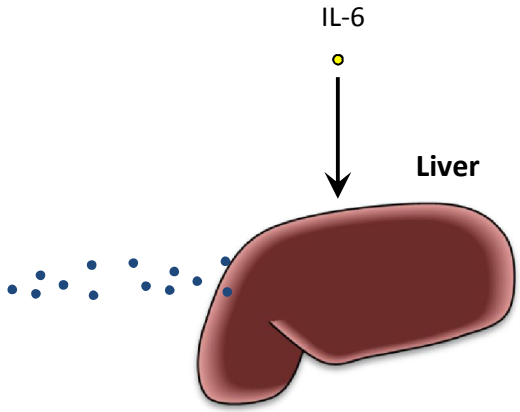
Tpo: quand la doser?



Thrombocytosis / High Tpo

Réactionnelle

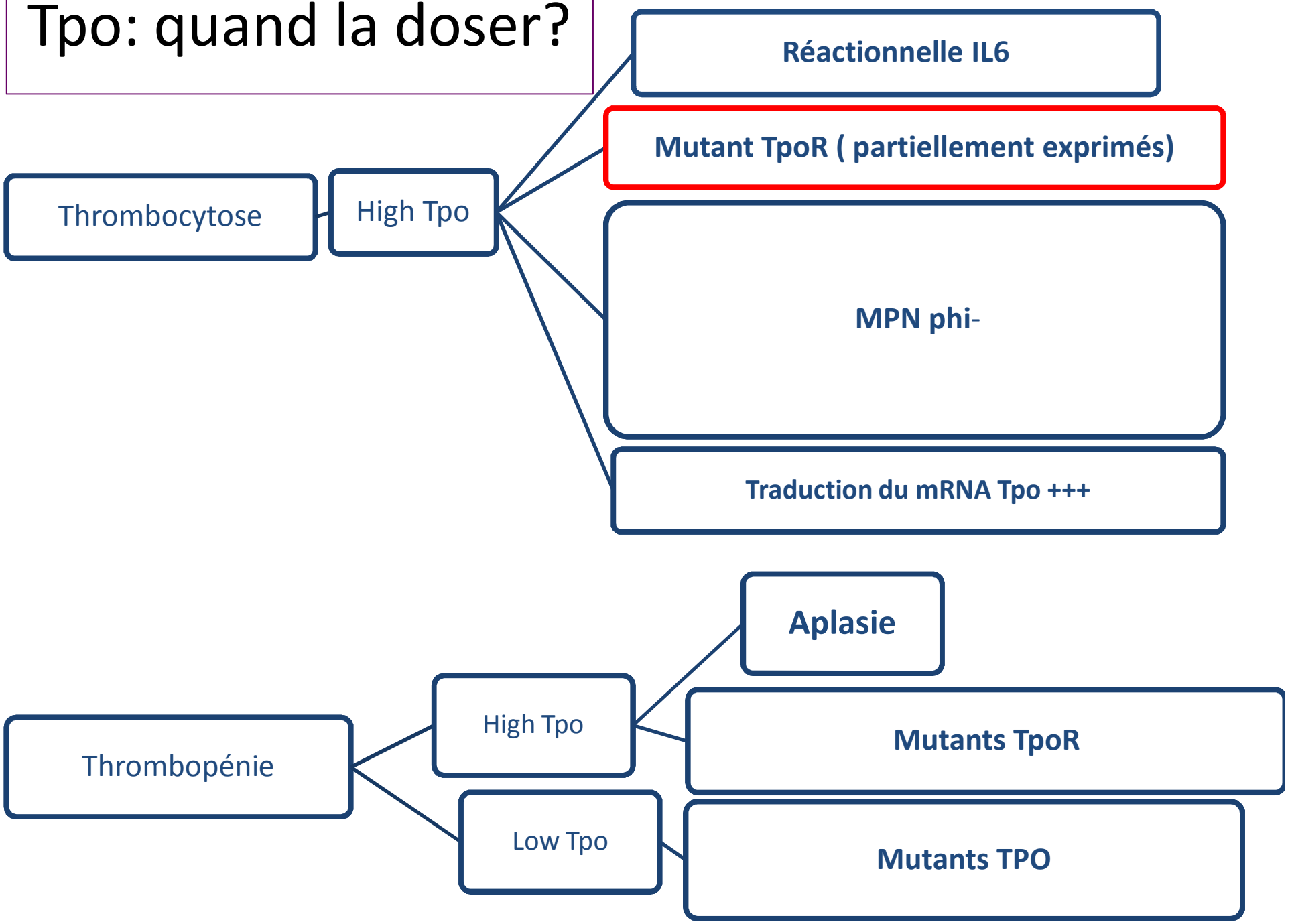




Tpo level (pg/ml)are increased in reactive thrombocytosis

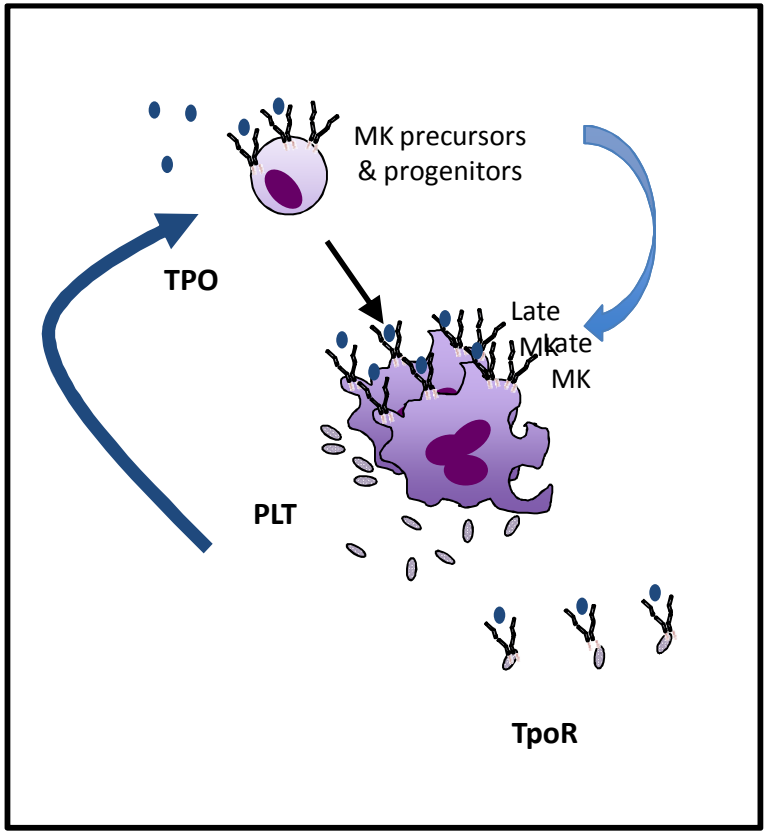
Cerrutti *et al.* British journal of haematology 1997

Tpo: quand la doser?

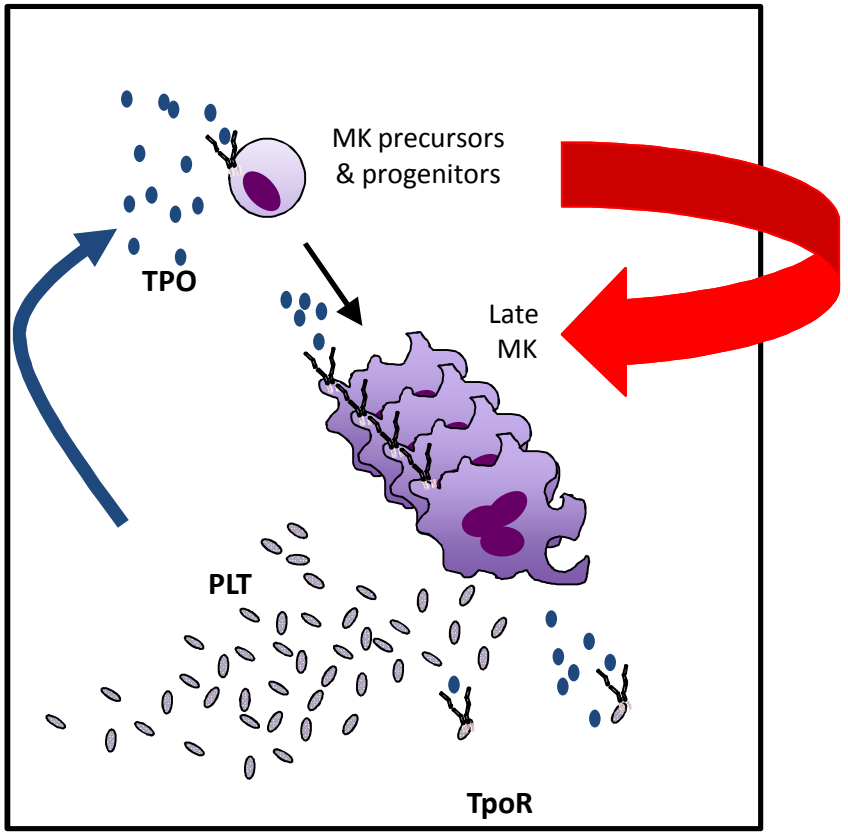


Thrombocytosis / High Tpo

Mpl WT

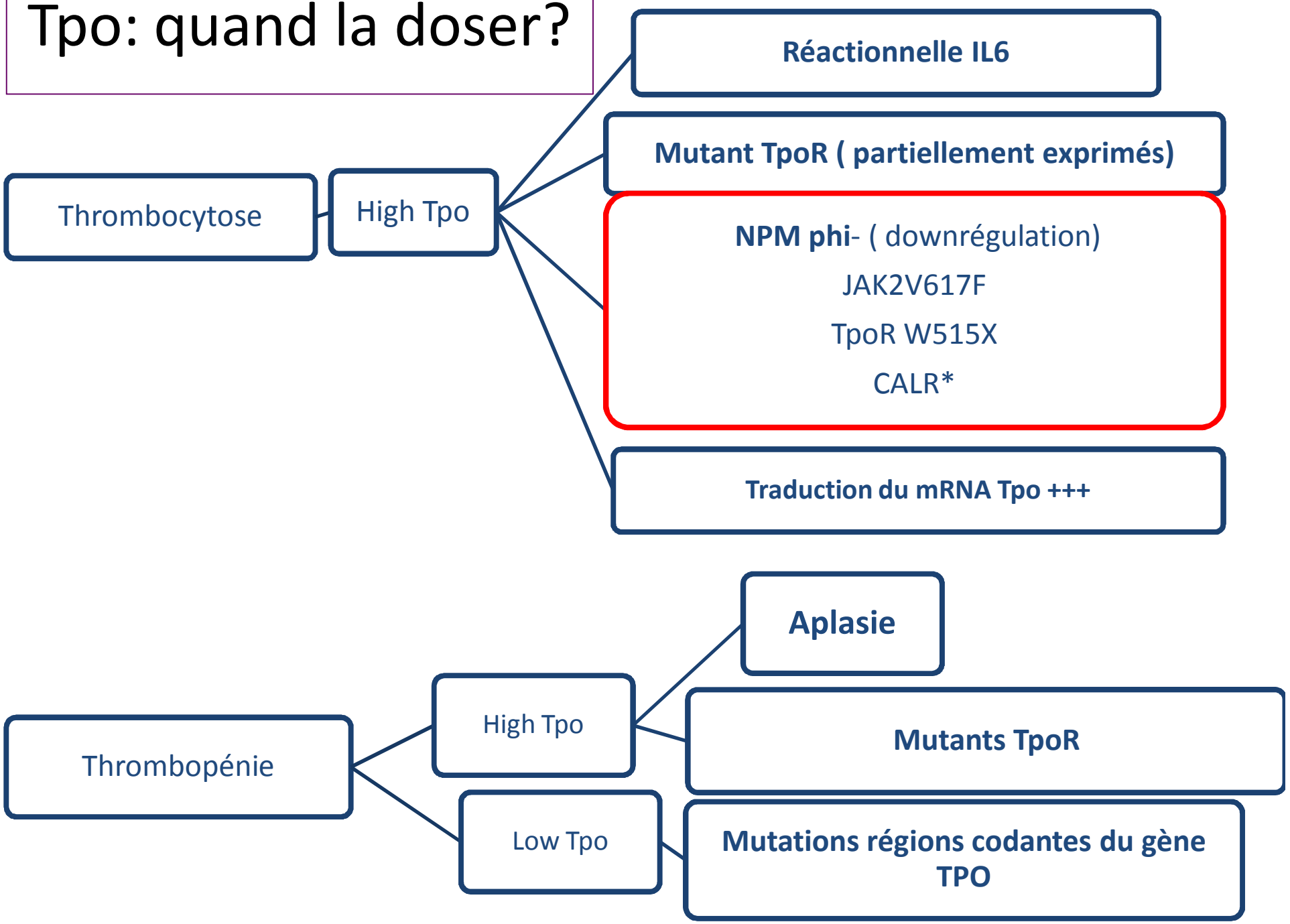


Mpl P106L / K39N

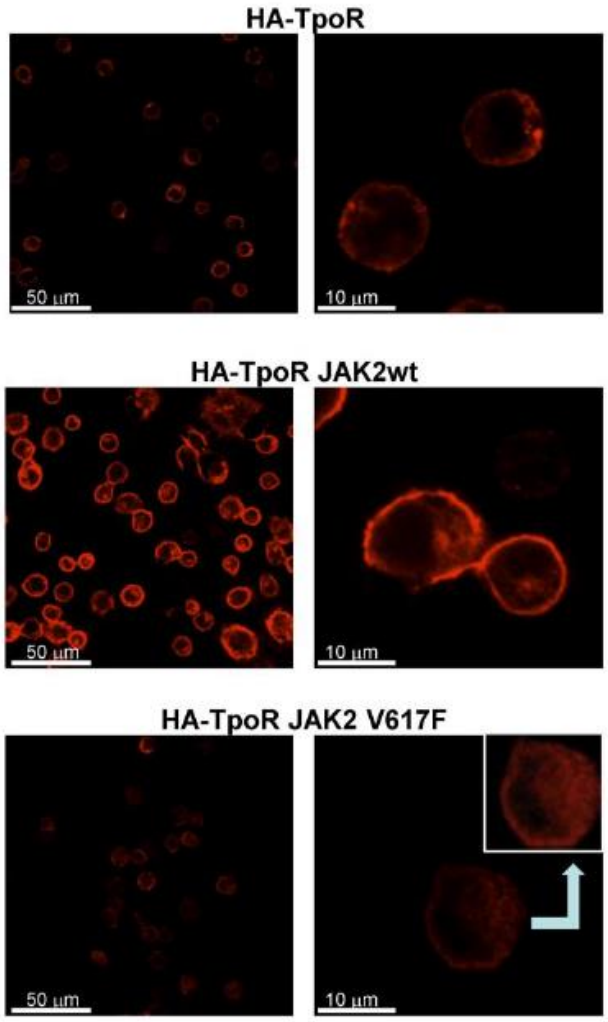


Adapted from Favale *et al* Blood 2016

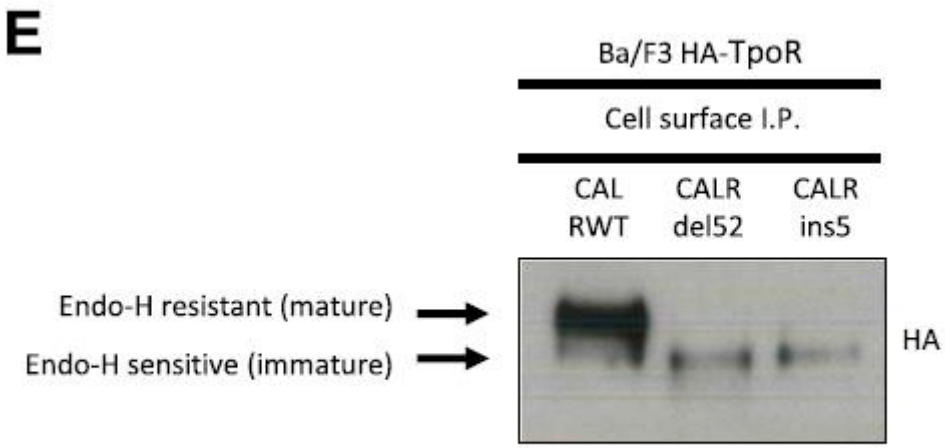
Tpo: quand la doser?



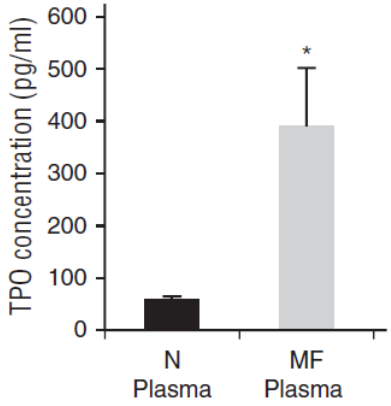
Both JAK2V617F and CALR induce a MPL trafficking defect ➔ More circulating Tpo



Pecquet *et al* Blood 2012

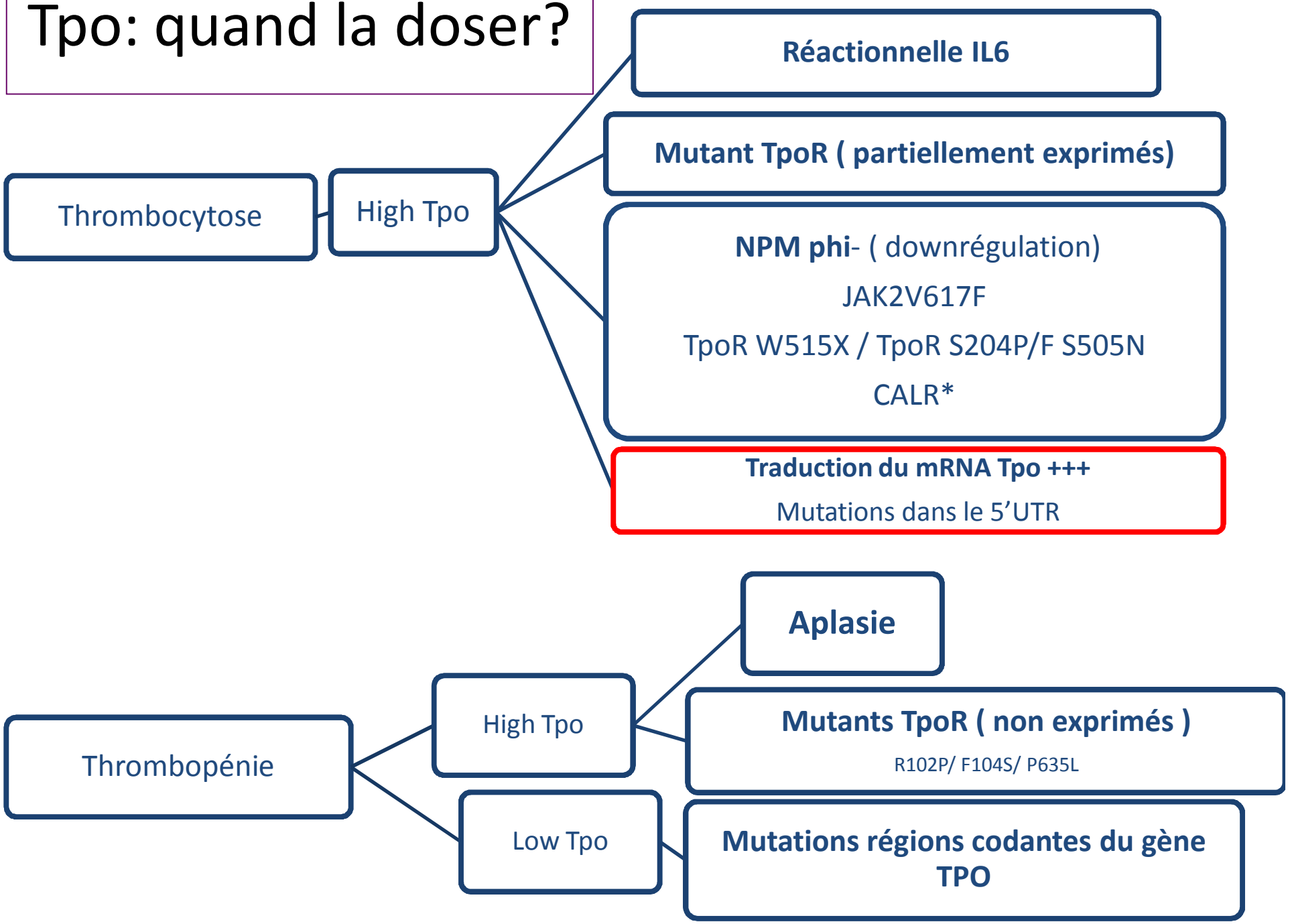


Chachoua *et al* Blood 2016



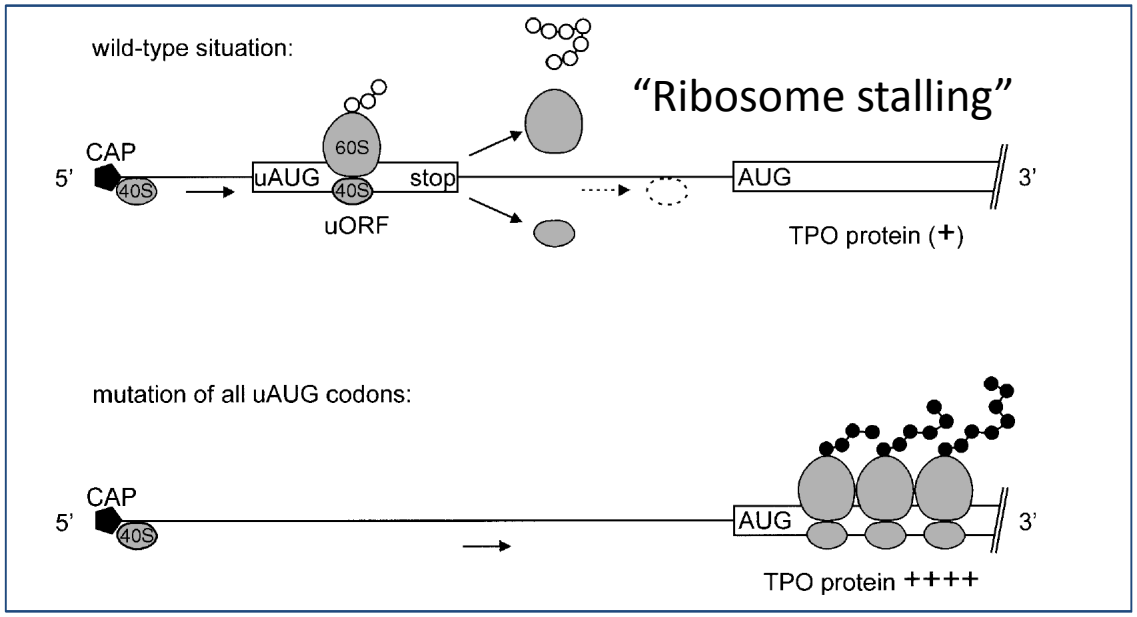
WANG et al BLOOD, 30 JUNE 2016

Tpo: quand la doser?



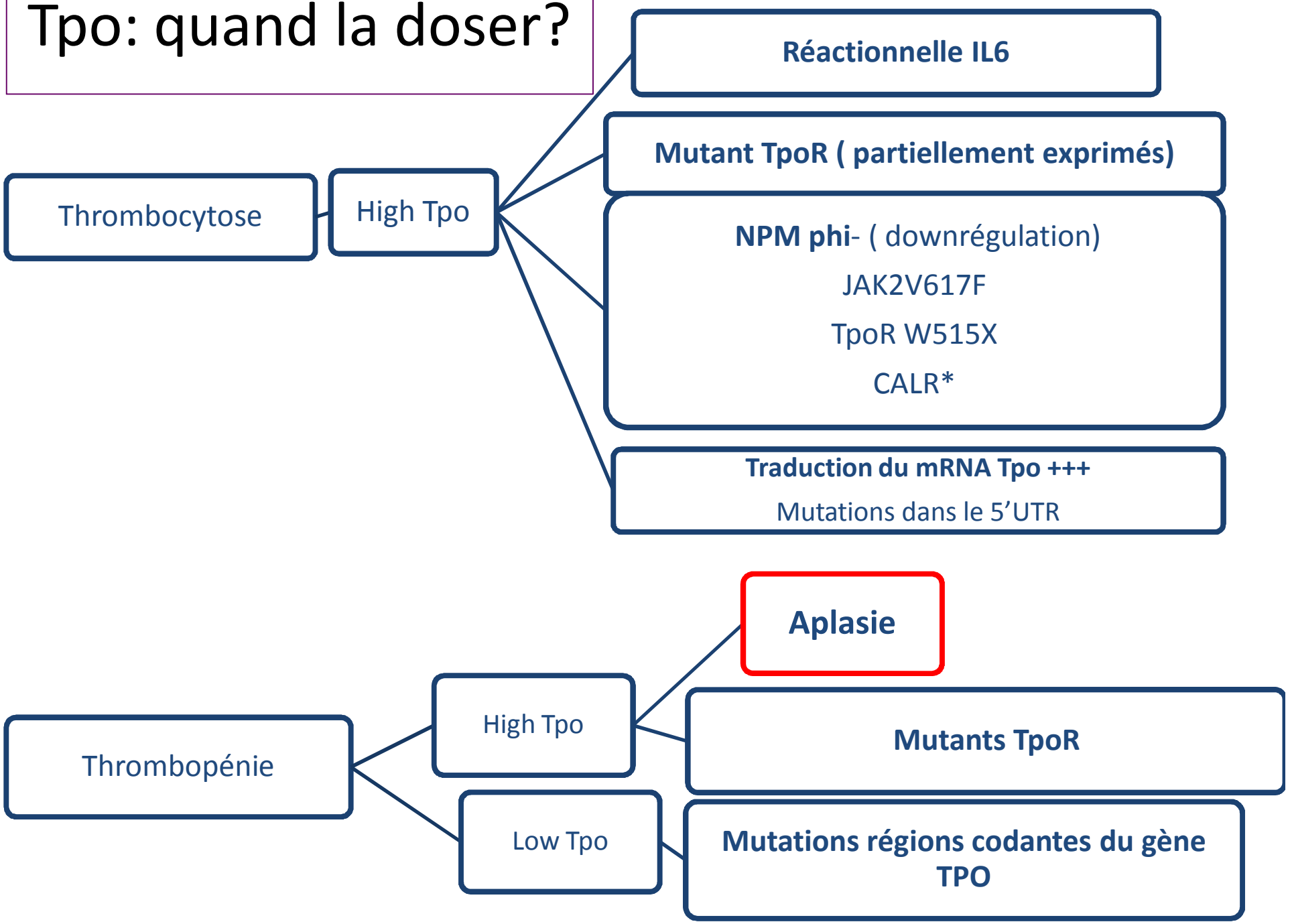
Derepression of Tpo mRNA translation Causes Familial Essential Thrombocythemia

Authors	Paper	Year	Gene mutation	Consequence	
Wiestner	Nat Genet	1998	Tpo G>C in intron 3	Loss of uORF-mediated repression	
Kondo	Blood	1998	Tpo deletion of G in 5'UTR	Loss of uORF-mediated repression	
Jorgensen	Blood	1998	TpoA>G	not studied.	
Ghilardi	Blood	1999	Tpo deletion of G in 5'UTR	Loss of uORF-mediated repression	7-fold more TPO



Ghilardi and skoda Blood 1998 and 1999

Tpo: quand la doser?



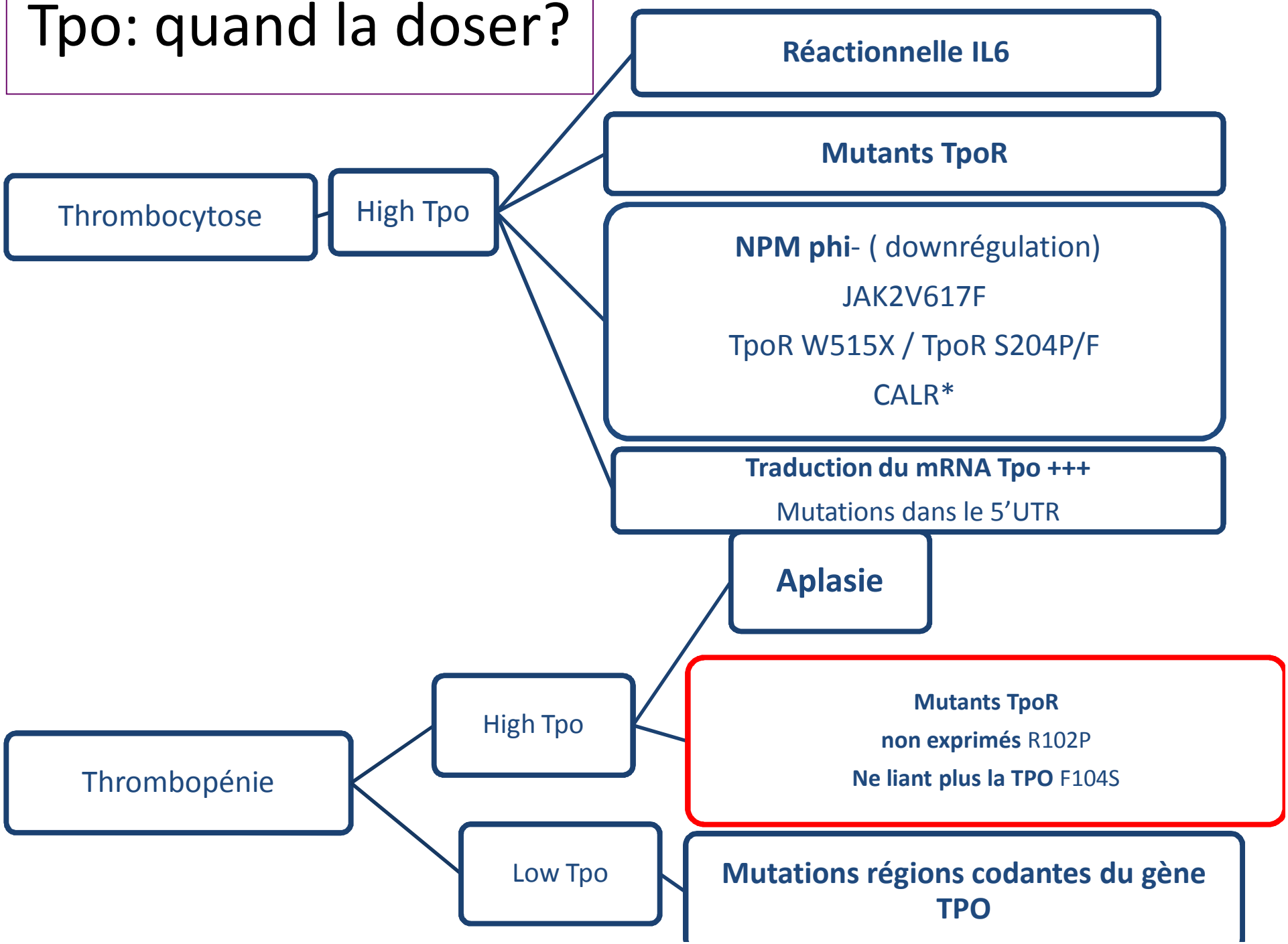
Anémie aplastique /bone marrow failure

Subject characteristic	Very severe AA	Severe AA	Non severe AA	Blood donors
Number	11	23	18	45
Median age in years (range)	19 (11-58)	29 (11-75)	30 (12-54)	33 (23-46)
Gender (M/F)	10/1	19/4	15/3	40/5
Median TPO in pg/ml (range)	2,765 (625-6 541)	1,190 (672.1-7 651)	1,111.5 (761.1-2 289.2)	121.1 (81.25-237.7)
Median platelet count × 10^9/l (range)	14 (7-34)	18 (9-24)	27 (14-36)	193 (130-332)

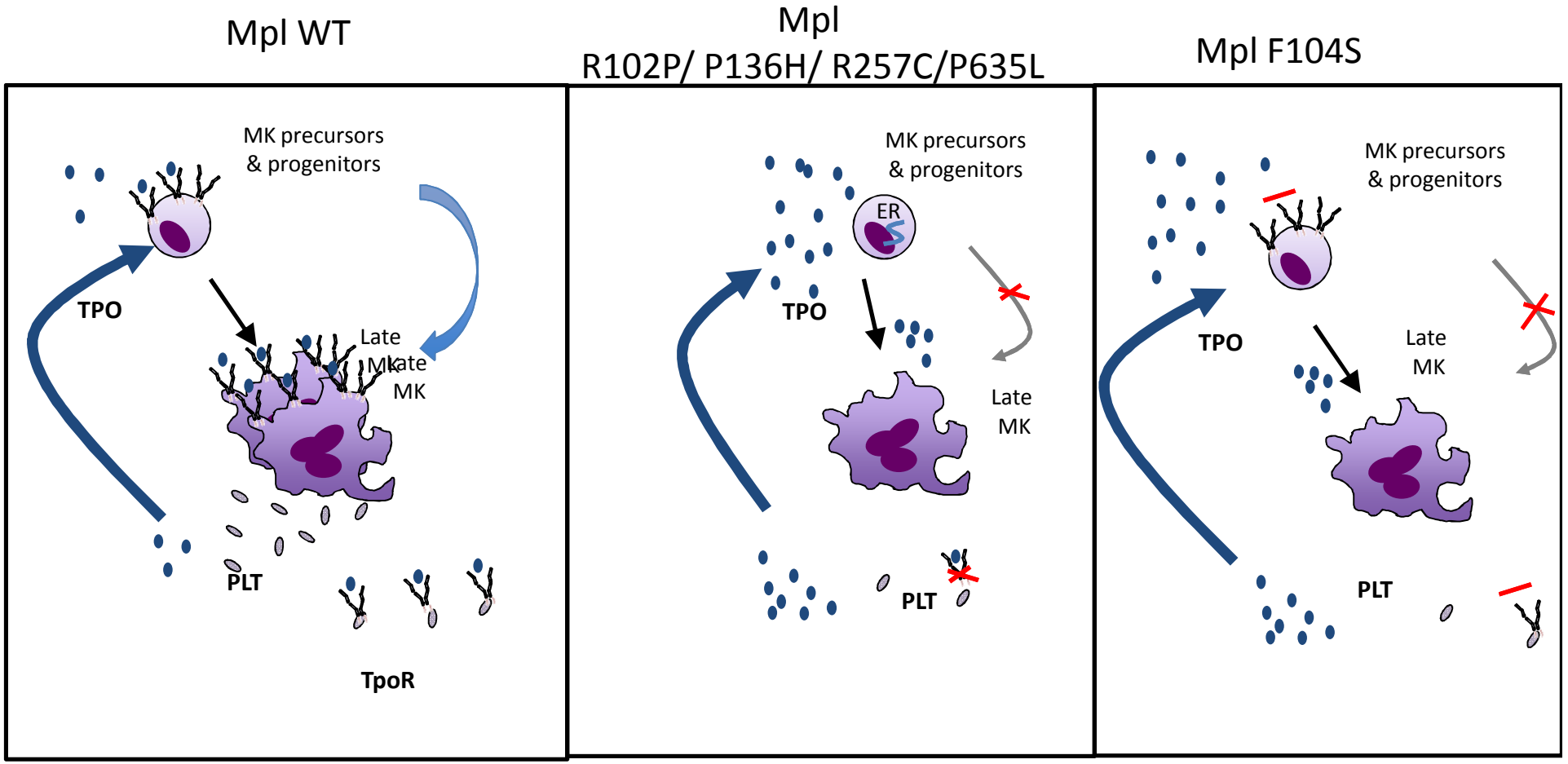
Singh et al. *Asian Journal of Transfusion Science*. 2015

TPO levels in aplastic anemia patients were significantly higher than in healthy blood donors; however, in aplastic anemia patients TPO levels were significantly higher only in patients with very severe disease.

Tpo: quand la doser?

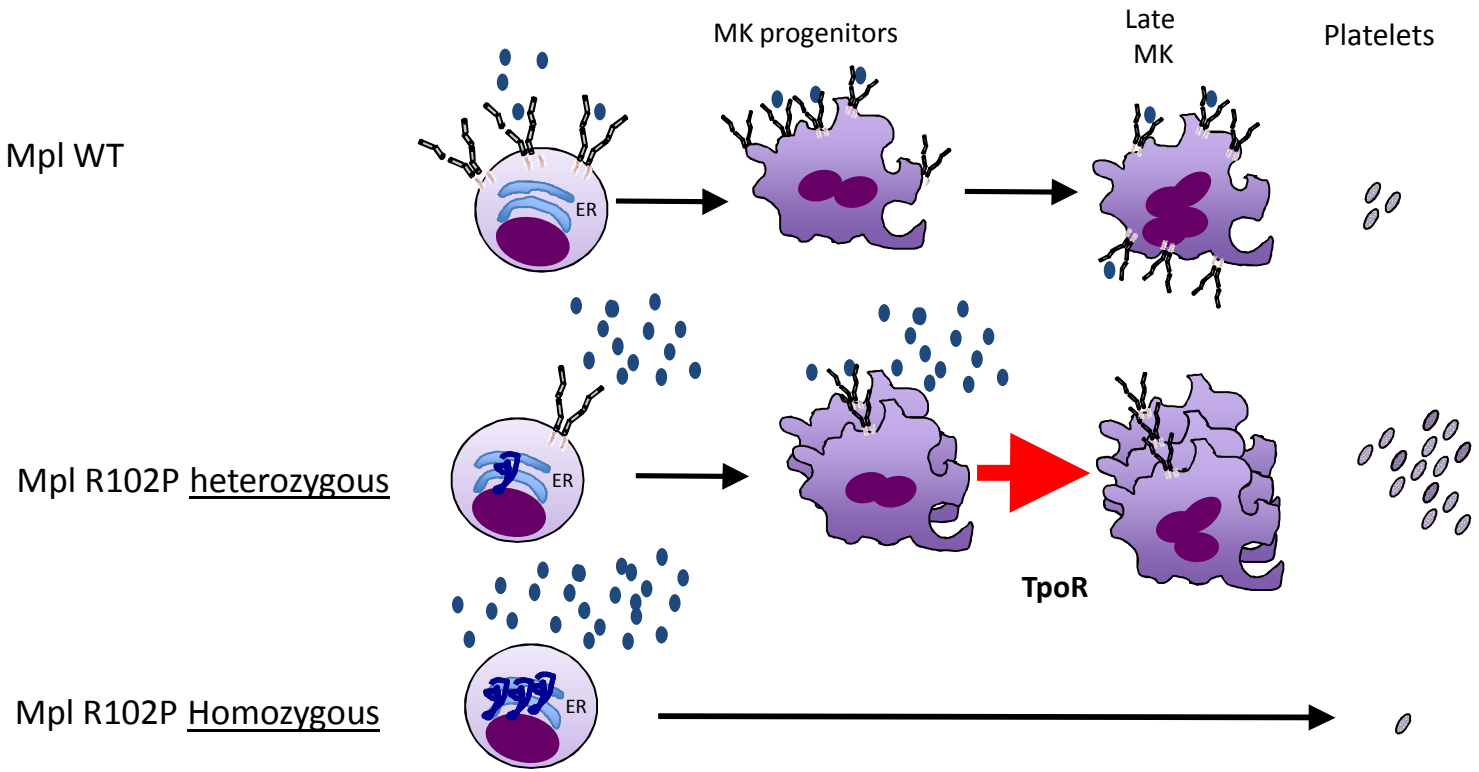


Thrombocytopenia / High Tpo



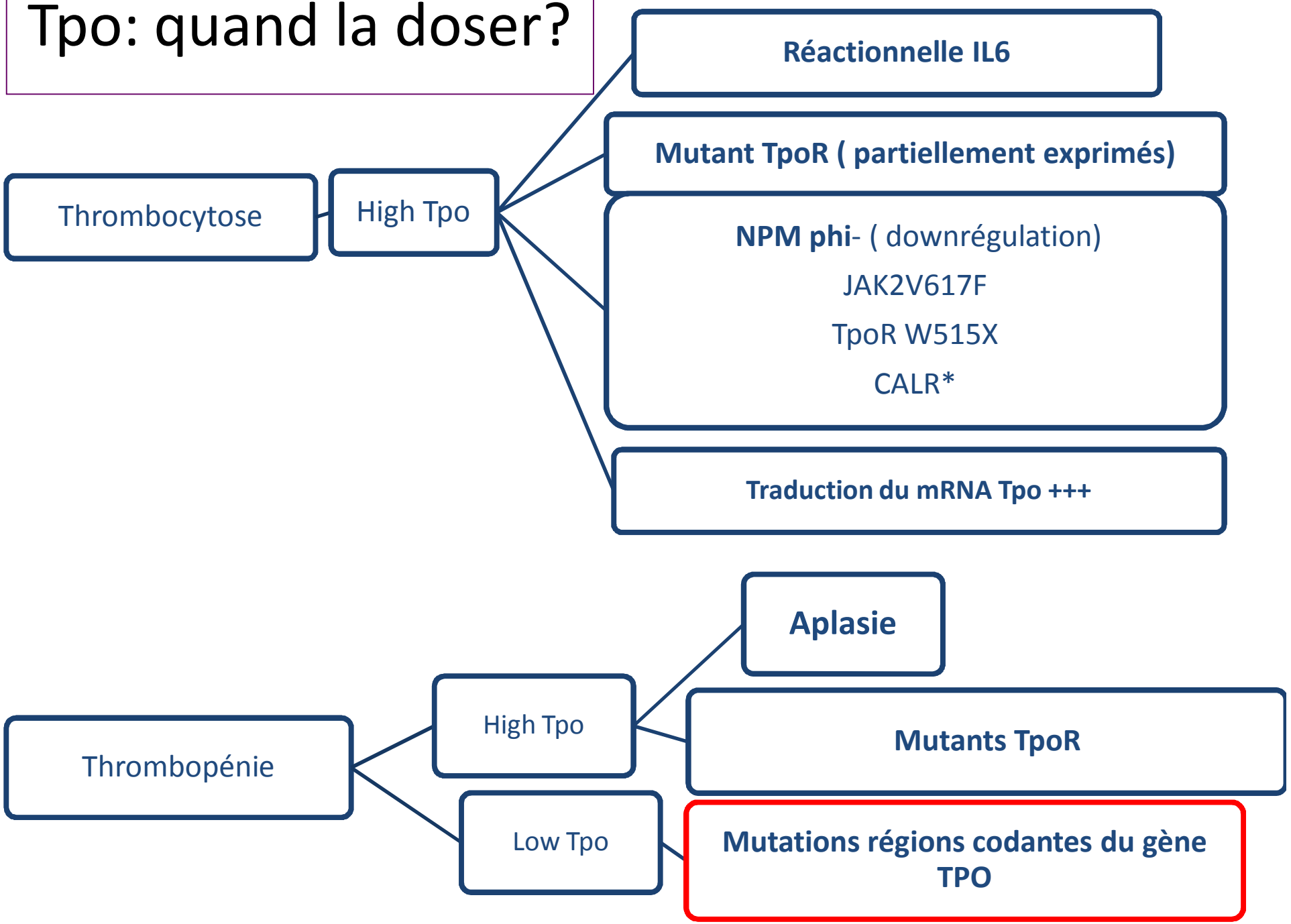
Adapted from Favale *et al.* Blood 2016 / Fox *et al.* Exp Hematol 2010/ Tijssen Br J Heamatol. 2008

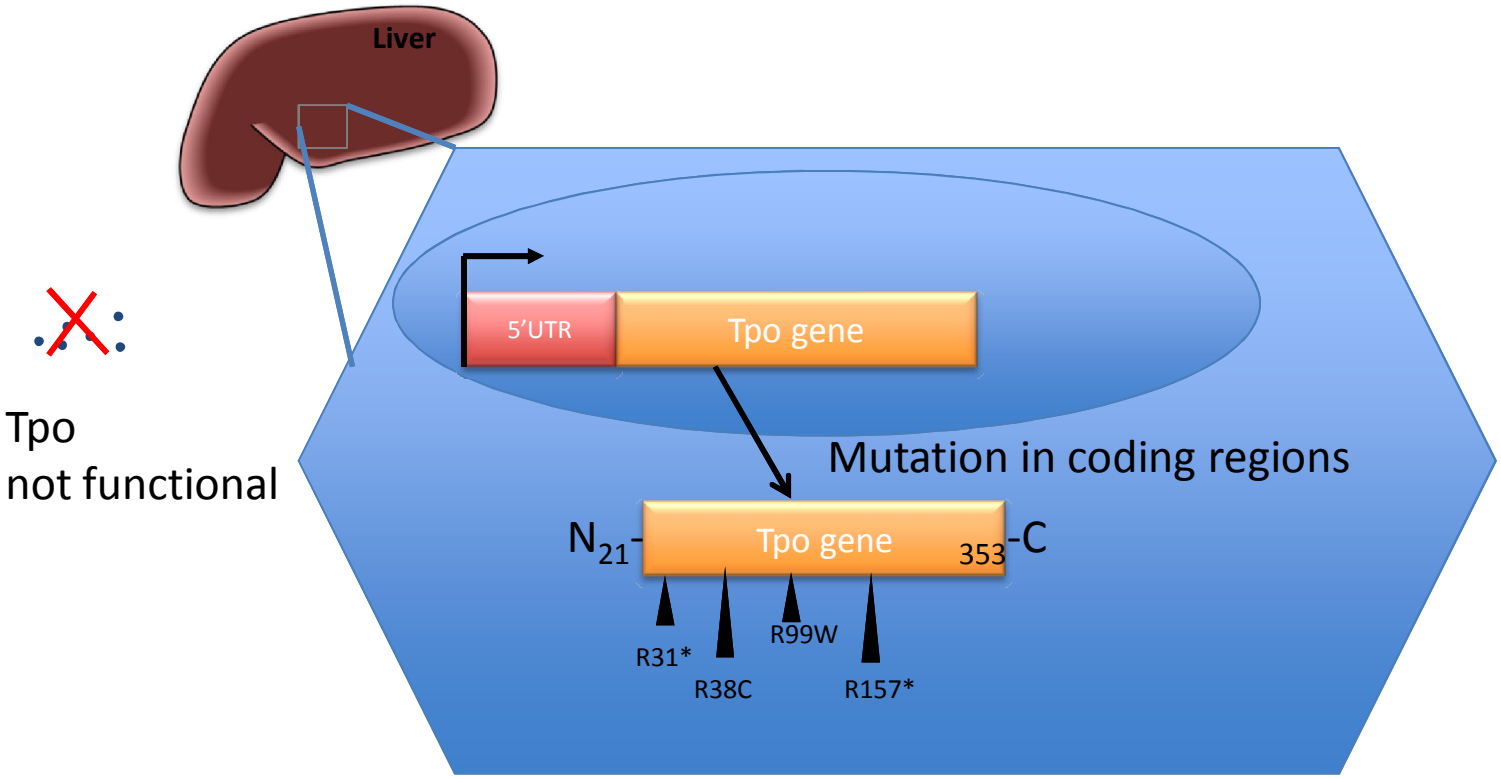
Homozygote vs hétérozygote



Adapted from Christine Bellané chantelot Front endocrinology 2017

Tpo: quand la doser?





adapted from Plo et al Front endocrinology 2017

Merci pour votre attention

Acknowledgments

Stefan N. Constantinescu **Ludwig Cancer Research Brussels**

Damien Gruson **Cliniques Universitaires Saint Luc Bruxelles**

Jean-Michel Cirriez Cliniques Notre Dame de Grâce Gosselies

Anne qui garde les enfants!

RhuEpo cross reactivity

Recombinant EPO Cross-reactivity Study Results.

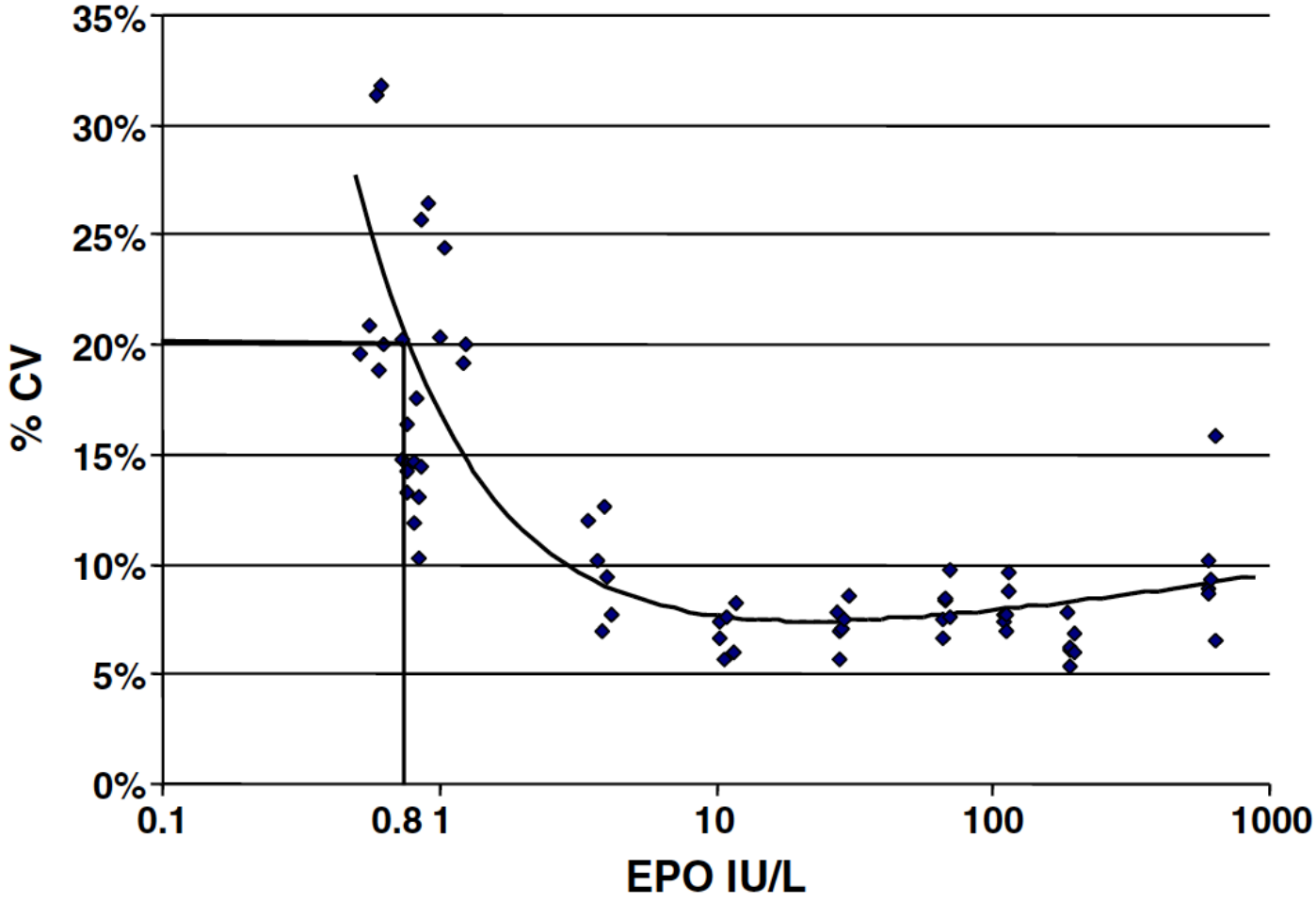
	Immulite 2000 Mono-Mono Assay Diluted Sample IU/l	Immulite 2000 Mono-Mono Assay Corrected for Dilution kIU/l	Access 2 Diluted Sample IU/l	Access 2 Corrected for Dilution kIU/l
Epoetin-Alfa 2000 kIU/l				
1:1000	>750	ND[a]	>824	ND
1:2000	541	1082	650	1300
1:10,000	99.1	991	128	1280
1:20,000	48.5	970	63	1260
1:40,000	23.6	944	32	1280
Mean (% Recovery)		997 (49.9)		1280 (64.0)
Darbopoetin alfa 25 mg/l (4150 kIU/l)[b]				
1:1000	>750	ND	>824	ND
1:2000	337	674	579	1158
1:10,000	62.0	620	102	1020
1:20,000	27.0	540	48	960
1:40,000	13.0	520	24	960
Mean (% Recovery)		588 (14.2)		1024 (24.7)

[a] Not determined.
[b] 166 IU/μg × 25 mg/l [4].

reduced recovery of darbopoetin with the new Immulite 2000 EPO assay

Owen et al Clinica Chimica Acta (2011)

Limit of quantification of the Immulite Siemens Epo Assay



Owen et al clinical biochemistry 2014